Gonadal growth and regression in Japanese quail (*Coturnix coturnix japonica*) and the effect of gonadotropin-releasing hormone (GnRH) on luteinising hormone (LH) and ovarian growth

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This is to certify that the research carried out for my doctoral thesis entitled: "Gonadal growth and regression in Japanese quail (*Coturnix coturnix japonica*) and the effect of gonadotropin-releasing hormone (GnRH) on luteinising hormone (LH) and ovarian growth" in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Turitea Campus, Palmerston North, New Zealand is my own work and that the thesis material has not been used in part or in whole for any other qualification.

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Certificate of regulatory compliance

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(a) is the original work of the candidate, and reference to work other than that of the candidate, has been appropriately acknowledged by appropriate attribution in the text and/or in the acknowledgements;

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Abstract

Improvements in breeding success are needed for conservation of endangered birds such as the New Zealand kakapo. A potential method to stimulate breeding is treatment with exogenous hormones. Hormone treatment is used in captive breeding programmes for endangered mammals but reliable techniques are not available for birds. Gonadotropin releasing hormone (GnRH), the principal hormone controlling reproduction, has been used to induce ovarian growth and ovulation in seasonally anoestrous mammals. The goal of the research in this thesis was to determine the potential of GnRH for hormone treatment in birds.

The Japanese quail (*Coturnix coturnix japonica*) was used in the current research. Female quail held outdoors with male quail and with access to nesting materials and nest sites showed clear seasonal patterns in the width of the cloacal opening (indicative of oviduct development) and FSH concentrations, whilst LH concentrations were low during winter and increased during spring and summer. Prolactin concentrations were elevated in birds incubating eggs in nests and birds caring for young. Photoperiodically induced gonadal growth and regression were described in detail for male and female quail under controlled conditions. Testicular and ovarian growth was preceded by increased LH and FSH concentrations and accompanied by increased gonadal steroid concentrations.

Administration of various types of GnRH stimulated luteinising hormone (LH) secretion in sexually regressed female Japanese quail. LH responses to cGnRH-II were greater than those to cGnRH-I. Low doses of buserelin stimulated similar LH responses to cGnRH-II, whilst high doses of buserelin and D-Lys^{6}Trp^{7}Tyr^8-GnRH induced sustained LH secretion. Single daily injections of various doses of cGnRH-II, buserelin or D-Lys^{6}Trp^{7}Tyr^8-GnRH in saline or polyvinylpyrrolidone (PVP) did not induce elevated baseline LH or stimulate ovarian growth. Repeated injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH did not increase LH concentrations over a short-term period. Continuous infusion of D-Lys^{6}Trp^{7}Tyr^8-GnRH by osmotic mini-pump severely blunted the LH response and did not stimulate ovarian growth.

Future studies using quail exposed to marginally stimulatory photoperiods will offer the opportunities to determine the effects of GnRH in birds under conditions which mimic photoperiod changes during the breeding season. Further studies on the potential development of a hormone treatment programme will continue to offer a promising future for endangered avian species including the New Zealand kakapo.

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1. General Introduction and Literature Review

Seasonal reproduction in birds is regulated by physiological mechanisms that are common among avian species, although the timing of breeding may vary between species and between individuals of the same species. Reproductive activity and/or egg laying does not occur every year in the severely endangered New Zealand kakapo (*Strigops habroptilus*), although successive breeding seasons have been documented (Cockrem, 1995). The reasons for the infrequent breeding seasons are not known and a comprehensive conservation effort is currently being pursued in order to increase breeding frequency and productivity of breeding attempts (Cresswell, 1996). One possible method for improving the breeding success of the kakapo is to develop a programme whereby reproductive output is increased using exogenous reproductive hormones. Programmes administering exogenous hormones have been successfully implemented in breeding programmes for endangered mammalian species. The aims of the research presented in this thesis were to determine the seasonal gonadal and endocrine patterns of the model species, the New Zealand strain of the Japanese quail and to determine whether an exogenous GnRH treatment regime would stimulate ovarian growth in non-reproductive quail.

1.1 Photoperiodic control of reproduction in birds

1.1.1 Seasonality

Reproduction in most temperate zone avian species occurs during spring and summer and is mediated by both neural and endocrine responses to environmental stimuli. Environmental factors that control seasonal breeding have been classified as ultimate

and proximate factors (Baker, 1938). Ultimate environmental factors are those that select individuals that produce young at the optimum time for survival. The main ultimate factor in birds is the availability of food for the feeding of young and post-fledgling survival (Wingfield, 1983). Proximate environmental factors are those that regulate the timing of breeding so that the probability of the survival of young is high. Wingfield (1983) divided proximate factors into four categories: initial predictive information, supplementary information, synchronizing and integrating information and modifying information.

Initial predictive information causes the physiological preparation of birds to breed in anticipation of the ensuing breeding season. This information initiates gonadal development but does not on its own induce the nesting or egg laying phases of reproductive activity. Annual variation in photoperiod is the primary source of initial predictive information for birds outside of the tropics (Follett, 1984) and provides a constant basis by which reproductive activity is timed. Other initial predictive information for birds can be provided by endogenous circannual rhythms of reproductive growth and regression, and rainfall.

Supplementary information causes the fine tuning of reproduction to local conditions and takes cues from factors such as the establishment and possession of a territory, social interactions and the availability of specific food sources. Once nesting activity has been initiated synchronizing and integrating information are utilised in order to regulate the sequence of breeding activities. Nesting activity involves a series of physiological and behavioural events such as nest building, mating, oviposition, incubation and rearing of young which must be regulated for breeding to be successful. Modifying information includes a wide range of factors known to disrupt activity at any stage of the reproductive cycle such as predation, loss of mate, loss of nest and adverse weather conditions. Both ultimate and proximate factors are necessary for successful reproduction to occur in birds by influencing the neuroendocrine and peripheral endocrine regulation of the hypothalamic-pituitary-gonadal axis.

1.1.2 Photoperiodism

Photoperiodism is defined as the ability of an organism to measure daylength and the direction of daylength as a basis for regulating seasonal changes in physiology and

behaviour (Brandstatter, 2003). The annual cycle of most birds involves two physiological states in response to changing daylength. During winter when daylength is short the gonads are immature or regressed and the concentrations of reproductive hormones are low. As the photoperiod increases a species-specific threshold with regard to the duration of the daily light period will be reached, leading to growth and maturation of the gonads via increased synthesis and secretion of reproductive hormones. This state of responsiveness to long daylengths is known as photosensitivity (Wada, 1979; Nicholls *et al.,* 1983). Eventually birds become unresponsive to daylengths that had previously induced gonadal growth. This state is known as photorefractoriness and leads to gonadal regression.

Photorefractoriness is initiated by the same long days that cause photostimulation and appears to be "programmed" into the hypothalamic-pituitary-gonadal axis at this time (Reinert and Wilson, 1996a) although it takes several weeks to develop. The photorefractory period prevents breeding which could be detrimental to both adult and young birds and is of particular value in migratory species where breeding needs to be terminated so that postnuptial moult and fattening can occur whilst food sources remain abundant.

Photorefractoriness has been documented in many temperate zone passerine species including starlings (*Sturnus vulgaris*), tree sparrows (*Spirella arborea*) and white crowned sparrows (*Zonotrichia leucophrys oriantha*) (for review see Nicholls *et al.,* 1988). In these species gonadal regression is maintained as long as the birds experience long daylengths and gonadal growth cannot be induced by photostimulation. These birds are absolutely refractory species. Absolute photorefractoriness can only be dissipated by exposure to a period of short daylengths, such as those encountered during autumn.

In contrast Japanese quail undergo a relative refractory state characterised by the lack of spontaneous gonadal regression on fixed artificial long daylengths. As long as daylength is long, gonadal maturation is maintained indefinitely. Gonadal regression does however occur in these birds but only when daylength is shortened (Nicholls *et al.,* 1988) and in contrast to absolutely refractory species, gonadal growth can be induced during any stage of gonadal regression if the birds are transferred to long daylengths.

For the vast majority of avian species the physiological responses during photostimulation and photorefractoriness are asymmetrical with changes in daylength and as such require a complex neural and endocrine control system.

1.2 Neuroendocrine regulation of reproduction in birds

Reproductive activity in birds is governed by a neuroendocrine system involving three main components: (1) encephalic photoreceptors that are coupled to an internal circadian clock to measure daylength, (2) the GnRH neuronal system and (3) the peripheral endocrine system (Figure 1.1).

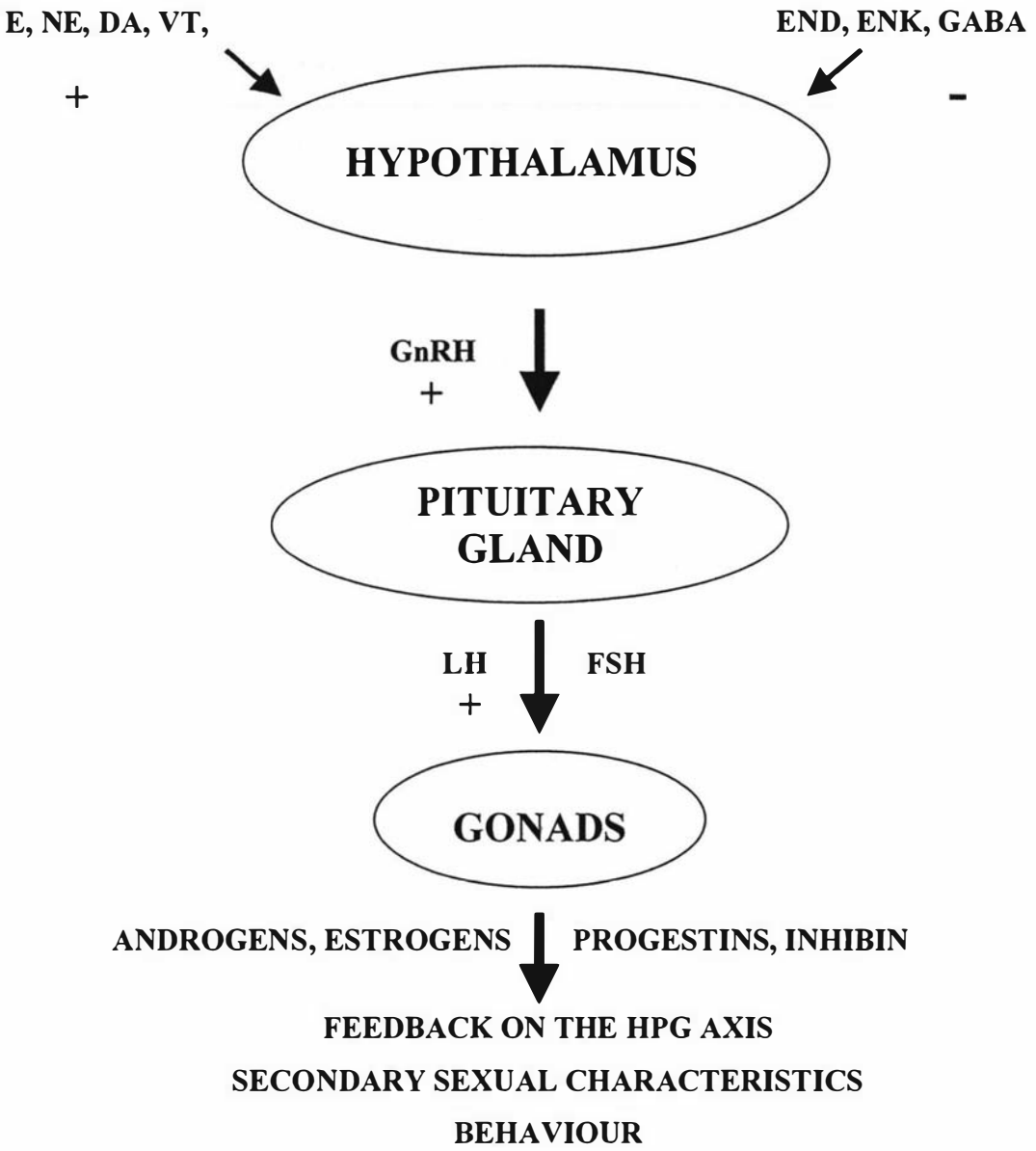

Figure 1.1. Diagram of the hypothalamic-pituitary-gonadal axis. In response to stimulatory photoperiodic conditions, gonadotropin-releasing hormone-I (GnRH-I) neurons secrete GnRH which stimulates the pituitary gland to synthesise and secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH bind to receptors within the testes or the ovary and stimulate the production of the gonadal steroids – androgens, estrogens, progestins and inhibin. The steroids participate in the regulation of the HPG axis through feedback systems and support the development and maintenance of secondary sexual characteristics and behaviour. Further control of the GnRH-I system is provided by neuropeptides and neurotransmitters – epinephrine (E), norepinephrine (NE), dopamine (DA), vasotocin (VT), β-endorphin (END), enkephalin, (ENK) and the γ-aminobutyric acid-ergic (GABA) systems.

1.2.1 Encephalic photoreceptors and the circadian clock

Daylength is measured in birds and mammals by photoreceptors and a circadian clock. In birds photic cues stimulate retinal, pineal and encephalic photoreceptors thought to be located in the lateral septum and the preoptic areas of the hypothalamus. Photic information from these receptors is organised for circadian pacemaking through the interaction of a multiple oscillary system consisting of three autonomous oscillators – the retina, pineal gland and a hypothalamic oscillator (Brandstatter, 2003). Although both the retina and pineal gland are photoreceptors, contain separate circadian clocks and produce melotonin, neither are essential for seasonal photoperiodic responses in birds (Siopes and Wilson, 1974; Simpson *et al.,* 1983; Siopes and Underwood, 1987; Siopes and El Halawani, 1989; Wilson, 1991). The encephalic receptors provide the necessary input to the hypothalamic biological clock that generates a circadian rhythm of photoinducibility (Follett *et al.,* 1981). The exact location of this hypothalamic clock is unknown. Recent studies in quail and sparrows have focussed on two promising sites – the medial suprachiasmatic nucleus and the lateral visual suprachiasmatic nucleus (Dawson, 2001).

Photostimulation of the neural pathways associated with the encephalic photoreceptors and the hypothalamic clock result in the transduction of photoperiodic information to the cGnRH-I system. The mechanisms by which this occurs are not fully understood, although recent studies suggest there are direct interactions between the photoreceptors and the cGnRH-I neurones. Microscopy studies revealed that the cGnRH-I neurones and processes are scattered among photoreceptor cells in the lateral septum, and immunocytochemical studies indicate opsin-positive terminals form axon dendritic synapses on to cGnRH-I dendrites in the lateral septum and the preoptic area of the hypothalamus (Saldanha *et al.,* 2001).

1.2.2 GnRH neuronal system

Neural control of seasonal breeding in birds is mediated via the septo-preoptic-infundibular cGnRH-I system (Ball and Hahn, 1997). In response to neural signals from the avian circadian system hypothalamic cells release GnRH (also known as luteinising hormone releasing hormone, LHRH). Nine GnRH structures have been reported for vertebrates and two for invertebrates (Figure 1.2).

GnRH										
Mammal	pGLU	HIS	TRP	SER	TYR	GLY	LEU	**ARG**	PRO	GLY
Chicken-I	pGLU	HIS	TRP	SER	TYR	GLY	LEU	**GLN**	PRO	GLY
Sea Bream	pGLU	HIS	TRP	SER	TYR	GLY	LEU	**SER**	PRO	GLY
Catfish	pGLU	HIS	TRP	SER	**HIS**	GLY	LEU	**ASN**	PRO	GLY
Salmon	pGLU	HIS	TRP	SER	TYR	GLY	**TRP**	**LEU**	PRO	GLY
Dogfish	pGLU	HIS	TRP	SER	**HIS**	GLY	**TRP**	**LEU**	PRO	GLY
Chicken-II	pGLU	HIS	TRP	SER	**HIS**	GLY	**TRP**	**TYR**	PRO	GLY
Lamprey-III	pGLU	HIS	TRP	SER	**HIS**	**ASP**	**TRP**	**LYS**	PRO	GLY
Lamprey-I	pGLU	HIS	**TYR**	SER	**LEU**	**GLU**	**TRP**	**LYS**	PRO	GLY
Tunicate-I	pGLU	HIS	TRP	SER	**ASP**	**TYR**	**PHE**	**LYS**	PRO	GLY
Tunicate-II	pGLU	HIS	TRP	SER	**LEU**	**CYS**	**HIS**	**ALA**	PRO	GLY

Figure 1.2. Comparison of eleven GnRH peptides. Bold type amino acids indicate changes with respect to the mammalian form. From (Sherwood *et al.*, 1997)

The structure of each peptide is highly conserved in length (10 amino acids) and in the NH2- (residues one, two and four) and COOH- (residues nine and 10) terminal sequences. In birds two forms of GnRH have been isolated and described. Chicken GnRH-I (cGnRH-I) differs from mammalian GnRH (mGnRH) at position eight where glutamine is substituted for arginine (King and Millar, 1982a; 1982b) and chicken GnRH-II (cGnRH-II) differs from both mammalian GnRH and cGnRH-I by three amino acids (Miyamoto *et al.*, 1984). Chicken GnRH-II was first identified in the chicken and has since been found to be widely distributed (for review see Sherwood *et al.*, 1997; Dunn and Millam, 1998) and the most strongly conserved GnRH peptide in vertebrates. Although the nomenclature for GnRH molecules in recently published literature refers to mGnRH and cGnRH-II as GnRH I and GnRH II respectively (Kauffman, 2004; Neil *et al.*, 2004), the terms mGnRH, cGnRH-I and cGnRH-II will be used in this thesis.

Although exogenous cGnRH-I and –II stimulate gonadotropin secretion in *in vitro* preparations of avian pituitary tissue and *in vivo* (Millar and King, 1984; Miyamoto *et al.*, 1984; Chou *et al.*, 1985; Hattori *et al.*, 1986a; Millar *et al.*, 1986; Sharp *et al.*, 1986; Sharp *et al.*, 1987; 1990), the physiological role of cGnRH-II is not clear and the vast majority of studies to date report that cGnRH-I is the primary GnRH form in birds that regulates reproduction. However the molecular details concerning the role and regulation of GnRH, GnRH receptors and their respective genes continue to be revealed.

1.2.2.1 Molecular biology of GnRH

1.2.2.1.1 cGnRH-I gene

cGnRH-I arises from the post-translational processing of a precursor molecule, proGnRH-GAP, comprising of a signal peptide, the decapeptide, a cleavage site and a GnRH-associated peptide (GAP). The DNA coding sequences for some GnRH pre-molecules have been identified – (mGnRH prepropeptide in human, mouse, and rat; salmon GnRH prepropeptide in species of bony fish; cGnRH-I prepropeptide in chicken; cGnRH-II prepropeptide in species of bony fish; catfish GnRH-I and –II prepropeptide in African catfish; sea bream prepropeptide in species of bony fish; reviewed in King and Millar (1997). The cGnRH-I prepropeptide has approximately 50 % homology with mammalian prepropeptide and 25 % homology with the teleost prepropeptide (Dunn *et al.*, 1993).

The cGnRH-I gene contains four exons. The first exon encodes the 5'-untranslated region. The second exon encodes the signal peptide, cGnRH-I decapeptide, the enzymatic amidation and precursor processing site and the first amino acid sequence of the GAP. The third exon encodes the middle portion of the GAP and the fourth exon encodes the carboxyl terminus of the GAP and the 3'-untranslated region.

The signal peptide region in the cGnRH-I gene consists of 23 amino acids which is the same length as most mammalian signal peptide regions except in the mouse where it is shorter by two amino acids (Dunn and Millam, 1998). Generally signal peptides have a hydrophobic core followed by a sequence of alternating polar and non-polar amino acids. The cGnRH-I prepropeptide has a hydrophobic region, 15 amino acids in length followed by the carboxyl terminal region (Dunn and Millam, 1998). Within the terminal region lies the signal peptide cleavage sequence. When comparing the different forms of GnRH (mammal, chicken I and II, salmon, sea bream and catfish I and II) there is low conservation in both signal and associated peptides (King and Millar, 1997).

Once the signal peptide has been cleaved the cGnRH-I molecule is further processed. This involves pyrolisation of the N-terminal glutamic residue and C-terminal amidation (King and Millar, 1982a; 1982b). The processing enzymes required for this process have not been identified in birds, although the process is thought to be similar for all

GnRH molecules. In general the cleavage and processing of this region involves hydrolysis and digestion by specific enzymes. The mature GnRH-peptide produced is highly conserved and the homologies between peptides are shown in Table 1.

Table 1.1. Homologies (percentage) for amino acid sequence of mature GnRH peptides including the processing site. From (King and Millar, 1997).

mGnRH	92	92	83	75	83
	cGnRH-I	92	83	75	83
		Sea bream	83	75	83
			Salmon	83	75
				cGnRH-II	83
					Catfish

The GAP identified in both chicken and turkey is the same length as that found in mammals (56 amino acids) although the molecules are considerably less conserved compared to their concomitant endocrine peptides (Dunn *et al.,* 1993). The physiological importance of GAP in any species is unclear. In mammals there is evidence that the GAP functions as a conformational assistant. In hypogonadal mice the third and fourth exons which encode the majority of GAP are deleted from the mGnRH gene, whilst the second exon which encodes mGnRH remains intact. These mice do not secrete GnRH and gonadotropin concentrations are not detectable suggesting that full-length transcripts in the correct reading frame are required for the processing of the GnRH peptide (Seeburg *et al.,* 1987). The GAP may have a role in the intracellular processing of preproGnRH or an endocrine role which is thought be a recent phenomenon in evolutionary terms (Dunn and Millam, 1998).

The gene encoding cGnRH-I is transcribed in the hypothalamus and in several extrahypothalamic sites including the gonads. In the Japanese quail the total amount of cGnRH-I mRNA in the gonads is significantly greater than in the preoptic area that contains the cGnRH-I cell bodies (Baines *et al.,* 1999). The sequence of the cGnRH-II gene in birds has not been reported to date. In humans the prepro-GnRH-II gene consists of a 5'- untranslated exon and three coding exons with the mature peptide encoded within exon one (White *et al.,* 1998).

1.2.2.1.2 Degradation of GnRH

Enzymatic degradation of cGnRH-I in chickens generates two main degradation fragments. A post-proline cleaving enzyme produces cGnRH-I $^{1-9}$ and an endopeptidase that cleaves between Tyr^5 and Gly^6 produces the more prevalent cGnRH-I^{1-5} (Advis *et al.*, 1985; Contijoch *et al.*, 1990). Degradation of cGnRH-I to cGnRH$^{1-9}$ may be a necessary precursor to the production of GnRH$^{1-5}$ (Lew *et al.*, 1994). Measurement of GnRH degradation using high-performance liquid chromatography (HPLC) demonstrated the highest GnRH degrading activity occurred in samples from the infundibulum where cGnRH-I content was highest whilst degradation of cGnRH-I outside of the hypothalamus was the lowest (Advis *et al.*, 1985). Additionally cGnRH-I degrading activity was greater in laying than out-of-lay hens suggesting cGnRH-I degradation changes with the physiological state of the animal (Advis *et al.*, 1985).

1.2.2.1.3 Distribution of GnRH immunoreactive cells and fibres

Immunohistochemical studies completed in the 1970s and early 1980s in avian species used antisera raised against mGnRH and found the most numerous populations of GnRH neurons to be located in the septal-preoptic region and anterior hypothalamus (for review see Mikami, 1986). During the late 1980s and early 1990s immunohistochemical studies using antisera specific for cGnRH-I or cGnRH-II provided detailed distribution maps (Mikami *et al.*, 1988; Millam *et al.*, 1993; van Gils *et al.*, 1993).

GnRH neurones migrate from the nasal region into the brain during early embryonic development (Witkin *et al.*, 2003). The general distribution of cGnRH-I cells and fibres falls into three main groups (for review see Ball and Hahn, 1997). The first and best studied group begins in the preoptic area then continues caudo-dorsally into the lateral septum. The perikarya send fibres to both the anterior and posterior regions of the median eminence. The second group extends laterally from the first group and caudo-dorsally into the rostral sections of the lateral hypothalamic region. The last and the smallest group of cGnRH-I cells and fibres is scattered throughout the olfactory bulb. Chicken GnRH-I fibres are also present in the medial thalamic area, nucleus subhabenularis medialis, tuberal hypothalamus, caudal lateral hypothalamus and in the hippocampus.

The initial immunohistochemical studies using antisera specific for cGnRH-II located cGnRH-II containing cell bodies in the mesencephalon and in the caudal extent of the lateral hypothalamus and a complex distribution of fibres in the telencephalon and diencephalon (Mikami *et al.*, 1988; Millam *et al.*, 1993). These studies did not observe cGnRH-II immunoreactivity in the median eminence. van Gils *et al.* (1993) detected immunoreactive cGnRH-II fibres in the median eminence. As this was the only study that had reported cGnRH-II immunoreactive fibres in the avian median eminence, the findings remained controversial. The presence of cGnRH-I and –II in the median eminence has been further confirmed by light and electron microscopy (D'Hondt *et al.*, 2001 cited in Clerens *et al.*, 2003) and by HPLC combined with radioimmunoassays of separate chromatography fractions (Clerens *et al.*, 2003). The role of cGnRH-II in the median eminence remains unclear but confirmation of its presence has revived the possibility that cGnRH-II may play a role in the regulation of reproductive events.

GnRH-immunoreactive elements not observed when cGnRH-I or –II antisera are utilised have also been reported in the nucleus lateralis anterior thalami (Blahser, 1983). These elements may represent evidence for the presence of a third species of GnRH in birds.

1.2.2.1.4 GnRH receptors

The existence of a receptor for GnRH in birds was first inferred from the increases in gonadotropin concentrations in response to exogenous administration of various types of synthetic mammalian and avian GnRH solutions. These responses are similar to those seen in mammals given exogenous GnRH indicating that the binding properties of the avian GnRH receptor (GnRH-R) are similar to those of the mammalian GnRH receptor. It appears that all of the vertebrate GnRHs stimulate gonadotropin release in all vertebrates but that the specificities of the GnRH-Rs differ (King and Millar, 1997), although relatively few of the GnRH-Rs have been investigated in detail.

The cloned chicken GnRH-R cDNA encodes for a 375 amino acid polypeptide that contains seven hydrophobic putative transmembrane domains connected by three cytosolic and three extracellular loops, extracellular amino-terminal and cytosolic carboxyl terminal domains (Sun *et al.*, 2001). The extracellular amino-terminal contributes to ligand recognition and binding while the intracellular carboxyl-terminal

domain contributes to effector binding, the propagation of signalling events as well as desensitisation (Dohlman *et al.,* 1987). The cGnRH-R has the structural characteristics of the largest group of cell surface receptors, the serpentine family of receptors (Sealfon and Weinstein, 1997). This group of receptors transmit their messages through heterotrimeric G-proteins and are commonly known as G-protein coupled receptors (GPCRs). GnRH-Rs have been identified in numerous tissues including the pituitary, testis, ovaries and placenta (for review see McArdle *et al.,* 2002).

Three groups of GnRH receptors have been identified in vertebrates. The first known as type I GnRH-Rs include all known mammalian pituitary GnRH receptors except the primate type II GnRH-Rs and have a higher affinity for cGnRH-I than cGnRH-II. The second group (type II) includes all the receptors from non-mammalian vertebrates and the type-II primate GnRH-Rs and have a higher affinity for cGnRH-II than cGnRH-I. A third distinct type (type III) was recently characterised in fish and amphibians.

The mammalian GnRH-R is unique amongst these receptors in that it possesses a short intracellular third loop and has no common cytoplasmic carboxyl-terminal domain (c-terminal tail)(Sealfon and Weinstein, 1997; McArdle *et al.,* 2002). C-terminal tails and the third loop have been shown to be involved in internalisation and desensitisation and as such these receptors do no undergo short-term homologous desensitisation (Davidson *et al.,* 1994a, 1994b; McArdle *et al.,* 2002). The cGnRH-R possesses a c-terminal tail and has low homology with the human GnRH-R (59 %) (Illing *et al.,* 1999; Troskie *et al.,* 2000).

1.2.2.1.5 GnRH ligand-receptor interaction

The molecular interactions that occur between the cGnRH-I ligand and cognate receptor are currently being investigated. These interactions are thought to be similar to those which occur between the mGnRH ligand and receptor leading to the activation of second messenger systems (McArdle *et al.,* 2002). GnRH activation of its receptor in mammals stimulates GTP binding proteins (G-proteins), which in turn activate phospholipase C (McArdle *et al.,* 2002). G-proteins are heterotrimeric proteins composed of an α subunit that binds guanine nucleotides and a dimer that consists of β and γ subunits. Phospholipase C hydrolyses phosphatidylinositol-4, 5-biphosphate (PIP_2) producing inositol-1, 4, 5-triphosphate (IP_3) and diacylglycerol (DAG) leading to

the activation of various protein kinase C (PKC) subspecies (Dunn and Millam, 1998). cGnRH-I stimulation of chicken cells *in vitro* causes increases in cellular PIP_2 and IP_3 suggesting the mammalian model of ligand-receptor stimulated second messenger activation also occurs in birds (Davidson *et al.,* 1988). PKC, IP_3 and DAG function as second messengers and have been shown to stimulate LH release in chicken cells *in vitro* (Davidson *et al.,* 1987; Johnson and Tilly, 1991).

The activities of cGnRH-I are dependent on Ca^{2+} that is an important intracellular messenger. cGnRH-I induces immediate intracellular Ca^{2+} mobilisation followed by extracellular Ca^{2+} influx which occur via the mobilisation of two distinct calcium pools (Dunn and Millam, 1998). Extracellular Ca^{2+} enters the cell through multiple voltage-gated and non-voltage sensitive calcium channels in the plasma membrane whilst IP_3 releases Ca^{2+} from intracellular stores via a specific IP_3 receptor (Naor, 1990). Both intracellular and extracellular Ca^{2+} are involved in the secretion of LH and FSH. The increase in intracellular Ca^{2+} is thought to be responsible for the spike phase release of LH (Naor, 1990) whilst the extracellular Ca^{2+} influx in association with phosphokinase C participates in the sustained plateau phase of gonadotropin secretion. Removal of calcium from the incubating medium of chicken pituitary tissue reduces both the spike phase and plateau phase of GnRH-stimulated LH release in tissue from immature and mature male and female birds (Davidson *et al.,* 1988; Liu *et al.,* 1995). Activation of PKC and Ca^{2+} are among the important events that mediate GnRH action in pituitary cells of birds as seen in mammals.

Two aspects of the GnRH ligand-receptor interaction that are of interest and appear to be unique compared to other Ca^{2+} mobilising ligands are the abilities of GnRH to up-regulate its own receptors at physiological concentrations and to prime the anterior pituitary to augment GnRH stimulation.

G-protein coupled receptor-mediated responses are generally desensitised by sustained or intense stimulation. Studies on GnRH receptor desensitisation reported that the absence of a c-terminal tail in mGnRH-Rs is correlated with an absence of rapid homologous desensitisation of PLC activation (McArdle *et al.,* 1995). The absence of the c-terminal tail in mGnRH-Rs and its presence in non-mammalian vertebrate GnRH-Rs suggests this may have physiological correlates in mammals and non-mammals. The

absence of the tail may prevent rapid desensitisation and internalisation of mGnRH-Rs in order to allow a protracted LH surge over several hours which is required for ovulation in mammals (Pawson *et al.*, 1998). In contrast galliforms such as quail and chickens have one day cycles which include a shorter LH surge of no more than 120 min (Furr *et al.*, 1973a). The functional dichotomy between types I and II GnRH-Rs may exist within species as well as between species. (Neill *et al.*, 2001) recently observed desensitisation in the type II primate GnRH-Rs under conditions in which a type I human GnRH-R did not undergo desensitisation. Comparative studies have confirmed that the functional distinctions such as differing ligand specificity and propensity for desensitisation and internalisation between type I and II GnRH-Rs do not reflect differences in receptor density and are observed at physiological receptor numbers (Hislop *et al.*, 2001).

Desensitisation of G-protein receptors is regulated by several mechanisms including uncoupling from their cognate G-proteins, receptor internalisation and down-regulation of receptor gene expression. In type I receptors desensitisation is associated with downstream adaptive responses such as a GnRH-stimulated reduction in the number of Ins (1,4,5) P_3-Rs during desensitisation of G-stimulated Ca^{2+} mobilisation from intracellular stores (McArdle *et al.*, 1996). The relevance of the effects of desensitisation of gonadotropin secretion has not yet been established.

1.2.2.2 Pattern of cGnRH-I secretion

The patterns of GnRH secretion into the hypophyseal portal vessels of several mammalian species have been determined using cannulation or microdialysis of the median eminence region. In mammals the secretion of GnRH is pulsatile and driven by an oscillatory system located in the central nervous system (Karsch *et al.*, 1984; Moenter *et al.*, 1992). High frequency GnRH pulses are associated with a shift toward increased LH secretion whereas low frequency GnRH pulses are associated with increased FSH secretion (Schwartz, 1995). Pulsatile secretion of cGnRH-I has not been determined *in vivo* in birds although LH secretion is pulsatile (Wilson and Sharp, 1975a; Gledhill and Follett, 1976; Urbanski, 1984; Vizcarra *et al.*, 2004) and this is thought to reflect episodic GnRH neuronal activity. Additionally cGnRH-I secretion from the medial-basal preoptic area of Japanese quail has been shown to be pulsatile *in vitro* (Li *et al.*, 1994).

Although cGnRH-I secretion has not been described in photosensitive and photorefractory birds hypothalamic GnRH content has been measured. In the European starling, the absolute photorefractoriness model species, hypothalamic cGnRH-I content in dissected hypothalami exhibited significant changes in birds in different reproductive states (Dawson *et al.,* 1985). Photostimulation is associated with an increase in cGnRH-I content and photorefractoriness with a decrease in content and in the number and size of the cGnRH-I immunoreactive cell bodies (Foster *et al.,* 1987; Goldsmith *et al.,* 1989). Chicken GnRH-I content increased in photorefractory starlings on short days as the refractory state dissipated and photosensitivity was induced. Juvenile starlings also show an increase in cGnRH-I content when photosensitivity is being attained (Goldsmith *et al.,* 1989) affirming the importance of short day exposure for the dissipation of photorefractoriness. The decrease in cGnRH-I content and in the precursor molecule for GnRH, proGnRH-GAP, during photorefractoriness occurs after gonadotropin secretion has declined and gonadal regression has been induced (Goldsmith *et al.,* 1989; Parry *et al.,* 1997). This suggests that in the starling the secretion of cGnRH-I is inhibited resulting in decreased gonadotropin synthesis and secretion and gonadal regression whilst hypothalamic content remains high, and that the cGnRH-I neuronal system is not the primary site of reproductive inhibition during photorefractoriness (Dawson *et al.,* 1985). Only after the gonads have regressed is there a decline in the hypothalamic content of cGnRH-I. The mechanisms responsible for the decline in cGnRH-I are not known but appear to be associated with an increase in synaptic input to the cGnRH-I cells (Parry and Goldsmith, 1993). This neural input may drive changes in transcription or post-translational processing of precursor GnRH molecules resulting in inhibited cGnRH-I production (Parry *et al.,* 1997).

In contrast to absolutely photorefractory species, little is known about changes in the cGnRH-I system in relatively photorefractory species. Japanese quail show no decline in hypothalamic cGnRH-I when relatively photorefractory (Foster *et al.,* 1988) nor do they show any changes during the first days of photostimulation (Creighton and Follett, 1987). Concentrations of cGnRH-I in micro-dissected hypothalamic regions of turkey hen were also not different between reproductive states (Millam *et al.,* 1995). Furthermore the secretion of cGnRH-I appears to be inhibited in quail on short days as hypothalamic cGnRH-I expression is higher in quail transferred from long days to short

days for 25 day that have regressed gonads compared with chronically long day exposed birds (Foster *et al.*, 1988).

1.2.3 Peripheral endocrine system

1.2.3.1 Gonadotropins

The avian gonadotropins LH and FSH are heterologous dimeric glycoproteins composed of non-covalently bound α and β subunits (Ishii, 1993). The α subunit is the same for both hormones and is encoded by a single gene in all species examined. The α subunit is thought to confer little or no biological activity whilst the β subunit confers the unique biological specificity for each hormone. Both LH and FSH are synthesised in and secreted from adenohypophyseal parenchymal cells under the influence of GnRH. GnRH also controls LH-β and FSH-β mRNA gene expression (Kubokawa *et al.*, 1994; Kikuchi *et al.*, 1998; Shen and Yu, 2002).

GnRH-induced LH and, to a lesser extent, FSH secretion is pulsatile in both male and female avian species (fowl, Wilson and Sharp, 1975a; Japanese quail, Gledhill and Follett, 1976; Urbanski, 1984; Hatanaka and Wada, 1988; cockerel, Sharp and Gow, 1983; turkey, Bacon *et al.*, 1991; Chapman *et al.*, 1994). Although the endocrine mechanisms which regulate the secretion of FSH in birds are poorly understood, observations of the differential secretion of LH and FSH have led to proposals for separate releasing factors for the gonadotropins. The presence of both cGnRH-I and –II in almost all species studied thus far leads to the question is cGnRH-II the FSH-releasing hormone? Recent immunohistochemical studies showed that LH and FSH reside almost exclusively in separate populations of gonadotropes in the chicken pituitary (Proudman *et al.*, 1999). When compared to cGnRH-I, Millar *et al.* (1986) found cGnRH-II had preferential FSH-releasing activity in chicken pituitary cells *in vitro* and the recent findings of cGnRH-II neurons and fibres in the median eminence (discussed earlier) support the possibility of cGnRH-II playing a role in reproduction. Preferential cGnRH-II stimulated FSH release has been observed in rams (Millar, 2001). Krishnan *et al.* (1993) reported increased LH but no change in FSH concentrations in chickens treated with cGnRH-II, whereas Hattori *et al.* (1986a) observed cGnRH-I-stimulated increased FSH *in vivo* and *in vitro* in male Japanese quail.

Several lines of evidence suggest that differential LH and FSH secretion patterns may be due to factors other than releasing hormones. Although both LH- and FSH-immunoreactive cells are present in both lobes of the pituitary, FSH-ir cells are less numerous and largely absent from the outer margin of the pituitary compared to LH-ir cells which are evenly distributed throughout the pituitary (Proudman *et al.,* 1999). Additionally FSH secretion during specific stages of the reproductive cycle is regulated by inhibin and activin as seen in mammals (Johnson and Wang, 1993; Vanmontfort *et al.,* 1994; Vanmontfort *et al.,* 1995). Inhibin blocks pituitary release and activin stimulates FSH release (Cogburn *et al.,* 2000).

The effects of various forms of GnRH stimulation on LH have been intensively studied but relatively little is known of the effects of GnRH on FSH secretion. To determine a role for cGnRH-II in stimulating FSH secretion further investigations on basic FSH physiology are required in addition to physiological investigations on cGnRH-II as cGnRH-II secretion patterns and rates have not been defined.

1.2.3.1.1 Gonadotropins in male birds

Gonadotropins play important roles in the maturation of the gonads and initiation of steroidogenesis. In male birds the gonadotropins exert their effects by binding to specific membrane receptors on Leydig and Sertoli cells in the testes. LH stimulation of Leydig cells causes the synthesis and secretion of androgens, principally testosterone and androstenedione (Sharp *et al.,* 1977). Testosterone is secreted in a pulsatile pattern that closely follows LH secretion patterns (Bacon *et al.,* 1991), and in the adult male bird testosterone is essential for the maintenance of the excurrent ducts, spermatogenesis and the expression of reproductive behaviours. FSH acts upon the Sertoli cells and is essential for the maintenance of spermatogenesis and testosterone potentiated FSH-induced effects on Sertoli cells during spermatogenesis (Tsutsui and Ishii, 1978).

1.2.3.1.2 Gonadotropin stimulation of ovarian function in female birds

In female birds LH induces steroidogenesis in both hierarchal and non-hierarchal ovarian follicles and more importantly induces ovulation of the F1 follicle. The active ovary of a laying quail or hen consists of numerous small follicles (1-8 mm in diameter) and a hierarchy of five to seven large yellow follicles (>8 mm in diameter). These large

follicles are classified based on diameter and order for ovulation and are known as F1 (largest follicle and first to ovulate), F2 (second largest which ovulates on the following day) through to F5-F7.

Follicle growth occurs in three phases (Decuypere *et al.*, 1999). The first phase involves slow growth over several months to a year followed by the second phase of increasingly rapid growth consisting of the deposition of yolk protein. The third phase known as the rapid growth phase occurs during the final 7-11 days before ovulation. During this phase follicle diameter increases and the majority of yolk and lipids are deposited (Imai, 1983).

The steroidogenic tissues of the ovarian follicle are the granulosa and theca layers (theca interna and theca externa). The thecal layer preferentially metabolises steroids via the Δ^5 pathway regardless of the maturational stage, with pregnenolone (P_5) converted to dihydroepiandrosterone (DHEA) and then to androstenedione, testosterone and estradiol. The thecal layer also expresses cytochrome P_{450} cholesterol side-chain cleavage (P_{450}scc), an enzyme in the steroid synthesis pathway (Kowalski *et al.*, 1991; Johnson *et al.*, 1996). Granulosa cells tend to use the Δ^4 pathway when the follicle is mature to primarily produce progesterone (P_4) (Lee *et al.*, 1998).

The gonadotropins are the major extra-ovarian regulators of ovarian function and responsiveness of the follicles to gonadotropins changes with follicular maturation. In the hen the small preovulatory follicles are more responsive to FSH whilst the large follicles are more responsive to LH (Hernandez *et al.*, 2001). FSH is considered to be responsible for follicular recruitment and growth. In hens daily FSH injections increase the numbers of white and small yellow follicles and decrease atretic follicles without affecting the follicles of the hierarchy (Palmer and Bahr, 1992). Additionally FSH but not LH stimulates P_4 secretion from granulosa cells of the F5 follicle (Hammond *et al.*, 1981) by inducing the synthesis of P_{450} scc (Li and Johnson, 1995; Tilly *et al.*, 1991). In contrast LH but not FSH stimulates the granulosa cells from the F1 to produce P_4 that is primarily used as a precursor for androgen and estradiol production in the thecal layer (Asem and Hertelendy, 1985).

Ovarian function is also influenced by growth factors that play key roles in steroidogenesis and are involved in follicular recruitment. Genes for both insulin-like growth factors –I and –II (IGF-I and –II) are expressed in theca and granulosa cells (Onagbesan *et al.,* 1999a). Several studies in galliforms have demonstrated the stimulatory effects of IGF-I and –II on granulosa and theca cell proliferation and enhancement of P_4 production by granulosa cells and androstenedione by theca cells (Roberts *et al.,* 1994; Onagbesan and Peddie, 1995; Onagbesan *et al.,* 1994; 1999a, 1999b). An IGF-II variant expressed in the granulosa cells of a small number of small yellow follicles occurs when these follicles are being selected for recruitment into the follicular hierarchy (Armstrong and Hogg, 1996). As only a small number of follicles are recruited into the hierarchy it has been suggested that only the follicles expressing the IGF-II variant are recruited and follicles that do not express the variant become atretic.

Recruitment of follicles into the hierarchy is accompanied by a dramatic increase in the α-subunit of inhibin produced by the granulosa cells (Safi *et al.,* 1998). IGFs have been shown to be involved in the changes of expression level of the alpha subunits of inhibins in granulosa cells of chicken ovaries (Vanmontfort *et al.,* 1992; Safi *et al.,* 1998).

Another growth factor, which is of importance, is epidermal growth factor (EGF) produced by the germinal disc, the counterpart of the mammalian oocyte. EGF promotes growth and prevents atresia of follicles by stimulating proliferation of granulosa cells thus ensuring survival of the follicle. EGF also prevents the premature maturation of the follicle by delaying differentiation (Hernandez *et al.,* 2001).

1.2.3.1.3 Ovulation

Once a follicle has attained the F1 position in the hierarchy it is ready to ovulate. In domestic fowl oviposition occurs in a sequence over successive days followed by a pause of one or more days (Kamiyoshi and Tanaka, 1983). The interval between ovipositions is 25-27 hours and a sequence usually finishes when an ovulation occurs approximately eight hours after the first ovulation in the sequence. (Hernandez *et al.,* 2001). Ovulation requires significant changes in the plasma concentrations of gonadotropins and ovarian steroids. The first increase in LH occurs 14-10 hours prior

to ovulation (Etches and Cheng, 1981) followed by the first increase in P_4 (Decuypere *et al.*, 1999). The significance of the LH increase has not been determined although the progesterone increase is thought to be part of the stimulus for the preovulatory LH surge that occurs 8-6 hours prior to ovulation. Androgen and oestrogen concentrations peak 10-6 hours and 6-3 hours respectively prior to ovulation as a result of increased secretion from the four largest follicles (Johnson, 2000). These increases do not appear to be involved in the direct induction of LH or ovulation. Estradiol is thought to be necessary in the priming of the positive feedback effects that P_4 exerts on LH, whilst the role of testosterone is yet to be established (Kamiyoshi and Tanaka, 1983). Just prior to ovulation the production of androgens and estrogens decline (Mori *et al.*, 1985), removing their inhibitory effects on P_4 secretion.

The increased P_4 concentrations 6-4 hours prior to ovulation exert a positive feedback effect on LH release (Wilson and Sharp, 1976) by acting at receptors within the preoptic region of the hypothalamus increasing cGnRH-I which in turn triggers the preovulatory LH surge (Decuypere *et al.*, 1999). During a spontaneous preovulatory LH surge there is no change in cGnRH-I content suggesting P_4 not only stimulates cGnRH-I release (Dunn *et al.*, 1996) but synthesis as well. The increased LH concentrations stimulate further production of P_4 within the F1 follicle that drives the positive feedback loop until the F1 eventually ruptures (Etches, 1996).

The mechanisms controlling the regulation of gonadotropin-induced steroidogenesis during follicular growth and ovulation are not fully understood. The numbers of FSH receptors in granulosa cells decrease in hierarchal follicles as they mature (Etches and Cheng, 1981; Ritzhaupt and Bahr, 1987) and recent studies found mRNA for the FSH receptor to be more abundant in granulosa cells from less developed follicles in the hierarchy whereas mRNA for the LH receptor was more abundant in the F3-F1 follicles (Johnson *et al.*, 1996; You *et al.*, 1996). Additionally Kikuchi and Ishii (1992) found that as the F2 matures the affinity of the LH receptors in the thecal cells increased three-fold as the number of receptors decreased. These changes did not occur in the granulosa layer. As a consequence there was a decrease in LH binding which precisely corresponded with the change from oestrogen to P_4 production in the follicles (Doi *et al.*, 1980; Mori and Kantou, 1987). It is hypothesised that the decrease in LH receptor number and binding decreases the sensitivity of thecal tissue to LH which decrease the

synthesis and secretion of androgens and oestrogen (Kikuchi and Ishii, 1992). As a consequence the inhibitory effects of androgens and oestrogen on P_4 are reduced allowing P_4 concentrations to increase and ovulation to occur.

1.2.3.2 Gonadal steroid feedback

In mammals gonadal steroids modulate hypothalamic GnRH secretion and exert negative feedback effects on gonadotropin secretion by altering both GnRH secretion and pituitary responsiveness to GnRH (Ellis and Turek, 1979). Gonadal steroids have been shown to depress LH concentrations in the plasma of sexually mature male quail (Davies, 1976) and chickens (Williams and Sharp, 1977; Etches, 1996). The inhibitory feedback of endogenous steroids is removed by castration and restored with the administration of exogenous hormones (Follett *et al.,* 1972; Wilson and Follett, 1974; Deviche *et al.,* 1980; El Halawani *et al.,* 1983). It is thought to be mediated at the hypothalamus (Cusick and Wilson, 1972; Stetson, 1972; Etches, 1996) as cGnRH-I content in the hypothalamus is increased after castration (Knight *et al.,* 1983b; Sharp, 1990). Chronic treatment of cultured male chicken hypothalami with oestrogen also decreased cGnRH-I release, although the acute oestrogen treatment increased cGnRH-I release (Li *et al.,* 1994).

Gonadal steroids also alter LH release in birds at the anterior pituitary level. Preincubation of anterior pituitary cells from male quail with 17β-estradiol did not affect GnRH-induced LH secretion (Connolly and Callard, 1987) and in chicken cells, LH release was either augmented (Bonney and Cunningham, 1977) or blocked (Luck and Scanes, 1980). In contrast testosterone and 5α-dihydrotestosterone both inhibited LH secretion in quail (Connolly and Callard, 1987) and rooster (King *et al.,* 1988) cells. In hens P_4 can have a negative or positive (during ovulation) feedback effect on LH secretion, whilst elevated oestradiol inhibits LH secretion. Administration of tamoxifen, a selective oestrogen receptor modulator increases cGnRH-I content (Wilson *et al.,* 1990).

The recently described dodecapeptide, GnIH, and prolactin provide additional regulation of gonadotropin synthesis and secretion.

1.2.3.3 Gonadotropin-inhibitory hormone (GnIH)

Gonadotropin-inhibitory hormone (GnIH) is a novel 12 residue peptide that directly inhibits LH release from cultured Japanese quail pituitary glands *in vitro* in a dose-dependent manner (Tsutsui *et al.*, 2000). Southern blotting analysis of reverse-transcriptase mediated PCR products demonstrated a specific expression of the gene encoding GnIH in the diencephalon including the hypothalamus (Satake *et al.*, 2001). Immunoreactivity for GnIH has been observed in the paraventricular nucleus and the median eminence of Japanese quail (Tsutsui *et al.*, 2000), song sparrows (*Melospiza melodia*) and house sparrows (*Passer domesticus*) (Bentley *et al.*, 2003). In the median eminence of sparrows the GnIH-immunoreactive fibres are localized in close proximity to GnRH neurones suggesting GnIH may act at the hypothalamic level, although synapses between the two have not yet been determined.

Further studies are required to determine what environmental factors affect GnIH secretion and what the physiological and behavioural effects of GnIH secretion may be. The fact that GnIH directly affects gonadotropin release suggests GnIH may play an important role in the regulation of the avian annual cycle of reproduction.

1.2.4 Prolactin

Vasoactive intestinal peptide (VIP) is well established as the hypothalamic releasing factor for the stimulation of prolactin secretion in birds (for review see Sharp *et al.*, 1998). The VIP neuronal system receives input from the hypothalamic biological clock to transduce photoperiodic information for the control of prolactin secretion in a similar manner to the relationship between the biological clock and the GnRH neuronal system. It would therefore seem plausible that the neural connections linking the VIP and GnRH neuronal system to the biological clock would share some common components.

Photoperiodic control of prolactin secretion in birds is similar to mammals in that it is stimulated by long photoperiods and inhibited by short photoperiods. Photostimulation causes an increase in prolactin concentrations once a critical daylength is reached in Japanese quail (Goldsmith and Hall, 1980), starlings (Ebling *et al.*, 1982), turkey hens (El Halawani *et al.*, 1984) and bantams (Sreekumar and Sharp, 1998a) in the laboratory and in wild birds (starlings, Dawson and Goldsmith, 1982; ring doves, Buntin *et al.*,

1988; great tits Silverin and Goldsmith, 1997). The critical daylength for prolactin release in bantams (between 10-12 hours of light per day (Sreekumar and Sharp, 1998b) is similar to that for gonadotropin release.

Prolactin secretion during photostimulation is concurrent with increasing LH concentrations and continues to increase after LH concentrations have reached maximum values (Dawson and Goldsmith, 1982). Maximal prolactin concentrations in birds occur during incubation and the start of the care of nestlings. Prolactin concentrations then decrease rapidly in species with precocial young and slowly in birds with altricial young. In birds not incubating eggs or caring for young, seasonally high concentrations of prolactin in response to increasing daylength are associated with photorefractoriness (Nicholls *et al.,* 1988) and with decreased concentrations of LH and gonadal steroids (for review see Sharp *et al.,* 1998). It has been suggested that the maximal concentrations of prolactin induce the gonadal regression observed during photorefractoriness. This hypothesis was tested in European starlings immunized against VIP to suppress photoinduced prolactin secretion. Immunisation of photostimulated birds prevented prolactin release and retarded testicular regression but did not inhibit regression (Dawson and Sharp, 1998). These results show seasonal prolactin increases are temporally related to the onset of photorefractoriness but do not play a direct causal role in starlings.

1.2.5 The role of thyroid hormones in seasonal reproduction

Numerous studies have focussed on the role thyroid hormones play in avian reproduction. Studies on the effects of thyroidectomy on seasonal gonadal responses in birds have shown clear but contradictory effects between and within species depending on the timing of thyroidectomy in relation to reproductive stage. In some studies photostimulated gonadal growth was unaffected by thyroidectomy (Goldsmith and Nicholls, 1984) whereas in others it was impaired (Chaturvedi and Thapliyal, 1983; Follett and Nicholls, 1984; Thapliyal and Lal, 1984; Lien and Siopes, 1989; Dawson, 1998). Gonadal regression associated with photorefractoriness and postnuptial moult is normally prevented by thyroidectomy (Wieselthier and van Tienhoven, 1972) but in some studies photorefractoriness was accelerated (Lal and Thapliyal, 1982; Dawson, 1998). Exogenous thyroxine (T4) administration to birds maintained on non-

stimulatory conditions can mimic the effects of long photoperiods (Follett and Nicholls, 1988; Follett *et al.,* 1988; Goldsmith and Nicholls, 1992) and augment testicular growth in tree sparrows moved to stimulatory daylengths (Wilson and Reinert, 1999). These studies support the suggestion that T4 plays an essential role during photostimulated gonadal growth and have led to studies which suggest the increase in T4 programmes the hypothalamic-pituitary-gonadal axis for the subsequent breeding cycle including photorefractoriness and moult (but not the initial activation of the cycle; Reinert and Wilson, 1996a; Wilson and Reinert, 2000).

The effects of thyroidectomy on gonadal responses take several weeks to fully develop (Wieselthier and van Tienhoven, 1972; Goldsmith and Nicholls, 1984; Wilson and Reinert, 1993; 2000) suggesting the responses are not immediately due to the absence of thyroid hormones. The delayed responses may represent the lack of a programming role of thyroid hormones during the initiation of photostimulation (Wilson and Reinert, 1995; 1999). In contrast Bentley *et al.* (1997) suggests the development of photorefractoriness does not depend upon the photoperiodically induced increase of T4 in intact starlings. Thyroidectomized starlings injected with exogenous T4 to maintain subphysiological T4 levels became photorefractory and molted suggesting the mere presence of T4 is required for the appropriate photoperiodic reproductive responses to photostimulation to occur.

The mechanisms by which thyroid hormones interact with the reproductive axis are not known. However studies thus far suggest that thyroid hormones act at the level of the hypothalamus (Follett *et al.,* 1988; Wilson and Reinert, 2000; Yoshimura *et al.,* 2003) although thyroid hormone action at a peripheral site cannot be excluded. Two studies provide evidence of a central control site. In thyroidectomized male American tree sparrows low doses of thyroid hormones injected intracerebroventricularly restored all components of seasonality whereas the same doses administered subcutaneously did not (Wilson and Reinert, 2000). Similarly infusion of T3 into the third ventricle by an osmotic minipump induced testicular growth in a dose dependent manner in Japanese quail held on short days (Yoshimura *et al.,* 2003).

1.3 Photoperiodic responses of Japanese quail

The photoperiodic responses of Japanese quail are the most extensively studied of any bird species (for review see Robinson and Follett, 1982; Follett and Nicholls, 1984; Follett and Pearce-Kelly, 1990). The small size of the quail, rapid sexual maturation and adaptability to a wide range of husbandry conditions has made the species popular as a laboratory animal for photoperiodic studies and behavioural, developmental and genetic research.

Annual cycles of gonad size and gonadotropin concentrations have been described in domesticated Japanese quail of the United Kingdom and Japanese strains held in outdoor cages (Follett and Maung, 1978; Robinson and Follett, 1982; Wada *et al.*, 1992). These strains of Japanese quail originate from birds genetically selected for specific photoperiodic response traits (United Kingdom) or from commercially bred stocks produced for egg or meat production (Japanese). The New Zealand strain of the Japanese quail has been bred to produce birds for both meat and egg production. Annual changes in gonad size and reproductive hormones have not been characterised in the New Zealand strain.

The photoperiodic responses to natural environmental conditions in the United Kingdom and Japanese strains are similar. During the increasing daylengths of spring, gonadotropin concentrations increased when daylengths were between 11.9 – 13 hours of light followed by gonadal development. Gonadal growth was rapid and was completed within six weeks. During late summer as daylength decreased, gonadotropin concentrations decreased and gonadal regression occurred followed by a period of refractoriness during winter when daylength was short and temperatures low.

Although these studies have measured gonadal and gonadotropin changes in birds exposed to natural environmental conditions, the endocrinology of the annual reproductive cycle has not been described for Japanese quail living in a group on the ground with access to nesting material and nest sites.

1.3.1 Gonadal growth on artificial long daylengths

In the laboratory male Japanese quail raised on long daylengths from hatch attain sexual maturity between five-six weeks of age (Stein and Bacon, 1976). Spermatozoa are first observed in the testes and vas deferens between 26-30 days after hatch (Mather and Wilson, 1964; Ottinger and Brinkley, 1979). Sexual maturity was also reached within a similar time frame in sexually immature, adult male quail transferred to maximally stimulatory daylengths (20L:4D) with gonadotropin concentrations significantly increased during the first week of photostimulation (Follett, 1976).

Increased testicular size is associated with increased androgen concentrations to stimulate the expression of reproductive behaviours such as crowing and strutting (Wada, 1981) and with secondary sexual characteristics such as the cloacal gland. The cloacal gland is an androgen dependent gland located in the dorsal lip of the cloaca that is stimulated by testosterone, androstenedione and 5α-dihydrotestosterone (Massa *et al.*, 1980). There is a high correlation between the size of this gland and testicular size and activity (Sachs, 1967; Siopes and Wilson, 1975; Follett and Maung, 1978; Massa *et al.*, 1980). The measurement of this gland is a quick and simple process and has been utilised as a convenient external indicator of testicular activity.

Ovarian growth in Japanese quail has been reported to be variable in comparison to the more uniform growth observed in male birds and there have been fewer studies of this gender. Female quail raised on long days (> 16 hours) from hatch begin laying eggs as early as five weeks of age (Stein and Bacon, 1976), with the majority of birds beginning to lay between eight-nine weeks of age (Wilson *et al.*, 1962; Brain *et al.*, 1988). In the latter studies small yolk filled follicles (3 – 5 mm diameter) were first observed at five weeks of age. The New Zealand strain of Japanese quail raised on long daylengths from hatch begin laying between six – eight weeks of age (Bennett, 2002; Chua, 2003). However, the development of the follicular hierarchy in quail has not been described.

The width of the cloacal opening in female Japanese quail is correlated with the diameter of the oviduct (Bennett, 2002; Chua, 2003) and provides useful information about the development of the reproductive tract.

1.3.2 Gonadal growth on artificial short daylengths

There has been a range of results from studies on gonadal growth of Japanese quail in response to short daylengths. These variations are due to both photoperiod and the strain of quail used. Testicular growth was retarded for between 15 – 25 weeks in quail raised from hatch on 6L:18D (Follett and Farner, 1966; Siopes and Wilson, 1974.; Stein and Bacon, 1976) and for up to 10 weeks in quail raised on 8 L: 16D (Konishi *et al.*, 1965). This delay in testicular growth was due to low gonadotropin concentrations as quail raised on non-stimulatory photoperiod respond to exogenous ovine LH (McFarland *et al.*, 1964). Oishi and Konishi (1983) showed that whilst some quail maintained immature cloacal gland areas on 8L others developed fully mature cloacal glands suggesting differences in photoperiodic responses within a population also occur.

Ovarian growth and the onset of lay was also delayed in female quail raised on 6 L or 8 L from hatch (Siopes and Wilson, 1974; Stein and Bacon, 1976; Brain *et al.*, 1988). Stein and Bacon (1976) found females raised on 6L did not attain full maturity until 19 weeks of age whilst hatch mates raised on 14 L were laying at an average age of six weeks.

Transferring sexually immature quail from 16 L to 8 L at three weeks of age also results in diverse gonadal responses. Gonadal growth was prevented indefinitely in male quail (S.Ishii pers. comm. cited in Chua, 2003) and female quail (Wakabayashi *et al.*, 1992) although gonadal growth was observed in the majority of both sexes of the New Zealand strain of quail after seven weeks of short days (Chua, 2003). Gonadal inhibition in immature Japanese quail can however be achieved through the manipulation of environmental stimuli such as temperature in addition to photoperiod. Chua (2003) found that short days and low temperatures (10 °C) prevented gonadal development and gonadotropin increases in all males and in 83 % of females between three and 10 weeks of age, as reported for quail in Japan (Oishi and Konishi, 1978; Wada, 1993). The mechanism by which gonadal inhibition on short days and low temperatures occurs is not known but appears to be the result of the additive effects of photoperiod and temperatures as low temperatures alone do not inhibit the reproductive axis of Japanese quail (Oishi and Konishi, 1978).

1.3.3 Gonadal regression in quail transferred from long to short daylengths

Transferring sexually mature Japanese quail from long to short days results in a variety of responses ranging from complete gonadal regression (Eroschenko and Wilson, 1974; Stein and Bacon, 1976; Follett *et al.*, 1977; Robinson and Follett, 1982; Urbanski and Follett, 1982a) to partial or no regression (Oishi and Konishi, 1983; Tsuyoshi and Wada, 1992; Wada *et al.*, 1990; 1992; Wada, 1993; Chua, 2003). Gonadal regression occurs rapidly as a result of decreased gonadotropin concentrations (Urbanski and Follett, 1982a; Robinson and Follett, 1982; Delville *et al.*, 1985). Egg production ceases within one to two weeks after birds are transferred to short days (Wilson *et al.*, 1965; Stein and Bacon, 1976). Studies using the Japanese strain of quail showed that transferring mature male quail from long to short days did not suppress LH to reproductively quiescent concentrations (Wada *et al.*, 1990; Tsuyoshi and Wada, 1992; Wada, 1993). This strain of quail requires both short days and low temperatures (10 ° C) to fully suppress LH concentrations and cause gonadal regression. The effects of environmental temperatures on gonadal regression are thought to be mediated by increased thyroid hormone secretion, although the mechanism by which this occurs is not known. Gonadal regression in the New Zealand strain of the Japanese quail has not yet been studied. As the New Zealand strain of the Japanese quail do not appear to show strong photoperiodic responses, it is likely that this strain will also require both short days and low temperatures for complete regression to be attained.

The endocrine mechanisms involved in the control of reproduction in birds and the known photoperiodic responses of the model species used in these studies have been discussed in the preceding section. Treatment with GnRH should stimulate the hypothalamic-pituitary-gonadal axis in birds, as has been observed in studies on mammals. Studies of GnRH treatment in birds will be discussed in the next section.

1.4 Hormonal stimulation using GnRH in birds

Treatment with exogenous GnRH has been used to increase reproductive output and control breeding schedules of several domestic mammalian species. Treatment with GnRH can induce ovulation in seasonally anoestrous mares (Hyland *et al.*, 1987; Johnson, 1987; Turner and Irvine, 1991; Becker and Johnson, 1992), ewes (McLeod *et al.*, 1982; Wright *et al.*, 1983) and cows (Riley *et al.*, 1981, Vizcarra *et al.*, 1997; 1999;

Barros *et al.*, 2000; Martinez *et al.*, 2000). GnRH has also been used to stimulate reproductive activity in non-domestic mammalian species such as rats, hamsters, and rabbits (Humphrey *et al.*, 1973) and in the rhesus monkey (Belchetz *et al.*, 1978). In human artificial insemination and *in vitro* fertilisation protocols, GnRH agonists have been used to improve ovarian responses to ovary stimulation (Barbieri and Hornstein, 1999). Agonists administered during the luteal phase, before giving ovulation induction hormones, significantly improves pregnancy success and live birth numbers in humans and primates due to enhanced stimulation and control of the preovulatory LH surge (Barbieri *et al.*, 1999).

There has been limited hormonal stimulation research in birds using GnRH. The majority of studies in which exogenous GnRH has been administered have focussed on the GnRH-induced gonadotropin response. A single injection of synthetic GnRH (4 – 20 μg) can significantly increase plasma LH concentrations in Japanese quail (Davies and Collins, 1979; Hattori *et al.*, 1986a), cockerels and chickens (Furr *et al.*, 1973b; Bonney *et al.*, 1974; Wilson and Sharp, 1975b; Johnson and van Tienhoven, 1981; Sharp and Lea, 1981; Tanabe *et al.*, 1981; Clarke and Ottinger, 1987, Wilson *et al.*, 1989), turkeys (Burke and Cogger, 1977; El Halawani *et al.*, 1987) and white crowned sparrows (Wingfield *et al.*, 1979; Wingfield and Farner, 1993; Osugi *et al.*, 2004). The GnRH-induced LH responses varied both between species and within species treated during different physiological reproductive states. GnRH-induced gonadotropin secretion is greatest in sexually immature birds and diminishes as birds mature (Bonney *et al.*, 1974; Knight *et al.*, 1985; Wilson *et al.*, 1989). This may reflect functional changes of the feedback control systems in the maturing HPG axis.

Several *in vitro* and *in vivo* studies have compared the ability of both cGnRH-I and cGnRH-II to release LH and/or FSH in birds. The LH response to a single injection of exogenous mGnRH or chicken GnRH-I is immediate and short–lived and is not thought to be long enough to stimulate the initiation of ovarian growth in birds. In some studies cGnRH-II was approximately six-fold more potent than cGnRH-I in releasing LH and/or FSH (Miyamoto *et al.*, 1984; Millar *et al.*, 1986; Sharp *et al.*, 1986; King *et al*, 1988; Delobelle *et al.*, 1995) or equipotent (Hattori and Ishii, 1984; Chou *et al.*, 1985; Hattori *et al.*, 1986a) Porcine GnRH was slightly more active than cGnRH-I in LH releasing ability in broody bantams (Sharp *et al.*, 1986). cGnRH-I and mGnRH were

equipotent in stimulating LH release from chicken pituitary cells (King and Millar, 1982a; Millar and King, 1983; Milton *et al.,* 1983; Millar *et al.,* 1986).

1.4.1 Stimulation of ovarian development in birds using GnRH

Few studies have investigated the stimulation of ovarian development in birds with exogenous GnRH. Attempts to induce ovarian development in non-laying poultry with injections of GnRH have thus far been unsuccessful. Treating hens daily for 14 days with 3.4 μg of mGnRH did not result in increased ovarian weight or follicular development (Reeves *et al.,* 1973). Burke and Cogger (1977) treated turkeys twice daily with cGnRH-I for six days and also reported no increase in ovarian, oviductal or pituitary weights. In contrast Minoia *et al,* (1984) reported moderate development of ovarian follicles and oviducts of pheasants and partridges after five, 10 and 15 days of treatment with 3.8 μg mGnRH every eight hours. However, sample sizes on each day were small (n = 2) and birds were held under conditions of natural light. The experiment began on short daylengths although the changes in natural daylength during the study were not reported.

Superactive analogues and continuous release implants have also been used to enhance and prolong GnRH-induced LH secretion in birds. A single intravenous injection (10 μg/bird) of long acting avian (D-Arg^6-cGnRH-II) and mGnRH (buserelin) analogues resulted in a sustained LH release for approximately eight hours in broody bantams (Sharp *et al.,* 1986). However, repeated injection of both hormones at 7 – 9 hourly intervals for nine days failed to maintain elevated plasma LH concentrations and subsequently caused ovarian regression. GnRH administered in polyacrylamide gel implants to turkeys also stimulated LH release for up to eight hours before concentrations returned to baseline levels (Burke and Cogger, 1977). It is thought the decreased plasma LH concentrations resulted from pituitary desensitisation to GnRH stimulation.

1.4.2 Stimulation of ovulation in birds using GnRH

Mammalian and avian GnRH have been used to induce normal and premature ovulations in chickens and quail. Premature or normal ovulations were induced in some birds treated with 5 – 10 μg GnRH whilst doses between 20 and 40 μg administered

between 15 and 12 hours before the next expected ovulation caused almost all birds to ovulate up to six hours before the next expected ovulation (van Tienhoven and Schally, 1972; Reeves *et al.,* 1973; Tanaka and Kamiyoshi, 1976; Johnson and van Tienhoven, 1981; Sekiguchi and Imai, 1987; Onagbesan and Peddie, 1988). In contrast Bonney *et al.* (1974) did not stimulate ovulation in laying hens using 20 μg mammalian GnRH.

Daily injections of either 1 or 10 μg buserelin in laying hens for 12 days synchronized the timing of most ovipositions and did not affect the rate of lay (Sterling and Sharp, 1984). Plasma LH concentrations in these birds increased significantly after injection of buserelin on both days 1 and 12 which suggested there was no reduction in pituitary responses to the analogue. This is in contrast to the loss of reproductive function observed after chronic buserelin administration in mammals (Johnson *et al.,* 1976; Fraser *et al.,* 1980; Schmidt-Gollwitzer *et al.,* 1982).

1.4.3 Conclusion

The effectiveness of GnRH for the stimulation of increased gonadotropin concentrations leading to ovarian development remains unclear. Previous studies varied in the type of GnRH used, doses, frequency and duration of treatment and the method of hormone delivery, in addition to different species of birds used in various reproductive status. It is clear however, that both mammalian and avian GnRHs are capable of stimulating gonadotropin release, and could potentially induce ovarian development.

1.5 Outline of thesis

The overall aim of the research described in this dissertation was to determine whether GnRH could be used to stimulate ovarian growth leading to ovulation and egg-laying in Japanese quail. A protocol developed in quail could then be utilised to develop a treatment programme to stimulate reproduction in endangered birds.

This research was conducted using the New Zealand strain of the Japanese quail (*Coturnix coturnix japonica).* Japanese quail have been extensively studied as they reach sexual maturity within four - six weeks of age, are easily housed and maintained and display consistent reproductive behavioural patterns. The Japanese quail was a good model species for this research as reproductive activity of Japanese quail can be

easily manipulated through changes in photoperiod and temperature and the patterns and rates of gonadal growth and regression could be easily determined for comparative studies in which exogenous hormones would be used to stimulate gonadal growth.

Pregnant mare serum gonadotropin (PMSG) has previously been used in our laboratory to stimulate ovarian growth and ovulation in quail. However responses to PMSG treatment were very variable and frequently led to overstimulation of quail ovaries. GnRH was therefore chosen for the present study because it is the principal hormone responsible for the control of reproduction in mammals and birds, is easily obtained and administered to birds, and a variety of GnRH analogues with varying activities are commercially produced.

The following questions were addressed in this thesis:

1. Does the New Zealand strain of the Japanese quail have an annual cycle of gonadal growth and regression, gonadotropin, prolactin and thyroid hormone concentrations when housed outdoors and able to express natural behaviours under natural conditions?
2. What are the patterns and rates of changes in gonad size and reproductive hormone concentrations in quail during photoperiodically-induced gonadal growth and regression?
3. Can exogenous GnRH stimulate endogenous LH in Japanese quail?
4. Can a single daily injection of GnRH dissolved in saline or a vehicle designed to delay absorption induce ovarian development?
5. How does the frequency of GnRH administration affect LH secretion?
6. Does a continuous low dose of GnRH stimulate LH secretion and ovarian development?

Chapter 2 describes an experiment in which the annual reproductive cycle was determined whilst chapter 3 describes a series of experiments in which the patterns and rates of gonadal growth and regression and the associated reproductive hormones were determined. Chapter 4 describes a series of experiments that were designed to answer questions 3 – 4 outlined above. Chapter 5 describes an experiment designed to answer question 5 and chapter 6 describes an experiment in which osmotic minipumps were

used to deliver continuous doses of GnRH. In chapter 7, the general discussion, the results from chapters 2 – 6 as well as future directions for this research are discussed.

2 Annual reproductive cycle of female Japanese quail held in a semi-natural environment

2.1 Introduction

It is well established that the annual changes in daylength play an important role in the regulation of reproductive cycles in vertebrates (Follett, 1984; Wingfield *et al.*, 1997). Additionally, environmental cues such as temperature, food and rainfall are also essential for the regulation of seasonal breeding. In birds, as in other vertebrates, the reproductive cycle involves changes in both physiology and behaviour for the individual to develop from sexual maturation through to rearing of young. The integration of these changes is largely ensured by changes in various pituitary and gonadal hormones whose secretion is in turn influenced by environmental and behavioural cues (Dawson and Goldsmith, 1982).

In seasonally breeding birds there are annual cycles of gonadal growth and regression and of plasma concentrations of luteinising hormone (LH) and follicle stimulating hormone (FSH). LH and FSH stimulate gonadal growth and maturation, the initiation of gonadal steroid secretion, and egg laying in female birds (Sharp *et al.*, 1998). Pronounced annual cycles of prolactin are also observed, with prolactin generally low during autumn and winter when daylength is short and temperatures low in ring doves (Buntin, 1988), starlings (Dawson and Goldsmith, 1982) and great tits (Silverin and Goldsmith, 1997). After photostimulation prolactin concentrations increase and are maximal during incubation and during the start of caring for young in turkeys (Scanes *et al.*, 1979; Burke and Dennison, 1980), starlings (Dawson and Goldsmith, 1982) and ring doves (Buntin, 1988; for review see Sharp *et al.*, 1998). In birds not incubating eggs or caring for young, seasonally high concentrations of prolactin in response to increasing

daylength are associated with photorefractoriness (Nicholls *et al.,* 1988) which in turn is associated with decreases in plasma LH and gonadal steroids (Sharp *et al.,* 1998). The thyroid hormones have also been implicated as playing a permissive role in allowing long daylengths to activate photorefractoriness, terminate reproduction and perpetuate the annual cycle in the Japanese quail and the starling (Follett *et al.*, 1988; Nicholls *et al.*, 1988; Dawson, 1989).

Photoperiodic responses in Japanese quail maintained under fixed daily photoperiods in the laboratory have been characterised in detail primarily for males (Follett, 1976; Follett and Maung, 1978; Davies and Follett, 1980; Peczely *et al.*, 1980; Robinson and Follett, 1982; Brain *et al.*, 1988, Nicholls *et al.*, 1988; Follett and Pearce-Kelly, 1990; Wada *et al.,* 1990, 1992; Wada, 1993; Tsuyoshi and Wada, 1992; Wakabayashi *et al.*, 1992; Chua, 2003). However, there is limited information on the annual reproductive cycle of Japanese quail maintained under natural photoperiods and temperatures.

Annual cycles in gonad size and gonadotropin concentrations have been described in Japanese quail held outdoors under natural photoperiods and temperature in the United Kingdom and Japanese strains of quail. In experiments in Bangor (53°15'N) during 1975-1978 and Bristol (51°27'N) during 1979-1980 where quail were held under natural weather conditions in an outdoor aviary with a run and in individual cages in a greenhouse (respectively), gonadal growth began when daylengths were between 11.9 and 13 hours. Gonadal regression was first observed during late summer when daylength was approximately 14 hours (Follett and Maung, 1978; Robinson and Follett, 1982). Similar results were found in male Japanese quail held in individual cages and exposed to natural weather conditions in Ichikawa City (35°45'N) in Japan. LH increased when daylength exceeded 12 hours and decreased when daylength was 14 hours (Wada *et al.,* 1992). The endocrinology of the annual cycle has not been described in female Japanese quail that are able to express natural behaviours such as nest building and incubation behaviours.

Behavioural studies of Japanese quail in environments where nesting materials were provided have not consistently observed full reproductive behaviours from courtship to the raising of young (Rothstein, 1966; Hess *et al.,* 1976; Buchwalder and Wechsler, 1997; Schmid and Wechsler, 1997). Reproductive behaviours such as nest building and

the incubation of eggs are said to have been bred out of this domestic species (Ball and Balthazart, 2002). However, Nichols *et al.* (1992) found Japanese quail placed in a semi-natural environment exhibited a behavioural repertoire similar to that seen in wild quail.

The goal of the current experiment was to characterise the pattern and timing of gonadal growth and regression and changes in plasma concentrations of reproductive hormones in female Japanese quail maintained in a semi-natural environment where natural behaviours could be expressed. The experiment was conducted to determine the endocrine and gonadal changes occurring during gonadal growth under natural conditions as a reference for laboratory experiments where gonadal growth and regression would be stimulated under artificial conditions.

The aims of the experiment were as follows:

1. To determine the annual patterns and timing of gonadal growth and regression in female Japanese quail able to express normal behaviours when maintained in a semi-natural environment under natural weather conditions.

2. To determine the annual patterns and timing of change of plasma LH, FSH, prolactin, thyroxine and triiodothyronine in female Japanese quail able to express normal behaviours when maintained in a semi-natural environment under natural weather conditions.

2.2 Materials and methods

2.2.1 Animals and housing

Ten female and two male three week old Japanese quail (*Coturnix coturnix japonica*) of wildtype plumage were purchased from a commercial source (Rangitikei Game Birds, Bulls, New Zealand). The birds had been reared under a long day photoperiod (15 hours light: 9 hours dark) at ambient temperatures (20 – 25 °C). The quail were sexually immature at the time of purchase.

Quail were identified with two coloured leg bands (Table 2.1) and transferred to a large outdoor courtyard (14 (length) x 10 (width) x 8 (height) m) in the Veterinary Science building, Massey University, Palmerston North, New Zealand (40°21'S, 175°37'E). The total number of quail that could be housed was limited by the size of the courtyard. The inclusion of the two male quail ensured natural mating would occur in some quail and allow a description of the breeding season of female Japanese quail that are sexually active, able to build nests and raise young under natural conditions.

Table 2.1. Legband identification of quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.

Legband identification of female quail	Legband identification of male quail
White White (WW)	Red Yellow (RY)
Green Yellow (GY)	Green Green (GG)
Pink Pink (PP)	
Green Red (GR)	
Green White (GW)	
Blue Blue (BB)	
Green Pink (GP)	
Green Blue (GB)	

The courtyard was covered with a light-gauge galvanized wire fencing made with hexagonal mesh to prevent entry of passerine birds. The courtyard floor consisted mainly of natural soil surrounded by a 75 cm wide concrete edge and was planted with grasses, shrubs, four large stands of pampas grass (2 - 3 m height) and two berry producing trees. Birds were given quail food (Harvey Farms quail layer mash) and water *ad libitum*. The food dispenser was situated under a metal shelter. Additional live food (worms, insects and fresh greens) was freely available. Birds were exposed to natural weather conditions and were able to express normal reproductive behaviours and nesting activity. The quail could take cover both in the vegetation and under the metal shelter covering the food dispenser.

2.2.2 Experimental design

The experiment started in January 1999 and concluded in January 2000. Behavioural observations were made from inside the courtyard without the use of a hide and recorded by hand into a notebook. Behaviour was systematically observed for two hours each week, one hour in the morning (0900 - 1000 h) and one hour in the afternoon (1600 - 1700 h) by two observers. Casual observations were recorded daily. During the one hour observation periods general activities (feeding, drinking, preening,

dustbathing, walking/running, flying, pecking/scratching, vocalisations), social interactions (pairing, pecking, chasing) and sexual behaviour (grasping female neck feathers, mounting, copulation) were recorded but not quantified. Nest building and incubation behaviour were also recorded along with the location of each nest and the number of eggs within each nest. Behavioural activity was recorded so that birds could be classified as nest building, incubating, caring for young or not participating in these activities at the time of blood sample collection.

Body weight (Mettler P1200 scales; ± 0.1 g) and cloacal measurements (vernier callipers; ± 0.1 mm) were recorded in all birds every four weeks from six weeks of age when the birds had reached sexual maturity. In males the cloacal gland area was determined by multiplying the maximum width and the maximum length of the cloacal protuberance. The cloacal gland in male quail is an androgen dependent gland (Balthazart *et al.*, 1979; Massa *et al.*, 1980) that increases in size and increases production of cloacal foam as circulating androgen levels increase, providing an external indicator of reproductive status. In females, the width of the cloacal opening was measured. The cloacal opening of female quail is the combined endpoint of the gastrointestinal tract and the oviduct. As the oviduct develops, the diameter of the cloacal opening increases, providing a practical external indicator of reproductive tract growth.

Blood samples were collected by puncture of the ulnar vein with a 27 gauge needle and approximately 800-900 µl of blood was collected into a into a heparinized 1 ml tube every four weeks from six to 54 weeks of age between 0900 and 1100 hours. Blood was expelled into a heparinized 1 ml polystyrene tube and kept on ice until centrifugation. Samples were centrifuged at 1 900 g for 15 minutes (Beckman TJ-6 centrifuge). Plasma was removed with a glass Hamilton syringe, stored in 1.5 ml polypropylene Eppendorf tubes and frozen at – 20 °C until assay.

2.2.3 Radioimmunoassay

Luteinising hormone (LH), follicle stimulating hormone (FSH), prolactin, thyroxine (T4) and triiodothyronine (T3) concentrations in plasma samples were determined by radioimmunoassay. Gonadal steroids were not measured in this experiment, as there was not sufficient plasma to do so. Plasma samples were thawed and spun at 14 000 g

for five minutes (IEC Micromax ventilated microcentrifuge OM3590) in a 1.5 ml Eppendorf tube to separate lipid from the plasma before assay. Clear plasma was removed from underneath the lipid using a glass Hamilton syringe.

Hormone concentrations for samples that measured below the sensitivity of the assay were assigned a value equal to the sensitivity of the assay.

2.2.3.1 Luteinising hormone (LH)

Luteinising hormone concentrations in plasma were measured directly by Dr Mitoshi Kikuchi (Department of Biology, Waseda University, Tokyo) using a modification of the double- antibody radioimmunoassay for chicken LH described by Hattori and Wakabayashi (1979). Purified chicken LH (CANOMS12442B; Kikuchi and Ishii, 1989) was used for radioiodination, with a crude chicken LH fraction (AGC112B; Kikuchi and Ishii, 1989) for the reference standard (Silverin *et al.*, 1999), and an anti-avian LH antiserum (AL-MH#1) (Hattori and Wakabayashi, 1979).

25 μl of plasma or standard was incubated overnight at 4°C with 400 μl of 0.1% gelatin in PBS (PBSG), 100 μl of diluted antiserum (1:100,000) in 1% normal rabbit serum (0.05M EDTA-PBS) (Hattori and Wakabayashi, 1979). 100 μl of 131I-LH in 0.1% PBSG was added the day after incubation and incubated overnight at 4°C. A goat anti-rabbit γ-globulin serum (H-4) was added to all tubes and then mixed before the supernatant was aspirated and the pellet counted (Hattori and Wakabayashi, 1979). The LH content in each sample was expressed in terms of the weight of CANOMS12442B.

The limit of sensitivity of the radioimmunoassay defined as the concentration of LH that corresponded to the percentage binding two standard deviations from the mean % bound of zero control tubes was 0.06 ng/ml. The intra-assay coefficient of variation provided by Dr M. Kikuchi was 6.2%. Quail plasma produced dilution curves parallel to chicken LH standard curves.

2.2.3.2 Follicle stimulating hormone (FSH)

Follicle stimulating hormone concentrations in quail plasma were measured by Dr Mitoshi Kikuchi using a modification of the double-antibody radioimmunoassay for chicken FSH described by Sakai and Ishii (1983). Purified chicken FSH

(AGCSQS11132D; Kikuchi and Ishii, unpublished data) was used for radioiodination (Silverin *et al.,* 1999). Samples were analysed in duplicate in a single assay.

25 µl of standard or plasma diluted with gelatin-PBS (0.01 M disodium phosphate buffer, pH 7.5 containing 0.15 M NaCl, 0.1 % gelatin and 0.01 % merthiolate) and 25 µl of antiserum diluted with NRS-EDTA-PBS (0.01 M disodium phosphate buffer, pH 7.5 containing 0.15 M NaCl, 0.05 M disodium EDTA, 1 % normal rabbit serum and 0.01 % merthiolate) were mixed in an assay tube (5 x 55 mm) and incubated at 4 °C for two days. 100 µl of anti-rabbit γ-globulin in diluent (EDTA-PBS, 0.01 disodium phosphate buffer, pH 7.5 containing 0.15 M NaCl, 0.05 M disodium EDTA and 0.01 % merthiolate) was added and the mixture incubated at 4 °C for 24 hours. 500 µl of cold gelatin PBS was added and the tubes were centrifuged at 3 000 g for 20 minutes. The supernatant was removed by aspiration and the remaining pellets were counted in an automatic gamma counter.

The sensitivity of the radioimmunoassay for FSH was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 0.02 ng/ml. Serial dilutions of quail plasma in assay buffer produced curves parallel to chicken FSH standard curves (Sakai and Ishii, 1983). The intra-assay coefficient of variation was 6.2 %. Cross reactivity of the FSH antiserum is minimal with highly purified chicken LH preparations (Sakai and Ishii, 1983).

2.2.3.3 Prolactin

Prolactin concentrations in quail plasma were measured by Dr Richard Talbot (Roslin Institute, Edinburgh, Scotland) using a radioimmunoassay described by Talbot and Sharp (1994). Purified chicken prolactin was used for radioiodination (Talbot and Sharp, 1994). Samples were assayed in duplicate in a single assay.

50 µl of standard or plasma diluted with buffer (0.05 M sodium phosphate buffer containing 0.155 M NaCl, 0.1 % sodium azide, 0.01 M disodium EDTA and 2 % horse serum) containing antibody 31/1 at a dilution of 1:8 000 were mixed in polystyrene test tubes (6 x 50 mm) and incubated at 4 °C overnight. 50 µl of label was added and mixed and the mixture incubated at 4 °C overnight.

50 µl of donkey anti-rabbit serum and 50 µl of normal rabbit serum were added and mixed and allowed to stand at 4 °C for 16 hours before centrifugation at 2 000 g for 20 minutes. 50 µl of a 6 % solution of insoluble starch was added to each tube to prevent the disturbance of the precipitate during aspiration. Tubes were centrifuged at 2 000g for 10 minutes, the supernatant was removed by aspiration and the remaining pellets were counted in a LKB Wallac 1277 Gammamaster.

The sensitivity of the assay was 0.08 ng/ml. Serial dilutions of quail plasma in assay buffer produced curves parallel to chicken prolactin standard curves. The intra-assay coefficient of variation was 9.8 %.

2.2.3.4 Thyroxine (T4)

Thyroxine hormone concentrations in quail plasma were measured by Dr Masaru Wada (Tokyo Medical and Dental University, Ichikawa, Chiba 272, Japan) using a modification of a radioimmunoassay (Tasaki *et al.*, 1986) that has previously been used to measure T4 concentrations in Japanese quail (Wada, 1993). Samples were assayed in duplicate in a single assay.

1 µl of plasma, 50 µl of 0.11 M barbital buffer (containing NaN_3 (0.1 %) and bovine γ-globulin (0.25 %); pH 8.6) and 50 µl of diluted (38-fold dilution) anti-thyroxine serum (Lot 01628; Wien Laboratories Inc., New Jersey) were mixed and incubated at room temperature for three hours. 50 µl of [L-^{125}I] thyroxine (New England Nuclear, Boston; approximately 14 000 cpm) was added to each tube and incubated overnight at 4 °C. 200 µl of chilled barbital buffer and 500 µl of 20 % polyethylene glycol were added to each tube and incubated at 0 °C for 20 minutes. Samples were then centrifuged at 3 000 g (refrigerated centrifuge) and the supernatant removed by aspiration. The remaining pellets were counted in an automatic gamma counter.

The sensitivity of the radioimmunoassay for T4 was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 3 pg/ml of thyroxine in quail plasma. Mean intra-assay and inter-assay coefficients of variation were 2.1 and 13.5 % respectively. Cross reaction of the antibody with T3 was reported by Wien Laboratories Inc. as < 3.4 %.

2.2.3.5 Triiodothyronine (T3)

Triiodothyronine hormone concentrations in quail plasma were measured by Dr Masaru Wada using a modification of a radioimmunoassay (Tasaki *et al.*, 1986) that has previously been used to measure T3 concentrations in Japanese quail (Wada, 1993). Samples were assayed in duplicate in a single assay.

100 µl of plasma, 100 µl of 0.2 M borate buffer (containing bovine serum albumin (0.5 %), NaN_3 (0.05 %) and 8-anilino-1-naphthalene-sulfonic acid sodium salt (0.25 %); pH 8.5) and 100 µl of diluted (38-fold dilution) T3 antiserum (Endocrine Sciences, California) were mixed and incubated at room temperature for three hours. 100 µl of [L-3, 5, 3' ^{125}I] triiodothyronine (New England Nuclear, Boston; approximately 14 000 cpm) was added to each tube and incubated overnight at 4 °C. 1 ml of a dextran-coated charcoal suspension was added to each tube and then samples were centrifuged at 3 000 g and the supernatant removed by aspiration. The remaining pellets were counted in an automatic gamma counter.

The sensitivity of the radioimmunoassay for T3 was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 6 pg/ml of T3 in quail plasma. Intra-assay coefficient of variation was reported as 7.4 %. Cross reaction of the antibody with T4 was reported by Endocrine Sciences, California as < 2.7 %.

2.2.4 Meteorological data

Daylengths at Palmerston North, New Zealand including civil twilight of dawn and dusk were calculated for 1 January 1999 to 31 January 2000 from the Astronomical Applications Department database of the US Naval Observatory, Washington DC, USA (Figure 2.1A). Civil twilight is defined to begin in the morning and end in the evening when the centre of the sun is geometrically six degrees below the horizon. This is the limit when twilight illumination is sufficient for terrestrial objects to be clearly distinguished and is longer than the period between actual sunrise and sunset.

Data for daily temperature and rainfall from 1 January 1999 to 31 January 2000 were obtained from the National Climate Database (Observation station E05363,

AgResearch, Palmerston North) maintained by the National Institute of Water and Atmosphere Research Limited, Wellington, New Zealand (Figure 2.1B-C).

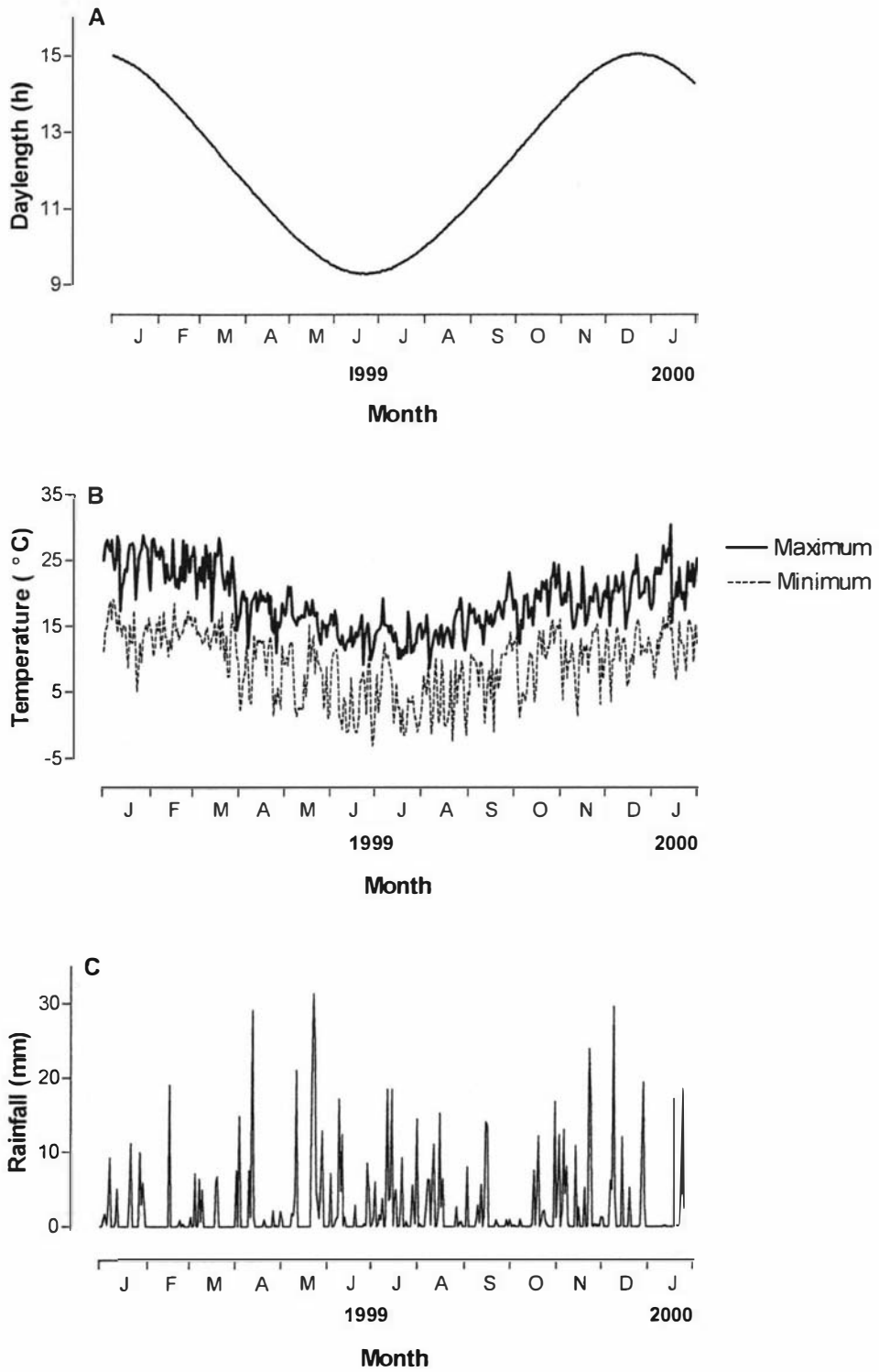

Figure 2.1. Annual cycle of daylength (including civil twilight) (A), minimum and maximum daily air temperatures (B) and daily rainfall (C) for Palmerston North, New Zealand (40° 21' S, 175° 37' E) from 1 January 1999 to 31 January 2000.

2.2.5 Statistics

Data were transformed to logarithms to reduce heteroscedascity where necessary. All variables were tested for homogeneity of variance using the Levenes test. When variances were homogenous across age for a single variable repeated measures ANOVA followed by Univariate F-tests were used to examine differences between ages. When variances were not homogenous across ages Friedmans non-parametric ANOVA and paired-samples t-tests were performed on selected data to determine differences between ages. Single measures ANOVA and independent-samples t-tests were performed on prolactin data at selected ages to determine differences between nesting and non-nesting quail. Body weights and the widths of the cloacal opening at three and six weeks of age were analysed using paired samples t-tests. A value of $p < 0.05$ was taken as statistically significant and indicated by an asterisk (*). Analyses were performed using Systat Version 8 (SPSS Inc., 1988).

Data are presented as individual points (raw data) or as means ± standard error (group data) using GraphPad Prism Version 3.0 (GraphPad Software Inc., 1999).

2.3 Results

Mortality during the experiment was 16.7 % (2 of 12 female birds). One bird (YY) died on 25 May 1999 and the other (RR) on 24 June 1999. Post mortem showed both birds were egg bound. Data from birds that did not survive the entire experiment were excluded from statistical analyses and graphs of mean group data and presented separately in individual data graphs. Data from the two male quail were presented in individual data graphs.

2.3.1 Reproductive activity

2.3.1.1 Male behaviour

Sexual behaviour was initiated in male quail between four and five weeks of age beginning with crowing. Crowing is a loud two-three syllable vocalization. During the year both male quail crowed periodically and were answered with cricket calls from the female quail when social groups were established around eight weeks of age.

Aggressive interactions between the two male quail began around five weeks of age and involved chasing, neck stretching, pecking and grabbing of neck feathers. This behaviour was observed throughout the year but was more frequent during autumn and spring-early summer when gonadotropin concentrations were high.

The male quail were polygamous and courtship behaviours such as strutting, wing flapping and tidbitting (pecking and scratching at the ground whilst emitting food calls, Etches, 1996) were directed toward groups of females and neck-grabbing or rear-approach and mounting attempts were directed at individual female birds.

Each male established his own social group of females and was observed performing maintenance activities such as foraging, preening, dust bathing and mating with the females in his own group (Table 2.2). Of particular interest was a pair bond formed between GG and WW within the first month that continued through to the end of the year. Both males attempted to court females from the other's social group that resulted in aggressive chasing, face pecking and feather pulling between the males.

Table 2.2. Social groups of quail maintained outdoors in a semi-natural environment from 11 January 1999 to 6 January 2000.

Group	Quail
1	RY (male), RR, YY, GY, GP*
2	GG (male), WW, GB, BB, GP*
	GR and PP were observed in the presence of other females but not generally within the male-female social groups.
	GP* was observed in groups 1 and 2 during the year.

2.3.1.2 Female behaviour

2.3.1.2.1 Egg laying

Egg laying began when the birds were six weeks of age (Figure 2.2). The first eggs found in the courtyard were soft-shelled and scattered throughout the habitat. At nine weeks of age four birds began incubating clutches of eggs in nests and by 13 weeks of age all females had begun incubating eggs in nests.

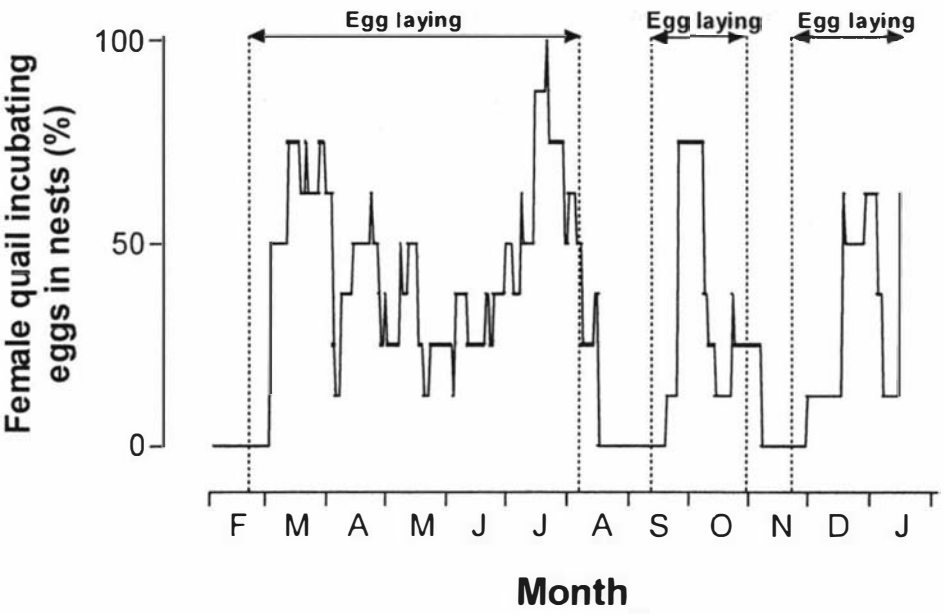

Figure 2.2. Periods of egg laying and daily percentage of female quail incubating eggs in nests. Quail were maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.

The proportion of females laying and incubating eggs varied throughout the year. All birds laid eggs between 22 February and 6 August followed by a period where almost no eggs were laid (7 August – 10 September). Two birds did however, lay eggs between 18 – 20 August then ceased. All birds then laid eggs from the 10 September, ceased laying between 1 – 21 November then continued through to the end of the experiment.

2.3.1.2.2 Nest building

Nests were built by females in large clumps of grass (75.5 %), at the base of the pampas bushes (17.8 %) or within a large pile of dried, decomposing weeds (6.7 %). Female quail used their bodies to hollow out a shallow bowl into which the eggs were laid. The female then walked around the bowl gathering dried grasses, plant fibres and twigs in her beak and threw these behind her in the direction of the bowl. These materials were then incorporated into the sides of the bowl forming a rim that prevented eggs from rolling out of the nest. Where the nest was utilized by two or more birds (see section 2.3.1.2.4 Communal nesting), one would sit on the nest adding materials to the rim whilst the other females would collect materials from the immediate surrounds. Females nesting in the grass pulled long blades of grass over the nest creating a canopy that sheltered and camouflaged the nest. This was repeatedly destroyed as birds moved on and off the nest. Maintenance of the nest with additional materials occurred throughout incubation.

2.3.1.2.3 Clutch size and incubation period

The number of eggs found in nests was variable. Quail tended to lay one egg per day in the nest until the clutch was complete. Clutch size in nests where one bird had laid ranged from 5 to 16 eggs (mean = 9.6 ± 1.1). The mean number of eggs found in nests that had eggs from more than one female was 13 ± 1.7 (see section 2.3.1.2.4 Communal nesting).

Incubation periods ranged between 15 and 23 days. All females incubating eggs developed brood patches and were observed turning eggs within the nest with their beaks and feet. During incubation females would leave the nest for 2 – 20 minute periods several times per day to eat, dust-bathe and preen. Birds approached and left their nests quietly and indirectly under the surrounding vegetation. Towards the end of

incubation females became aggressive towards all other birds. Generally vocalizations by incubating females were rare, although avoidance trills were directed toward approaching quail.

All females attempted to incubate eggs in a nest three or more times during the year. 71 % of all attempts to incubate eggs to hatch failed. Three birds (WW, GW, GR) successfully incubated their first nest of eggs to hatch. These three birds did not hatch chicks again although all attempted to. GY successfully incubated her second nest of eggs to hatch followed by three more broods. BB hatched broods on her third and sixth attempts and PP was successful on her sixth attempt. GB and GP did not hatch any chicks. All eggs that hatched were the result of a single female incubating the eggs during the last six days before hatch. Three of the nine nests where eggs hatched were at some stage incubated by more than one female.

Male quail were observed sitting quietly beside nests and interfering with nesting females. GG pecked at eggs underneath nesting females and both males chased females off nests and as the females returned to nests. On one occasion GG was observed sitting on a nest where he had developed a close pair bond with WW, one of two females incubating eggs within that nest. On this occasion GG prevented the other female from nesting and aggressively pecked at her until she retreated.

2.3.1.2.4 Communal nesting

Communal nesting was observed throughout the year. This involved more than one female laying and incubating eggs in a single nest. In some of the shared nests the females would for a short time, co-exist and share incubating and nest maintenance duties, although the vast majority of birds on shared nests would experience daily aggressive interactions. Females pecked and nudged one another and pulled eggs out from underneath one another and out of the nest. These daily disturbances resulted in some females deserting the incubation of eggs at a nest where another female was present and in some cases the communal nest was abandoned by all females incubating eggs.

2.3.1.2.5 Time and frequency of renesting

Renesting time after hatching chicks was 37.9 ± 9.45 days (range 8 – 95 days) compared with a renesting time after desertion of the nest of 32.4 ± 5.80 days (range 4 – 153 days). Renesting times were not significantly different between birds that had hatched chicks or deserted nests (Independent samples t-test, t = - 1.38,df = 8, p = 0.206). Two birds that deserted nests began to share another nest within 24 hours of deserting the first nest.

2.3.1.2.6 Hatchability of eggs and chick mortality

Data for hatchability were based on the nine nests that were successfully incubated to hatch. During the study six female quail produced nine natural broods with 2 – 5 chicks per brood. No broods were hatched during the same time period so only one group of chicks was present in the courtyard at any one time. Hatch success was 46.7 %. In nests where only one female laid hatchability was 61 % (n = 6 nests) and was higher (Independent samples t-test, t = - 14.75, df = 3, $p < 0.005$*) than in nests where more than one quail had laid eggs (hatchability = 40.1 %, n = 3 nests). Pipping and hatching were not observed on any occasion.

35 chicks were hatched during the study. No chicks survived for longer than 11 days post-hatch due to the poor parenting skills of the mother quail.

2.3.1.2.7 Parent-young bond

Females kept chicks in the nest for the first 24 hours and then spent time in covered areas where the female kept the chicks under her breast or tucked under her wing. Females and chicks were observed scratching for food amongst other quail approximately 48 hours after hatch. Mother quail were very aggressive toward other females and males. Female and male quail that were approached by chicks were apprehensive and often ran away. Fostering of chicks was not observed.

2.3.2 Body weight

Body weight increased gradually in female quail during the year (Figure 2.3; One way repeat measures ANOVA, $F_{12, 84} = 7.73$, $p < 0.000$*). As female quail matured and began to incubate eggs, wide ranges of body weights were observed (see Appendix 1.1 for individual data). Body weights of male quail also increased gradually during the year (Figure 2.4).

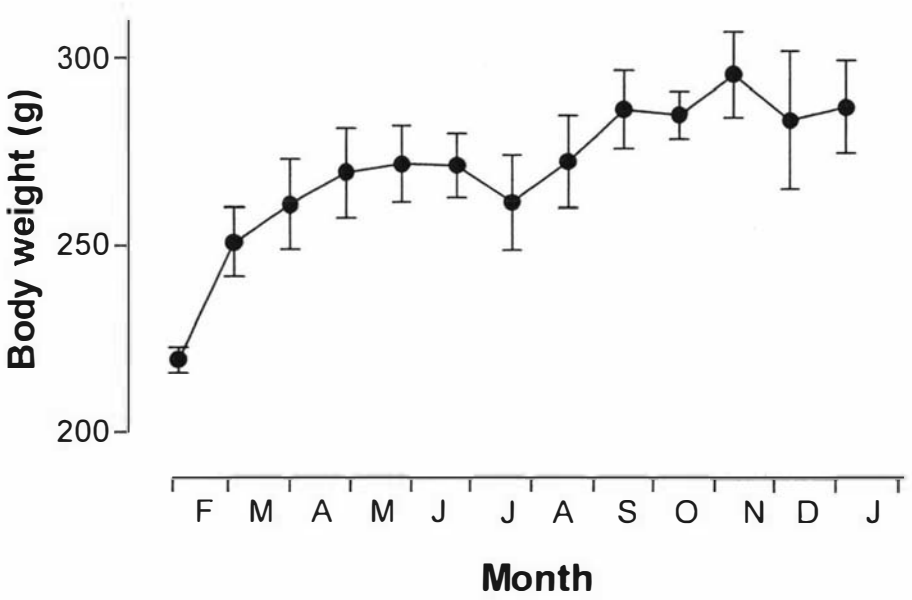

Figure 2.3. Changes in mean body weight of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as mean ± standard error.

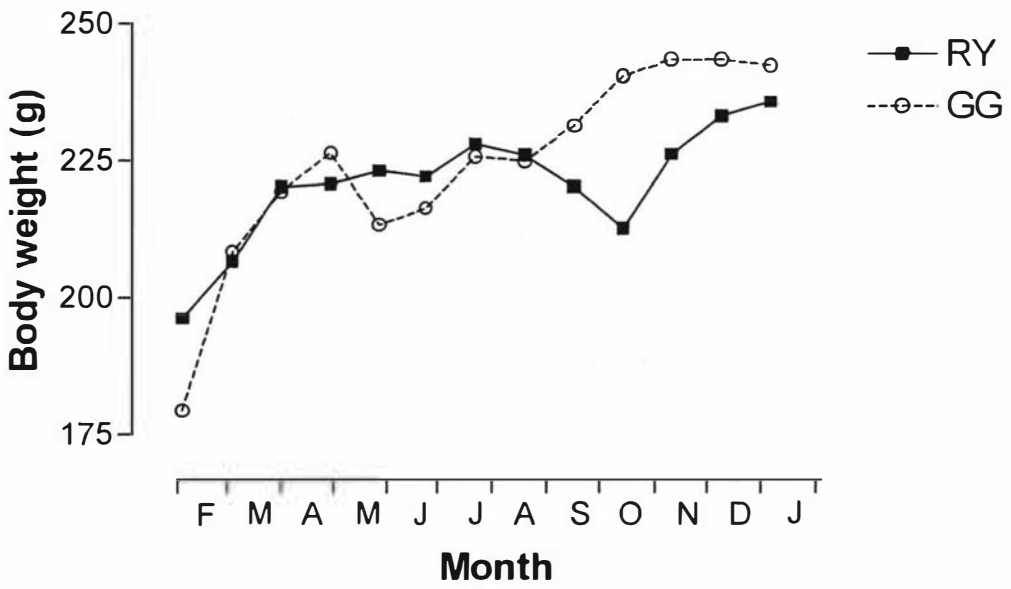

Figure 2.4. Individual body weights of male quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as individual data points.

2.3.3 Width of the cloacal opening

Changes in the mean width of the cloacal opening of female quail showed a seasonal cycle, which followed the changes in the annual daylength cycle (Figures 2.5 and Appendix 1.2 for individual data). The cloacal changes during autumn and spring lagged behind the changes in daylength. Cloacal opening decreased between February and May (see Table 2.3 for statistics) coinciding with a decrease in daylength from 15 to 10 h 19 min. Minimum mean cloacal opening occurred during May and June (9.81 ± 0.41 and 10.06 ± 0.35 mm respectively) when daylength was shortest (mean = 10 h 45 min; range = 10 h 19 min – 11 h 21 min) and daily temperatures were decreasing to minimum annual values in June-July. Cloacal openings then increased and from September 1999 to January 2000 were similar to those measured in February 1999 (see Table 2.3 for statistics).

Individual profiles for female and male quail are shown in figures 2.6 – 2.9. Changes in the width of the cloacal opening of most female quail showed a clear seasonal cycle. There was no clear seasonal change in the cloacal gland area of male quail.

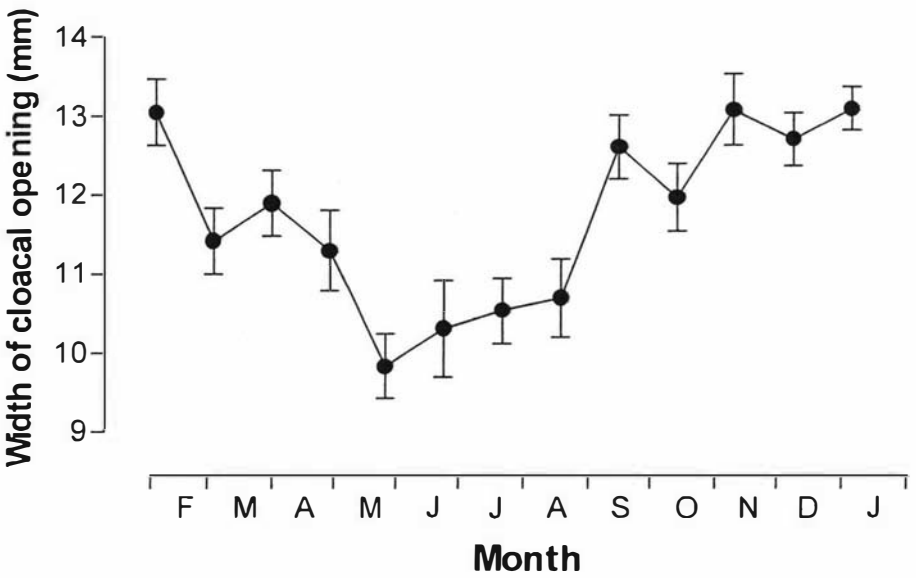

Figure 2.5. Mean cloacal opening diameters of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as mean ± standard error.

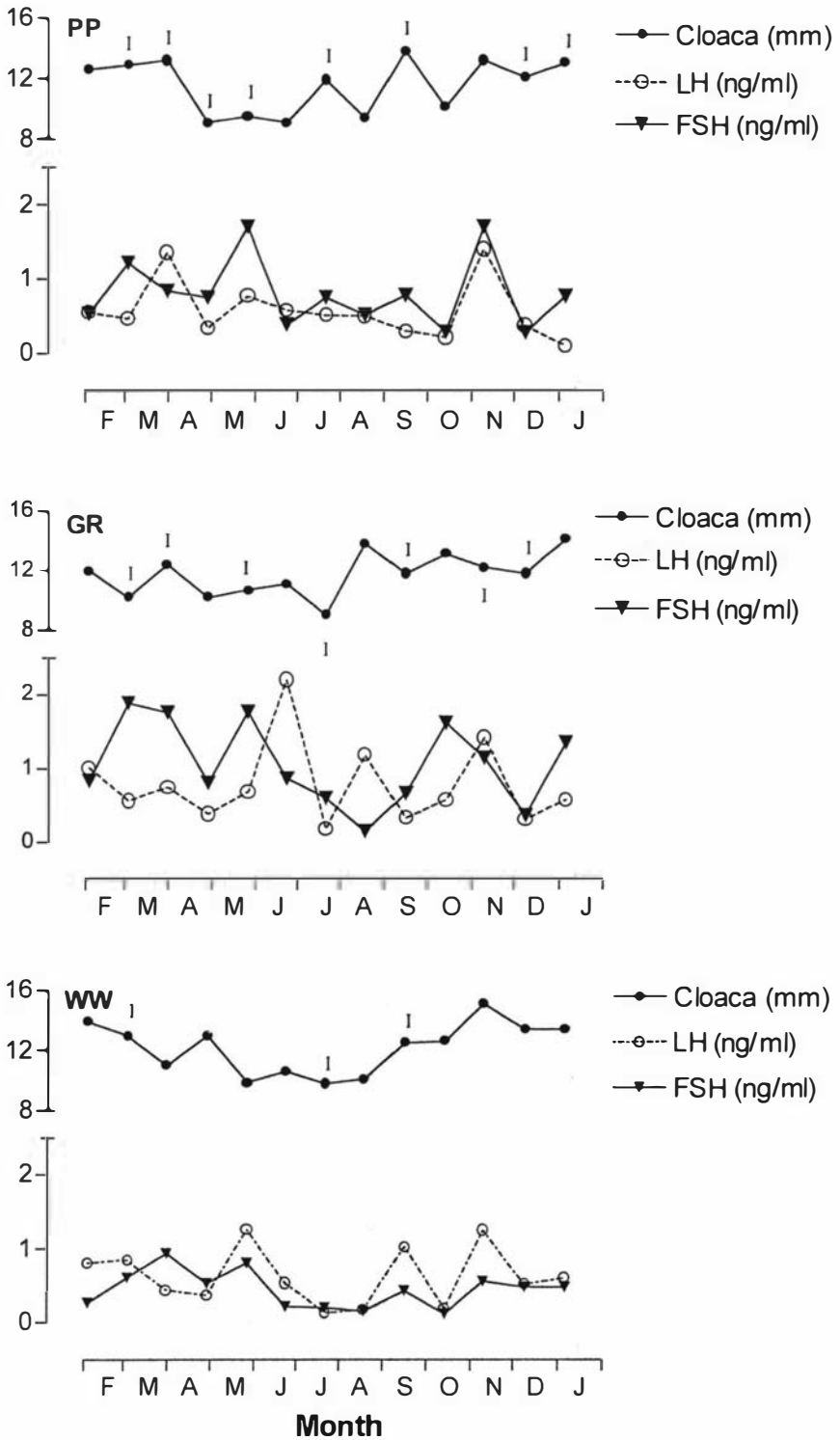

Figure 2.6. Individual profiles of the width of the cloacal opening, LH and FSH in female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. "I" indicates birds that were incubating eggs at the time of the collection of the blood sample.

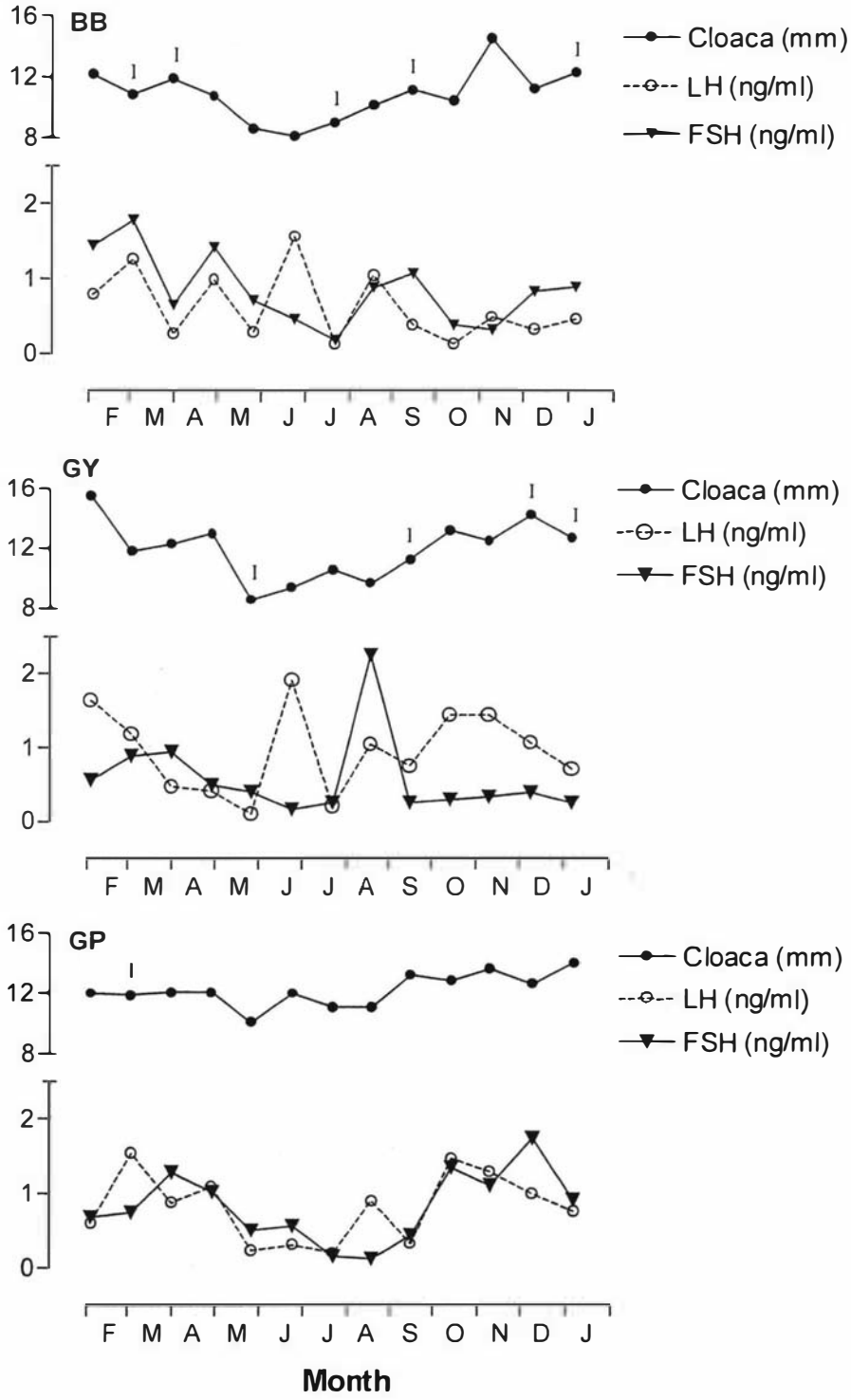

Figure 2.7. Individual profiles of the width of the cloacal opening, LH and FSH in female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. "I" indicates birds that were incubating eggs at the time of the collection of the blood sample.

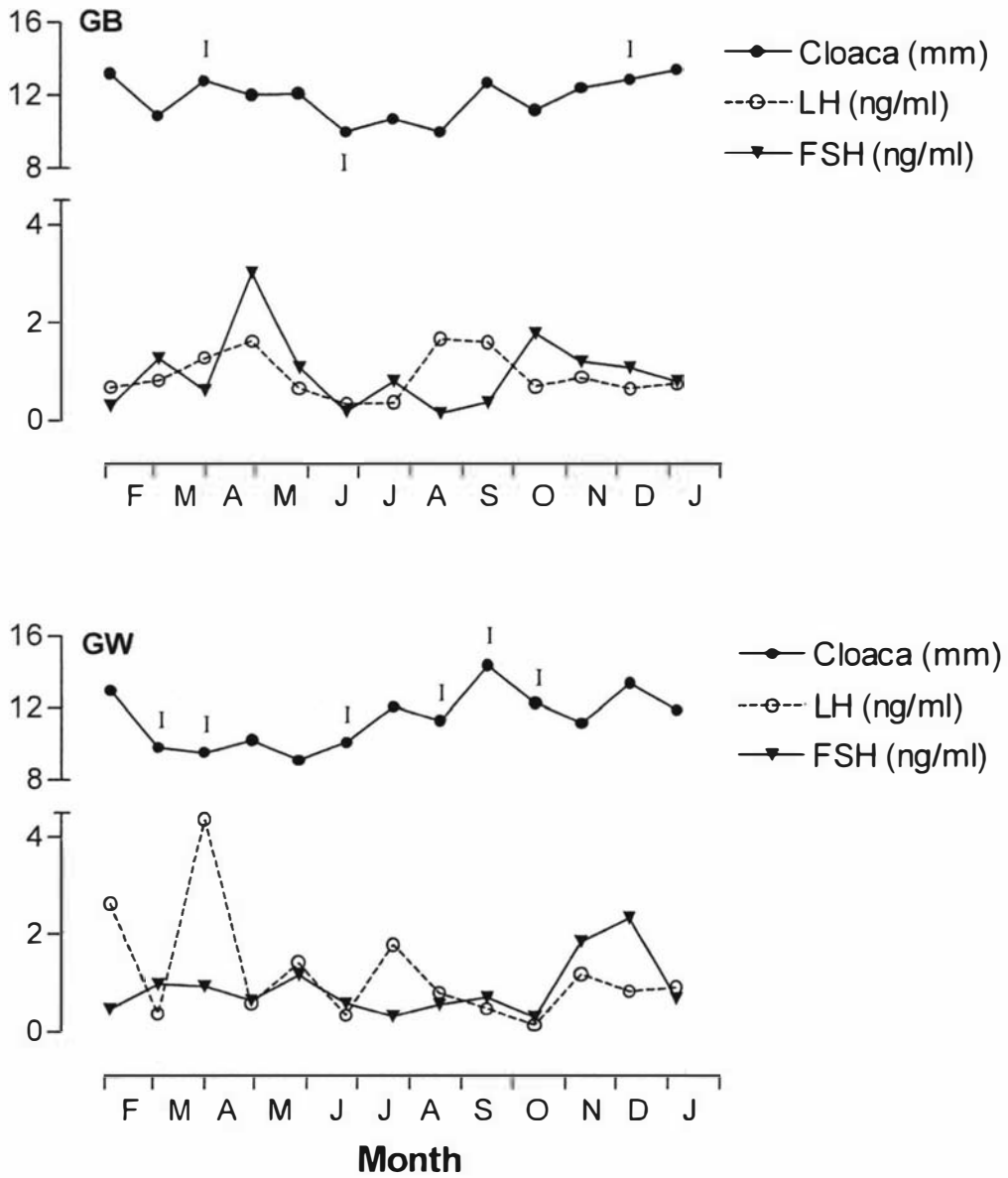

Figure 2.8. Individual profiles of the width of the cloacal opening, LH and FSH in female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. "I" indicates birds that were incubating eggs at the time of the collection of the blood sample

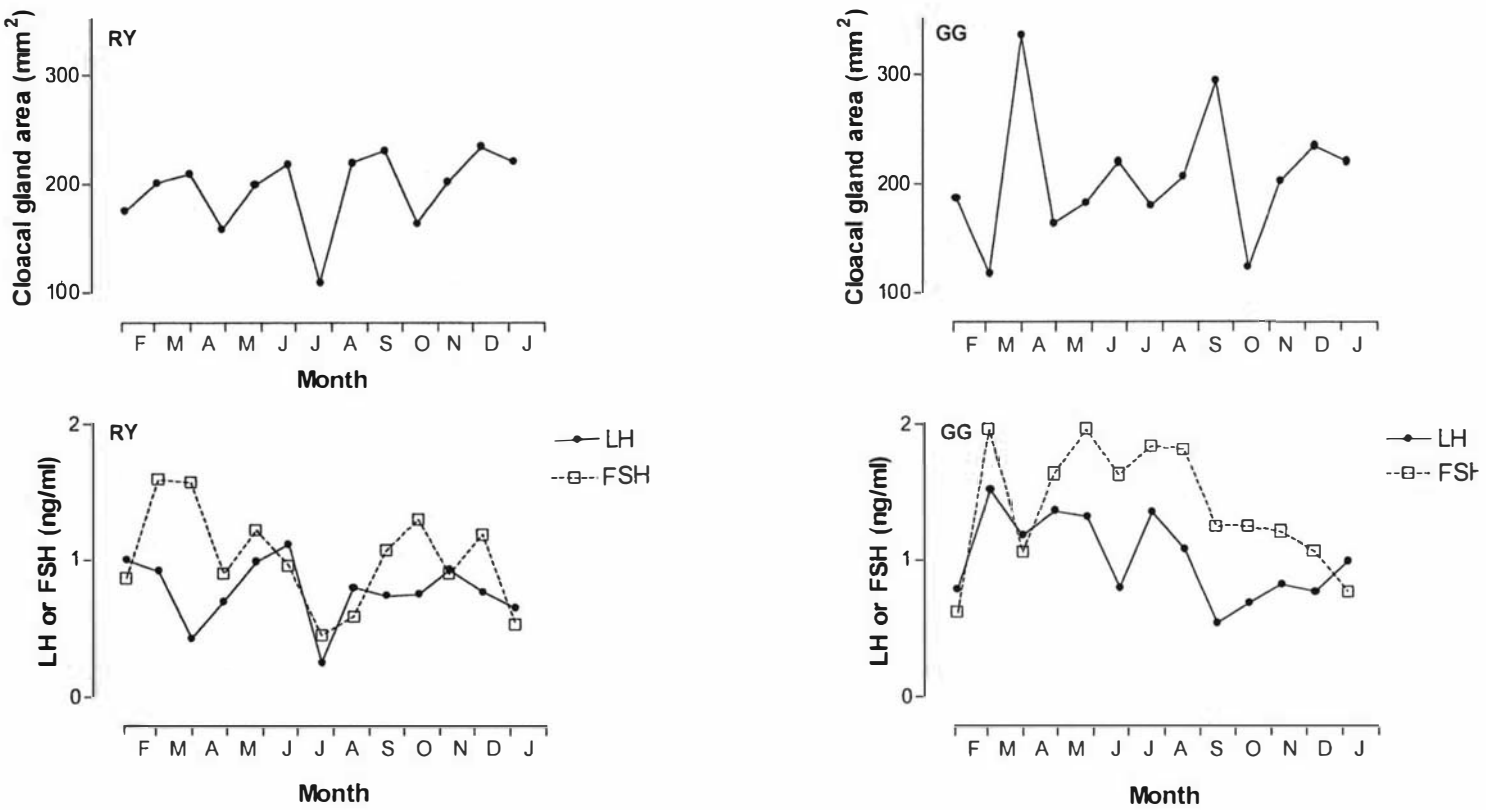

Figure 2.9. Individual profiles of the cloacal gland area, LH and FSH in male quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Note different scales on y axes.

Table 2.3. One-way repeat measures ANOVA for the width of the cloacal opening of female quail maintained outdoors in a semi-natural environment.

	Width of cloacal opening		
Effect	**F**	**df**	***p***
Time	8.43	12, 84	0.000*
Contrasts			
4 February 1999 vs 27 May 1999	24.80	1, 7	0.002*
4 February 1999 vs 16 September 1999	0.46	1, 7	0.518
4 February 1999 vs 6 January 2000	0.04	1, 7	0.844

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of post-hoc comparisons between sampling times.

2.3.4 Luteinising hormone (LH)

There was no clear seasonal cycle in mean LH concentrations in female quail (Figures 2.10 and Appendix 1.3 for individual data) although LH tended to be lower in most birds in mid winter compared to spring-summer. Mean LH concentrations gradually declined between February and July (see Table 2.4 for statistics) followed by fluctuations through to the end of the year. Annual LH cycles were clearly seen in some birds (GY, GP, GB) (Figures 2.7 and 2.8). GW had dramatically elevated LH concentrations on 1 April (Figure 2.8) and this was not included in mean group data or statistical analyses. At the time of sampling GW was not incubating and had just completed a post-nuptial molt after hatching chicks three weeks earlier.

There was no clear seasonal cycle in LH concentrations in male quail (Figure 2.9). Changes in LH concentration in RY were similar to the general pattern seen in female quail and LH was lower in winter than spring and summer.

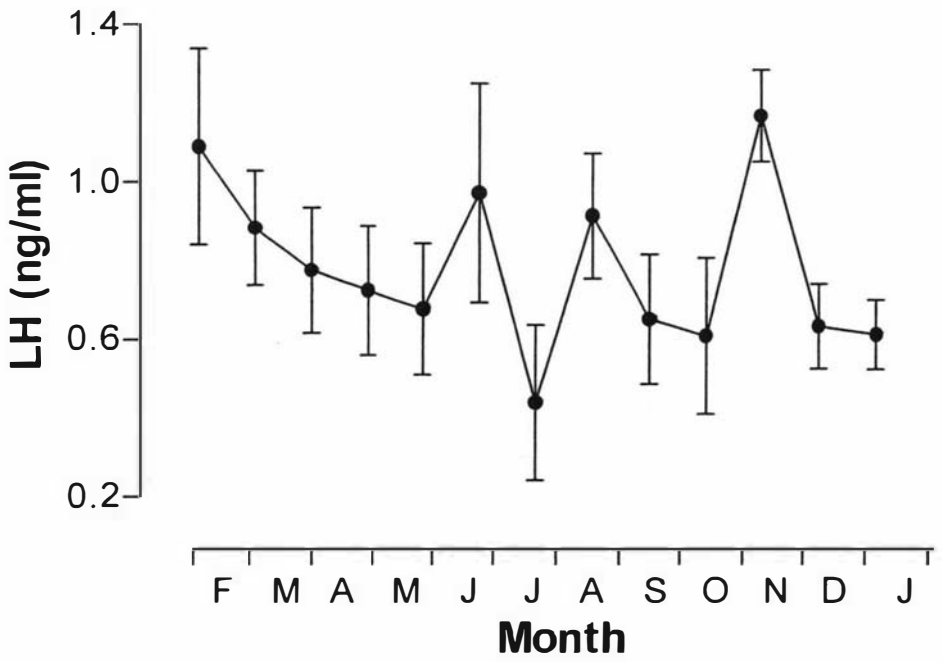

Figure 2.10. Mean LH concentration of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as mean ± standard error.

Table 2.4. One-way repeat measures ANOVA for mean LH concentrations in female quail maintained outdoors in a semi-natural environment.

		LH	
Effect	**F**	**df**	*p*
Time	2.22	12, 84	0.018*
Contrast			
4 February 1999 vs 22 July 1999	20.18	1, 7	0.003*

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining row shows the result of a post-hoc comparison between sampling times.

2.3.5 Follicle stimulating hormone (FSH)

There was a clear annual cycle in FSH concentrations in female quail (Figures 2.11 and Appendix 1.4 for individual data). FSH increased from February to March 1999, remained elevated between March and May as daylength decreased then decreased to minimum values in June-July (0.43 ± 0.08 and 0.41 ± 0.10 ng/ml; see Table 2.5 for statistics). FSH increased in August but was not significantly elevated until November when concentrations were not different from initial concentrations in February.

FSH annual profiles for individual female quail tended to follow the pattern observed for mean FSH concentrations (Figures 2.6 – 2.8). High FSH concentrations occurred during summer-autumn followed by decreased concentrations during winter and increasing FSH concentrations during spring-early summer. One male quail RY also showed this profile whilst GG maintained elevated FSH concentrations during winter before decreasing during spring to low concentrations.

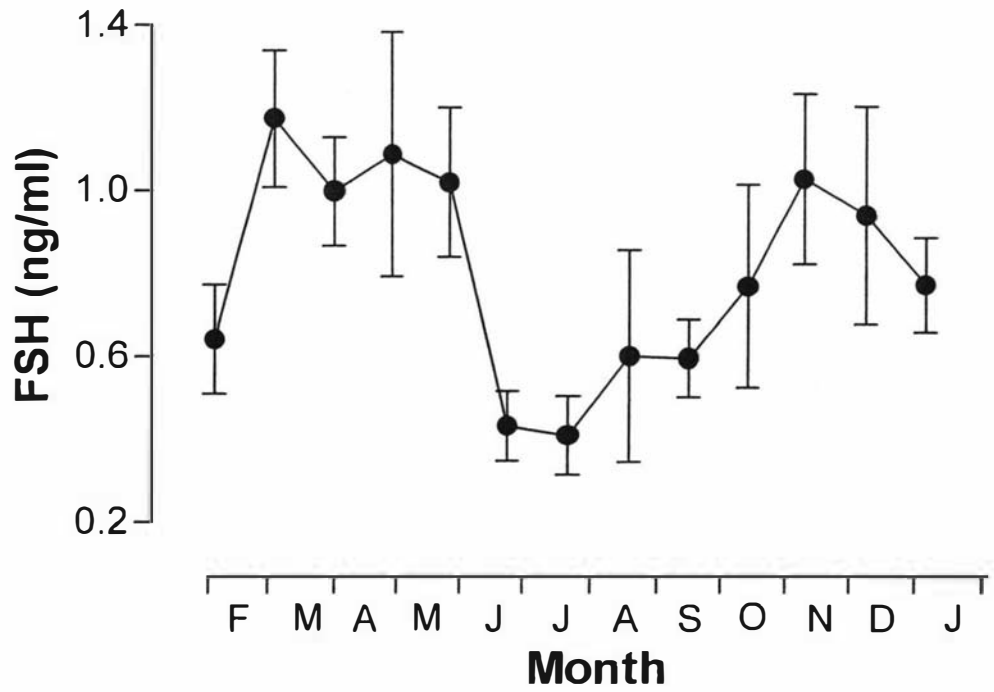

Figure 2.11. Changes in mean FSH concentration of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as mean ± standard error.

Table 2.5. Friedmans non-parametric ANOVA for mean FSH concentrations in female quail maintained outdoors in a semi-natural environment.

Effect	Test statistic	df	P
Time	34.88	12	0.000*
Contrasts			
4 Feb vs 4 March 1999	2.52		0.012*
4 Feb vs 11 Nov 1999	1.54		0.123
27 May vs 24 June 1999	2.38		0.017*
24 June vs 22 July 1999	- 0.28		0.779
22 July vs 11 November 1999	2.52		0.012*

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of post-hoc comparisons between sampling times.

2.3.6 Prolactin

There was no clear annual cycle in prolactin concentrations in female quail (Figures 2.12 and 2.13; One-way repeat measures ANOVA $F_{12, 84} = 2.38$, $p < 0.011$*) with a large range of concentrations measured at each sampling period with the exception of February and November (see Appendix 1.5 for individual data). Prolactin concentrations were similar in quail incubating eggs and caring for young and were higher in these two groups than in birds not incubating eggs (Figure 2.14; see Table 2.6 for statistics).

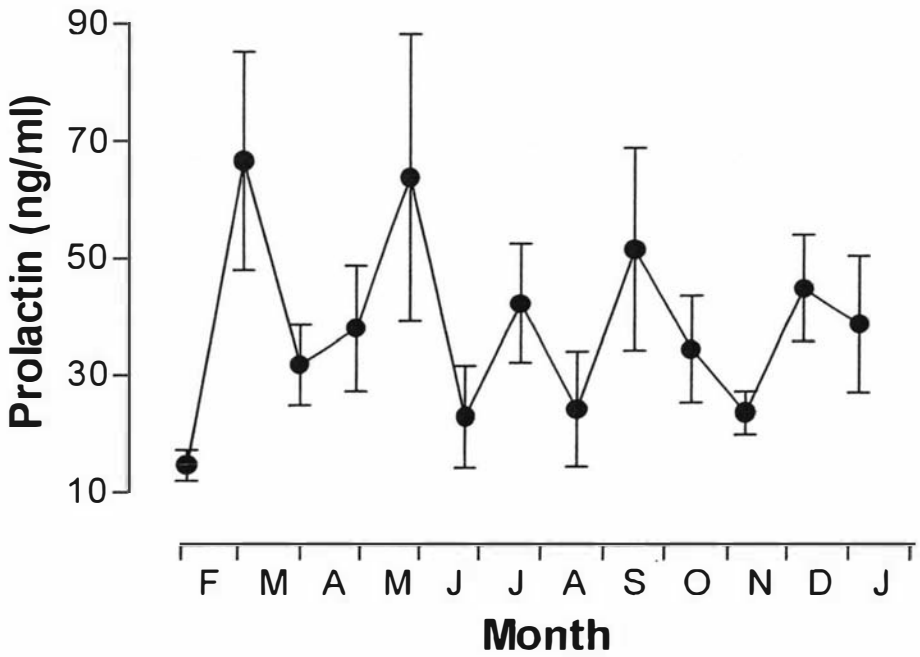

Figure 2.12. Changes in mean prolactin concentration of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as mean ± standard error

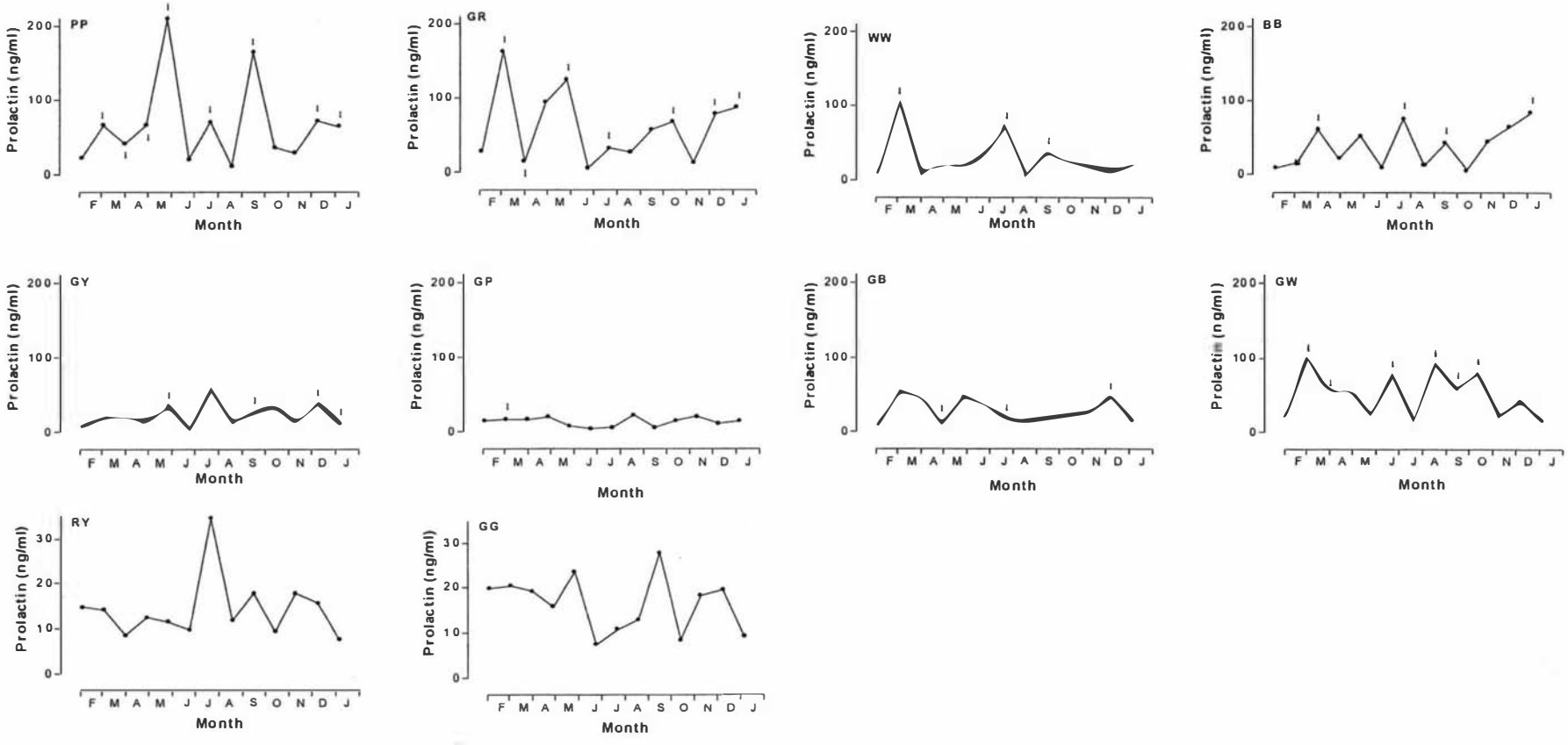

Figure 2.13. Individual profiles of prolactin concentrations in female and male quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as individual data points. "I" indicates birds that were incubating eggs at the time of the collection of the blood sample

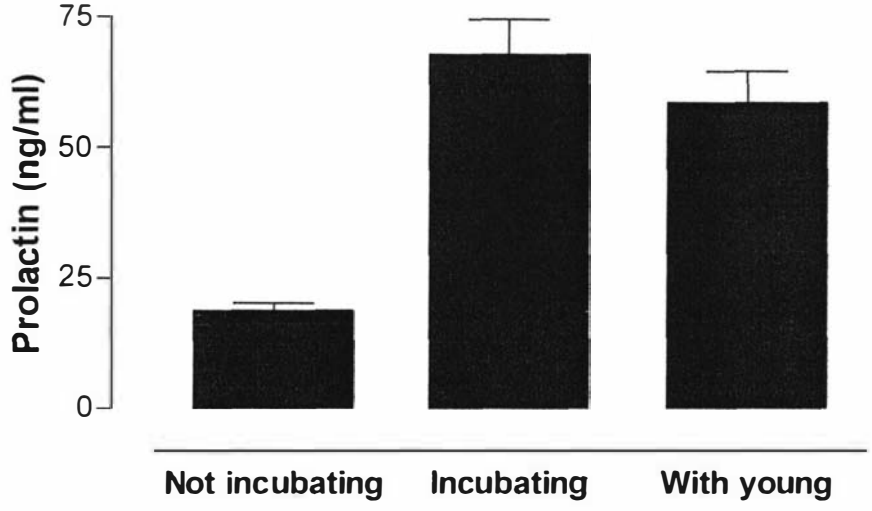

Figure 2.14. Prolactin concentrations in female quail that were not incubating eggs, incubating eggs or caring for young maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as mean ± standard error.

Table 2.6. One-way single measures ANOVA for mean prolactin concentrations in female quail maintained outdoors in a semi-natural environment.

Effect	F	df	*p*
Time	41.13	2, 101	0.000*
Contrasts			
Not incubating vs Incubating			0.000*
Not incubating vs caring for young			0.025*
Incubating vs caring for young			1.000

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of post-hoc comparisons between sampling times.

There was no annual cycle in prolactin concentrations in male quail (Figure 2.13).

2.3.7 Thyroxine (T4)

There was no clear annual cycle in T4 concentrations in female quail (Figure 2.15; One-way repeat measures ANOVA $F_{12, 84} = 1.25$, $p = 0.265$). Individual T4 concentrations showed a wide range of values each month with the exception of June and September when most birds had low T4 concentrations (Figures 2.16 and Appendix 1.6 for individual data).

Both male quail showed increased T4 concentrations during autumn as daylength decreased followed by lower levels in winter (Figure 2.17). T4 concentrations then increased between October and December as daylengths increased before declining in January 2000.

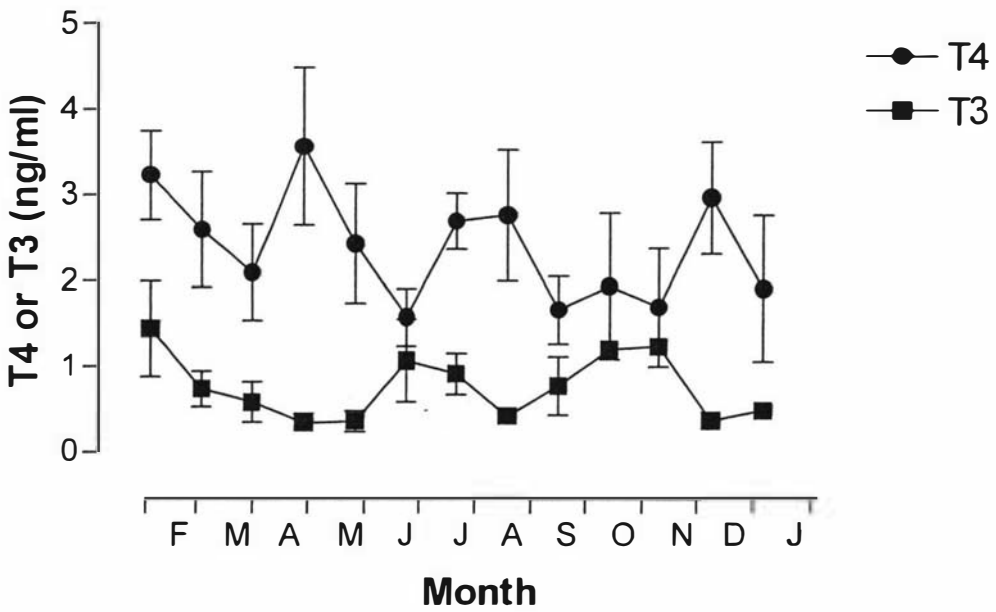

Figure 2.15. Changes in mean T4 and T3 concentrations of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Results are shown as mean ± standard error.

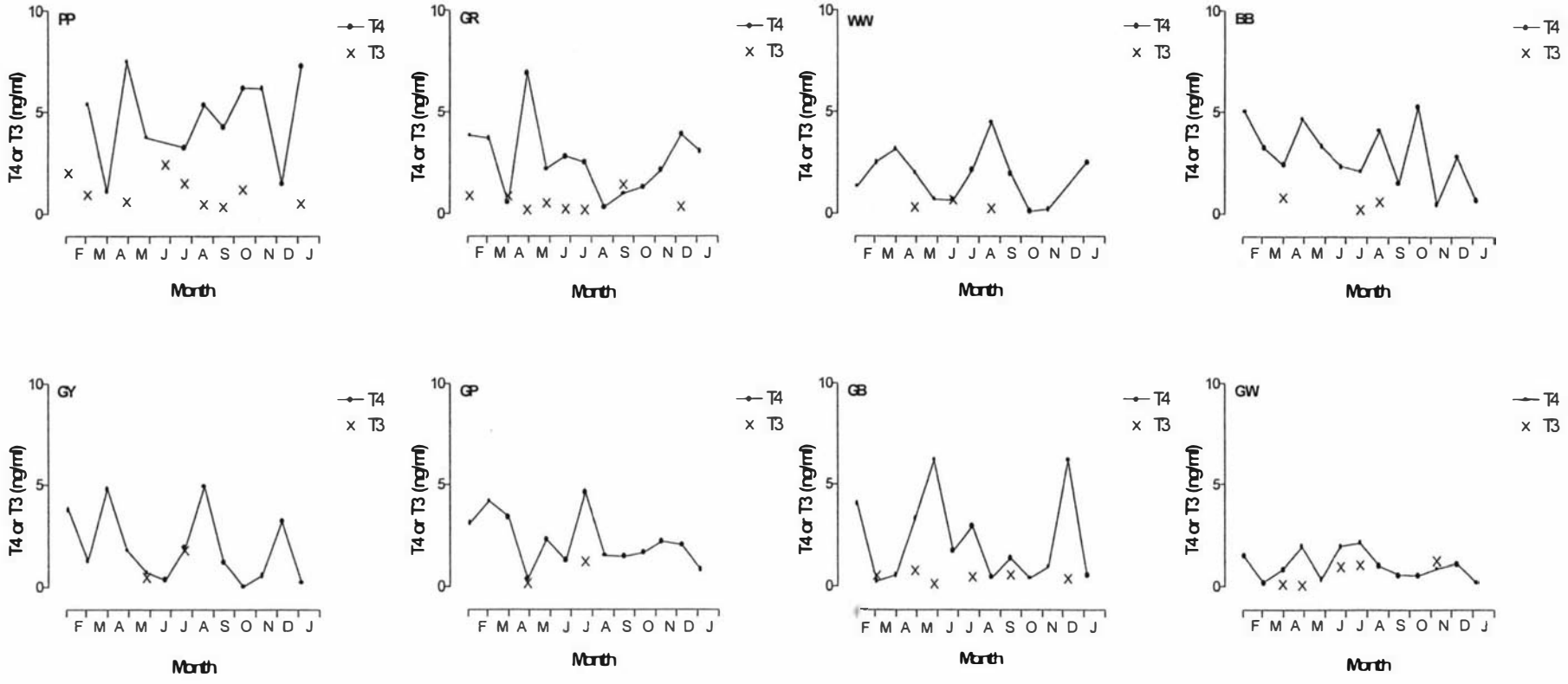

Figure 2.16 Individual profiles of T4 and T3 concentrations in female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.

2.3.8 Triiodothyronine (T3)

Sample sizes for T3 each month were small (between 1 – 7) as there was insufficient plasma for T3 assays for many of the birds. Mean and individual data are shown (Figures 2.15, 2.16, 2.17 and Appendix 1.7 for individual data), but there are insufficient data to determine whether there was an annual cycle of T3.

2.3.9 Molt

Molt of the primary feathers in quail was observed on 16 occasions and generally occurred in female quail following the desertion of a nest or after the hatching of chicks. Molt began within 3 – 13 days following the end of incubation and was completed within 14 – 22 days. Both males molted once during the year (RY in July and GG in September).

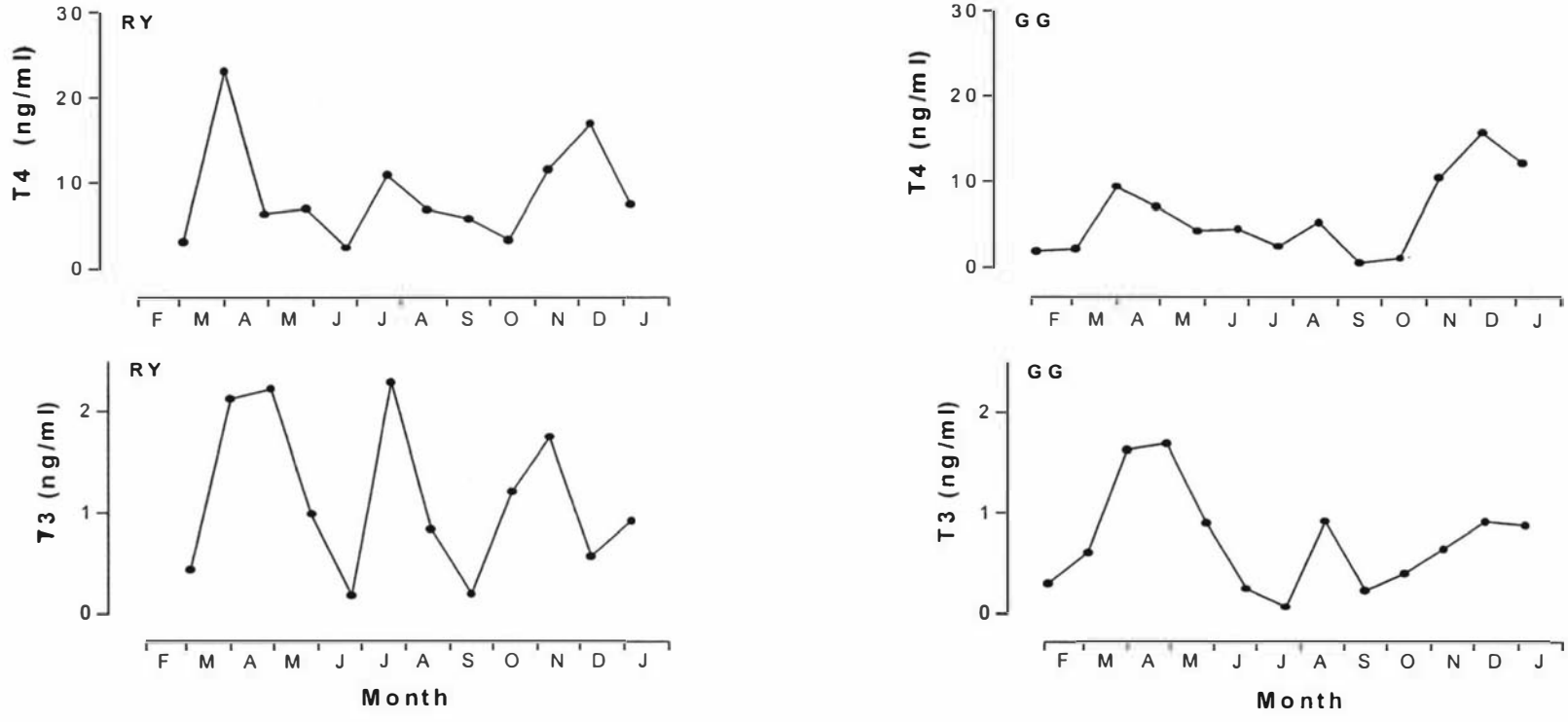

Figure 2.17 Individual profiles of T4 and T3 concentrations in male quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Note different scales on y axes.

2.3.10 Summary of results

The New Zealand strain of the Japanese quail laid eggs in clutches, built nests and became broody (terminated egg production, incubated eggs and cared for young) when held in a semi-natural environment under natural conditions. Egg laying ceased following decreases in LH and FSH concentrations and coincided with low prolactin concentrations. Clear seasonal changes in mean cloacal diameter and plasma FSH concentrations were seen in female quail whilst LH concentrations decreased during late summer and autumn to be lower in winter compared with spring and summer concentrations (Figure 2.18). Prolactin concentrations were higher in female Japanese quail incubating eggs or caring for young compared with birds that were not participating in either activity, whilst no annual cycle was seen in either T4 or T3.

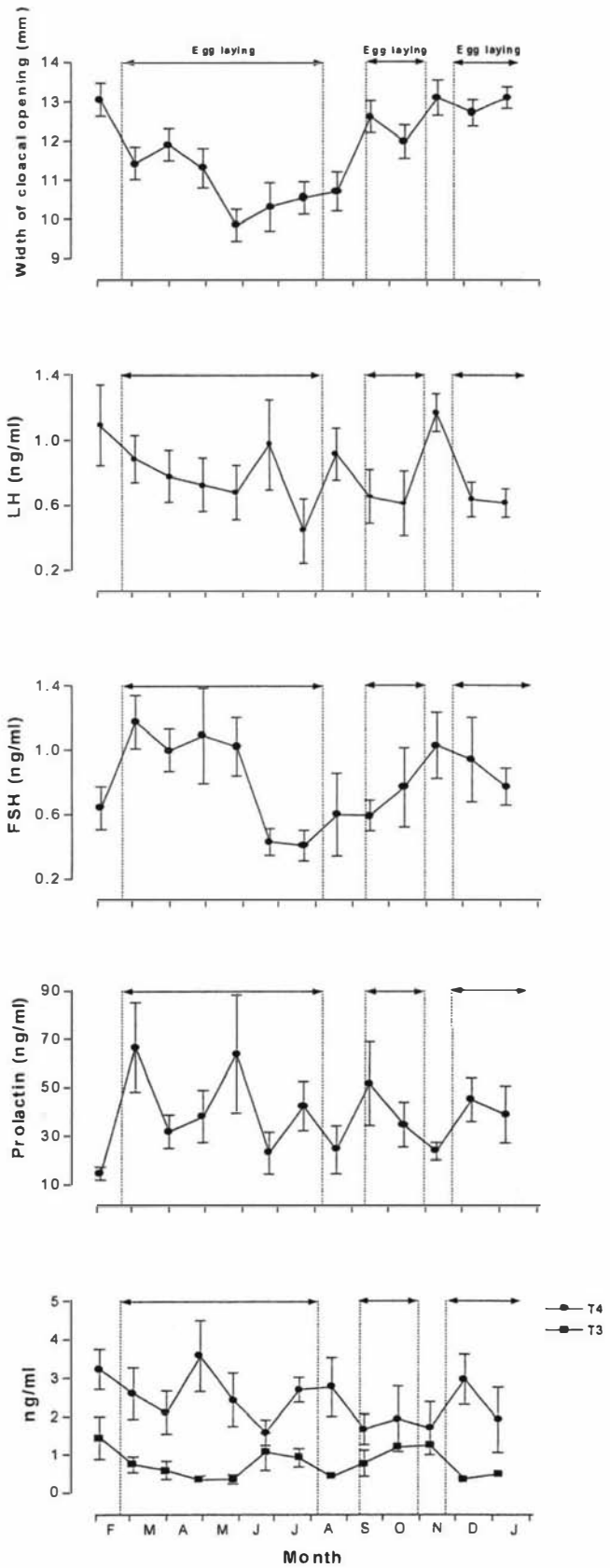

Figure 2.18. Summary graphs of variables measured in female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000. Note different scales on y axes.

2.4 Discussion

2.4.1 Behaviour

Japanese quail held in a semi-natural environment under natural conditions displayed an extensive repertoire of behaviour. Sexual maturation occurred between four to six weeks of age as indicated by crowing in males and egg-laying in females. Crowing is androgen-dependent and does not occur in sexually immature or castrated male quail unless they are treated with testosterone (Adkins *et al.*, 1980). Reproductively active male quail generally crow prior to locating a mate or upon being separated from her (Potash, 1972). Male aggressive and sexual behaviour was more frequently observed during autumn and late spring-summer coincident with high gonadotropin concentrations. This was not surprising as the activation of both appetitive and consummatory male behaviours are induced by the metabolism of androgens into 5α-reduced androgens followed by aromatisation into estrogenic metabolites (Balthazart *et al.*, 2003).

Quail tended to lay one egg per day in the nest until the clutch was complete, similar to results reported in previous studies (Orcutt and Orcutt, 1976; Nichols *et al.*, 1992). Clutch sizes in the present study were similar to those reported for wild-derived and domesticated quail (single bird nests = 10, shared nests = 12) by Nichols *et al.* (1992). Review of the sparse literature available on quail maintained in semi-natural environments found nest-building, egg laying in clutches and incubation behaviour has been observed in some studies (Stevens, 1961; Orcutt and Orcutt, 1976; Nichols *et al.*, 1992; Schmid and Wechsler, 1997) but not others (Rothstein, 1966; Hess *et al.*, 1976) even when birds were housed in ample space with a range of nesting materials available. In the present study quail had a clear preference for laying eggs and building nests under cover. Studies in which reproductive behaviour was investigated in domestic strains of Japanese quail held in semi-natural environments also reported that the hens built their nests in concealed sites (Stevens, 1961; Orcutt and Orcutt, 1976; Nichols *et al.*, 1992; Schmid and Wechsler, 1997). Furthermore, studies in which birds were provided with a choice of cover type reported quail preferred cover types with small entrances and no openings to the sides or on the top for egg laying (Buchwalder and Wechsler, 1997; Schmid and Wechsler, 1998). The preference of quail to lay and nest in secluded sites may have evolved to reduce the risk of predation by birds of prey.

It may therefore be more important for quail to have secluded areas in which shallow scratched out depressions in the ground can be made for nesting than access to ample nesting materials.

Communal nesting was observed in the present study. It was also observed by Nichols *et al.* (1992) in both wild-derived and domestic Japanese quail, but not by Hess *et al.* (1976). Although communal nesting may be a natural and somewhat opportunistic occurrence it does not appear to be effective in increasing the number of progeny as hatch success was lower in shared nests compared to nests incubated by solo females. It could be suggested that communal nesting results from high bird density, although both inter- and intra-specific nest parasitism have been documented in many gallinaceous species (Platt, 1968; Gratson, 1989; Westemeier and Esker, 1989).

The incubation period in the current study ranged between 15 and 23 days which was somewhat longer and more varied than observed by Orcutt and Orcutt, (1976; 16.5 – 17 days). The major difference between the two studies is that quail in the latter study were housed in pairs with no other birds.

There appears to be no quantitative studies of hen and chick relationships for Japanese quail (Mills *et al.,* 1997). In the present study six females produced nine broods supporting earlier observations that quail hatch multiple broods per year (Stevens, 1961; Nichols *et al.,* 1992; Schmid and Wechsler, 1997). None of the 33 chicks survived for longer than 11 days in the present study. The reasons for this are unknown. Examination of deceased juveniles showed no indication of disease or physiological defects. Hess *et al.* (1976) reported mortality rates of chicks were positively correlated with increases in population and were associated with a high incidence of attacks on chicks by male quail. This was not observed in the current study but may have occurred outside of observation periods. In the current study hens with chicks were observed trampling on chicks when attacking other adult quail that came close. As these birds were yearlings and from domesticated stocks raised for generations in wire cages, the appropriate parenting skills may not have been developed and possibly would develop and mature as further broods were raised.

2.4.2 Endocrinology

The first changes in the width of the cloacal opening (indicative of changes in ovarian and oviducal size) and LH concentrations occurred at the beginning of the year after daylength had decreased from 15 to 13.5 hours. As daylength decreased during autumn and winter, mean cloacal diameter decreased and by late winter (August) was minimal in all but one female bird whilst mean LH concentrations were significantly decreased by July. In contrast FSH concentrations increased during autumn before rapidly decreasing between May-June and remained low through to the end of July. The first indication of recrudescence was the increased LH concentrations between July and August following an increase in daylength from 10.4 – 11.3 hours. Gonadal growth indicated by increased cloacal diameter was first observed one month later in September followed by a more gradual increase in FSH that was not significantly elevated until November.

In earlier studies male Japanese quail held under natural photoperiods showed cycles of gonadotropin and testicular mass that were skewed with respect to photoperiod (Follett and Maung, 1978; Follett and Robinson, 1980; Robinson and Follett, 1982; Wada *et al.*, 1992). In these studies gonadal growth began in spring when daylengths exceeded 11.3 hours of light with an increase in LH and regression occurred in late summer when daylength lay between 14 – 15 hours of light. These results along with those of the present study show that gonadal regression occurred at a daylength that was greater than that required for gonadal growth during spring, meaning the quail had become insensitive to daylengths that were once stimulatory, a phenomenon defined as photorefractoriness.

The pattern of increased LH secretion during early spring confirmed results in the same species (Follett and Maung, 1978; Follett and Robinson, 1980; Robinson and Follett, 1982; Wada *et al.*, 1992) and in wild-derived European quail held under natural conditions (Boswell *et al.*, 1993). In these studies LH concentrations increased at daylengths of 11.3 to 13 hours compared to 10.4 to 11.3 hours in the present study. The results of the earlier studies concur with experiments designed to determine the photosensitive phase for LH release in quail held under artificial conditions. Night interruption experiments showed that gonadotropin secretion increased in male Japanese quail when exposed to a 30 minute light pulse between 13 and 15 hours after the onset

of the main photoperiod (4L:20D, 8L:16D, 11L:13D) (Wada, 1979; 1981). LH secretion increased in gonadectomized male Japanese quail exposed to a single four hour light pulse when given 10 to 16 hours after the onset of the main photoperiod 8L:16D (Nicholls *et al.,* 1983). The reason for the earlier increase in LH secretion in the present study compared to earlier studies may be due to exposure of the birds to artificial light from offices adjoining the courtyard. It is possible that although natural daylength was below the photoinducible threshold, artificial light during the dark period may have caused the earlier increase in gonadotropin secretion.

FSH concentrations continued to increase after LH and cloacal diameter had begun to decrease in autumn. Unlike LH concentrations which increase rapidly in female and male Japanese quail upon photostimulation (Nicholls *et al.,* 1973; Gibson *et al.,* 1975). FSH concentrations in comparison have been shown to increase more gradually as seen in white crowned sparrows (Wingfield *et al.,* 1997). The high FSH concentrations seen as daylengths declined may have been an artifact of photostimulation. Additionally, the persistence of high levels of gonadotropins in quail transferred to short days known as 'carry-over' has been observed in birds held under artificial conditions (Davies and Follett, 1980). It is thought to reflect a delay by the hypothalamo-hypophysial system in reducing GnRH secretion since deafferentiation of the hypothalamus leads to the decrease of circulating LH within one day. FSH concentrations were not measured in that study. Gonadotropin carry-over is not observed in all bird species. In birds such as the rook, LH, FSH and testosterone concentrations increased together during photostimulation and decreased together during short days (Lincoln et al., 1980). Unlike the Japanese quail, rooks have a precise breeding season with absolute photorefractoriness and dependence upon a single food source a determinant of successful breeding.

In the current study prolactin concentrations increased following the photoinduced increase of LH during maturation as seen in studies in fowl (Scanes *et al.,* 1976), ruffed grouse (Etches *et al.,* 1979), turkey hens (Scanes *et al.,* 1979; Burke and Dennison, 1980; Gahali *et al.,* 2001) and European starlings (Ebling *et al.,* 1982). However, the highest prolactin concentrations observed in this study were in females incubating eggs and generally occurred when gonadotropin concentrations were low. In galliforms broody behaviour is characterized by high prolactin and low gonadotropin and ovarian

steroid concentrations. Results similar to those found in the current study have been reported in female grouse (Etches *et al.,* 1979), bantam hens (Sharp *et al.,* 1979), mallards (Goldsmith and Williams, 1980), turkeys (Burke and Dennison, 1980), ring doves (Goldsmith *et al.,* 1981), starlings (Dawson and Goldsmith, 1982) and Australian black swans (Goldsmith, 1982).

The high prolactin concentrations observed during incubation have been shown to have an antisteroidogenic effect on the ovary in chickens and turkeys (Rozenboim *et al.,* 1993; Dunn *et al.,* 1996). In the current study high prolactin concentrations were associated with low LH concentrations indicating that such a mechanism may occur in Japanese quail. In turkey hens prolonged exposure to high concentrations of prolactin have been shown to inhibit ovarian steroidogenic enzyme gene expression (cytochrome P_{450} 17α-hydroxylase and cytochrome aromatase mRNA) (Tabibzadeh *et al.,* 1995) and act on the neuroendocrine system to reduce hypothalamic GnRH and inhibit LH secretion (Rozenboim *et al.,* 1993). In domestic chickens the hypothalamic content of cGnRH-I mRNA increased when incubating hens were deprived of their eggs, and was significantly higher in laying than in incubating hens suggesting that a decrease in the expression of the cGnRH-I gene is a major factor in maintaining depressed LH secretion in incubating domestic chickens (Dunn *et al.,* 1996).

High prolactin concentrations in incubating birds are thought to be influenced by the tactile stimulation of the eggs in the nest. In the current study female quail with high levels of prolactin that subsequently intentionally or unintentionally deserted eggs in a nest had dramatically reduced concentrations of prolactin within days. The immediate decrease in prolactin and subsequent increase in LH after nest desertion allows for the immediate initiation of a new breeding attempt (Sharp *et al.,* 1998). Similar results have been reported in broiler breeder hens deprived of nests three days after the initiation of incubation (Richard-Yris *et al.,* 1998), and in turkeys and mallards several days after the hatch of chicks (Goldsmith and Williams, 1980; El Halawani, 1988). Furthermore denervation or anaesthetization of the brood patch in incubating mallards caused significantly decreased prolactin concentrations within 24 hours (Hall, 1987). Prolactin is believed to act synergistically with ovarian steroids, particularly estradiol (Dawson, 1983) to initiate vascularisation of the brood patch and the development of oedema and feather loss from the region (Etches, 1996).

Thyroid hormone concentrations undergo significant changes in association with seasonal breeding cycles in birds and are influenced by a variety of environmental factors in addition to photoperiod (Sharp and Klandorf, 1985). In the present study there was no clear T4 or T3 cycle. T4 concentrations were varied in winter and tended to be high when gonadotropin concentrations were low and vice versa. T3 concentrations were high when daylengths were short and temperature low and peaked when T4 concentrations were low. The first blood samples in this experiment were collected during late summer as birds approached sexual maturity making it difficult to establish whether the high T4 concentrations observed were a result of sexual maturation or related to the development of photorefractoriness. In birds with strong photorefractory responses, such as starlings and ptarmigan, T4 concentrations increased considerably toward the end of the reproductive period and peaked at the onset of photorefractoriness (Klandorf *et al.,* 1982; Dawson, 1984). The relative photorefractoriness observed in quail is similar in its T4 dependence to that of the absolute refractoriness of starlings (for review see Nicholls *et al.,* 1988), and when T4 is administered to birds on short daylengths can both induce and maintain Japanese quail in a photorefractory state (Follett *et al.,* 1988).

Molt occurred in female quail following the termination of broody behaviour and the decline of prolactin concentrations. Several studies have reported an association between the end of breeding and the start of molt. In adult Japanese and European quail held under natural conditions replacement of the primary feathers began after gonadal regression (Robinson, 1983; Boswell *et al.,* 1993). Prolactin administration has been observed to inhibit induced molting in laying hens (Millam and El Halawani, 1986). However, it is not clear whether increased prolactin concentrations inhibit molting directly or indirectly by influencing other hormones (Lien and Siopes, 1993).

2.5 Conclusion

This is the first study in which behavioural and endocrine changes have been described for female Japanese quail that could express natural behaviours in a semi-natural environment under natural conditions. The quail displayed an extensive and rich repertoire of behaviours with clear seasonal changes in mean cloacal diameter and plasma FSH concentrations seen in female quail. LH concentrations decreased during

late summer and autumn to be lower in winter compared with spring and summer concentrations. The results of this study will complement future studies of the photoperiodic control of reproduction in Japanese quail held under artificial conditions.

3 Photoperiodic control of gonadal growth and gonadal regression in male and female Japanese quail

3.1 Introduction

Japanese quail are seasonal breeders, with the timing of breeding influenced by several factors including daylength and temperature. In the laboratory dramatic testicular and ovarian responses to the manipulation of artificial photoperiods make Japanese quail ideal models for studies of reproductive system responses to photoperiodic changes. The photoperiodic changes and the additional supplementary information cues that accelerate or retard gonadal growth have been described in detail (Robinson and Follett, 1982; Follett and Pearce-Kelly, 1990; Dawson *et al.,* 2001). In most studies the transfer of Japanese quail to long daylengths has lead to an increase in circulating gonadotrophin concentrations and gonadal development and growth. Conversely, the few studies that have investigated gonadal regression have shown that transfer of quail to short daylengths or short daylengths and low temperatures decreases circulating plasma gonadotrophin levels, with prolonged exposure leading to gonadal regression.

Changes in plasma hormones in photostimulated quail have been described for LH, FSH (Follett, 1976; Follett and Maung, 1978) testosterone, estradiol, estrone and progesterone (P_4) (Peczely *et al.,* 1980; Brain *et al.,* 1988; for review see Nicholls *et al.,* 1988). Endocrine changes that occur during gonadal regression have been described for the gonadotrophins and testosterone (Delville *et al.,* 1985; for review see Nicholls *et al,* 1988) with recent studies on the role of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3), in controlling reproduction (Oishi and Konishi, 1978; Wada *et al.,* 1990; Tsuyoshi and Wada, 1992; Wada, 1992, 1993; Wakabayashi *et al.,* 1992). However, gonadotrophin and steroid hormone concentrations have not been measured together in quail during gonadal growth or gonadal regression.

Gonadal growth occurs on various daylengths, with the response being evident in males before females (Marshall, 1955). When Japanese quail of either sex were exposed to photoperiods greater than 11.5 hours plasma LH increased significantly in males 24 hours after the dawn of the first long day and after 48 hours in females (Nicholls *et al.,* 1973). This delay in females compared with males was only evident in photostimulated females undergoing gonadal development and growth for the first time, and was not apparent in females that had already undergone a cycle of gonadal growth and regression (Nicholls *et al.,* 1973). FSH concentrations were significantly elevated two days post-stimulation and peaked between days 9 - 11. Concentrations remained maximal for approximately one week (Follett, 1976). During this time there was a uniform increase in the size of both testes and ovaries leading to the release of testosterone, estrogens and P_4. The increased concentrations of gonadal steroids are thought to negatively feedback on the hypothalamus and pituitary causing a decrease in LH and FSH to concentrations that remain higher than those seen in birds held on short days (Follett, 1976). In male quail plasma testosterone increased rapidly after six days of photostimulation and remained high and consistent after 15 days (Follett, 1976).

Plasma prolactin increased in turkey hens (El Halawani *et al.,* 1984), male starlings (Ebling *et al.,* 1982) and bantams (Sreekumar and Sharp, 1998a; 1998b) following photostimulation. The increase in prolactin was initially slower than for LH and continued after LH concentrations were maximal (for review see Sharp *et al.*, 1998). This photostimulated increase in prolactin was accelerated by elevated ambient temperature in female turkeys (El Halawani *et al.*, 1984; Gahali *et al.*, 2001) and white crowned sparrows (Maney *et al.*, 1999), and retarded during exposure to cool temperatures in female turkeys (El Halawani *et al.*, 1984).

Growth and maturation of the gonads under short daylengths have been observed in male and female quail raised on photoperiods of six and eight hours of light per 24 hours, although growth was retarded with full sexual maturity attained at 15 – 24 weeks of age instead of five – six weeks of age (Follett and Farner, 1966; Follett and Sharp, 1969; Stein and Bacon, 1976; Follett and Maung, 1978; Oishi and Konishi, 1978). Additionally, an increase in ovary weight of quail raised on eight hours light for six months was observed by Brain *et al.* (1988) but no yolk-filled follicles were observed.

Gonadal regression does not occur in Japanese quail following prolonged exposure to long photoperiods as seen in absolute photorefractory species such as starlings where circulating LH concentrations decline whilst daylengths remain long. Gonadal function is maintained indefinitely in Japanese quail held on daylengths greater than 12 hours (Dawson *et al.*, 2001) and regression occurs only when the daily photoperiod is decreased (for review see Nicholls *et al.*, 1988).

There have been a range of results from studies of gonadal regression in Japanese quail in response to a decrease in photoperiod. Several investigators showed that the transfer of quail from daylengths of $\geq$ 20 hours of light to shorter daylengths resulted in decreased LH followed by complete gonadal regression (Eroschenko and Wilson, 1974; Follett *et al.*, 1977; Robinson and Follett, 1982; Urbanski and Follett, 1982b) whereas others reported no gonadal regression (Oishi and Konishi, 1978; Wada *et al.*, 1990; 1992; Tsuyoshi and Wada, 1992; Wada, 1993).

Under natural conditions the mechanism for the termination of LH release leading to gonadal regression at the end of the breeding season in Japanese quail appears to involve two factors – a decrease in daylength and a reduction in ambient temperature in late summer to early autumn (Wada *et al.*, 1992). Studies on the Japanese and New Zealand strains of quail show that both short days and low temperatures are required in the laboratory to induce gonadal regression (Oishi and Konishi, 1978) and inhibit gonadal growth (Wada *et al.*, 1990; Wada, 1993; Chua, 2003). When the Japanese strain of quail was transferred to 8 L:16 D and 8 – 10 °C plasma T4 concentrations decreased and T3 increased followed by a decrease in LH leading to gonadal regression. Low temperatures alone do not suppress plasma LH in male quail held on long daylengths (16L: 8D) (Tsuyoshi and Wada, 1992). The role which thyroid hormones play in the mechanisms which control gonadal regression has yet to be established.

The New Zealand strain of the Japanese quail that is used in our laboratory is bred for meat production by a commercial supplier under long daily photoperiods (15L: 9D). These birds require both short daily photoperiods (8L: 16D) and low temperatures to inhibit gonadal growth (Chua, 2003). Gonadal growth occurs under both long and short daily photoperiods at temperatures greater than 18 °C although it is slower on short days.

The goal of the current experiments was to characterise the patterns and rates of gonadal growth and regression and the changes in plasma concentrations of the reproductive hormones which occur during gonadal growth and regression in both male and female Japanese quail. The experiments were conducted to determine the endocrine and gonadal changes occurring during gonadal growth as a reference for experiments where gonadal growth would be stimulated using exogenous GnRH.

The aims of these experiments were as follows:

1. To determine the patterns and rates of gonadal growth and regression in male and female Japanese quail transferred from short days (8 L:16 D) at 10 °C to long days (16 L:8 D) at 20 °C and transferred from long days (16 L:8 D) at 20 °C to short days (8 L:16 D) at 10 °C.

2. To determine the patterns and rates of change of plasma LH, FSH, prolactin, T4, T3, testosterone (males), estradiol (females) and P_4 (females) in Japanese quail during gonadal growth and gonadal regression.

3.2 Materials and Methods

3.2.1 Animals and housing

Four and seven week old male and female Japanese quail (*Coturnix coturnix japonica*) with wildtype plumage were purchased from a commercial source (Rangitikei Game Birds, Bulls, New Zealand). The birds were reared under a long day photoperiod (15 hours light: 9 hours dark) at ambient temperatures (20 – 25 °C). The four week old quail were sexually immature and the seven week old quail were sexually mature.

Quail were identified with a numbered leg band and individually housed in cages, 35 cm (depth) x 25 cm (height) x 20 cm (width) in light and temperature controlled rooms in the Veterinary science building at Massey University. Artificial light for each room was provided by two 75 watt Phillips light bulbs which were controlled by an HPM Excel Light Switch and Timer (Cat # XL77OT). Sixteen hours of light and eight hours of dark (16 L:8 D; lights on 0800 h) and eight hours of light and sixteen hours of darkness (8 L:16 D; lights on 0800 h) were used as long and short photoperiods (long

and short day) respectively. Light intensity in each room was measured using a Minolta Illuminance meter and was 130 lux in cages at the top of each rack and 15 lux in cages near the floor of each rack (W.H. Chua, pers. comm.).

A Carrier temperature control unit controlled temperature in each room. Temperature in the short photoperiod room was 10 ± 2 °C and in the long photoperiod room was 20 ± 2 °C. An extractor fan provided ventilation for each room. Birds were given quail food (Harvey Farms quail layer mash) and water *ad libitum*.

3.2.2 Experimental Design

Separate experiments with male and female quail were conducted.

3.2.2.1 Determination of the pattern and rate of gonadal growth

Eighty four-week-old male or female quail were held under a short day photoperiod at 10 ± 2 °C until they reached seven weeks of age. This treatment allowed the quail to reach the age of sexual maturity while retaining undeveloped testes (males) or ovaries (females). At seven weeks of age 72 of the quail were transferred to a long day photoperiod at 20 ± 2 °C. This treatment stimulated the growth of the reproductive system. The birds were euthanased in groups of nine birds at 0, 5, 10, 15, 20, 25, 30 and 35 days after the transition to long days at 20 ± 2 °C. The remaining eight quail remained on a short day photoperiod at 10 ± 2 °C as a control group and were euthanased at 12 weeks of age (35 days after other birds were moved to long days). Each bird was euthanased within two minutes of being removed from its cage between 0900 – 1100 h.

3.2.2.2 Determination of the pattern and rate of gonadal regression

Eighty seven-week-old male or female quail were held under a long day photoperiod at 20 ± 2°C until they reached 10 weeks of age. This treatment allowed cloacal foam production (males) or egg production (females) to be recorded daily and to ensure that all birds were sexually mature. At 10 weeks of age 72 of the quail were transferred to a short day photoperiod at 10 ± 2 °C. This treatment caused the regression of the reproductive system. The birds were euthanased in groups of nine birds at 0, 5, 10, 15, 20, 25, 30 and 35 days after the transition to short days at 10 ± 2 °C. The remaining

eight quail remained on a long day photoperiod at 20 ± 2 °C as a control group and were euthanased at 15 weeks of age (35 days after transition). Each bird was euthanased within two minutes of being removed from its cage between 0900 – 1100 h.

All experimental procedures were approved by the Massey University Animal Ethics Committee.

3.2.3 Data Collection

3.2.3.1 Cloacal foam production and egg production

The presence or absence of cloacal foam underneath the cages of male quail was recorded daily. Individual egg laying records for female quail were noted each day.

3.2.3.2 Blood and tissue samples

Body weight (Mettler P1200 scales; ± 0.1 g) and cloacal measurements (vernier callipers; ± 0.1 mm) were recorded in all birds before euthanasia. In males the cloacal gland area was determined by multiplying the maximum width and the maximum length of the cloacal protuberance. In females, the width of the cloacal opening was measured.

Birds were euthanased by stunning followed by decapitation. Trunk blood was collected into heparinized 10 ml polypropylene centrifuge tubes and kept on ice until centrifugation. Samples were centrifuged at 1 900 g for 15 minutes (Beckman TJ-6 centrifuge). Plasma was removed with a glass Hamilton syringe, stored in 1.5 ml polypropylene Eppendorf tubes and frozen at – 20 °C until assay.

The reproductive organs were removed. Paired testis weights were weighed and recorded (Mettler Toledo AB104-S; ± 0.0001 g). Ovaries and oviducts were weighed and the numbers of yellow and large white (≥ 2.5 mm) follicles on each ovary were recorded. The diameters of the five largest follicles were measured using vernier callipers. Molting was assessed by checking fallen body feathers and primaries on the cage floor.

3.2.3.3 Rate of gonadal growth and regression

In photostimulated quail gonadal growth shows an approximately linear relationship between log gonadal weight and time (Farner and Wilson, 1957; Follett and Farner, 1966) and can be expressed as:

$$\text{Log } W_t = \log W_0 + kt$$

where W_0 is the gonadal weight at the beginning of treatment, Wt is the weight at t days and k is the logarithmic growth rate constant per day. The slope (k) is a useful quantitative measure of the effectiveness of any given artificial photoperiodic schedule in inducing testicular or ovarian growth and has been previously used to determine growth rates in quail (Follett and Farner, 1966; Follett and Maung, 1978), white crowned sparrows (Farner and Wilson, 1957) and American tree sparrows (Reinert and Wilson, 1996a; 1996b). In the current study rates of testicular or ovarian growth or regression with 95 % confidence limits were calculated by regression analysis.

3.2.4 Radioimmunoassays

Luteinising hormone (LH), follicle stimulating hormone (FSH), prolactin, testosterone, estradiol, progesterone (P_4), thyroxine (T4) and triiodothyronine (T3) concentrations in plasma samples were determined by radioimmunoassay. Plasma samples were thawed and spun at 14 000 g for five minutes (IEC Micromax ventilated microcentrifuge OM3590) in a 1.5 ml Eppendorf tube to separate lipid from plasma before assay. Clear plasma was removed from underneath the lipid using a glass Hamilton syringe.

Hormone concentrations for samples that measured below the sensitivity of each assay were assigned a value equal to the sensitivity of that assay.

3.2.4.1 Luteinising hormone (LH)

LH concentrations in quail plasma were measured by Dr Richard Talbot (Roslin Institute, Edinburgh, Scotland) using a double-antibody radioimmunoassay that has previously been used in domestic chickens and fowl (Sharp *et al.*, 1987; 1990). Purified chicken LH (PRC-AE1-s-1) was used for radioiodination (Sharp *et al.*, 1987). Samples were analysed in duplicate in a single assay.

40 µl of standard or plasma diluted with buffer (0.5 M PO_4 buffer pH 7.5, sodium chloride, sodium azide, Na_2-EDTA and bovine serum albumin; pH 7.0) and 50 µl of antibody 3/3 (Sharp *et al.*, 1987) diluted with buffer at a dilution of 1:160 000 were mixed in polystyrene test tubes (7 x 51 mm) and incubated at 4 °C overnight. 50 µl of label was added and mixed and the mixture incubated at 4 °C overnight.

100 µl of donkey anti-rabbit serum and 100 µl of normal rabbit serum were added and mixed and allowed to stand at 4 °C overnight before centrifugation at 2 000 g for 20 minutes. 50 µl of a 5 % solution of insoluble starch was added to each tube to prevent the disturbance of the precipitate during aspiration. Tubes were centrifuged at 2 000g for 10 minutes, the supernatant was removed by aspiration and the remaining pellets were counted in a LKB Wallac 1277 Gammamaster.

The sensitivity of the assay was 0.03 ng/tube. Serial dilutions of quail plasma in assay buffer produced curves parallel to chicken LH standard curves. The intra-assay coefficient of variation was less than 5%. Cross reactivity of the LH antiserum was reported as chicken FSH < 0.3 %, chicken prolactin < 0.7 % and negligible for chicken growth hormone preparations.

3.2.4.2 Follicle stimulating hormone (FSH)

FSH concentrations in quail plasma were measured by Dr Mitoshi Kikuchi (Department of Biology, Waseda University, Tokyo) using a modification of the double-antibody radioimmunoassay for chicken FSH described by Sakai and Ishii (1983). Purified chicken FSH (AGCSQS11132D; Kikuchi and Ishii, unpublished data) was used for radioiodination (Silverin *et al.*, 1999). Samples were analysed in duplicate in a single assay.

25 µl of standard or plasma diluted with gelatin-PBS (0.01 M disodium phosphate buffer, pH 7.5 containing 0.15 M NaCl, 0.1 % gelatin and 0.01 % merthiolate) and 25 µl of antiserum diluted with NRS-EDTA-PBS (0.01 M disodium phosphate buffer, pH 7.5 containing 0.15 M NaCl, 0.05 M disodium EDTA, 1 % normal rabbit serum and 0.01 % merthiolate) were mixed in an assay tube (5 x 55 mm) and incubated at 4 °C for two days. 100 µl of anti-rabbit γ-globulin in diluent (EDTA-PBS, 0.01 disodium phosphate

buffer, pH 7.5 containing 0.15 M NaCl, 0.05 M disodium EDTA and 0.01% merthiolate) was added and the mixture incubated at 4 °C for 24 hours.

500 µl of cold gelatin PBS was added and the tubes were centrifuged at 3 000 g for 20 minutes. The supernatant was removed by aspiration and the remaining pellets were counted in an automatic gamma counter.

The sensitivity of the radioimmunoassay for FSH was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 0.02 ng/ml. Serial dilutions of quail plasma in assay buffer produced curves parallel to chicken FSH standard curves (Sakai and Ishii, 1983). The intra-assay coefficient of variation was 6.2 %. Cross reactivity of the FSH antiserum is minimal with highly purified chicken LH preparations (Sakai and Ishii, 1983).

3.2.4.3 Prolactin

Prolactin concentrations in quail plasma were measured by Dr Richard Talbot (Roslin Institute, Edinburgh, Scotland) using a radioimmunoassay described by (Talbot and Sharp, 1994). Purified chicken prolactin was used for radioiodination (Talbot and Sharp, 1994). Samples were assayed in duplicate in a single assay.

50 µl of standard or plasma diluted with buffer (0.05 M sodium phosphate buffer containing 0.155 M NaCl, 0.1 % sodium azide, 0.01 M disodium EDTA and 2 % horse serum) containing antibody 31/1 at a dilution of 1:8 000 were mixed in polystyrene test tubes (6 x 50 mm) and incubated at 4 °C overnight. 50 µl of label was added and mixed and the mixture incubated at 4 °C overnight.

50 µl of donkey anti-rabbit serum and 50 µl of normal rabbit serum were added and mixed and allowed to stand at 4 °C for 16 hours before centrifugation at 2 000 g for 20 minutes. 50 µl of a 6 % solution of insoluble starch was added to each tube to prevent the disturbance of the precipitate during aspiration. Tubes were centrifuged at 2 000g for 10 minutes, the supernatant was removed by aspiration and the remaining pellets were counted in a LKB Wallac 1277 Gammamaster.

The sensitivity of the assay was 0.03 ng/tube. Serial dilutions of quail plasma in assay buffer produced curves parallel to chicken prolactin standard curves. The intra-assay coefficient of variation was 9.8 %.

3.2.4.4 Testosterone

Testosterone concentrations in quail plasma were measured by radioimmunoassay using a modification of the ImmuChem Double Antibody Testosterone ^{125}I RIA Kit (ICN Biomedicals Inc., USA; Cat # 07-189102) method. Samples were assayed in duplicate in a single assay.

20 µl of sex binding globulin inhibitor solution (SGBI, Cat # 07-166812), 100 µl of iodinated testosterone (Cat # 07-189121, approximately 5 000 cpm) and 100 µl of antibody (anti-testosterone raised in rabbit, Cat # 07-189113) were added to 10 µl of undiluted plasma, vortexed and incubated at 37 °C in a water bath for 120 minutes. 20 µl of second antibody (Cat # 07-166602) was then added to each tube and the tubes were vortexed and incubated at 37 °C in a water bath for a further 60 minutes. Samples were then centrifuged at 2 300 g (Heraeus Christ 5000S refrigerated centrifuge) for 15 minutes at 4 °C and the supernatant removed by aspiration. The remaining pellets were counted for five minutes in a LKB Wallac 1261 Multigamma Gamma Counter.

The sensitivity of the radioimmunoassay for testosterone was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 19 pg/ml (n = 1 assay) of testosterone in quail plasma. Serial dilutions of quail plasma in PBSG assay buffer (phosphate-buffered saline with gelatine; 0.1 M, pH 7.0) were parallel to the testosterone standard curve (n = 3; figure 3.1a). Recovery of testosterone added to quail plasma was 98.3 ± 4.8 %, 93.8 ± 4.8 % and 100.0 ± 4.2 % for three samples.

Solutions of testosterone in PBSG at concentrations that gave approximately 20, 50 and 80 % binding on the standard curve were used as high, medium and low quality controls. The mean concentrations of testosterone of these samples were 6122.2, 617.0 and 112.4 pg/ml respectively. Intra-assay coefficients of variation for these three samples were 9.8 %, 4.1 % and 6.9% (n=20) respectively.

Cross reactions of the antibody with other steroids were reported by ICN Biomedicals Inc. as: 5α-dihydrotestosterone (3.4 %), 5α-androstane-3β, 17β-diol (2.2 %), 11-oxotestosteorne (2.0 %), 6β-hydroxytestosterone (0.95 %), 5β-androstane-3β, 17β-diol (0.71 %), 5β-dihydrotestosterone (0.63 %), androstenedione (0.56 %), epiandrosterone (0.20 %), and others (< 0.01 %).

3.2.4.5 Estradiol

Estradiol concentrations in quail plasma were measured by radioimmunoassay using a modification of the ImmuChem Double Antibody Estradiol ^{125}I RIA Kit (ICN Biomedicals Inc., USA; Cat # 07-138102) method. Samples were assayed in duplicate in a single assay.

100 µl of iodinated estradiol (Cat # 07-138121, approximately 5 000 cpm) and 100 µl of antibody (anti-17β-estradiol raised in rabbit, Cat # 07-138113) were added to 10 µl of undiluted plasma, vortexed and incubated at 37 °C in a water bath for 90 minutes. 100 µl of the precipitate solution (Cat # 07-166624) was added to each tube and the tubes were vortexed for 30 s. Samples were then centrifuged at 2 300 g (Heraeus Christ 5000S refrigerated centrifuge) for 15 minutes at 4 °C and the supernatant removed by aspiration. The remaining pellets were counted for five minutes in a LKB Wallac 1261 Multigamma Gamma Counter.

The sensitivity of the radioimmunoassay for estradiol was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 37 pg/ml (n = 1 assay) of estradiol in quail plasma. Serial dilutions of quail plasma in assay buffer (PBSG; 0.1 M, pH 7.0) were parallel to the estradiol standard curve (n = 3; figure 3.1b). Recovery of estradiol added to quail plasma was 96.1 ± 12.3 %, 93.3 ± 9.7 % and 87.6 ± 8.7 % for three samples.

Solutions of estradiol in PBSG at concentrations that gave approximately 20, 50 and 80 % binding on the standard curve were used as high, medium and low quality controls. The mean concentrations of estradiol in these samples were 2454.1, 469.5 and 110.0 pg/ml respectively. Intra-assay coefficients of variation for these three samples were 11.4 %, 12.9 % and 15.4 % (n = 20) respectively.

Cross reactions of the antibody with other steroids were reported by ICN Biomedicals Inc. as estrone (20 %), estriol (1.51 %), estadiol-17α (0.68 %) and others (< 0.01 %).

3.2.4.6 Progesterone (P_4)

P_4 concentrations in quail plasma were measured by radioimmunoassay using a modification of the ImmuChem Double Antibody Progesterone ^{125}I RIA Kit (ICN Biomedicals Inc., USA; Cat # 07-170102) method. Samples were assayed in duplicate in a single assay.

20 µl of iodinated P_4 (Cat # 07-170121, approximately 5 000 cpm) and 50 µl of antibody (anti- progesterone raised in rabbit, Cat # 07-170113) were added to 10 µl of undiluted plasma, vortexed and incubated at 37 °C in a water bath for 60 minutes. 50 µl of the precipitate solution (Cat # 07-166624) was added to each tube and the tubes were vortexed for 30 s. Samples were then centrifuged at 2 300 g (Heraeus Christ 5000S refrigerated centrifuge) for 15 minutes at 4 °C and the supernatant removed by aspiration. The remaining pellets were counted for five minutes in a LKB Wallac 1261 Multigamma Gamma Counter.

The sensitivity of the radioimmunoassay for P_4 was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 15 pg/ml (n = 1 assay) of P_4 in quail plasma. Serial dilutions of quail plasma in assay buffer (PBSG; 0.1 M, pH 7.0) were parallel to the P_4 standard curve (n = 3; figure 3.1c). Recovery of P_4 added to quail plasma 98.7 ± 5.0 % and 95.0 ± 5.5 % for two samples.

Solutions of P_4 in PBSG at concentrations that gave approximately 20, 50 and 80 % binding on the standard curve were used as high, medium and low quality controls. The mean concentrations of P_4 in these samples were 13 373.6, 3005.0 and 230.6 pg/ml respectively. Intra-assay coefficients of variation for these three samples were 22.0 %, 8.4 % and 9.9 % (n = 10) respectively.

Cross reactions of the antibody with other steroids were reported by ICN Biomedicals Inc. as: 20α-dihydroprogesterone (5.41 %), desoxycorticosterone (3.80 %), corticosterone (0.70 %), 17α-hydroxyprogesterone (0.6 7%), pregnenolone (0.41 %), androstenedione (0.23 %), testosterone (0.16 %) and others (<0.01 %).

3.2.4.7 Thyroxine (T4)

Thyroxine concentrations in quail plasma were measured by radioimmunoassay using the DSL-3200 Active Thyroxine Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories, Inc., USA; Cat # DSL-3200) method. Samples were assayed in duplicate and randomly assigned between three assays.

200 µl of iodinated T4 (Cat # 3220, approximately 40 000 cpm) was added to 25 µl of undiluted plasma in an anti-T4-coated tube, vortexed and incubated for 60 minutes at room temperature on an orbital shaker (Chiltern Scientific SS70).

The supernatant was removed by simultaneous inversion of the tubes in a sponge rack. The tubes were struck sharply on absorbent material to facilitate complete drainage and allowed to drain on absorbent material for two minutes. The tubes were blotted to remove any droplets adhering to the rim before returning them to the upright position. 2.5 ml of diluted wash solution (buffered saline with a non-ionic detergent (Cat # 3230-5) diluted 50-fold with double distilled water) was added to each tube then decanted as previously described. The washing and decanting steps were repeated for a total of three washings. The fully drained tubes were counted for one minute on a LKB Wallac 1261 Multigamma Gamma Counter.

The sensitivity of the radioimmunoassay for T4 was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 5.5 pg/ml (n = 3 assays) of T4 in plasma. Serial dilutions of quail plasma in T4 Standard A (containing 0 µg/dL T4 in serum; Cat # 3201) were parallel to the T4 standard curve (n = 3; figure 3.1d). Recovery of T4 added to quail plasma was 95.1 ± 2.0 %, 94.4 ± 2.5 % and 98.4 ± 7.4 % for three samples.

Solutions of T4 in serum at concentrations that gave approximately 40 % and 20 % binding on the standard curve were used as high and low quality controls (Control level I and II; Cat # 3251 and Cat # 3252 respectively). The mean concentrations of these samples were 63.7 pg/ml and 126 pg/ml respectively. Intra assay coefficients of variation for these two samples were 2.9 % and 2.4 % (n = 20) respectively. Inter assay coefficients of variation for high and low quality controls were 8.9 % and 5.7 %.

Cross reactions of the antibody with other steroids (ratio of the T4 concentration to the concentration of the reacting compound at 50 % binding of the 0 μg/dL T4 standard) were reported by DSL Inc. as L-thyroxine (100 %), D-thyroxine (100 %), reverse triiodothyronine (1.18 %), triiodothyroacetic acid (1.13 %), L-triiodothyronine (0.91 %), diiodothyronine (0.03 %) and others (not detectable at 25 μg/dL).

3.2.4.8 Triiodothyronine (T3)

Triiodothyronine concentrations in quail plasma were measured by radioimmunoassay using the DSL-3100 Active Triiodothyronine Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories, Inc., USA; Cat # DSL-3100) method. Samples were assayed in duplicate and randomly assigned between four assays.

200 μl of iodinated T3 (Cat # 3120, approximately 45 000 cpm) and 100 μl of anti-T3 antibody (anti-T3 mouse monoclonal antibody in protein-based BSA buffer; Cat # 3110) were added to 50 μl of undiluted plasma in a GAMG-coated tube (goat anti-mouse gamma globulin; Cat # 3131), vortexed and incubated for 60 minutes at room temperature on an orbital shaker (Chiltern Scientific SS70). The supernatant was removed by simultaneous inversion with a sponge rack. The tubes were struck sharply on absorbent material to facilitate complete drainage and allowed to drain on absorbent material for two minutes. The tubes were blotted to remove any droplets adhering to the rim before returning them to the upright position. 2.5 ml of diluted wash solution (buffered saline with a non-ionic detergent (Cat # 8330-5) diluted 50-fold with double distilled water) was added to each tube then decanted as previously described. The washing and decanting steps were repeated for a total of three washings. The fully drained tubes were counted for one minute on a LKB Wallac 1261 Multigamma Gamma Counter.

The sensitivity of the radioimmunoassay for T3 was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 35.6 pg/ml (n = 5 assays) of T3 in quail plasma. Serial dilutions of quail plasma in T3 Standard A (containing 0 ng/dL T3 in human serum; Cat # 3101) were parallel to the T3 standard curve (n = 3; figure 3.1e). Recovery of T3 added to quail plasma was 97.8 ± 4.0 %, 97.4 ± 2.9 % and 107.0 ± 6.0 % for three samples.

Solutions of T3 in human serum at concentrations that gave approximately 30 % and 65 % binding on the standard curve were used as high and low quality controls (Control level I and II; Cat # 3151 and Cat # 3152 respectively). The mean concentrations of these samples were 466.3 pg/ml and 2398.1 pg/ml respectively. Intra-assay coefficients of variation for these two samples were 4.0 % and 1.5 % (n = 20) respectively. Inter-assay coefficients of variation for high and low quality controls were 3.6 % and 6.9 %.

Cross reactions of the antibody with other steroids (ratio of the T3 concentration to the concentration of the reacting compound at 50 % binding of the 0 ng/dL T3 standard) were reported by DSL Inc. as triiodo-L-thyronine (100 %), triiodo-L-thyronine (reverse) (0.004 %), L-thyroxine (0.003 %), D-thyroxine (< 0.001 %), triiodothyroacetic acid (2.76 %), 3,5-diiodo-L-thyronine (0.002 %), 3-monoiodo-L-tyrosine (0.001 %) and 3, 5-diiodo-L-tyrosine (0.001 %).

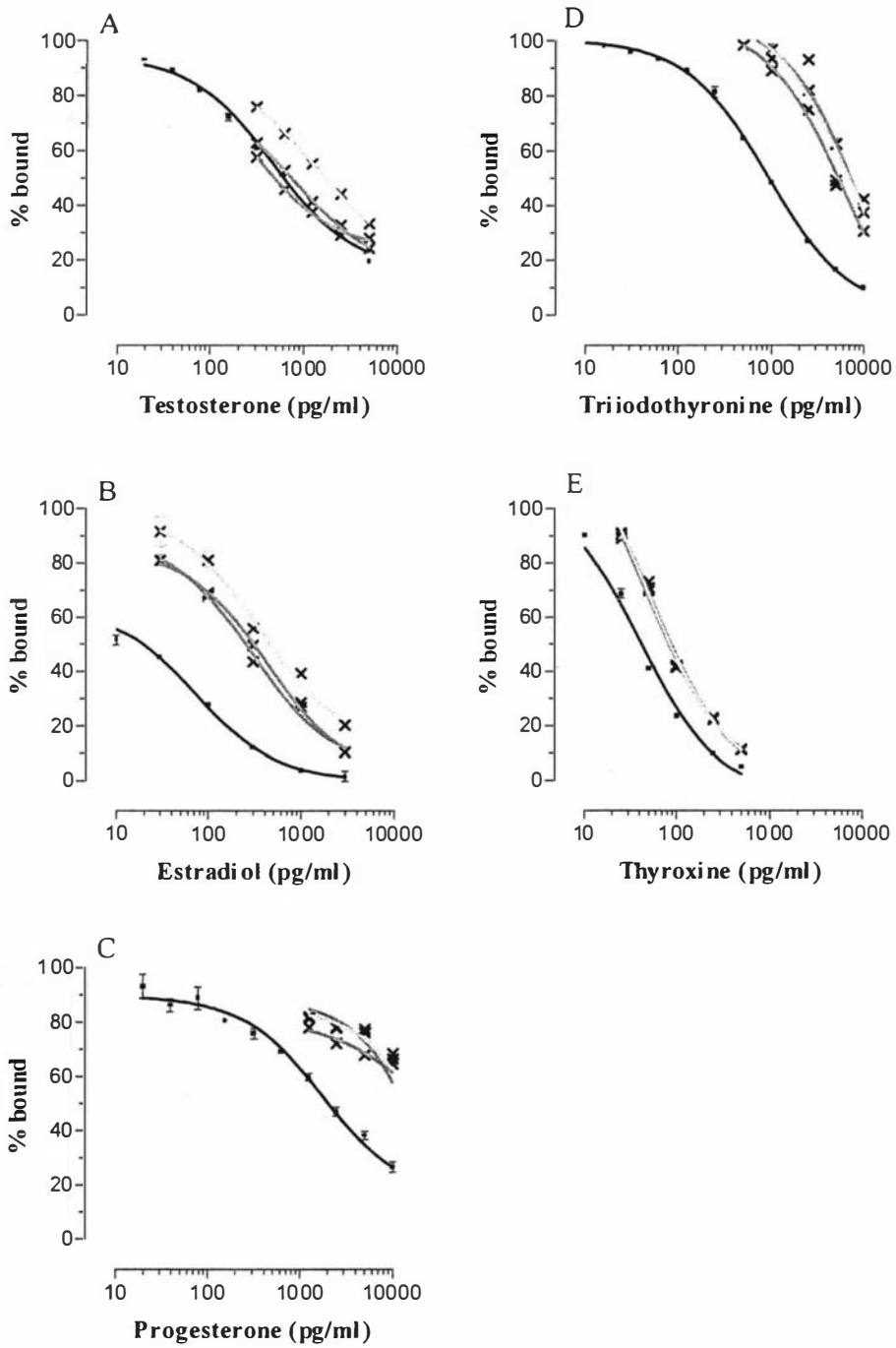

Figure 3.1. Parallelism demonstrated for Japanese quail plasma samples.
A: testosterone, B: estradiol, C: progesterone, D: thyroxine and E: triiodothyronine.
Curves with filled squares are standard curves and all other curves are samples.

3.2.5 Statistics

The experiment was arranged as a randomised block design with a factorial arrangement of treatment (short or long day photoperiod) and time as the main effects. Birds were placed at random, in cages on two racks consisting of four tiers of 10 cages per tier. Data were transformed to logarithms to reduce heteroscedascity where necessary. All variables were tested for homogeneity of variance using the Levenes test. Variables with equal variance were analysed using two way single measures ANOVA. The split-plot model included effects for treatment and time, and treatment x time as the main effects. ANOVA were followed by Bonferroni's (comparison of < 5 groups) or Tukey's (comparison of ≥ 5 groups) post hoc pairwise comparisons to determine differences between groups. Variables with unequal variance were analysed using Kruskal-Wallis non-parametric ANOVA followed by Mann-Whitney U-tests to determine differences between groups. Repeated measures ANOVA were used to compare changes in body weight and cloacal gland measurements over time in birds held on long daylengths at 20 °C or held on short daylengths at 10 °C. Correlations between variables were determined using linear regression to calculate r and r^2 values. A value of $p < 0.05$ was taken as statistically significant and indicated by an asterisk (*).

ANOVAs followed by post hoc tests and the non-parametric equivalents were performed using Systat Version 8 (SPSS Inc., 1988). Linear regression correlations between variables were performed using GraphPad Prism Version 3.0 (GraphPad Software Inc., 1999).

Data are presented as individual points (raw data) or as means ± standard error (group data) using GraphPad Prism Version 3.0 (GraphPad Software Inc., 1999).

3.3 Results

3.3.1 Gonadal growth and regression in male Japanese quail

3.3.1.1 Body weight

Body weight at the beginning of the experiment was similar in quail to be transferred to long or short days (Figure 3.2; see Table 3.1 for statistics). Body weight increased significantly in quail 15 days after transfer to long days and was similar to long day control quail after 20 long days. By day 35 birds transferred to long days were heavier than quail transferred to short days and did not differ in weight from either control group. Body weight did not change significantly in quail transferred from long to short days on days 0 and 35, although birds were significantly lighter on day 20 compared with day 0.

Body weight increased in short day control birds and long day control birds and did not differ between these groups on day 35 (254.7 ± 13.3 and 267.5 ± 7.0 g respectively; see Table 3.1 and 3.2 for statistics).

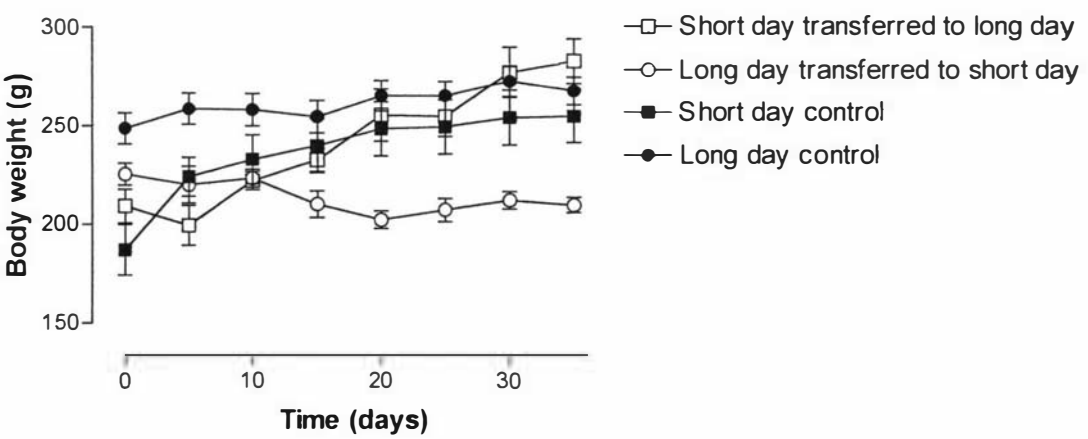

Figure 3.2. Changes in body weight of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 ℃ (short day control) and long days at 20 ℃ (long day control). Results are shown as mean ± standard error.

Table 3.1. Two-way single measures ANOVA for body weight of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	Body weight		
	F	df	*p*
Time	7.76	8, 130	0.000*
Treatment	42.42	1, 130	0.000*
Interaction between time and treatment	9.01	8, 130	0.000*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	1.11	1, 130	0.294
Day 0 vs day 10	1.58	1, 130	0.212
Day 0 vs day 15	4.85	1, 130	0.029*
Day 0 vs day 20	14.81	1, 130	0.000*
Day 0 vs day 25	15.69	1, 130	0.000*
Day 0 vs day 30	30.85	1, 130	0.000*
Day 0 vs day 35	34.91	1, 130	0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	28.22	1, 130	0.000*
Day 5 vs long day control	40.36	1, 130	0.000*
Day 10 vs long day control	15.03	1, 130	0.000*
Day 15 vs long day control	9.68	1, 130	0.002*
Day 20 vs long day control	1.65	1, 130	0.201
Day 25 vs long day control	1.83	1, 130	0.179
Day 30 vs long day control	0.06	1, 130	0.809
Day 35 vs long day control	0.36	1, 130	0.552
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	0.40	1, 130	0.533
Day 0 vs day 10	0.04	1, 130	0.848
Day 0 vs day 15	2.41	1, 130	0.123
Day 0 vs day 20	5.37	1, 130	0.022*
Day 0 vs day 25	3.34	1, 130	0.070
Day 0 vs day 30	1.68	1, 130	0.202
Day 0 vs day 35	2.31	1, 130	0.131
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	5.42	1, 130	0.020*
Day 5 vs short day control	8.49	1, 130	0.004*
Day 10 vs short day control	6.30	1, 130	0.013*
Day 15 vs short day control	14.30	1, 130	0.000*
Day 20 vs short day control	20.22	1, 130	0.000*
Day 25 vs short day control	16.32	1, 130	0.000*
Day 30 vs short day control	12.06	1, 130	0.001*
Day 35 vs short day control	14.07	1, 130	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	2.67	1, 130	0.105
Transfer to SD day 35 vs transfer to LD day 35	35.05	1, 130	0.000*
Short day control vs Long day control on day 35	1.77	1, 130	0.186

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.2. One-way repeated measures ANOVA for body weight of male quail held on long days at 20 °C (long day control) and held on short days at 10 °C (short day control).

Body weight – long days at 20 °C				Body weight – short days at 10 °C			
Effect	**F**	**df**	***p***	**Effect**	**F**	**df**	***p***
Time	12.81	7, 49	0.000*	Time	22.27	7, 42	0.000*
Contrasts				**Contrasts**			
Day 0 compared to:				**Day 0 compared to:**			
Day 5	5.87	1, 7	0.046*	Day 5	10.85	1, 6	0.017*
Day 10	22.00	1, 7	0.002*	Day 10	12.26	1, 6	0.013*
Day 15	11.22	1, 7	0.012*	Day 15	17.50	1, 6	0.006*
Day 20	67.00	1, 7	0.000*	Day 20	22.80	1, 6	0.003*
Day 25	55.34	1, 7	0.000*	Day 25	24.99	1, 6	0.002*
Day 30	104.73	1, 7	0.000*	Day 30	27.71	1, 6	0.002*
Day 35	49.40	1, 7	0.000*	Day 35	30.49	1, 6	0.001*

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.2 Cloacal gland area

Cloacal gland area had increased in most quail 10 days after transfer from short to long days and was significantly greater than in short day quail (Figures 3.3A and 3.4; see Table 3.3 for statistics). Cloacal gland area continued to increase in most quail and reached a similar size to long day control quail after 15 long days. By the end of the experiment all quail transferred to long days had glands similar in size to long day controls (see Table 3.3 for statistics). Cloacal gland area steadily decreased after transfer from long to short days and reached a similar size to short day control quail after 30 short days (Figure 3.3B; see Table 3.4 for statistics).

Cloacal gland area in long day control quail decreased between day 0 and five and remained constant thereafter (Figure 3.4; see Table 3.5 for statistics). One bird held on long days at 20 °C had a small cloacal gland (96.0 mm^2) that was only slightly larger than the glands of short day control quail. Cloacal gland areas of short day control quail remained small and did not change during the experiment (see Table 3.5 for statistics). On day 35 glands of short day control quail were significantly smaller than those of long day control quail (33.1 ± 4.7 and 298.2 ± 36.9 mm^2 respectively; see Table 3.6 for statistics).

Cloacal gland areas were strongly correlated with testis weight and correlated with testosterone concentration during gonadal growth and regression (Figures 3.5 and 3.6;

see Table 3.7 for statistics). Generally cloacal glands reached a reproductively mature size when testosterone concentrations were greater than 1 ng/ml.

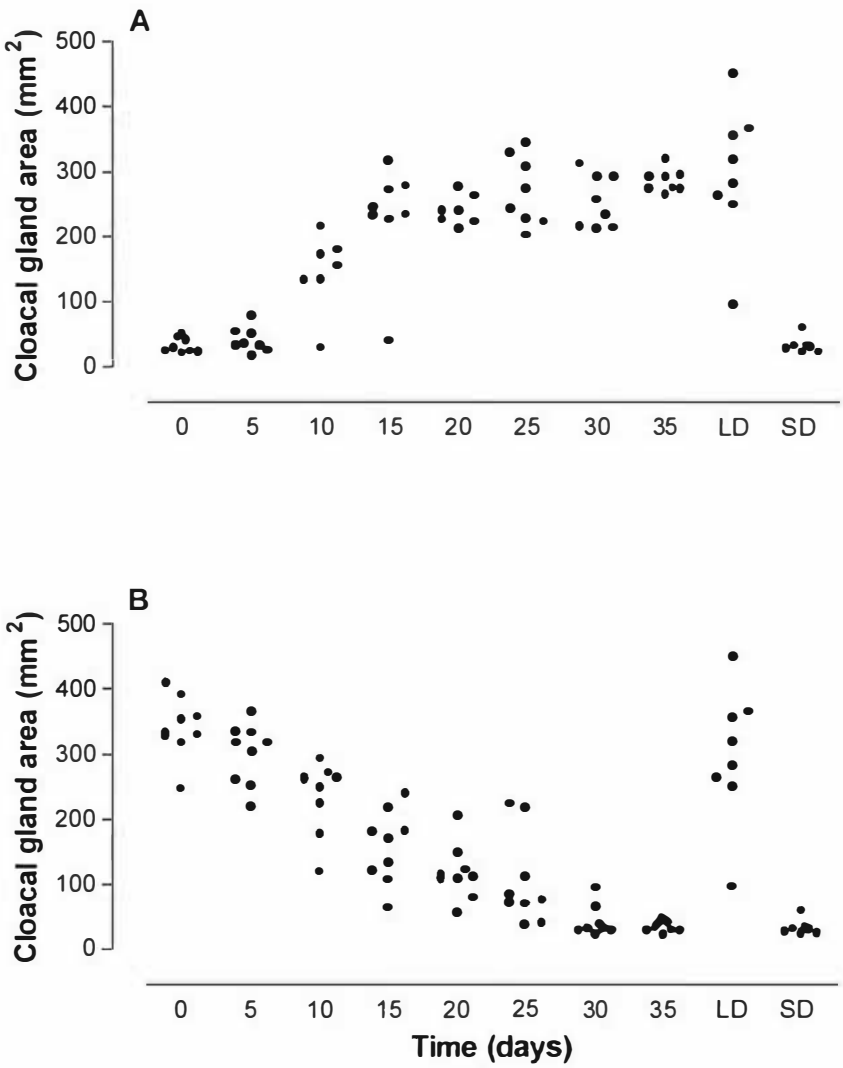

Figure 3.3. Cloacal gland areas of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) or short days at 10 °C (SD).

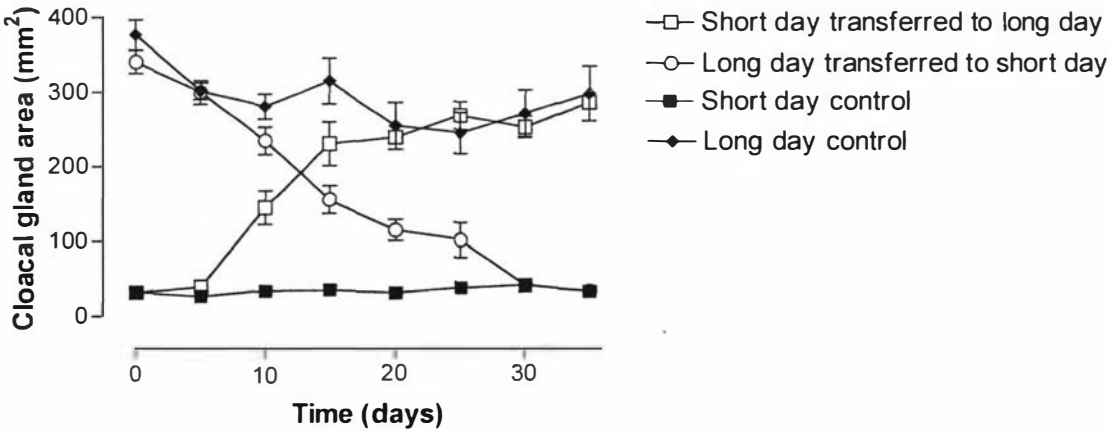

Figure 3.4. Changes in cloacal gland area of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results are shown as mean ± standard error.

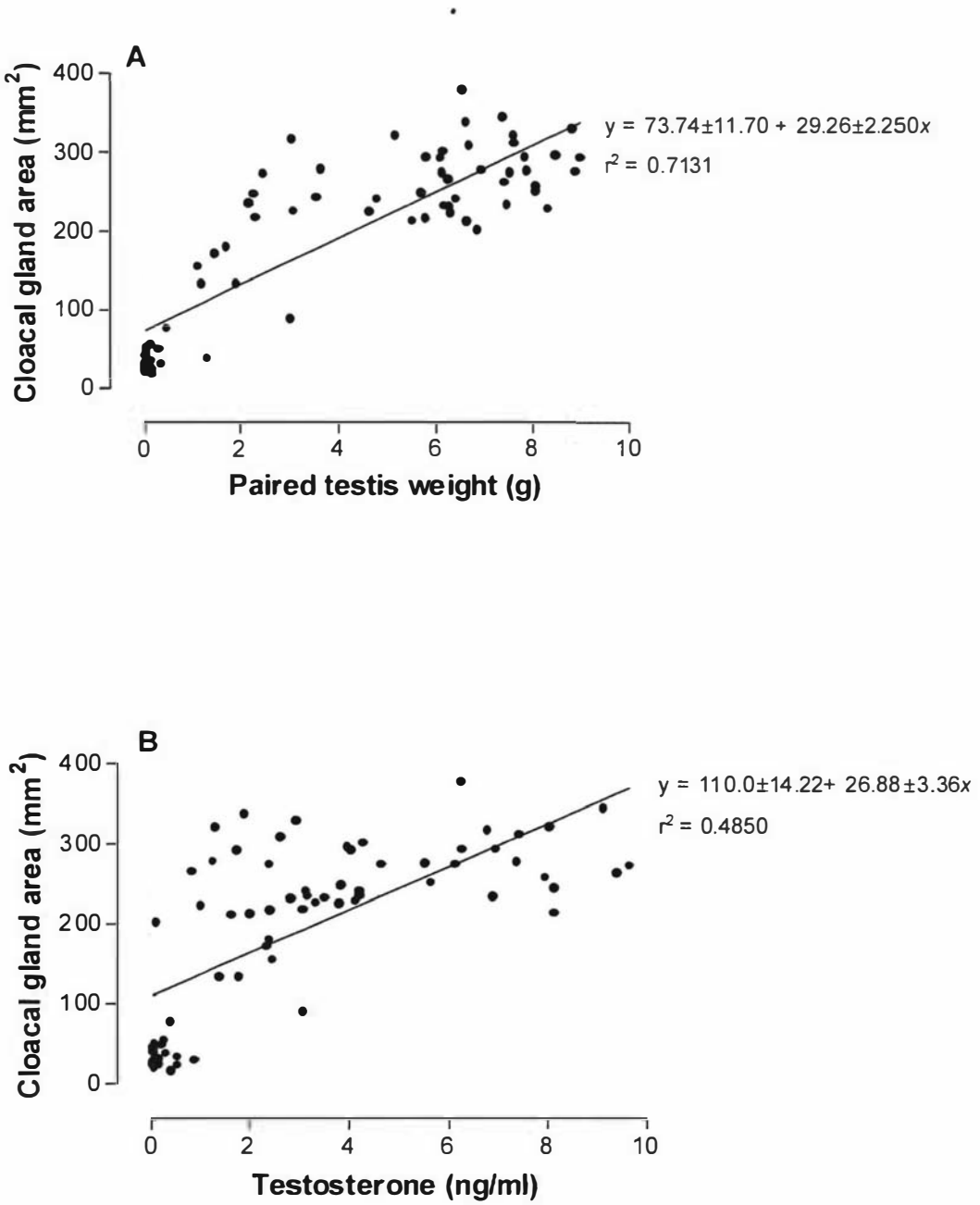

Figure 3.5. Relationships between cloacal gland area and paired testis weight (A) and cloacal gland area and testosterone (B) in quail transferred from short days to long days for 35 days.

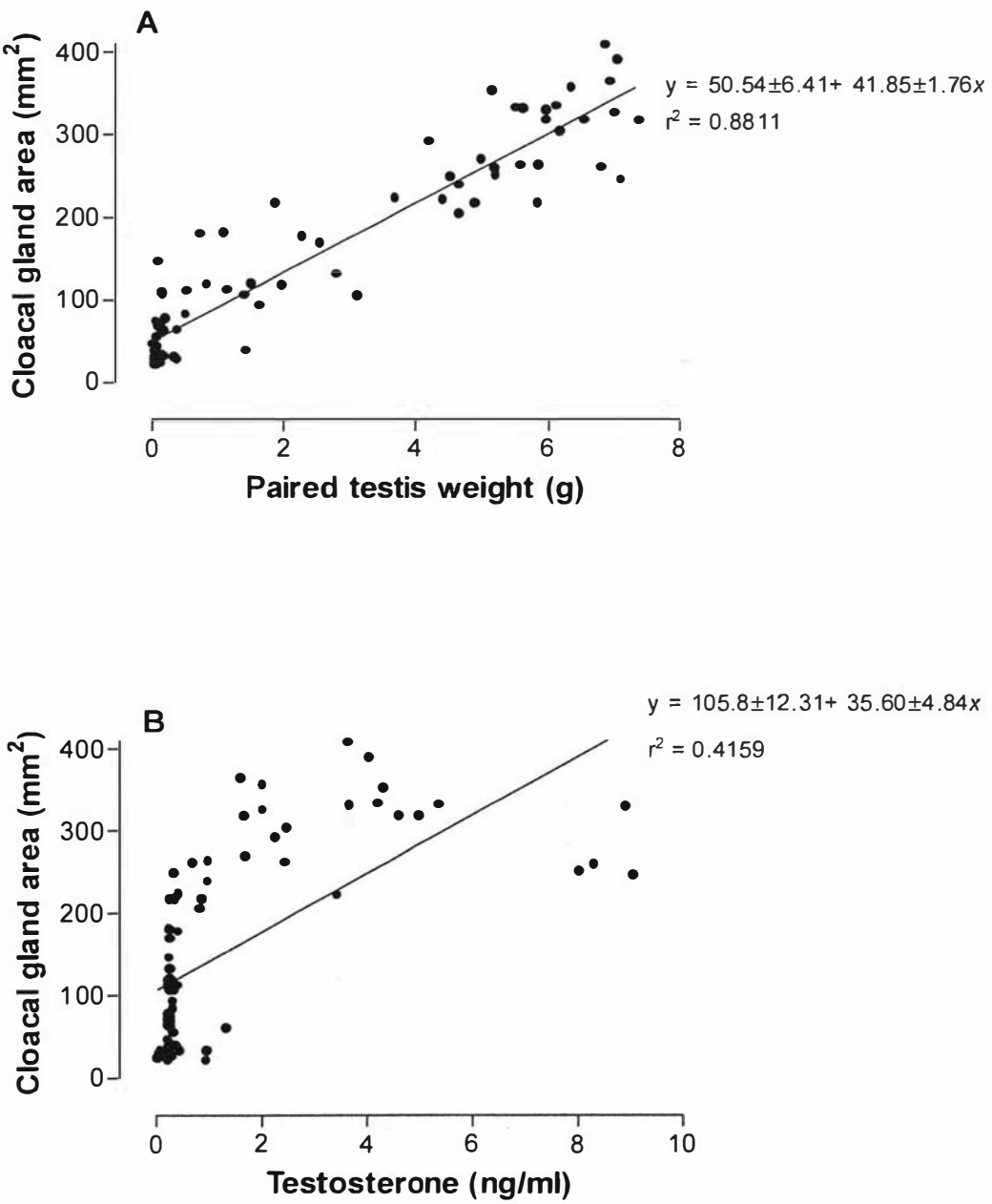

Figure 3.6. Relationships between cloacal gland area and paired testis weight (A) and cloacal gland area and testosterone (B) in quail transferred from long days to short days for 35 days.

Table 3.3. One-way single measures ANOVA for cloacal gland area of male quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

Effect	Cloacal gland area		
	F	df	*p*
Time	43.95	9, 76	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5			0.998
Day 0 vs day 10			0.000*
Day 0 vs day 15			0.000*
Day 0 vs day 20			0.000*
Day 0 vs day 25			0.000*
Day 0 vs day 30			0.000*
Day 0 vs day 35			0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control			0.000*
Day 5 vs long day control			0.000*
Day 10 vs long day control			0.055
Day 15 vs long day control			0.986
Day 20 vs long day control			1.000
Day 25 vs long day control			1.000
Day 30 vs long day control			1.000
Day 35 vs long day control			1.000

Table 3.4. One-way single measures ANOVA for cloacal gland area of male quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

Effect	Cloacal gland area		
	F	df	*p*
Time	49.50	9, 85	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5			0.999
Day 0 vs day 10			0.485
Day 0 vs day 15			0.001*
Day 0 vs day 20			0.000*
Day 0 vs day 25			0.000*
Day 0 vs day 30			0.000*
Day 0 vs day 35			0.000*
Quail transferred to short days (days 0 –35) compared to short day controls on day 35			
Day 0 vs short day control			0.852
Day 5 vs short day control			0.996
Day 10 vs short day control			1.000
Day 15 vs short day control			0.115
Day 20 vs short day control			0.001*
Day 25 vs short day control			0.000*
Day 30 vs short day control			0.000*
Day 35 vs short day control			0.000*

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.5. One way repeated measures ANOVA for cloacal gland area of male quail held on long days at 20 °C and male quail held on short days at 10 °C.

Cloacal gland area – long days at 20 °C				Cloacal gland area – short days at 10 °C			
Effect	**F**	**df**	**p**	**Effect**	**F**	**df**	**p**
Time	4.73	7, 49	0.000*	Time	0.91	7, 42	0.511
Contrasts				**Contrasts**			
Day 0 compared to:				**Day 0 compared to:**			
Day 5	10.04	1, 7	0.016*	Day 5			
Day 10	15.88	1, 7	0.005*	Day 10			
Day 15	3.39	1, 7	0.108	Day 15			
Day 20	8.98	1, 7	0.020*	Day 20			
Day 25	12.51	1, 7	0.010*	Day 25			
Day 30	7.68	1, 7	0.028*	Day 30			
Day 35	4.38	1, 7	0.075	Day 35			

NOTE: The first row shows the results of the one-way repeated measures ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.6. Two way single measures ANOVA for cloacal gland area on day 35 of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

Effect	Cloacal gland area		
	F	**df**	***p***
Time	0.75	2, 43	0.478
Treatment	53.00	1, 43	0.000*
Interaction between time and treatment	341.21	2, 43	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	297.30	1, 43	0.000*
Transfer to SD day 35 vs transfer to LD day 35	235.25	1, 43	0.000*
Short day control vs Long day control on day 35	192.92	1, 43	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.7. Regression analyses for male quail transferred from short days to long days (A) and for male quail transferred from long days to short days (B).

A	r^2	F	df	*p*
Cloacal gland area *versus* paired testis weight	0.7131	169.00	1, 68	< 0.0001*
Cloacal gland area *versus* T	0.4850	64.05	1, 68	< 0.0001*
T *versus* paired testis weight	0.3597	38.21	1, 68	< 0.0001*
Paired testis weight *versus* LH	0.0243	1.70	1, 68	0.1969
Paired testis weight *versus* FSH	0.1893	15.88	1, 68	0.0002*
T *versus* LH	0.0004	0.02	1, 68	0.8671
T *versus* FSH	0.0325	2.29	1, 68	0.1352
LH *versus* FSH	0.0318	2.24	1, 68	0.1394
LH *versus* T4	0.0762	5.53	1, 68	0.0217*
LH *versus* T3	0.0873	6.50	1, 68	0.0130*
T4 *versus* T3	0.0211	1.44	1, 68	0.2337

B	r^2	F	df	*p*
Cloacal gland area *versus* paired testis weight	0.8811	563.40	1, 76	< 0.0001*
Cloacal gland area *versus* T	0.4159	54.12	1, 76	< 0.0001*
T *versus* paired testis weight	0.4551	63.47	1, 76	< 0.0001*
Paired testis weight *versus* LH	0.5506	93.10	1, 76	< 0.0001*
Paired testis weight *versus* FSH	0.1111	9.50	1, 76	0.0029*
T *versus* LH	0.6589	146.80	1, 76	< 0.0001*
T *versus* FSH	0.1235	10.71	1, 76	0.0016*
LH *versus* FSH	0.2260	22.20	1, 76	< 0.0001*
LH *versus* T4	0.0935	7.01	1, 76	0.0101*
LH *versus* T3	0.0093	0.70	1, 76	0.4047
T4 *versus* T3	0.0018	0.14	1, 76	0.7140

3.3.1.3 Cloacal foam production

Quail transferred from short days to long days began to produce cloacal foam by day 10 when testosterone concentrations had increased significantly (Figure 3.7; see Table 3.8). All quail in this group were producing foam between days 20 – 35. Quail transferred to short days continued to produce cloacal foam for five days. By day 10 foam production decreased and had ceased in all quail by day 35 when testosterone concentrations were at their lowest.

All long day control quail were producing cloacal foam on day 0, although one bird had ceased foam production by day five. No quail held on short days at 10 °C produced cloacal foam.

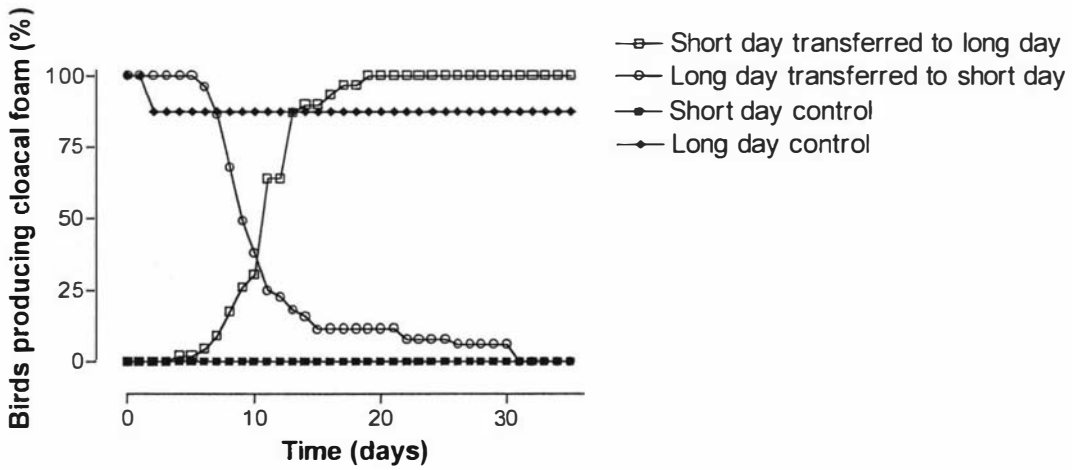

Figure 3.7. Changes in the number of male quail producing cloacal gland foam transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Table 3.8. Cloacal foam production and corresponding measurements for cloacal gland area and testosterone concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) or long days at 20 °C (long day control).

Day	n	Birds producing cloacal foam (%)	Mean cloacal gland area (mm^2)	Testosterone (ng/ml)
Male quail transferred from short days to long days				
0	8	0.0	31.7 ± 4.1	0.11 ± 0.06
5	8	0.0	39.4 ± 6.9	0.27 ● 0.06
10	7	29.0	145.7 ± 22.3	2.05 ± 0.28
15	8	87.5	230.9 ± 29.5	4.05 ● 1.05
20	7	100.0	239.8 ± 8.8	4.40 ± 1.12
25	8	100.0	270.0 ± 18.5	4.30 ± 1.06
30	8	100.0	252.9 ± 14.3	4.60 ± 0.99
35	8	100.0	286.0 ± 6.2	4.99 ± 0.86
Male quail maintained on long days at 20 °C				
Long day control	8	100.0	269.7 ± 31.3	4.13 ± 0.71
Male quail transferred from long days to short days				
Day	**n**	**Birds producing cloacal foam (%)**	**Mean cloacal gland area (mm^2)**	**Testosterone (ng/ml)**
0	9	100.0	342.8 ± 17.4	4.73 ± 0.87
5	9	100.0	300.1 ± 15.8	3.22 ● 0.84
10	9	44.4	235.2 ± 18.3	1.90 ± 0.85
15	9	87.5	156.2 ● 18.8	0.34 ± 0.08
20	9	11.1	115.6 ± 14.1	0.33 ± 0.06
25	9	11.1	102.5 ± 23.3	0.67 ± 0.35
30	8	12.5	41.4 ± 8.8	0.32 ± 0.09
35	9	0.0	34.2 ± 2.8	0.27 ± 0.04
Male quail maintained on short days at 10 °C				
Short day control	7	0.0	33.1 ± 4.7	0.40 ± 0.20

3.3.1.4 Paired testis weight

Paired testis weights had increased in some quail transferred to long days after five days and were significantly greater after 10 days than before transfer (Figures 3.8A and 3.9). Testis weights in quail transferred to long days after 20 days were similar to quail maintained on long days (see Table 3.9 for statistics). Mean paired testis weights of quail transferred to long days on day 35 were similar to those of long day control quail (7.1958 ± 0.3478 and 6.0970 ± 0.5458 g respectively). There was a wide range of paired testis weights within groups of birds on days 10 to 35. The rate of testicular growth (k) between days 0 and 20 in quail transferred to long days was calculated as k = 0.2599 ± 0.0286 (95 % confidence limits 0.2018 to 0.3180).

Paired testis weight in most quail transferred from long days to short days had decreased by day 10 and mean weights were significantly lower than before transfer at day 10. Paired testis weights reached a similar size to short day control quail after 20 days (Figure 3.8B; see Table 3.10 for statistics). One bird on day 30 and one on day 35 had not undergone full testicular regression (1.641 and 1.415 g). Both birds had ceased producing cloacal foam 10 days before sacrifice and both had small cloacal glands (93.6 and 39.0 mm^2 respectively).

Long day control quail had large testes ranging between 3 – 8g in weight. The testes of all short day quail were smaller than those of the long day control quail (see Table 3.11 for statistics).

Paired testis weights were correlated with testosterone during photostimulation (Figure 3.10; see Table 3.7A for statistics), were not related to LH and had an inverse relationship with FSH (Figure 3.10B-C). On transfer to short days paired testis weights were correlated with testosterone and LH (Figure 3.11A-B; see Table 3.7B for statistics). Testosterone and LH concentrations were low for testes < 4 g in weight. There was a weak inverse relationship between paired testis weight and FSH during gonadal regression (Figure 3.11C; see Table 3.7B for statistics).

Molt of the primary feathers in quail transferred from long days to short days was first detected on day 14 and proceeded rapidly to completion by day 24.

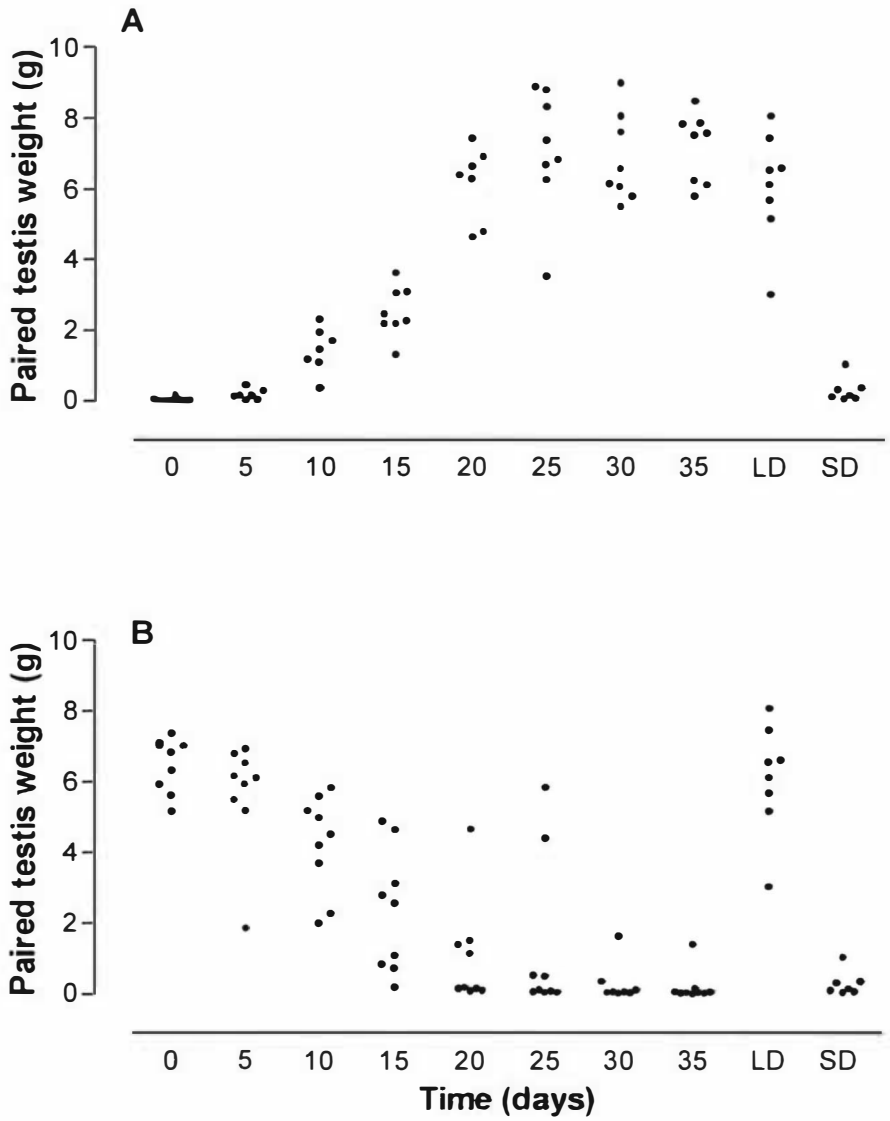

Figure 3.8. Paired testis weights of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

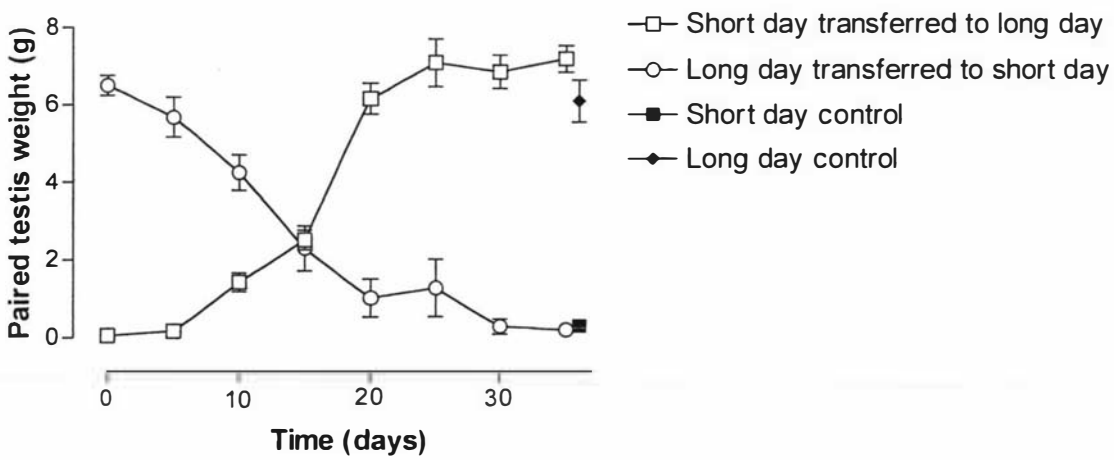

Figure 3.9. Changes in paired testis weights of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

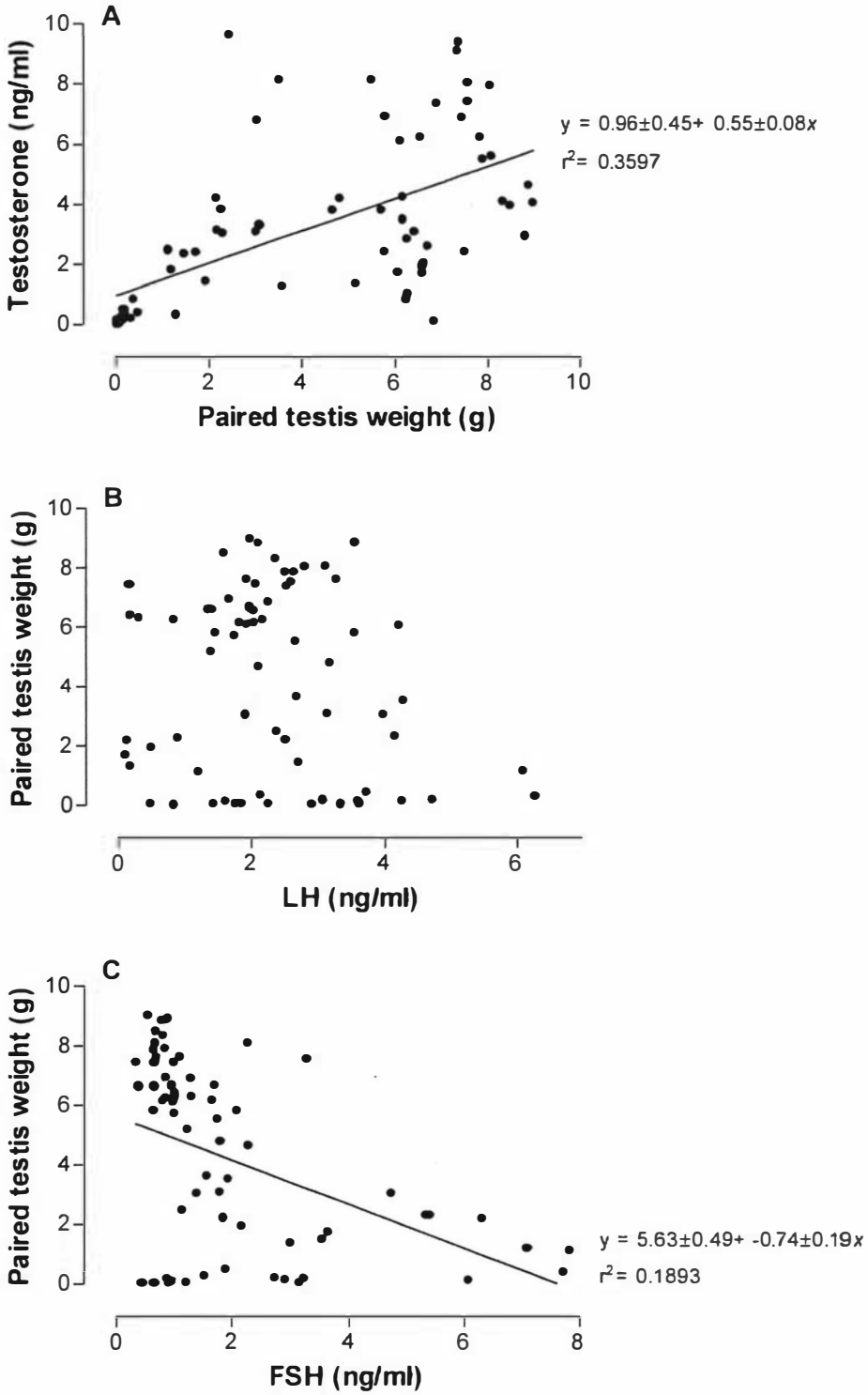

Figure 3.10. Relationships between paired testis weight and testosterone, LH and FSH (A-C) in quail transferred from short days to long days for 35 days. Note different scales on x axes.

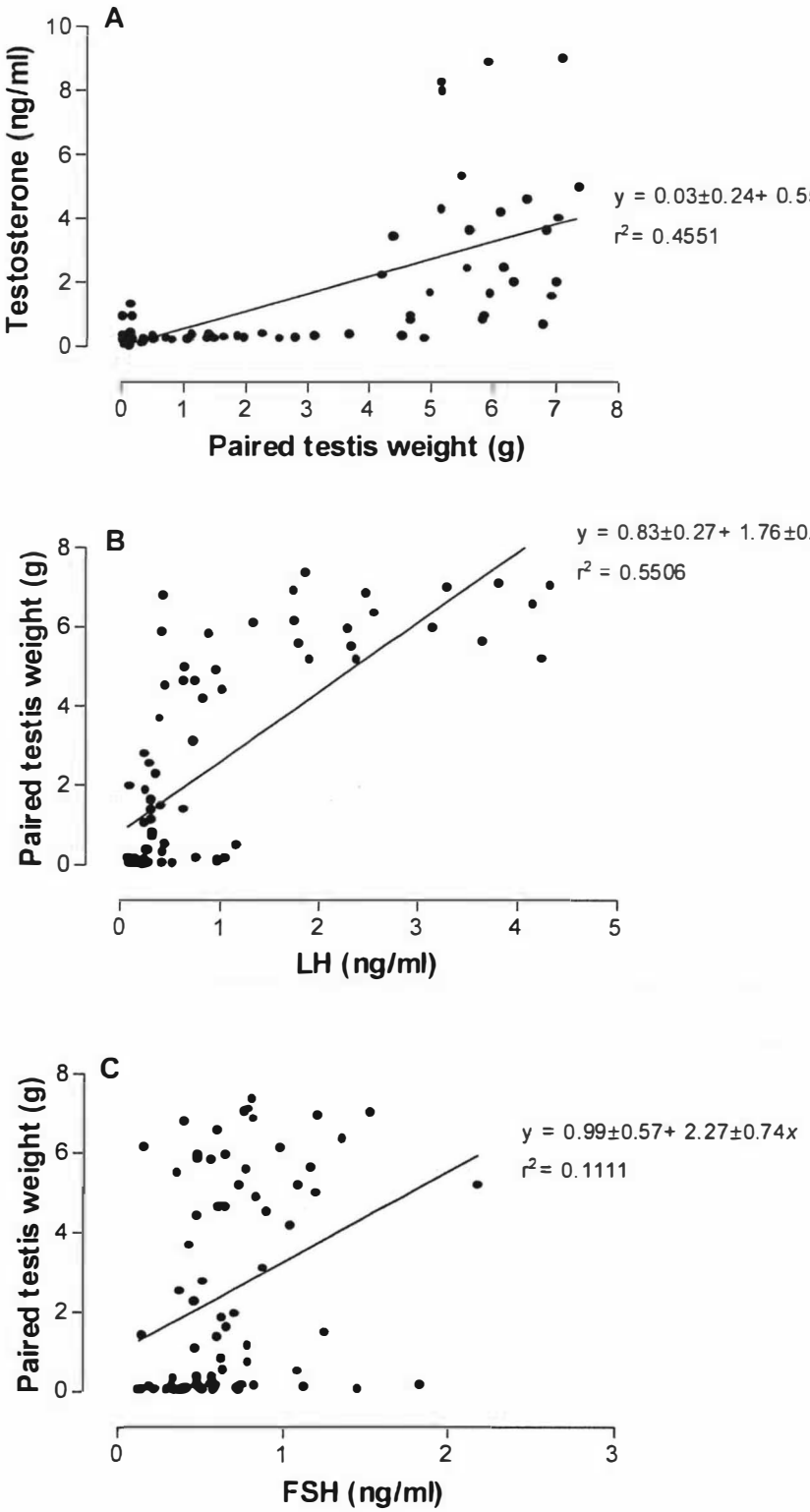

Figure 3.11. Relationships between paired testis weight and testosterone, LH and FSH (A-C) in quail transferred from long days to short days for 35 days. Note different scales on x and y axes.

Table 3.9. Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for paired testis weight of quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

Effect	Paired testis weight		
	K-W statistic	**df**	***p***
Time	52.6	7	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
	M-W statistic		***p***
Day 0 vs day 5	13.0		0.050
Day 0 vs day 10	0.0		0.000*
Day 0 vs day 15	0.0		0.000*
Day 0 vs day 20	0.0		0.000*
Day 0 vs day 25	0.0		0.000*
Day 0 vs day 30	0.0		0.000*
Day 0 vs day 35	0.0		0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	0.0		0.000*
Day 5 vs long day control	0.0		0.000*
Day 10 vs long day control	0.0		0.000*
Day 15 vs long day control	3.0		0.001*
Day 20 vs long day control	28.0		1.000
Day 25 vs long day control	17.0		0.130
Day 30 vs long day control	25.0		0.505
Day 35 vs long day control	18.0		0.161

Table 3.10. Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for paired testis weight of quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

Effect	Paired testis weight		
	K-W statistic	**df**	***p***
Time	58.7	7	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
	M-W statistic		***P***
Day 0 vs day 5	24.0		0.161
Day 0 vs day 10	4.0		0.000*
Day 0 vs day 15	0.0		0.000*
Day 0 vs day 20	0.0		0.000*
Day 0 vs day 25	0.0		0.000*
Day 0 vs day 30	0.0		0.000*
Day 0 vs day 35	0.0		0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	0.0		0.000*
Day 5 vs short day control	0.0		0.000*
Day 10 vs short day control	0.0		0.000*
Day 15 vs short day control	2.0		0.001*
Day 20 vs short day control	20.0		0.252
Day 25 vs short day control	30.0		0.918
Day 30 vs short day control	16.0		0.189
Day 35 vs short day control	12.0		0.042

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.11. Two-way single measures ANOVA for paired testis weight on day 35 of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

Effect	Paired testis weight		
	F	df	*p*
Time	2.80	2, 43	0.072
Treatment	30.63	1, 43	0.000*
Interaction between time and treatment	248.23	2, 43	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	224.61	1, 43	0.000*
Transfer to SD day 35 vs transfer to LD day 35	190.72	1, 43	0.000*
Short day control vs Long day control on day 35	106.19	1, 43	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.5 Luteinising hormone (LH)

LH concentrations in quail increased significantly between days 0 and five during photostimulation then returned to initial concentrations by day 10 (Figure 3.12A; see Table 3.12 for statistics). Mean LH concentrations remained unchanged from day 10 to the end of the experiment in these birds, with a wide range of LH concentrations within groups on days 0 to 20. LH concentrations decreased in most quail transferred from long days to short days by day five and reached similar concentrations to short day control quail after 10 days (Figure 3.12B). Mean LH concentrations in these birds remained unchanged through to day 35.

LH concentrations did not differ significantly between quail on short and long days at the beginning of the experiment, but were higher in long day control quail compared to short day control quail on day 35 (1.93 ± 0.16 and 0.58 ± 0.13 ng/ml respectively; Figure 3.13; see Table 3.12 for statistics).

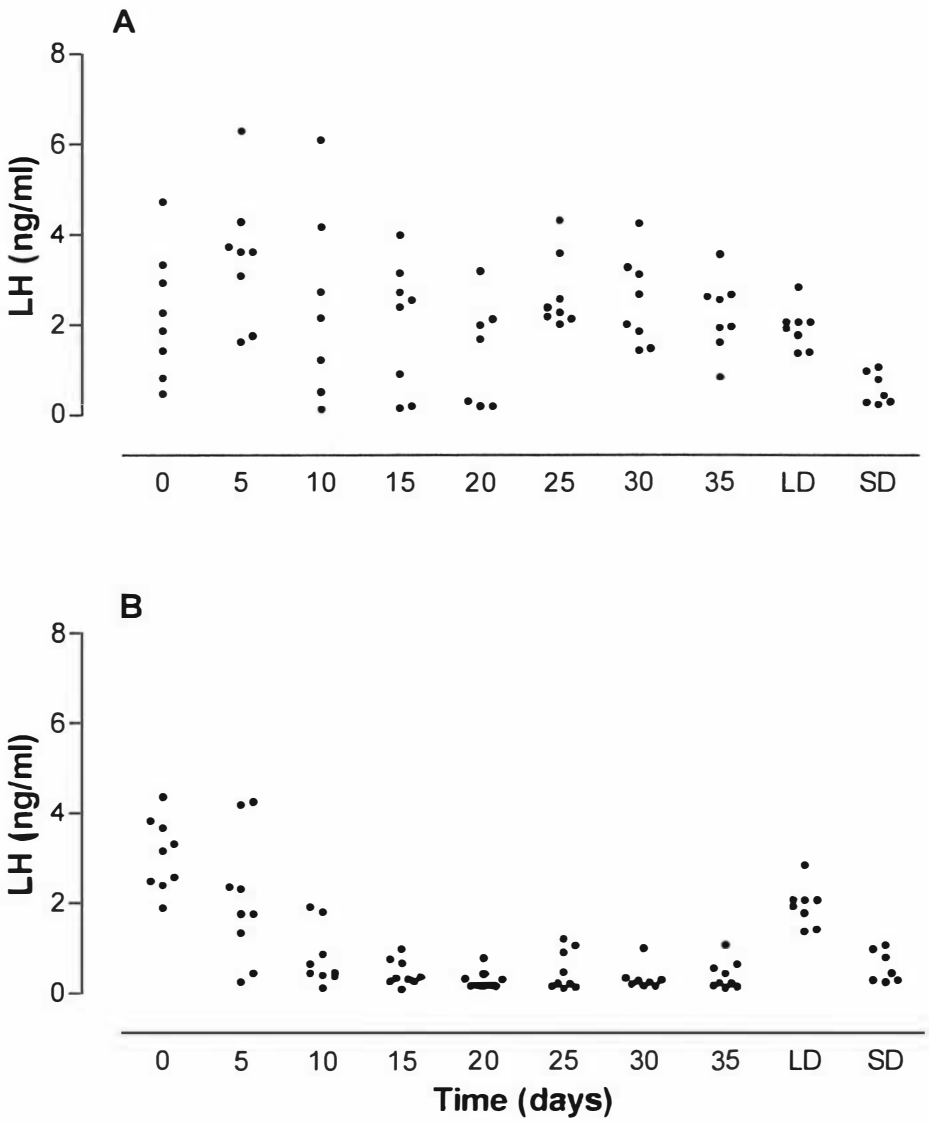

Figure 3.12. LH concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

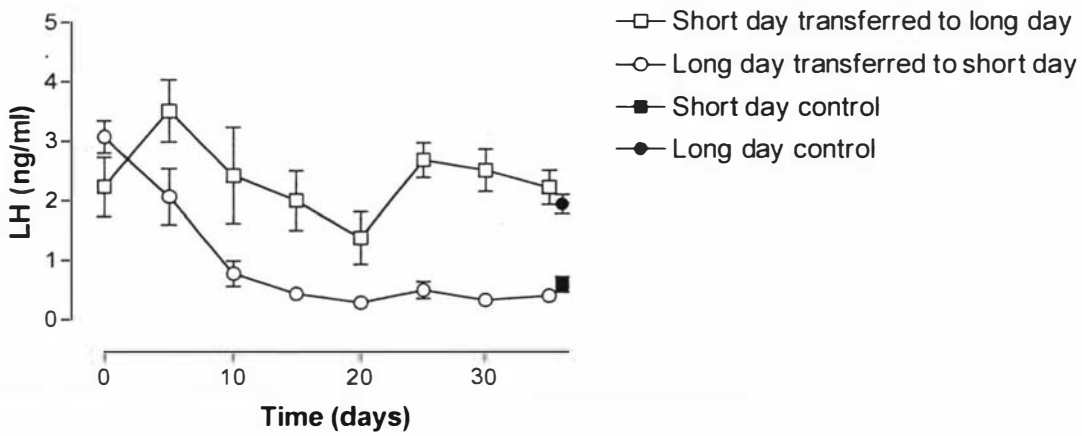

Figure 3.13. Changes in LH concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

Table 3.12. Two-way single measures ANOVA for LH concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	LH		
	F	df	p
Time	7.49	8, 130	0.000*
Treatment	73.81	1, 130	0.000*
Interaction between time and treatment	3.59	8, 130	0.001*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	6.68	1, 130	0.011*
Day 0 vs day 10	0.14	1, 130	0.705
Day 0 vs day 15	0.22	1, 130	0.641
Day 0 vs day 20	2.84	1, 130	0.094
Day 0 vs day 25	0.81	1, 130	0.368
Day 0 vs day 30	0.31	1, 130	0.581
Day 0 vs day 35	0.001	1, 130	0.974
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	0.37	1, 130	0.544
Day 5 vs long day control	10.19	1, 130	0.002*
Day 10 vs long day control	0.94	1, 130	0.335
Day 15 vs long day control	0.02	1, 130	0.888
Day 20 vs long day control	1.20	1, 130	0.275
Day 25 vs long day control	2.28	1, 130	0.133
Day 30 vs long day control	1.35	1, 130	0.248
Day 35 vs long day control	0.33	1, 130	0.566
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	4.67	1, 130	0.032*
Day 0 vs day 10	24.57	1, 130	0.000*
Day 0 vs day 15	32.35	1, 130	0.000*
Day 0 vs day 20	36.19	1, 130	0.000*
Day 0 vs day 25	30.99	1, 130	0.000*
Day 0 vs day 30	33.13	1, 130	0.000*
Day 0 vs day 35	33.34	1, 130	0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	25.27	1, 130	0.000*
Day 5 vs short day control	9.03	1, 130	0.003*
Day 10 vs short day control	0.15	1, 130	0.698
Day 15 vs short day control	0.09	1, 130	0.769
Day 20 vs short day control	0.36	1, 130	0.549
Day 25 vs short day control	0.03	1, 130	0.857
Day 30 vs short day control	0.26	1, 130	0.611
Day 35 vs short day control	0.14	1, 130	0.709
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	3.07	1, 130	0.082
Transfer to SD day 35 vs transfer to LD day 35	14.55	1, 130	0.000*
Short day control vs Long day control on day 35	7.08	1, 130	0.009*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.6 Follicle stimulating hormone (FSH)

FSH concentrations did not differ between quail transferred to long or short days on day 0. FSH concentrations increased significantly by day five of photostimulation reached a peak on day 10 then declined (Figures 3.14A and 3.15). FSH gradually decreased by day 20 to initial concentrations which were also similar to concentrations of long day control quail (see Table 3.13 for statistics). FSH concentrations decreased in most quail transferred to short days by day five and reached similar concentrations to short day control quail at this time (Figure 3.14B). FSH remained unchanged in these birds through to day 35.

FSH concentrations of long day control quail were significantly higher than those of short day controls on day 35 (0.98 ± 0.23 and 0.51 ± 0.11 ng/ml respectively) (Figure 3.15; see Table 3.13 for statistics).

FSH concentrations were not correlated to LH concentrations in quail undergoing gonadal growth but were during gonadal regression (Figure 3.16; see Table 3.7 for statistics).

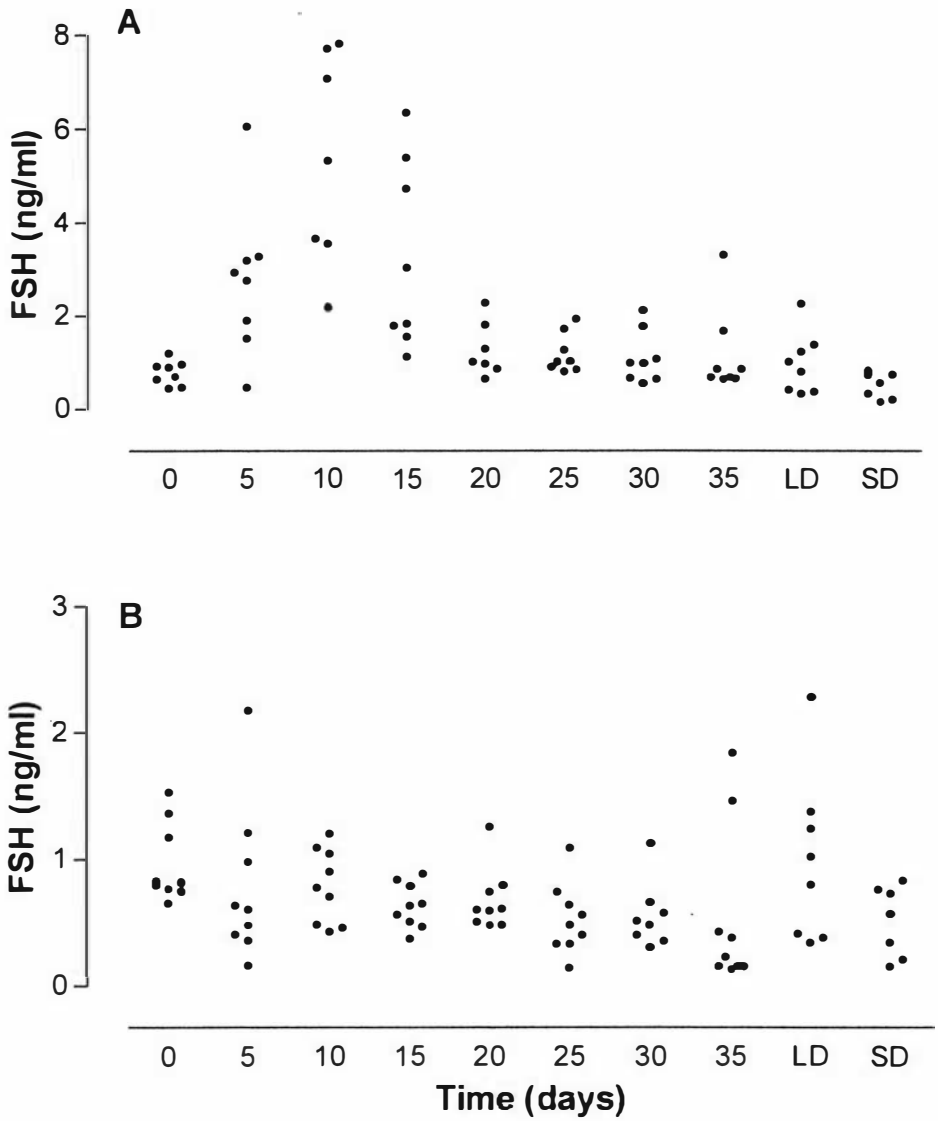

Figure 3.14. FSH concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD). Note different scales on y axes.

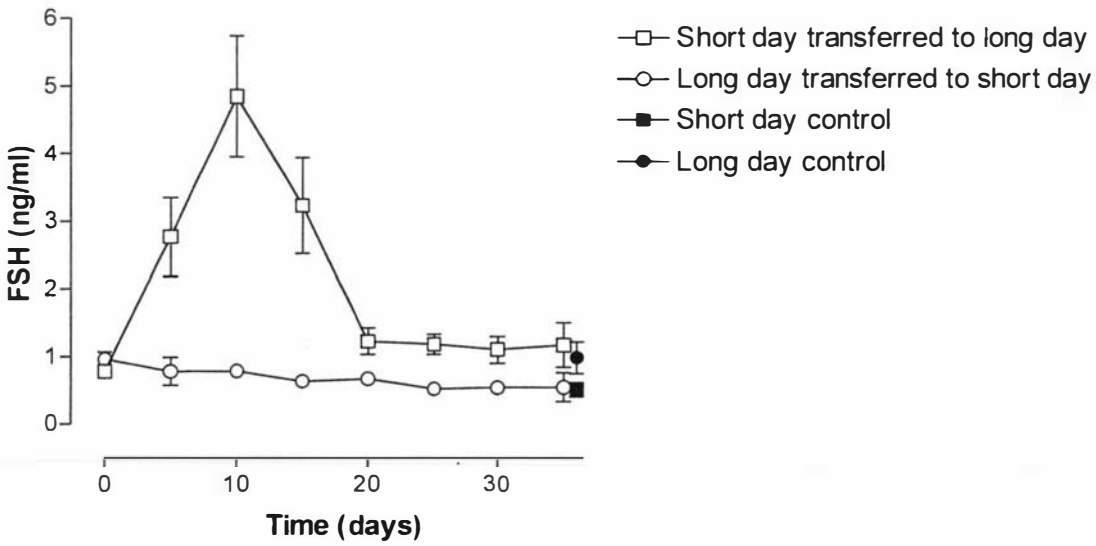

Figure 3.15. Changes in FSH concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

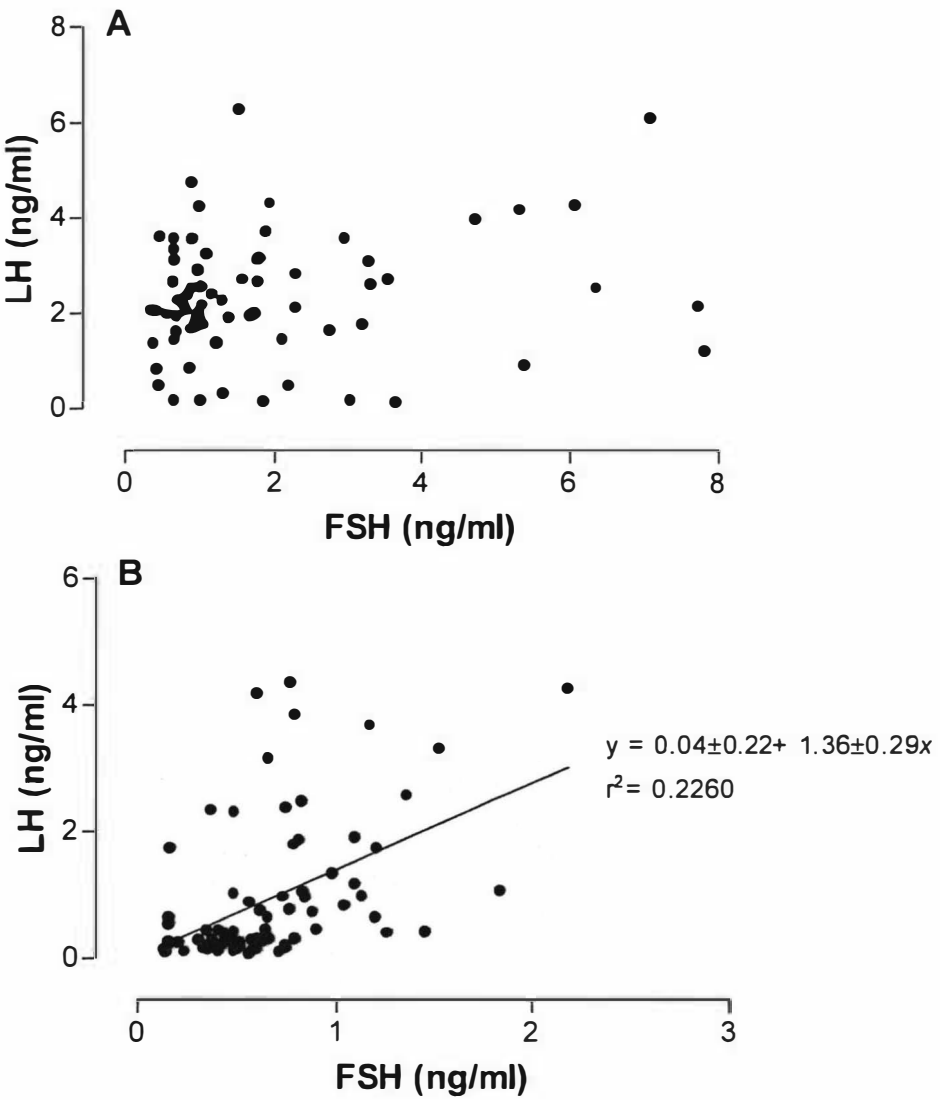

Figure 3.16. Relationships between LH and FSH in quail transferred from short days to long days (A) and in quail transferred from long days to short days (B) for 35 days. Note different scales on x and y axes.

Table 3.13. Two-way single measures ANOVA for FSH concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	FSH		
	F	df	*p*
Time	8.03	8, 130	0.000*
Treatment	99.11	1, 130	0.000*
Interaction between time and treatment	4.87	8, 130	0.000*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	15.97	1, 130	0.000*
Day 0 vs day 10	42.04	1, 130	0.000*
Day 0 vs day 15	21.39	1, 130	0.000*
Day 0 vs day 20	2.57	1, 130	0.112
Day 0 vs day 25	2.27	1, 130	0.134
Day 0 vs day 30	1.14	1, 130	0.289
Day 0 vs day 35	0.98	1, 130	0.323
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	0.10	1, 130	0.750
Day 5 vs long day control	12.36	1, 130	0.001*
Day 10 vs long day control	34.87	1, 130	0.000*
Day 15 vs long day control	16.95	1, 130	0.000*
Day 20 vs long day control	1.53	1, 130	0.219
Day 25 vs long day control	1.29	1, 130	0.258
Day 30 vs long day control	0.51	1, 130	0.477
Day 35 vs long day control	0.41	1, 130	0.522
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	2.49	1, 130	0.117
Day 0 vs day 10	0.69	1, 130	0.407
Day 0 vs day 15	2.40	1, 130	0.124
Day 0 vs day 20	1.85	1, 130	0.176
Day 0 vs day 25	7.04	1, 130	0.009*
Day 0 vs day 30	4.66	1, 130	0.033*
Day 0 vs day 35	15.21	1, 130	0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	7.21	1, 130	0.008*
Day 5 vs short day control	1.46	1, 130	0.229
Day 10 vs short day control	3.64	1, 130	0.059
Day 15 vs short day control	1.53	1, 130	0.219
Day 20 vs short day control	2.00	1, 130	0.160
Day 25 vs short day control	0.04	1, 130	0.839
Day 30 vs short day control	0.35	1, 130	0.558
Day 35 vs short day control	0.93	1, 130	0.338
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	0.70	1, 130	0.405
Transfer to SD day 35 vs transfer to LD day 35	15.75	1, 130	0.000*
Short day control vs Long day control on day 35	4.58	1, 130	0.034*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.7 Testosterone

Testosterone concentrations increased in most quail transferred to long days by day 10 and mean concentrations on day 10 did not differ significantly from those of long day control quail (Figure 3.17A; see Table 3.14 for statistics). Testosterone concentrations were highly variable in photostimulated quail from day 15 to the end of the experiment. Testosterone concentrations decreased in most quail after 10 short days and mean concentrations from day 15 did not differ from short day control quail (Figure 3.17B; see Table 3.14 for statistics). Short day control quail had lower mean testosterone concentrations than long day control quail on day 35 (0.40 ± 0.20 and 4.13 ± 0.71 ng/ml respectively; Figure 3.18; see Table 3.14 for statistics).

Testosterone concentrations were not related to LH or FSH concentrations during photostimulation (Figure 3.19; see Table 3.7A for statistics). Testosterone concentrations were strongly correlated with LH and very weakly with FSH concentrations during gonadal regression (Figure 3.20; see Table 3.7B for statistics).

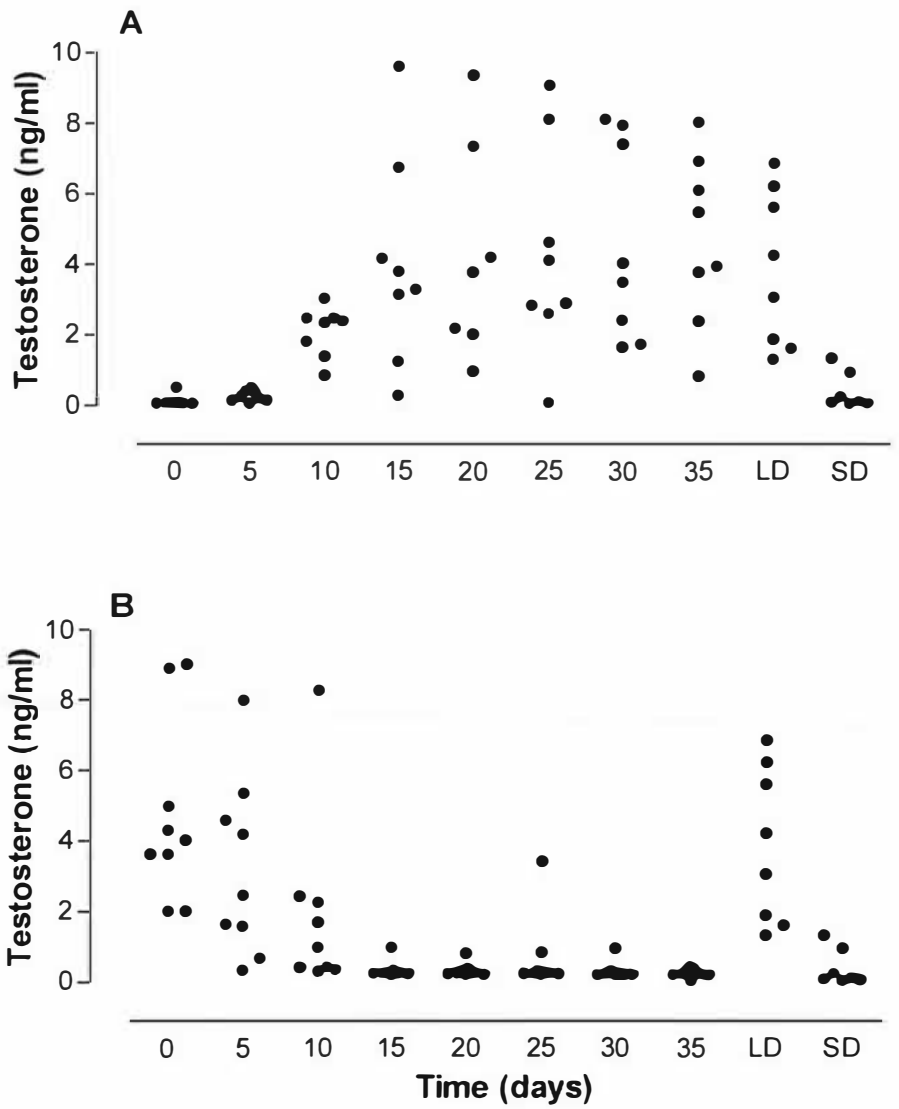

Figure 3.17. Testosterone concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

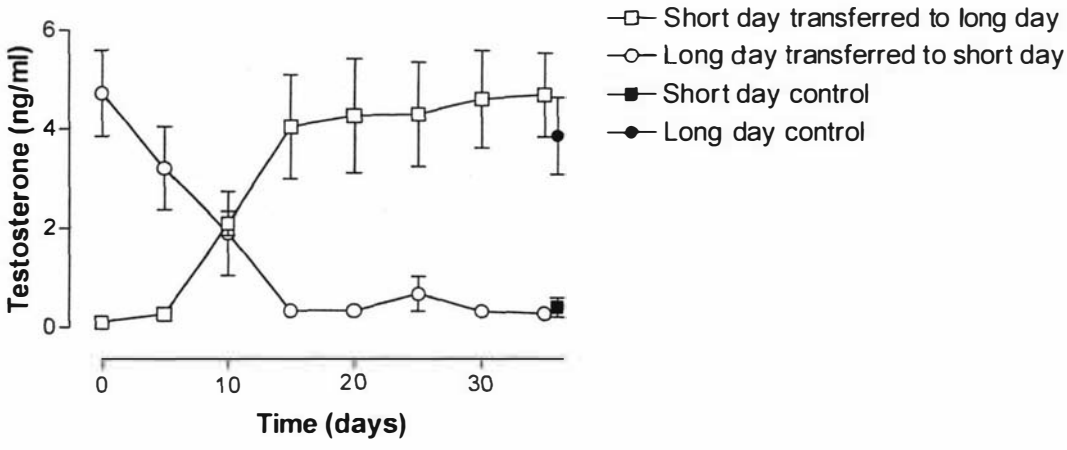

Figure 3.18. Changes in testosterone concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

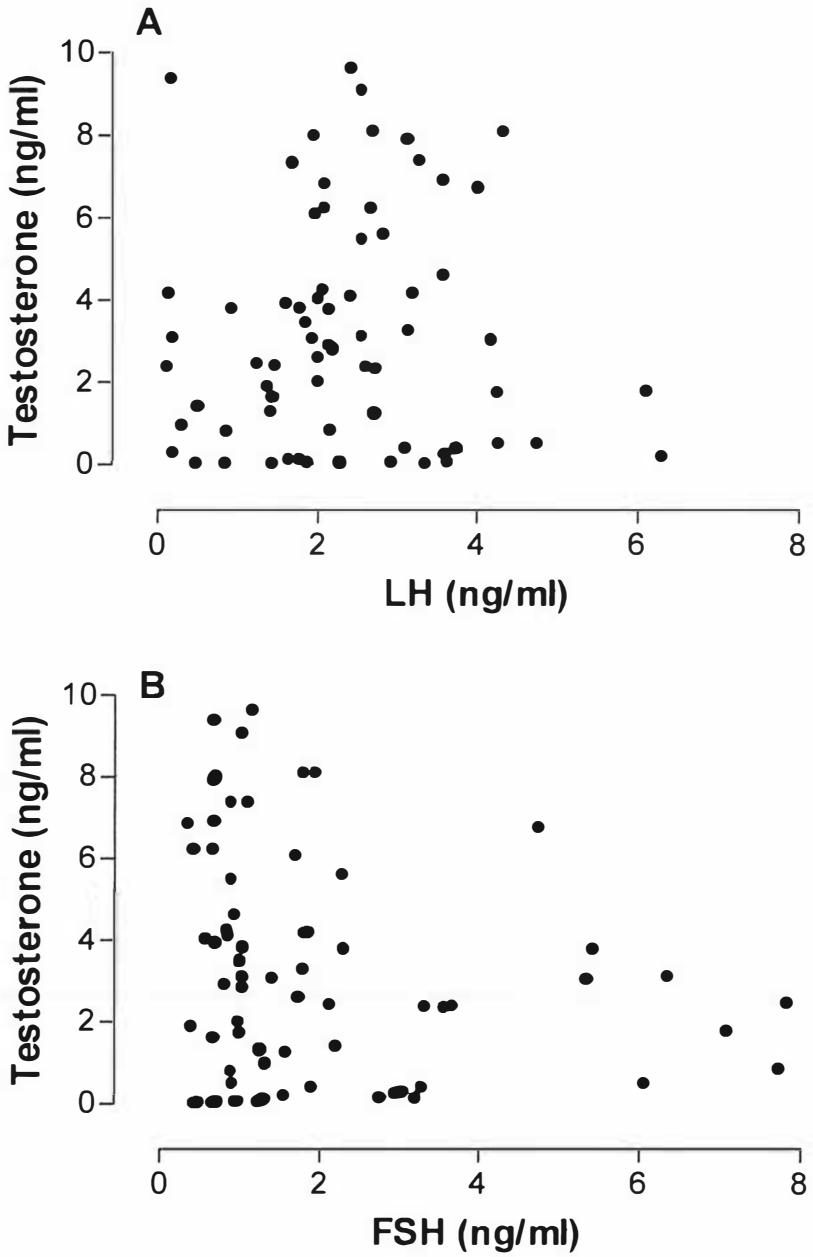

Figure 3.19. Relationships between testosterone and LH (A) and FSH (B) in male quail transferred from short days to long days for 35 days.

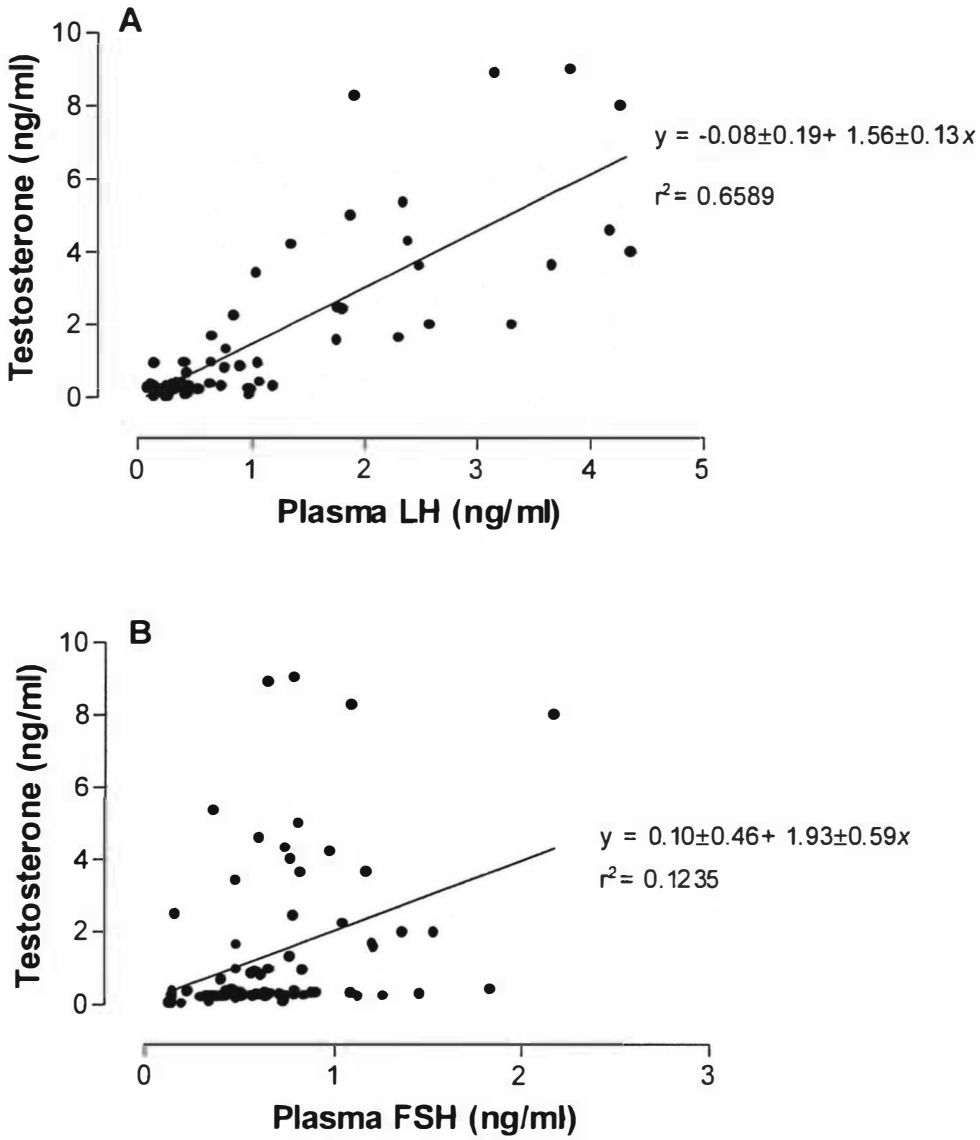

Figure 3.20. Relationships between testosterone and LH (A) and FSH (B) in male quail transferred from long days to short days for 35 days.

Table 3.14. Two way single measures ANOVA for testosterone concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	Testosterone		
	F	df	*p*
Time	1.08	8, 130	0.082
Treatment	54.45	1, 130	0.000*
Interaction between time and treatment	37.72	8, 130	0.000*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	8.38	1, 130	0.004*
Day 0 vs day 10	58.92	1, 130	0.000*
Day 0 vs day 15	78.34	1, 130	0.000*
Day 0 vs day 20	82.14	1, 130	0.000*
Day 0 vs day 25	76.17	1, 130	0.000*
Day 0 vs day 30	91.77	1, 130	0.000*
Day 0 vs day 35	95.44	1, 130	0.000*
Quail transferred to long days (days 0 –35) compared to long day controls on day 35			
Day 0 vs long day control	89.260	1, 130	0.000*
Day 5 vs long day control	42.950	1, 130	0.000*
Day 10 vs long day control	2.110	1, 130	0.149
Day 15 vs long day control	0.360	1, 130	0.552
Day 20 vs long day control	0.004	1, 130	0.949
Day 25 vs long day control	0.520	1, 130	0.473
Day 30 vs long day control	0.020	1, 130	0.895
Day 35 vs long day control	0.100	1, 130	0.748
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	2.47	1, 130	0.119
Day 0 vs day 10	12.21	1, 130	0.001*
Day 0 vs day 15	43.09	1, 130	0.000*
Day 0 vs day 20	42.43	1, 130	0.000*
Day 0 vs day 25	35.04	1, 130	0.000*
Day 0 vs day 30	43.42	1, 130	0.000*
Day 0 vs day 35	50.62	1, 130	0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	54.77	1, 130	0.000*
Day 5 vs short day control	35.19	1, 130	0.000*
Day 10 vs short day control	17.08	1, 130	0.000*
Day 15 vs short day control	1.59	1, 130	0.210
Day 20 vs short day control	1.71	1, 130	0.193
Day 25 vs short day control	3.47	1, 130	0.065
Day 30 vs short day control	1.04	1, 130	0.310
Day 35 vs short day control	0.56	1, 130	0.457
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	101.07	1, 130	0.000*
Transfer to SD day 35 vs transfer to LD day 35	21.49	1, 130	0.000*
Short day control vs Long day control on day 35	47.54	1, 130	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.8 Prolactin

There was no clear pattern in prolactin secretion in quail transferred to long days, with no significant changes in prolactin concentrations after 35 long days (Figures 3.21A and 3.22; see Table 3.15 for statistics). Prolactin concentrations decreased in quail transferred to short days and were similar to concentrations seen in short day control birds after 25 days (Figure 3.21B; see Table 3.15 for statistics).

Prolactin concentrations were similar between long day and short day control groups on day 35 (7.49 ± 2.49 and 5.18 ± 0.83 ng/ml respectively; see Table 3.15 for statistics).

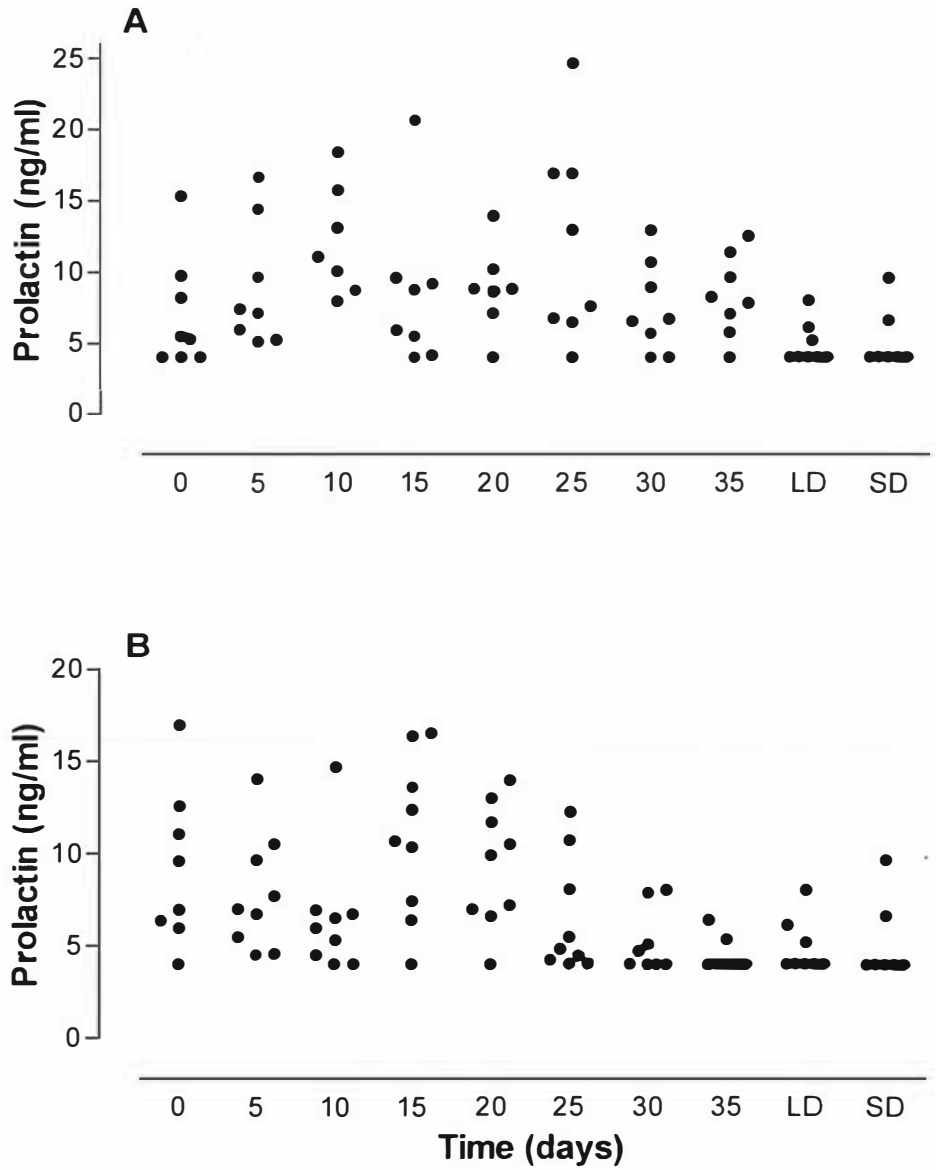

Figure 3.21. Prolactin concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD). Note different scales on y axes.

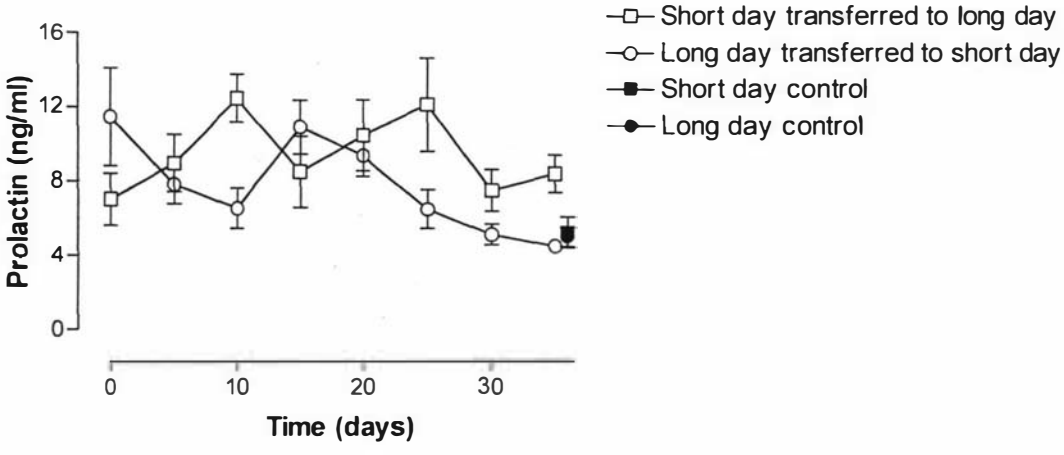

Figure 3.22. Changes in prolactin concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

Table 3.15. Two way single measures ANOVA for prolactin concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	Prolactin		
	F	df	*p*
Time	3.69	8, 130	0.001*
Treatment	5.15	1, 130	0.025*
Interaction between time and treatment	3.63	8, 130	0.001*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	1.58	1, 130	0.211
Day 0 vs day 10	8.12	1, 130	0.005*
Day 0 vs day 15	0.60	1, 130	0.440
Day 0 vs day 20	1.70	1, 130	0.194
Day 0 vs day 25	5.48	1, 130	0.021
Day 0 vs day 30	0.21	1, 130	0.646
Day 0 vs day 35	1.20	1, 130	0.275
Quail transferred to long days (days 0 –35) compared to long day controls on day 35			
Day 0 vs long day control	1.57	1, 130	0.212
Day 5 vs long day control	1.92	1, 130	0.168
Day 10 vs long day control	8.43	1, 130	0.004*
Day 15 vs long day control	0.86	1, 130	0.357
Day 20 vs long day control	2.03	1, 130	0.156
Day 25 vs long day control	5.87	1, 130	0.017*
Day 30 vs long day control	0.39	1, 130	0.533
Day 35 vs long day control	1.52	1, 130	0.220
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	1.89	1, 130	0.172
Day 0 vs day 10	5.52	1, 130	0.020*
Day 0 vs day 15	0.03	1, 130	0.864
Day 0 vs day 20	0.23	1, 130	0.631
Day 0 vs day 25	5.97	1, 130	0.016*
Day 0 vs day 30	10.03	1, 130	0.002*
Day 0 vs day 35	15.50	1, 130	0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	9.97	1, 130	0.002*
Day 5 vs short day control	3.51	1, 130	0.063
Day 10 vs short day control	0.92	1, 130	0.338
Day 15 vs short day control	11.01	1, 130	0.001*
Day 20 vs short day control	7.33	1, 130	0.008*
Day 25 vs short day control	0.76	1, 130	0.384
Day 30 vs short day control	0.01	1, 130	0.919
Day 35 vs short day control	0.27	1, 130	0.601
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	4.38	1, 130	0.038*
Transfer to SD day 35 vs transfer to LD day 35	8.51	1, 130	0.005*
Short day control vs Long day control on day 35	0.01	1, 130	0.918

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.9 Thyroxine (T4)

T4 concentrations did not differ at the beginning of the experiment between quail held on short or long days (Figure 3.23; see Table 3.16 for statistics). T4 increased significantly after 25 days of long days in birds moved to long days, whereas T4 declined after five days then remained relatively constant in birds moved to short days. Concentrations tended to be higher at the end of the experiment in birds maintained on long days compared with short days (Figure 3.24).

T4 concentrations were very weakly correlated with LH concentrations during gonadal growth and regression (Figure 3.25; see Table 3.7A-B for statistics).

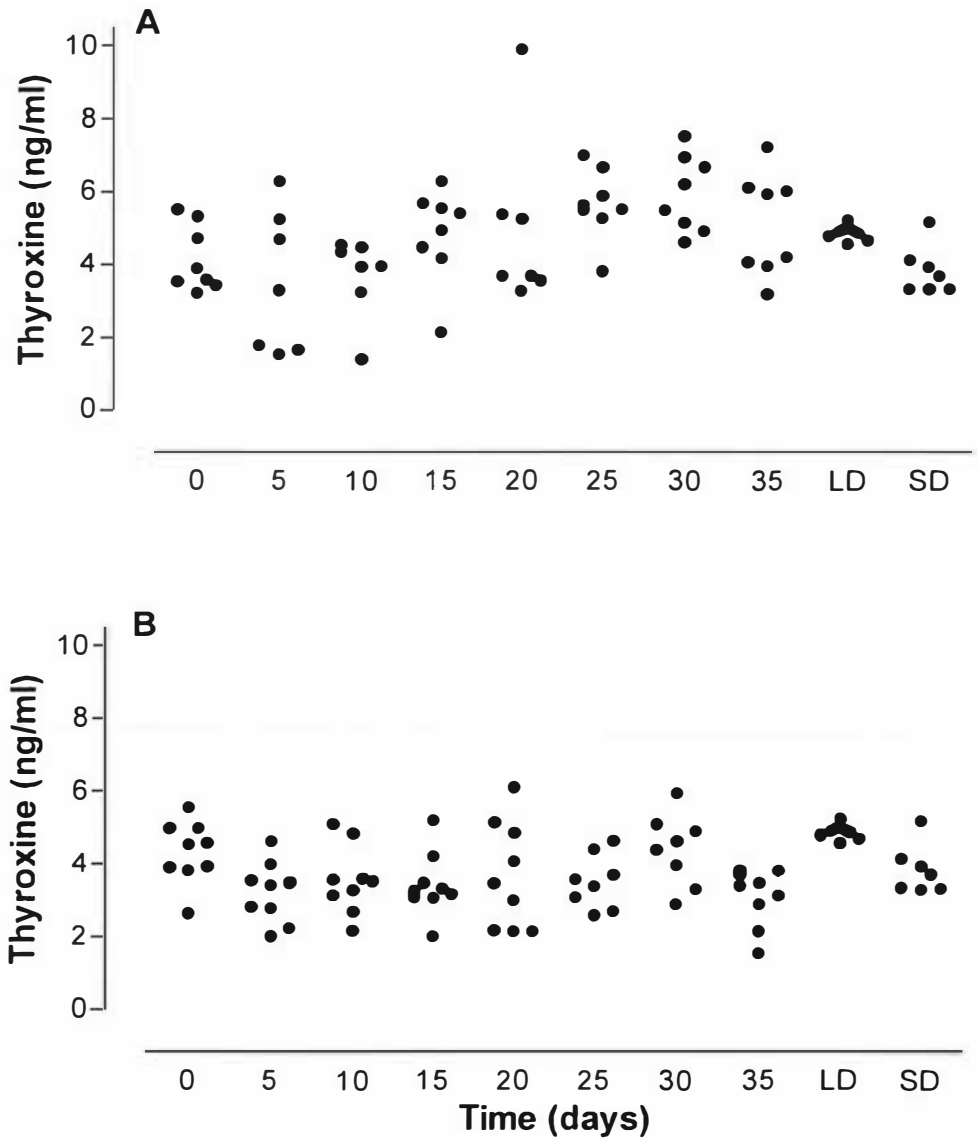

Figure 3.23. Thyroxine concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

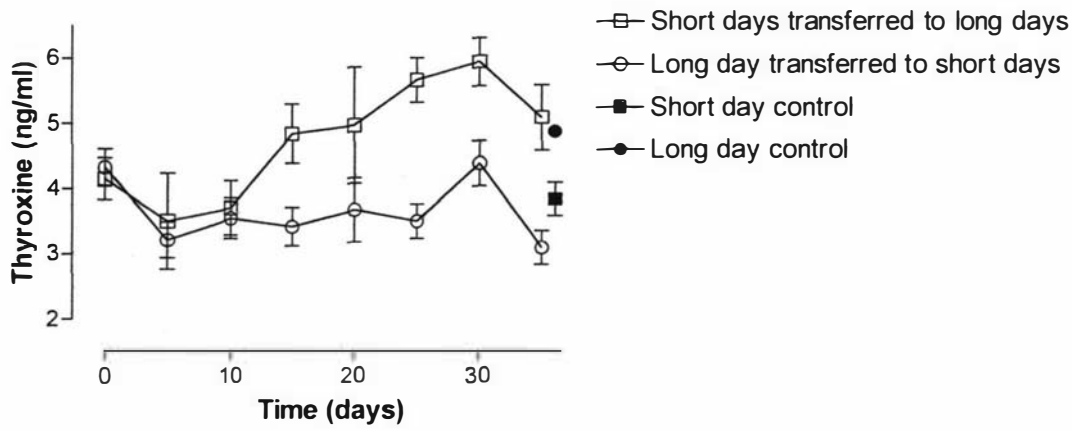

Figure 3.24. Changes in thyroxine concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

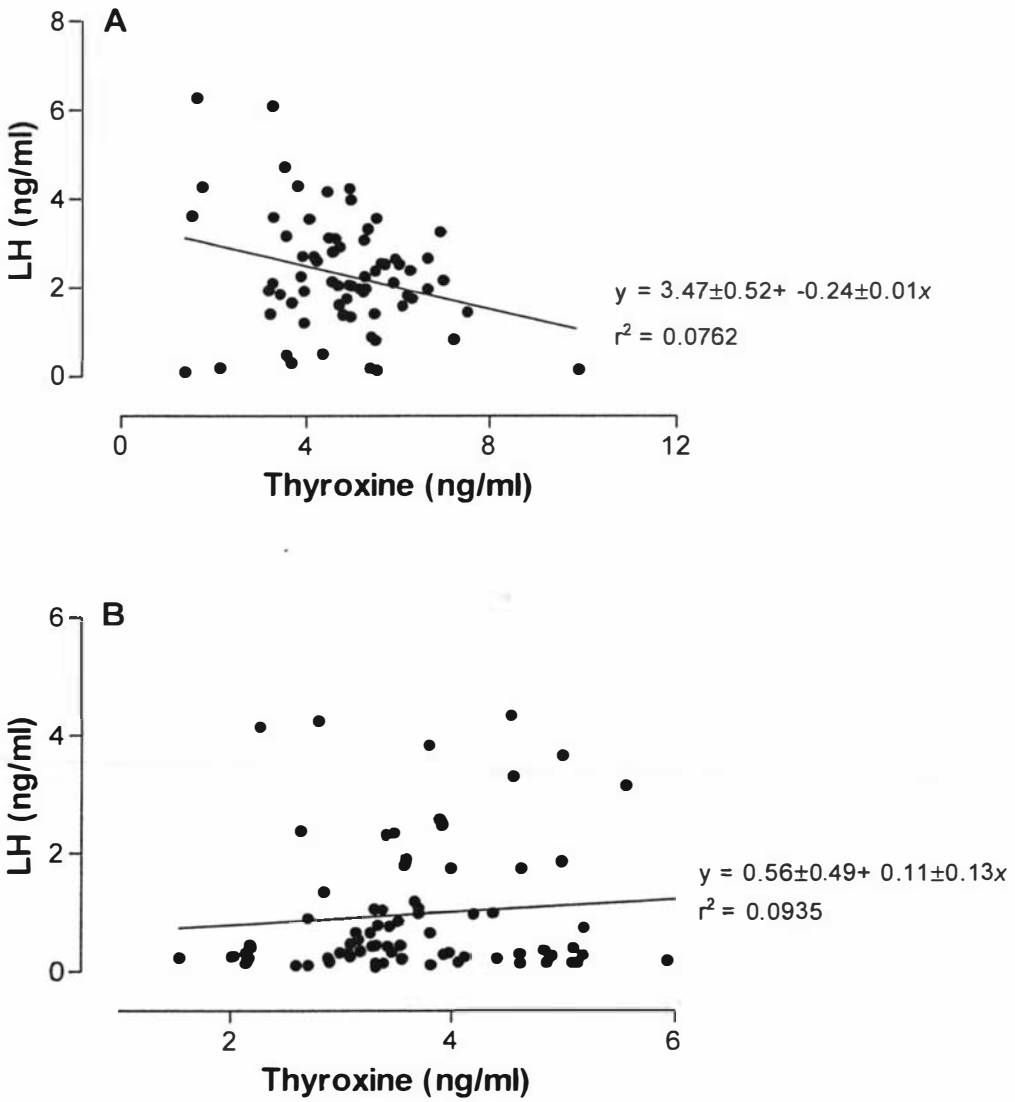

Figure 3.25. Relationships between thyroxine and LH in male quail transferred from short days to long days (A) and in male quail transferred from long days to short days for 35 days. Note different scales on x and y axes.

Table 3.16. Two-way single measures ANOVA for thyroxine concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	Thyroxine		
	F	df	*p*
Time	3.14	8, 130	0.003*
Treatment	22.88	1, 130	0.000*
Interaction between time and treatment	1.59	8, 130	0.135
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	1.75	1, 130	0.189
Day 0 vs day 10	0.95	1, 130	0.332
Day 0 vs day 15	0.77	1, 130	0.383
Day 0 vs day 20	0.65	1, 130	0.421
Day 0 vs day 25	4.38	1, 130	0.038*
Day 0 vs day 30	5.82	1, 130	0.017*
Day 0 vs day 35	1.58	1, 130	0.211
Quail transferred to long days (days 0 -35) compared to long day controls on day 35			
Day 0 vs long day control	1.43	1, 130	0.235
Day 5 vs long day control	6.33	1, 130	0.013*
Day 10 vs long day control	4.52	1, 130	0.035*
Day 15 vs long day control	0.10	1, 130	0.750
Day 20 vs long day control	0.12	1, 130	0.730
Day 25 vs long day control	0.81	1, 130	0.370
Day 30 vs long day control	1.49	1, 130	0.225
Day 35 vs long day control	0.004	1, 130	0.951
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	4.53	1, 130	0.035*
Day 0 vs day 10	2.12	1, 130	0.148
Day 0 vs day 15	2.88	1, 130	0.092
Day 0 vs day 20	2.26	1, 130	0.135
Day 0 vs day 25	1.09	1, 130	0.299
Day 0 vs day 30	0.007	1, 130	0.935
Day 0 vs day 35	5.91	1, 130	0.016*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	0.51	1, 130	0.477
Day 5 vs short day control	1.64	1, 130	0.203
Day 10 vs short day control	0.42	1, 130	0.518
Day 15 vs short day control	0.77	1, 130	0.384
Day 20 vs short day control	0.48	1, 130	0.489
Day 25 vs short day control	0..07	1, 130	0.794
Day 30 vs short day control	0.59	1, 130	0.442
Day 35 vs short day control	2.44	1, 130	0.121
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	0.08	1, 130	0.781
Transfer to SD day 35 vs transfer to LD day 35	11.37	1, 130	0.001*
Short day control vs Long day control on day 35	2.51	1, 130	0.115

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.10 Triiodothyronine (T3)

T3 concentrations decreased in quail transferred from short to long days after five days and from that day were similar to concentrations seen in quail on day 35 (Figure 3.26A; see Table 3.17 for statistics). Quail transferred to long days for 35 days had similar T3 concentrations to long day control quail (see Table 3.18 for statistics). T3 concentrations increased in quail 15 days after the birds were transferred to short days (Figure 3.26B; see Table 3.18 for statistics). T3 concentrations were similar from days 15 and 35 in these quail and on day 35 were similar to short day control quail (see Table 3.19 for statistics).

Short day control quail had higher concentrations of T3 than to long day control quail on day 35 (1.63 ± 0.34 and 0.75 ± 0.05 ng/ml respectively) (Figure 3.27; see Table 3.19 for statistics).

T3 concentrations were very weakly correlated with LH but not with T4 in quail undergoing gonadal growth and not correlated with LH or T4 in birds undergoing gonadal regression (Figures 3.28 and 3.29; see Table 3.7A-B for statistics).

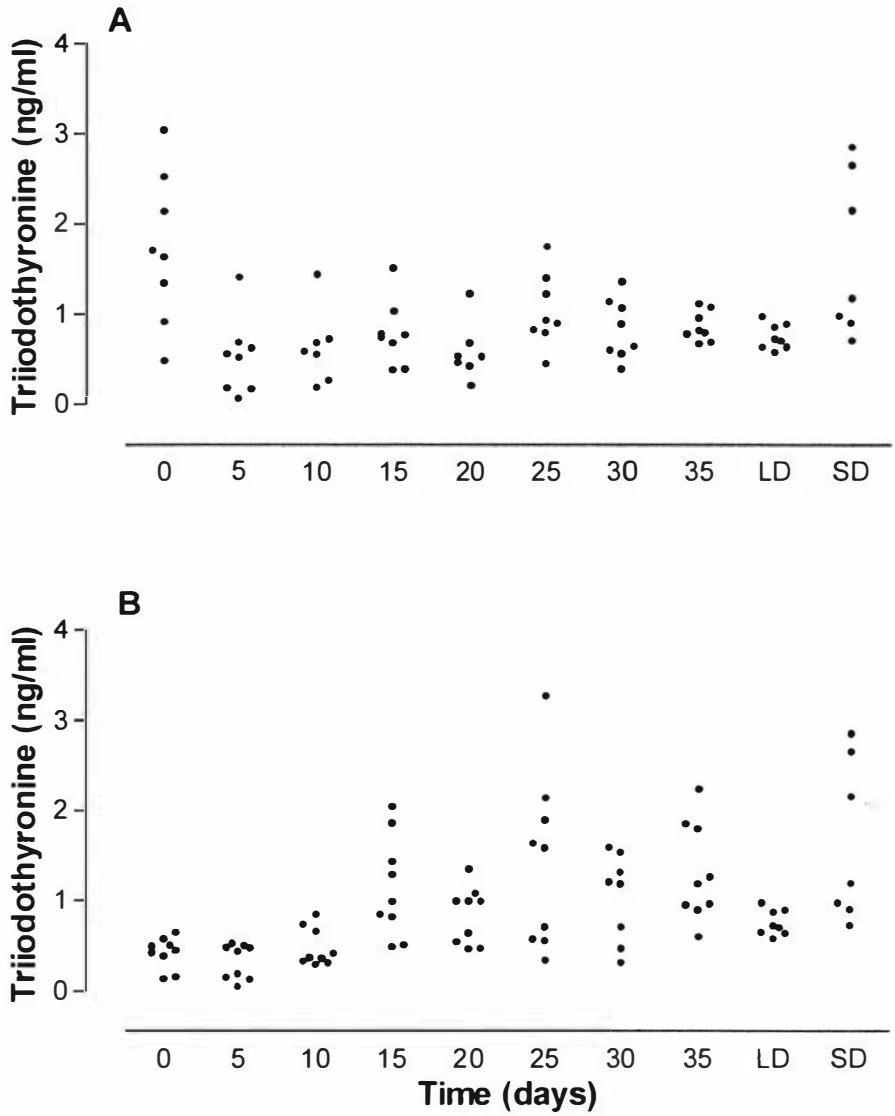

Figure 3.26. Triiodothyronine concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

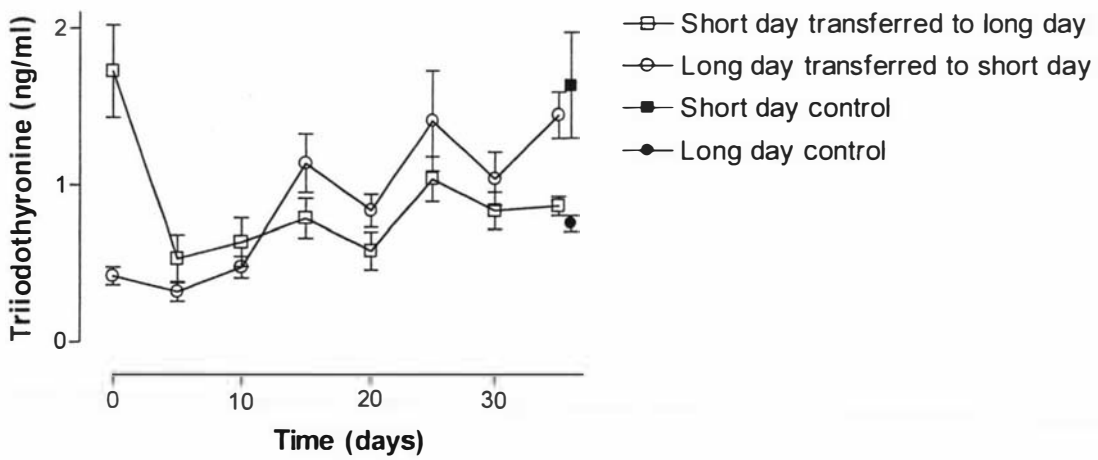

Figure 3.27. Changes in triiodothyronine concentrations of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

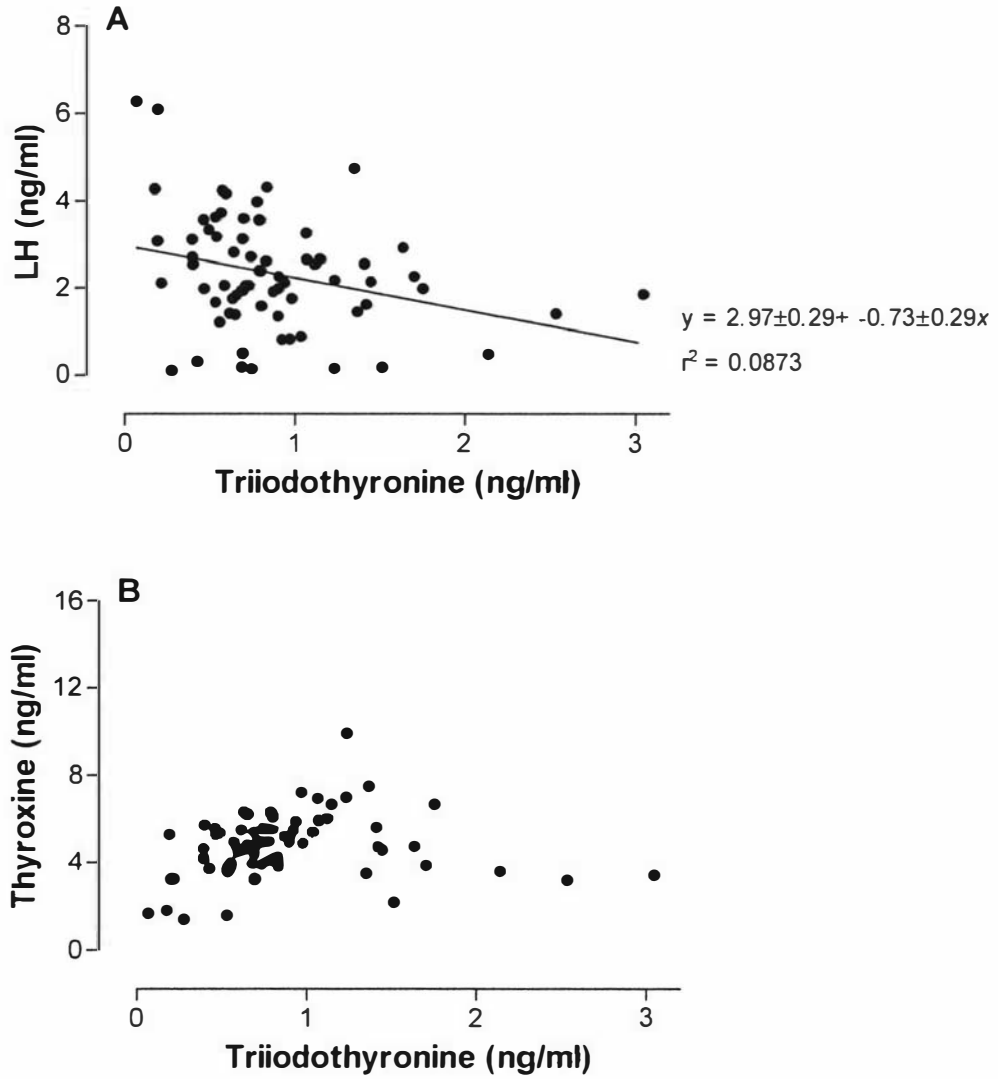

Figure 3.28. Relationships between triiodothyronine and LH (A) and thyroxine (B) concentrations in quail transferred from short days to long days. Note different scales on y axes.

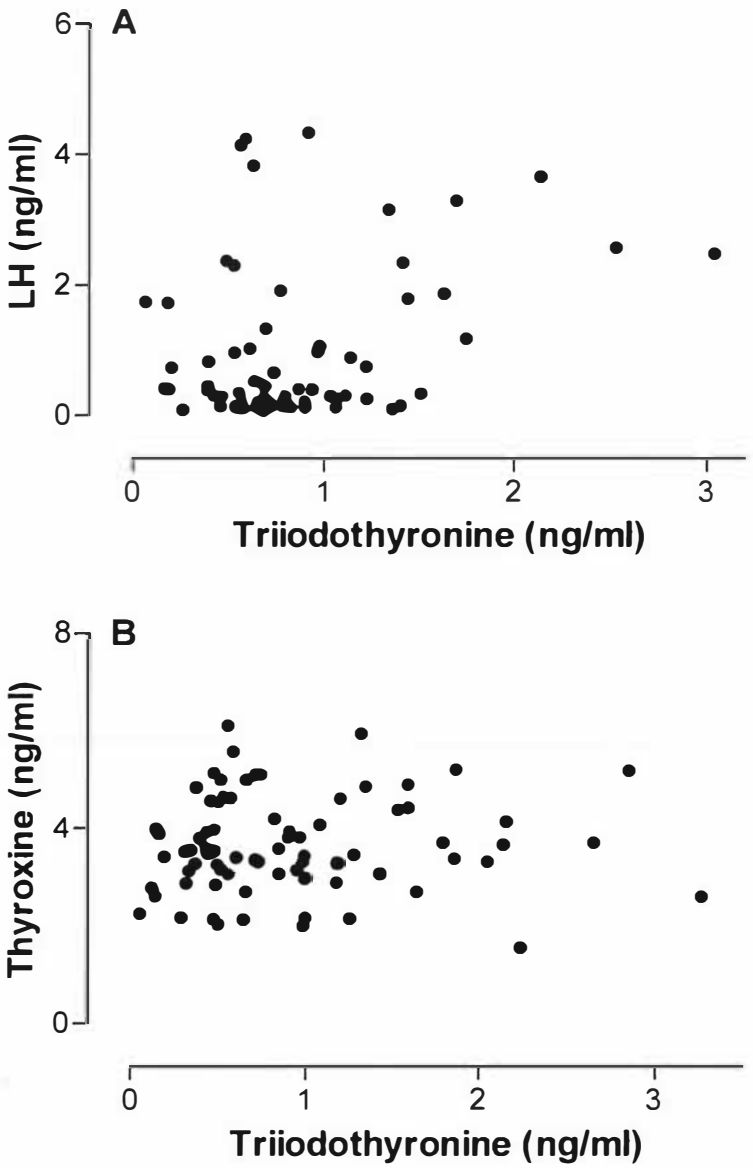

Figure 3.29. Relationships between T3 and LH (A) and T4 (B) concentrations in quail transferred from long days to short days. Note different scales on y axes.

Table 3.17. Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for triiodothyronine concentrations of male quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

Effect	Triiodothyronine		
	K-W statistics	df	*p*
Time	39.94	8	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
	M-W statistic		*p*
Day 0 vs day 5	7.0		0.007*
Day 0 vs day 10	7.0		0.014*
Day 0 vs day 15	9.0		0.015*
Day 0 vs day 20	5.0		0.006*
Day 0 vs day 25	15.0		0.083
Day 0 vs day 30	11.0		0.028*
Day 0 vs day 35	11.0		0.028*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	9.0		0.015*
Day 5 vs long day control	12.0		0.038*
Day 10 vs long day control	17.0		0.232
Day 15 vs long day control	30.0		0.878
Day 20 vs long day control	11.0		0.054
Day 25 vs long day control	16.0		0.105
Day 30 vs long day control	30.0		0.878
Day 35 vs long day control	20.0		0.234

Table 3.18 Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for triiodothyronine concentrations of quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

Effect	Triiodothyronine		
	K-W statistic	df	*p*
Time	39.94	8	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
	M-W statistic		*p*
Day 0 vs day 5	30.0		0.387
Day 0 vs day 10	39.0		0.931
Day 0 vs day 15	7.0		0.002*
Day 0 vs day 20	11.0		0.008*
Day 0 vs day 25	11.0		0.008*
Day 0 vs day 30	11.0		0.015*
Day 0 vs day 35	1.0		0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	0.0		0.000*
Day 5 vs short day control	0.0		0.000*
Day 10 vs short day control	2.0		0.001*
Day 15 vs short day control	21.0		0.299
Day 20 vs short day control	16.0		0.114
Day 25 vs short day control	23.0		0.408
Day 30 vs short day control	19.0		0.336
Day 35 vs short day control	25.0		0.536

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.19. Two-way single measures ANOVA for triiodothyronine concentrations on day 35 of male quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

Effect	Triiodothyronine		
	F	df	*p*
Time	2.49	2, 43	0.095
Treatment	0.99	1, 43	0.326
Interaction between time and treatment	24.79	2, 43	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	40.58	1, 43	0.000*
Transfer to SD day 35 vs transfer to LD day 35	2.56	1, 43	0.117
Short day control vs Long day control on day 35	7.99	1, 43	0.007*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.1.11 Summary of results for gonadal growth and regression

The transfer of male quail from short days and 10 °C to long days and 20 °C caused significant increases in LH, FSH, testosterone and testis size after five days and cloacal gland area after 10 days (Figure 3.30; see Table 3.20). T3 decreased after five days whereas T4 did not increase significantly until day 25 and prolactin did not undergo any consistent change.

Male Japanese quail transferred from long days and 20 °C to short days and 10 °C exhibited decreases in LH and FSH after five days and decreases in testosterone, testis weight and cloacal gland area after 10 days (Figure 3.31; see Table 3.20). T4 decreased after five days whilst T3 increased significantly by day 15. Prolactin decreased significantly after 10 days then rose before declining again.

Male quail held on long days and 20 °C for eight weeks as controls maintained large testes and continued to produce cloacal foam whilst birds held on short days and 10 °C did not undergo testicular growth (Table 3.20). Plasma concentrations of all hormones except T3 remained high in birds held on long days and low in birds held on short days. T3 was low on long days and high on short days.

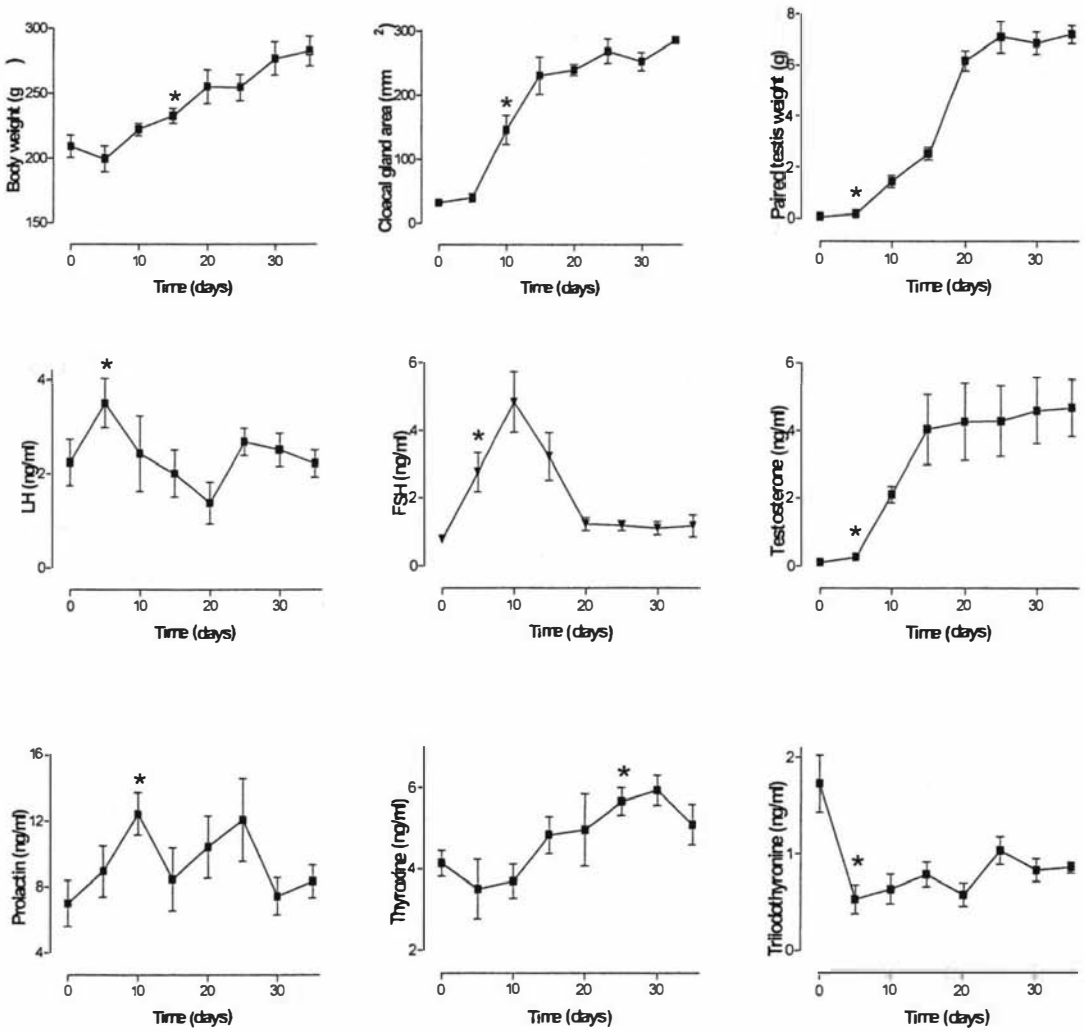

Figure 3.30. Summary of variables measured in male quail transferred from short days to long days for 35 days. The first significant change compared with day 0 is indicated with an asterisk for each variable. Results are presented as mean ± standard error. Note different scales on y axes.

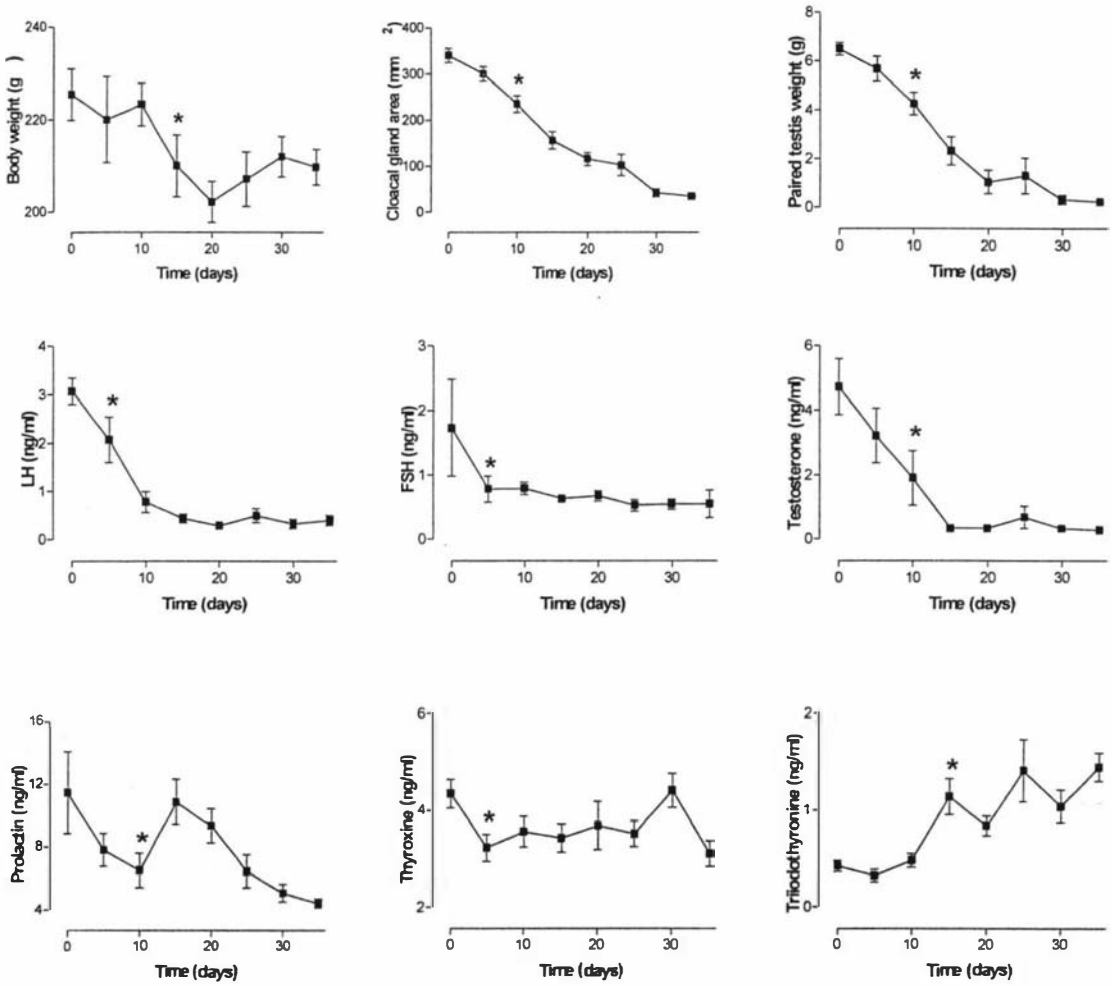

Figure 3.31. Summary of variables measured in male quail transferred from long days to short days for 35 days. The first significant change compared with day 0 is indicated with an asterisk for each variable. Results are presented as mean ± standard error. Note different scales on y axes.

Table 3.20. Summary of the timing of significant changes in variables measured in male quail transferred from short days to long days and in male quail transferred from long days to short days (first significant change compared with day 0 indicated in bold type for each variable).

Male quail transferred from short days to long days

Day	Birds producing foam (%)	Body weight (g)	Cloacal gland area (mm2)	Paired testis weight (g)	LH (ng/ml)	FSH (ng/ml)	T (ng/ml)	PRL (ng/ml)	T4 ng/ml	T3 ng/ml
0	0	209.2 ± 8.6	31.7 ± 4.1	0.06 ± 0.02	2.23 ± 0.50	0.77 ± 0.09	0.11 ± 0.06	7.00 ± 1.41	4.15 ± 0.32	1.73 ± 0.30
5	0	199.4 ± 10.1	39.4 ± 6.9	**0.18 ± 0.05**	**3.50 ● 0.52**	**2.76 ± 0.58**	**0.27 ± 0.06**	8.95 ± 1.55	3.87 ± 0.73	**0.53 ± 0.15**
10	29.0	222.0 ± 4.6	**145.7 ± 22.3**	1.44 ● 0.24	2.43 ± 0.81	5.35 ± 0.86	2.05 ± 0.28	**12.18 ± 1.45**	3.70 ± 0.42	0.64 ± 0.15
15	87.5	**232.4 ± 5.8**	230.9 ± 29.4	2.52 ± 0.25	2.00 ± 0.50	3.23 ± 0.71	4.05 ± 1.05	8.46 ± 1.92	4.84 ± 0.45	0.79 ± 0.13
20	100.0	255.2 ± 13.2	239.8 ± 8.7	6.17 ● 0.40	1.37 ± 0.44	1.27 ± 0.22	4.40 ± 1.12	8.80 ± 1.15	4.97 ± 0.89	0.58 ± 0.12
25	100.0	254.5 ± 10.2	270.0 ± 18.4	7.10 ● 0.62	2.68 ± 0.29	1.18 ± 0.15	4.30 ± 1.06	12.06 ± 2.51	**5.66 ± 0.34**	1.04 ± 0.14
30	100.0	275.8 ± 12.9	252.9 ± 14.3	6.86 ± 0.43	2.50 ± 0.35	1.10 ± 0.20	4.60 ± 0.99	7.44 ± 1.13	5.94 ± 0.37	0.83 ± 0.12
35	100.0	280.0 ± 10.1	286.0 ± 6.2	7.20 ± 0.35	2.22 ± 0.29	1.17 ± 0.33	4.99 ± 0.86	8.33 ± 1.00	5.09 ± 0.50	0.86 ± 0.06
Male quail maintained on long days										
Long day	100.0	271.3 ± 7.3	269.7 ± 31.3	6.10 ± 0.55	1.93 ± 0.16	0.98 ± 0.23	4.13 ± 0.71	7.49 ± 2.49	4.88 ± 0.07	0.75 ± 0.05

Male quail transferred from long days to short days

Day	Birds producing foam (%)	Body weight (g)	Cloacal gland area (mm2)	Paired testis weight (g)	LH (ng/ml)	FSH (ng/ml)	T (ng/ml)	PRL (ng/ml)	T4 ng/ml	T3 ng/ml
0	100.0	222.8 ± 5.5	342.8 ± 17.4	6.39 ± 0.26	3.22 ± 0.25	1.84 ± 0.84	4.70 ± 0.98	11.50 ± 2.98	4.24 ± 0.31	0.39 ± 0.06
5	100.0	220.0 ± 9.3	300.1 ± 15.8	5.68 ± 0.51	**2.07 ± 0.47**	**0.78 ± 0.21**	3.22 ± 0.84	7.80 ± 1.04	**3.22 ● 0.28**	0.32 ± 0.06
10	44.4	223.3 ± 4.6	**235.2 ± 18.3**	**4.26 ± 0.46**	0.77 ± 0.22	0.79 ● 0.10	**1.90 ± 0.85**	**6.50 ± 1.09**	3.56 ± 0.31	0.48 ± 0.07
15	11.1	210.0 ● 6.7	156.2 ± 18.7	2.31 ± 0.58	0.43 ● 0.10	0.63 ± 0.06	0.34 ± 0.08	10.86 ± 1.45	3.42 ± 0.29	**1.14 ± 0.19**
20	11.1	**202.1 ● 4.4**	115.6 ± 14.1	1.04 ± 0.49	0.28 ± 0.07	0.67 ± 0.08	0.33 ● 0.06	9.32 ± 1.11	3.67 ± 0.49	0.84 ± 0.10
25	11.1	207.1 ± 5.9	102.5 ± 23.3	1.29 ± 0.74	0.49 ± 0.14	0.52 ± 0.09	0.67 ± 0.35	6.44 ± 1.05	3.77 ± 0.35	1.41 ± 0.32
30	12.5	211.7 ± 4.3	41.4 ± 8.8	0.30 ● 0.20	0.32 ± 0.10	0.55 ± 0.09	0.32 ± 0.09	5.20 ● 0.61	4.39 ± 0.35	1.04 ± 0.17
35	0	209.7 ± 3.8	34.2 ± 2.8	0.21 ± 0.15	0.39 ± 0.10	0.55 ± 0.21	0.27 ± 0.04	4.42 ● 0.29	3.10 ± 0.26	1.30 ± 0.18
Male quail maintained on short days										
Short day	0	254.7 ± 13.3	33.1 ± 4.7	0.19 ● 0.05	0.58 ± 0.13	0.51 ± 0.11	0.40 ± 0.20	5.18 ± 0.83	3.84 ± 0.26	1.63 ± 0.34

3.3.2 Gonadal growth and regression in female Japanese quail

3.3.2.1 Body weight

Body weight increased in quail transferred from short to long days and reached similar weights to those of long day control quail by day 15 (Figure 3.32; see Table 3.21 for statistics). Body weight decreased in quail transferred from long to short days and did not differ from short day control quail after day 20. On day 35 body weights of quail transferred to short days were similar to short day control quail and were lighter than long day control quail.

Birds held on long days were heavier than birds held on short days at the start of the experiment (Figure 3.32; see Table 3.21 for statistics). Body weight increased in long day control quail and short day control quail (see Table 3.22 for statistics). Long day control quail were heavier than short day control quail on day 35 (see Table 3.21 for statistics).

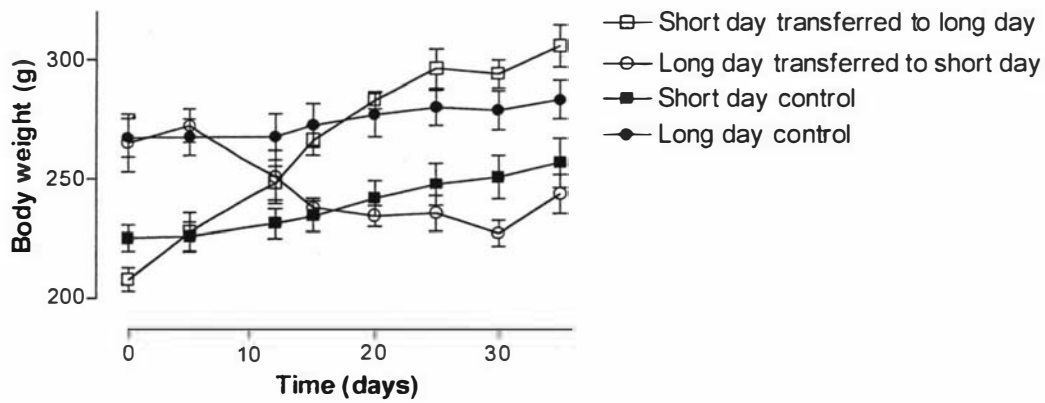

Figure 3.32. Changes in body weight of female birds transferred from short days to long days, birds transferred from long days to short days and birds held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results are shown as mean ± standard error.

Table 3.21. Two-way single measures ANOVA for body weight of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

	Body weight		
Effect	**F**	**df**	***p***
Time	5.02	8, 143	0.000*
Treatment	31.01	8, 143	0.000*
Interaction between time and treatment	18.27	8, 143	0.000*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	4.23	1, 143	0.041*
Day 0 vs day 12	17.09	1, 143	0.000*
Day 0 vs day 15	33.76	1, 143	0.000*
Day 0 vs day 20	54.59	1, 143	0.000*
Day 0 vs day 25	72.66	1, 143	0.000*
Day 0 vs day 30	66.80	1, 143	0.000*
Day 0 vs day 35	86.07	1, 143	0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	49.84	1, 143	0.000*
Day 5 vs long day control	25.64	1, 143	0.000*
Day 12 vs long day control	9.30	1, 143	0.003*
Day 15 vs long day control	2.03	1, 143	0.157
Day 20 vs long day control	0.01	1, 143	0.914
Day 25 vs long day control	1.05	1, 143	0.307
Day 30 vs long day control	0.76	1, 143	0.386
Day 35 vs long day control	3.08	1, 143	0.081
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	0.66	1, 143	0.419
Day 0 vs day 12	2.52	1, 143	0.115
Day 0 vs day 15	5.44	1, 143	0.021*
Day 0 vs day 20	7.28	1, 143	0.008*
Day 0 vs day 25	6.88	1, 143	0.010*
Day 0 vs day 30	11.13	1, 143	0.066
Day 0 vs day 35	3.42	1, 143	0.092
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	0.392	1, 143	0.532
Day 5 vs short day control	2.00	1, 143	0.160
Day 12 vs short day control	0.83	1, 143	0.363
Day 15 vs short day control	2.68	1, 143	0.104
Day 20 vs short day control	3.97	1, 143	0.048*
Day 25 vs short day control	3.68	1, 143	0.057
Day 30 vs short day control	6.93	1, 143	0.009*
Day 35 vs short day control	1.37	1, 143	0.245
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	30.58	1, 143	0.000*
Transfer to SD day 35 vs transfer to LD day 35	30.28	1, 143	0.000*
Short day control vs Long day control on day 35	5.09	1, 143	0.026*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.22. One-way repeated measures ANOVA for body weight of female quail held on long days at 20 °C (long day control) and held on short days at 10 °C (short day control).

Body weight – long days at 20 °C				Body weight – short days at 10 °C			
Effect	**F**	**df**	***p***	**Effect**	**F**	**df**	***p***
Time	5.850	7, 49	0.000*	Time	22.594	7, 49	0.000*
	Contrasts				**Contrasts**		
Day 0 compared to:				**Day 0 compared to:**			
Day 5	0.086	1, 7	0.788	Day 5	0.321	1, 7	0.589
Day 12	0.000	1, 7	0.986	Day 12	16.669	1, 7	0.005*
Day 15	2.592	1, 7	0.151	Day 15	10.333	1, 7	0.015*
Day 20	8.078	1, 7	0.025*	Day 20	33.995	1, 7	0.001*
Day 25	20.683	1, 7	0.003*	Day 25	31.351	1, 7	0.001*
Day 30	6.825	1, 7	0.035*	Day 30	36.622	1, 7	0.001*
Day 35	18.962	1, 7	0.003*	Day 35	33.655	1, 7	0.001*

NOTE: The first row of the table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.2 Width of cloacal opening

The width of the cloacal opening increased in quail transferred to long days after 12 days and was similar to that of quail on long days after 25 long days (Figure 3.33A; see Table 3.23 for statistics). In quail transferred to short days the width of the cloacal opening had decreased significantly in most quail after 25 short days (Figure 3.33B; see Table 3.24 for statistics). On day 35 individual cloacal opening diameters varied markedly in quail transferred to short days and the mean diameter was wider than that of short day control quail (see Table 3.24 for statistics).

The width of the cloacal opening increased in long day control quail and in short day control quail (Figure 3.34; see Table 3.25 for statistics). However cloacal opening diameter remained narrow in the short day controls and was significantly narrower that those of the long day controls on both days 0 and 35 (see Table 3.26 for statistics).

Strong relationships between the width of the cloacal opening and ovary and oviduct weights were observed during gonadal growth and regression (Figures 3.35 and 3.36; see Table 3.27 for statistics). These relationships indicate that quail with cloacal opening diameters < 9 mm have small ovaries and oviducts and quail with cloacal opening diameters > 9 mm possessed ovaries greater than 1 g in weight and oviducts greater than 0.5 g in weight.

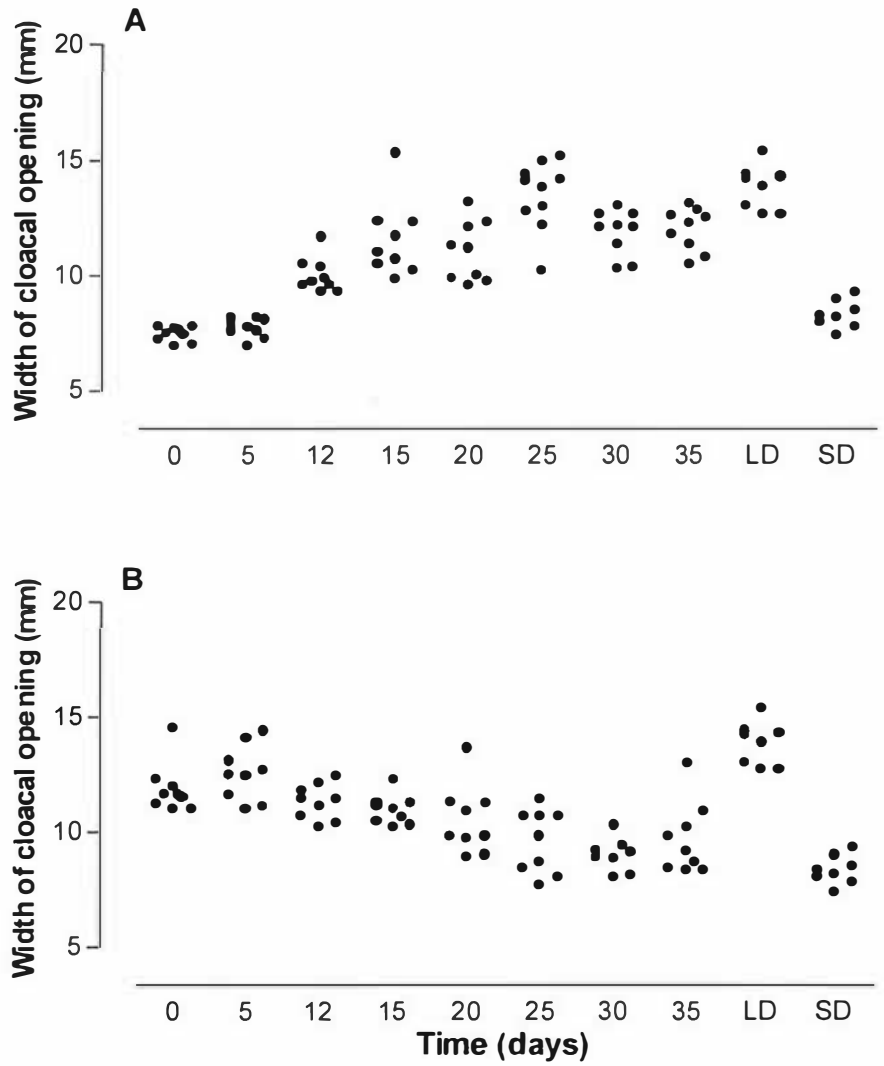

Figure 3.33. Width of cloacal opening of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

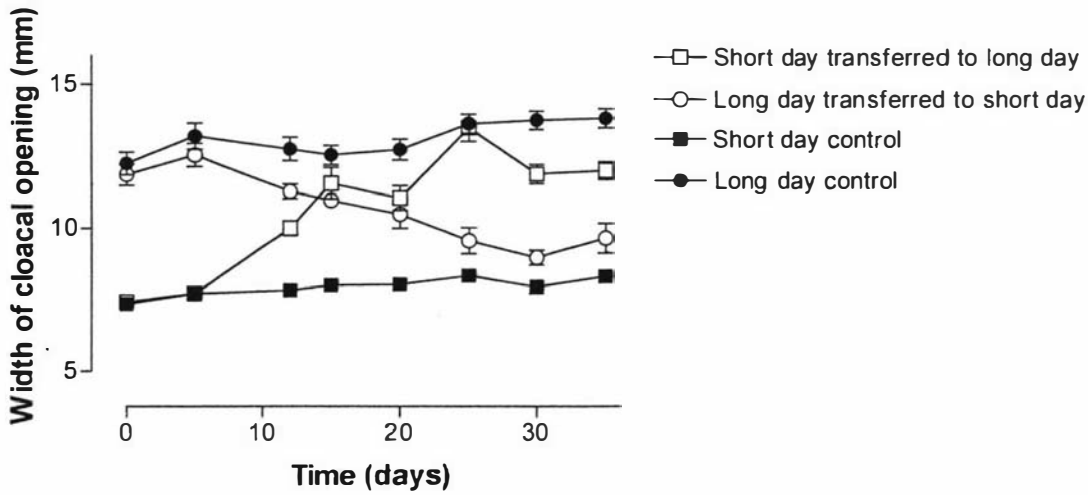

Figure 3.34. Changes in the width of the cloacal opening of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

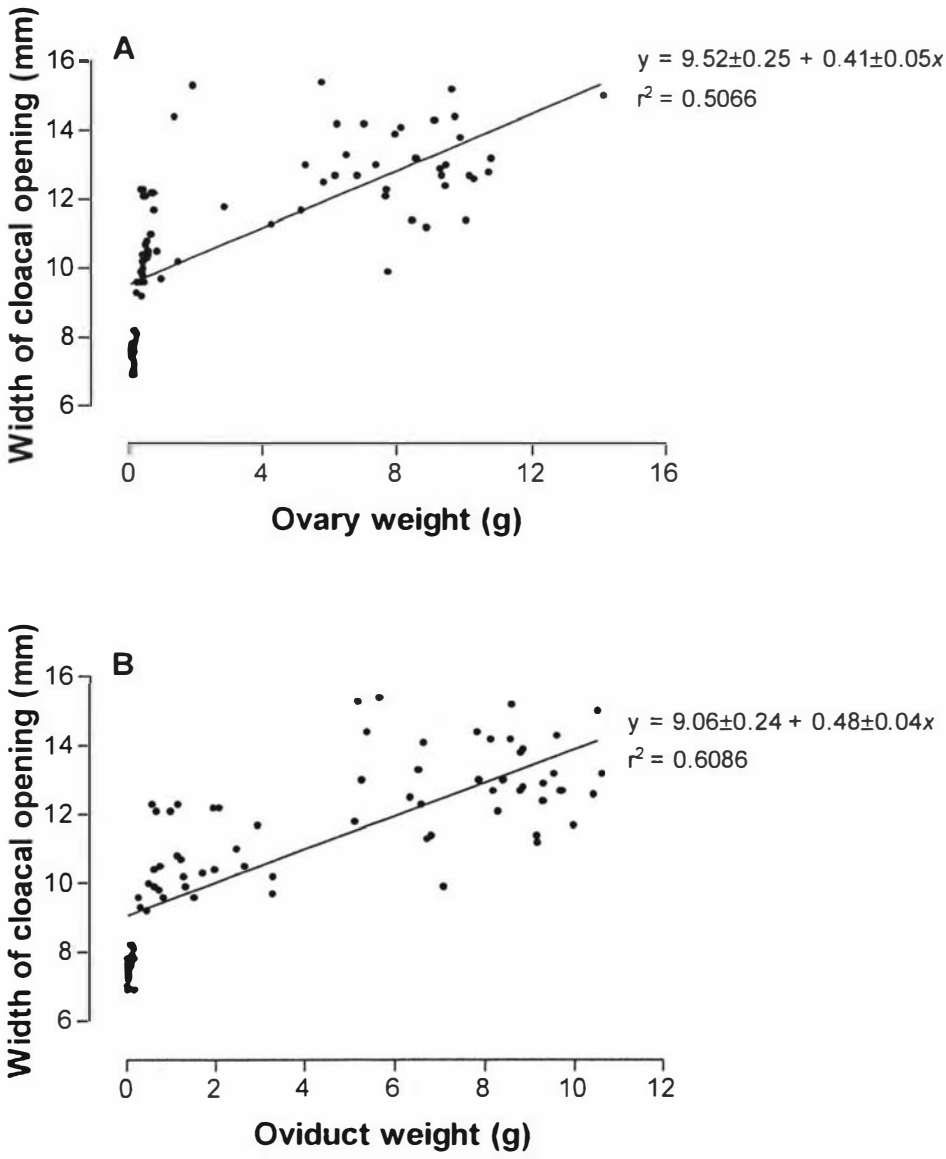

Figure 3.35. Relationships between the width of the cloacal opening and ovary weight (A), and the width of the cloacal opening and oviduct weight (B) of female quail transferred from short days to long days for 35 days. Note different scale on x axes.

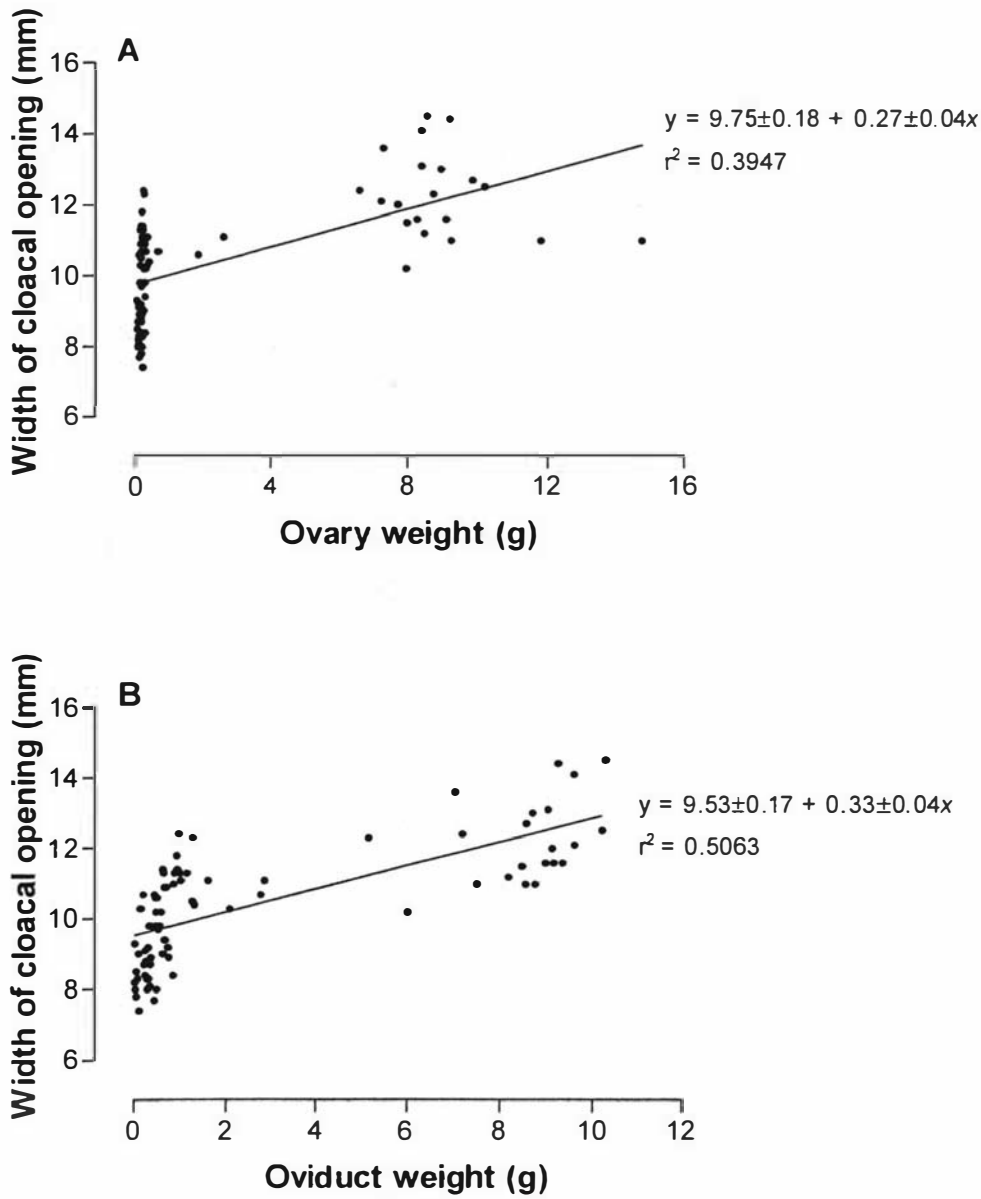

Figure 3.36. Relationships between the width of the cloacal opening and ovary weight (A), and the width of the cloacal opening and oviduct weight (B) of female quail transferred from long days to short days for 35 days. Note different scales on x axes.

Table 3.23. One-way single measures ANOVA for the width of the cloacal opening of female quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

Effect	Width of cloacal opening		
	F	df	*p*
Time	54.17	9, 80	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5			0.996
Day 0 vs day 12			0.000*
Day 0 vs day 15			0.000*
Day 0 vs day 20			0.000*
Day 0 vs day 25			0.000*
Day 0 vs day 30			0.000*
Day 0 vs day 35			0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control			0.000*
Day 5 vs long day control			0.000*
Day 12 vs long day control			0.000*
Day 15 vs long day control			0.003*
Day 20 vs long day control			0.000*
Day 25 vs long day control			1.000
Day 30 vs long day control			0.027*
Day 35 vs long day control			0.083

Table 3.24. One-way single measures ANOVA for the width of the cloacal opening of female quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

Effect	Width of cloacal opening		
	F	df	*p*
Time	19.70	9, 86	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5			0.945
Day 0 vs day 12			0.982
Day 0 vs day 15			0.775
Day 0 vs day 20			0.213
Day 0 vs day 25			0.001*
Day 0 vs day 30			0.000*
Day 0 vs day 35			0.002*
Quail transferred to short days (days 0 –35) compared to short day controls on day 35			
Day 0 vs short day control			0.015*
Day 5 vs short day control			0.347
Day 12 vs short day control			0.000*
Day 15 vs short day control			0.000*
Day 20 vs short day control			0.000*
Day 25 vs short day control			0.000*
Day 30 vs short day control			0.000*
Day 35 vs short day control			0.000*

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.25. One-way repeated measures ANOVA for the width of the cloacal opening of female quail held on long days at 20 °C (long day control) and held on short days at 10 °C (short day control).

Width of cloacal opening – long days at 20 °C				Width of cloacal opening – short days at 10 °C			
Effect	**F**	**df**	***p***	**Effect**	**F**	**df**	***p***
Time	6.14	7, 49	0.000*	Time	3.07	7, 49	0.003*
Contrasts				**Contrasts**			
Day 0 compared to:				**Day 0 compared to:**			
Day 5	7.864	1, 7	0.026*	Day 5	1.805	1, 7	0.228
Day 12	1.635	1, 7	0.242	Day 12	15.945	1, 7	0.007*
Day 15	0.684	1, 7	0.435	Day 15	20.956	1, 7	0.004*
Day 20	2.389	1, 7	0.166	Day 20	43.483	1, 7	0.001*
Day 25	16.105	1, 7	0.005*	Day 25	25.249	1, 7	0.002*
Day 30	20.648	1, 7	0.003*	Day 30	8.820	1, 7	0.025*
Day 35	19.284	1, 7	0.003*	Day 35	9.945	1, 7	0.020*

NOTE: The first row of the table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.26. Two-way single measures ANOVA for the width of the cloacal opening on day 35 of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

	Width of cloacal opening		
Effect	**F**	**df**	***p***
Time	13.02	2, 47	0.000*
Treatment	13.95	1, 47	0.001*
Interaction between time and treatment	131.39	2, 47	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	116.62	1, 47	0.000*
Transfer to SD day 35 vs transfer to LD day 35	31.96	1, 47	0.000*
Short day control vs Long day control on day 35	124.55	1, 47	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.27. Regression analyses for female quail transferred from short days to long days (A) and for female quail transferred from long days to short days (B).

A	r^2	F	df	*p*
Width of cloacal opening vs ovary weight	0.5066	82.13	1, 80	<0.0001*
Width of cloacal opening vs oviduct weight	0.6086	122.90	1, 80	<0.0001*
LH vs ovary weight	0.0411	3.39	1, 80	0.0694
FSH vs ovary weight	0.0917	7.98	1, 80	0.0060*
Estradiol vs ovary weight	0.0278	2.26	1, 80	0.1371
P_4 vs ovary weight	0.3812	48.67	1, 80	<0.0001*
Oviduct weight vs ovary weight	0.8985	699.60	1, 80	<0.0001*
LH vs oviduct weight	0.0473	3.87	1, 80	0.0527
FSH vs oviduct weight	0.0654	5.46	1, 80	0.0221*
Estradiol vs oviduct weight	0.0856	7.30	1, 80	0.0084*
P_4 vs oviduct weight	0.4598	66.40	1, 80	<0.0001*
FSH vs LH	0.1172	10.49	1, 80	0.0018*
LH vs estradiol	0.0107	0.86	1, 80	0.3579
LH vs P_4	0.0003	0.03	1, 80	0.8721
FSH vs estradiol	0.0851	7.41	1, 80	0.0080*
FSH vs P_4	0.0183	1.47	1, 80	0.2290
Estradiol vs P_4	0.2005	19.81	1, 80	<0.0001*
LH vs T4	0.1443	13.16	1, 80	0.0005*
LH vs T3	0.0648	5.40	1, 80	0.0227
T4 vs T3	0.0073	0.58	1, 80	0.4492

B	r^2	F	df	*p*
Width of cloacal opening vs ovary weight	0.3947	50.21	1, 77	< 0.0001*
Width of cloacal opening vs oviduct weight	0.5063	78.96	1, 77	< 0.0001*
LH vs ovary weight	0.5517	94.75	1, 77	< 0.0001*
FSH vs ovary weight	0.5120	80.80	1, 77	< 0.0001*
Estradiol vs ovary weight	0.6111	121.00	1, 77	< 0.0001*
P_4 vs ovary weight	0.7397	218.80	1, 77	< 0.0001*
Oviduct weight vs ovary weight	0.9091	770.50	1, 77	< 0.0001*
LH vs oviduct weight	0.5511	94.54	1, 77	< 0.0001*
FSH vs oviduct weight	0.5123	80.88	1, 77	< 0.0001*
Estradiol vs oviduct weight	0.5773	105.20	1, 77	< 0.0001*
P_4 vs oviduct weight	0.7253	203.3	1, 77	< 0.0001*
FSH vs LH	0.4785	70.64	1, 77	< 0.0001*
LH vs estradiol	0.4632	66.44	1, 77	< 0.0001*
LH vs P_4	0.4618	66.06	1, 77	< 0.0001*
FSH vs estradiol	0.4684	67.85	1, 77	< 0.0001*
FSH vs P_4	0.5417	91.00	1, 77	< 0.0001*
Estradiol vs P_4	0.4611	65.88	1, 77	< 0.0001*
LH vs T4	0.0012	0.000	1, 77	0.9931
LH vs T3	0.1250	11.00	1, 77	0.0014*
T4 vs T3	0.0318	2.53	1, 77	0.1157

3.3.2.3 Ovary weight

There was a small but significant increase in ovary weight five days after quail were transferred to long days (Figure 3.37A; see Table 3.28 for statistics). Some quail held on long days for more than 12 days exhibited full ovarian growth whilst other birds still had small ovaries on day 35 after transfer. The majority of quail transferred to long days for 35 days had ovary weights similar to those of long day control quail. Yolk-filled follicles were first observed in quail transferred to long days on day 12 when mean ovary weight was nearly 1 g (Table 3.31A). At this time birds were not laying eggs. Egg laying began 15 days after quail were transferred to long days and by day 35 50 % of birds were laying (Figure 3.41).

Linear regression analysis of ovary weights during gonadal growth between days 0 and 25 in quail transferred to long days revealed a logarithmic growth rate constant of k = 0.2481 ± 0.0484 (95 % confidence limits = 0.1509 to 0.3453).

Mean ovary weight had decreased markedly in most quail transferred to short days after 12 days and did not differ from short day controls from 15 days onwards (Figure 3.37B; see Table 3.29 for statistics). Gonadal regression did not occur in a few birds even after 35 short days. Yolk-filled follicles were not observed in the majority of quail transferred to short days on day 15 when mean ovary weight had decreased to 0.45 ± 0.18 g (Table 3.31B). Single birds with large ovaries on day 20 and 35 had large yolk-filled follicles and the bird on day 35 was still laying eggs.

Mean ovary weights of long day control quail were greater than short day control quail (7.69 ± 0.55 and 0.16 ± 0.03 g respectively; see Table 3.30 for statistics). The ovaries of short day control quail were small and contained small white follicles only, whereas ovaries of long day control birds contained yolk-filled follicles of a range of sizes (Table 3.31). All long day control quail laid eggs whereas no short day control quail laid eggs (Figure 3.41).

Ovary and oviduct weights were strongly correlated in birds transferred from short to long days, but relationships between ovary weight and LH, FSH, estradiol and progesterone concentrations were weak or non-existent in these birds (Figure 3.39; see Table 3.27A for statistics). Ovary weight was very strongly related with oviduct weight

and related with LH, FSH, estradiol and progesterone in birds transferred from long days to short days (Figure 3.40; see Table 3.27B for statistics).

Molt of the primary feathers in quail was first observed 11 days after transfer from long days to short days. Molt was completed by day 30.

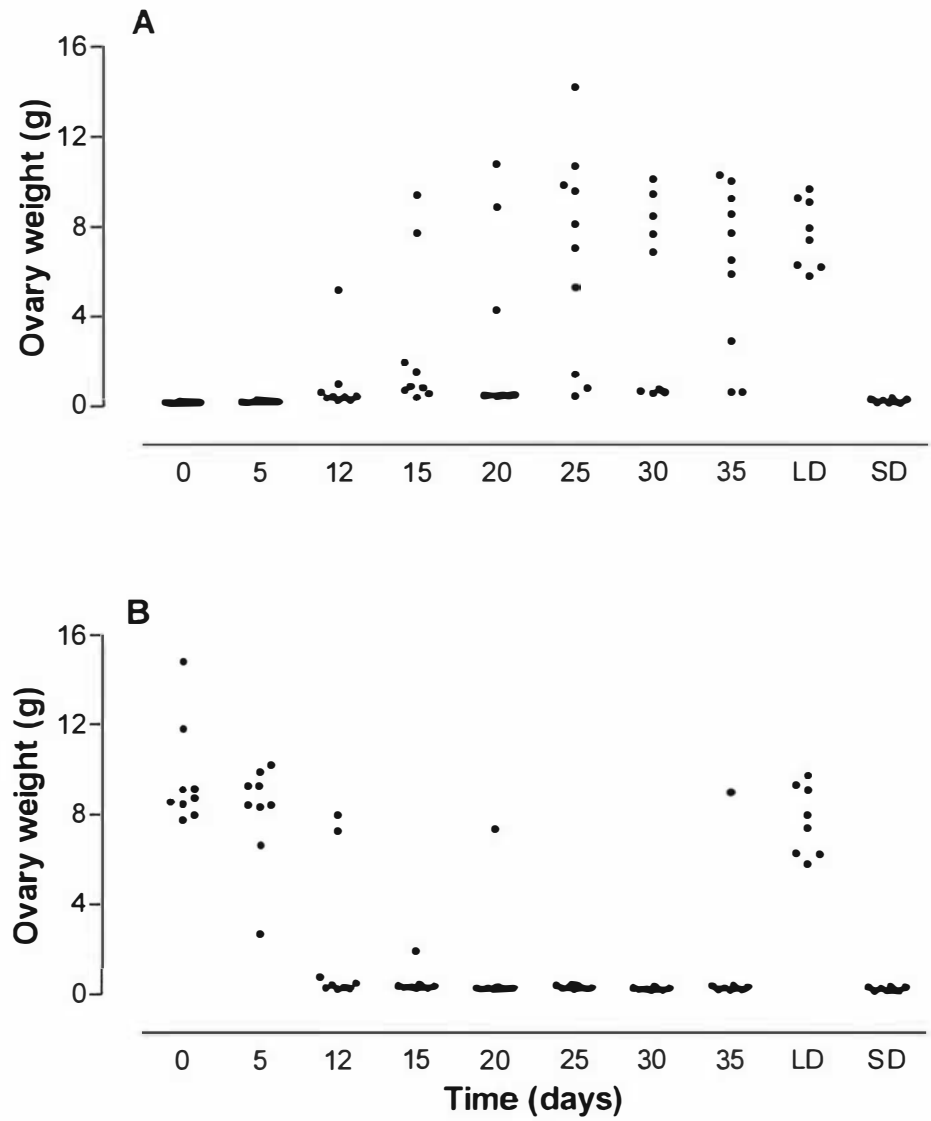

Figure 3.37. Ovary weights of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

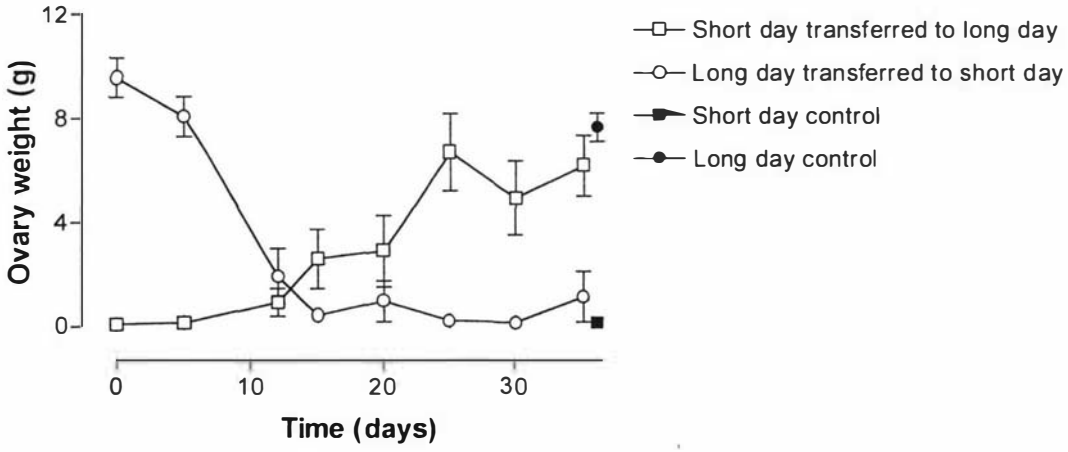

Figure 3.38. Changes in ovary weight of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

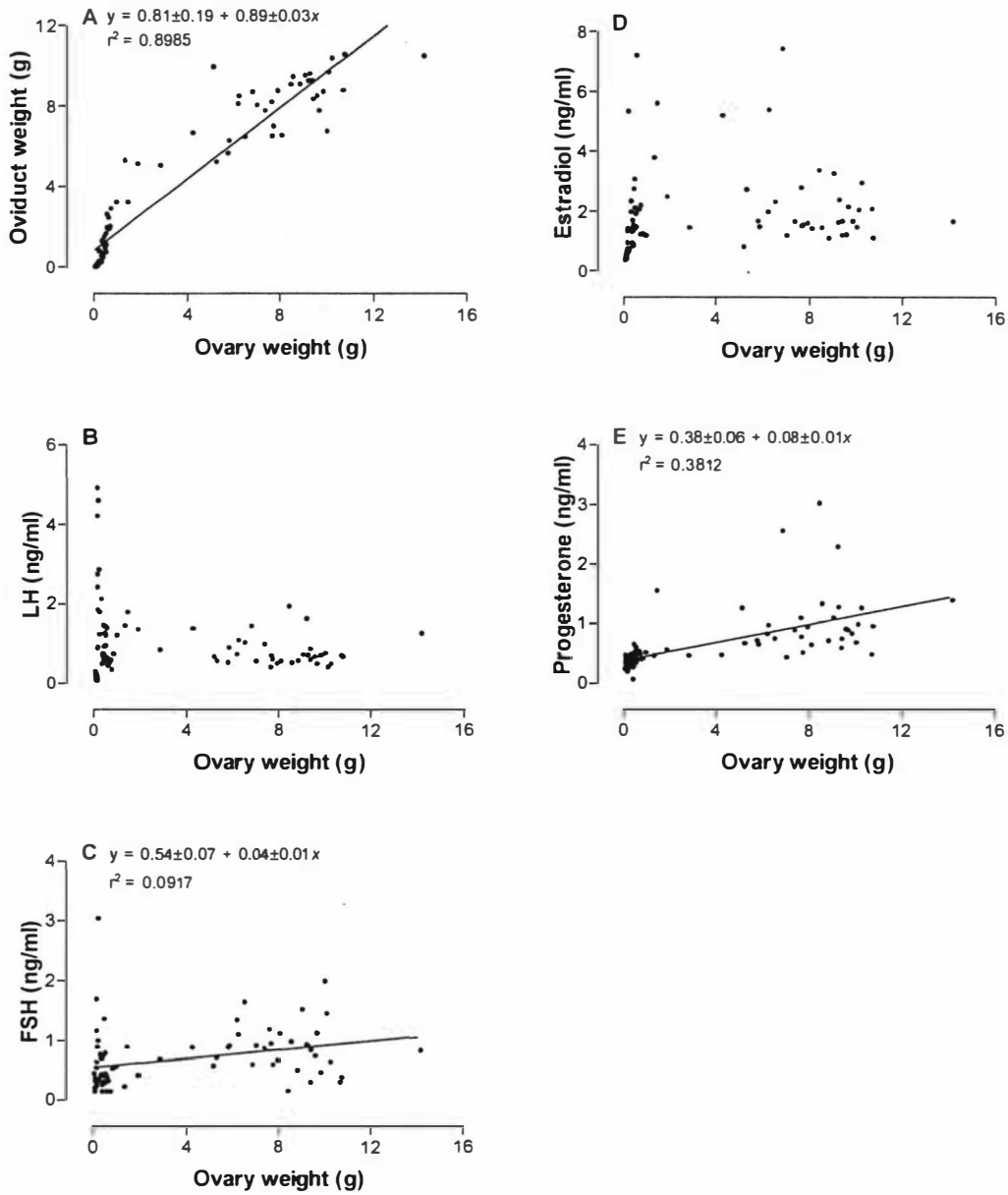

Figure 3.39. Relationships between ovary weight and oviduct weight (A), LH (B), FSH (C), estradiol (D) and P_4 (E) of quail transferred from short days to long days for 35 days. Note different scales on y axes.

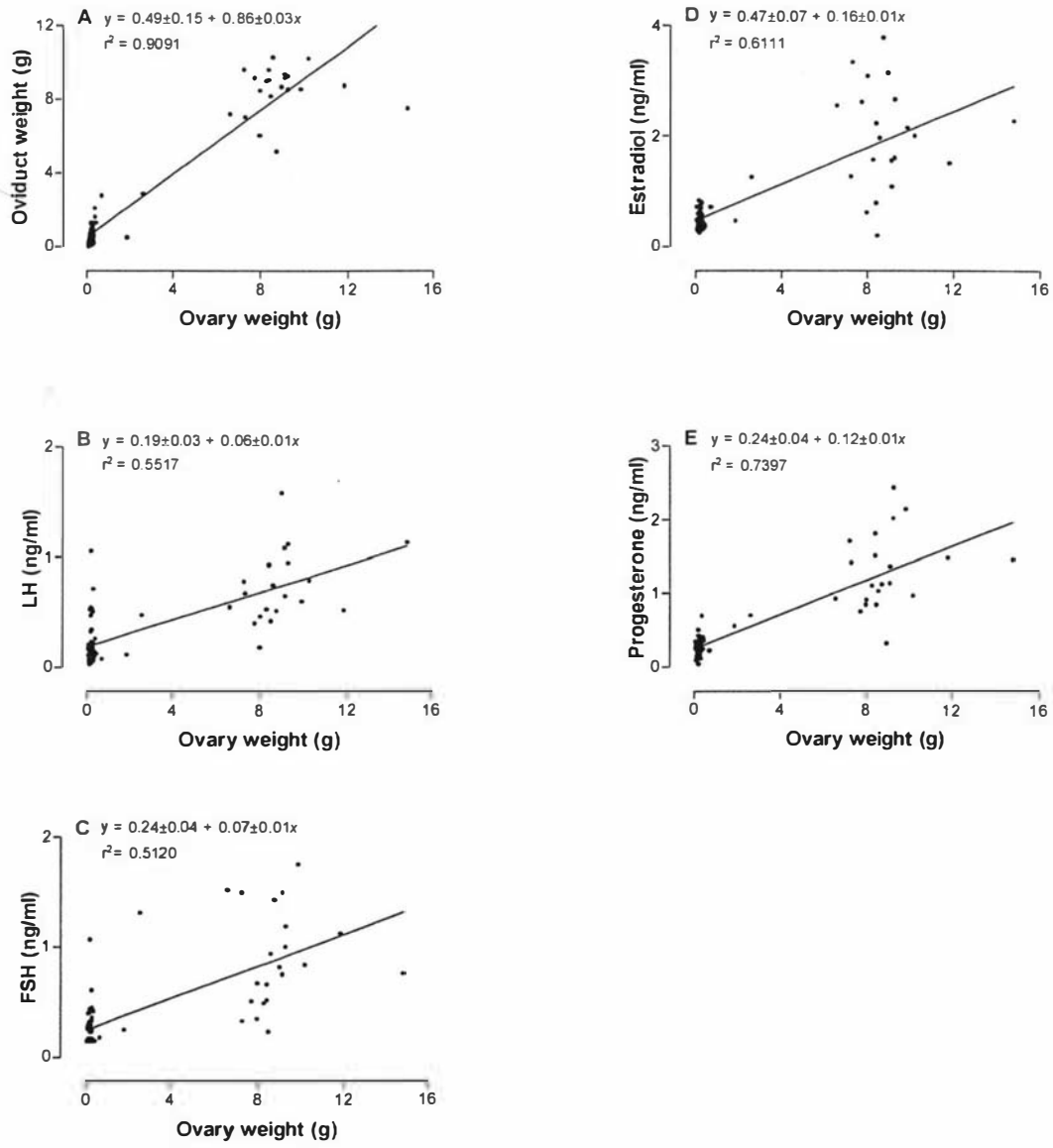

Figure 3.40. Relationships between ovary weight and oviduct weight (A), LH (B), FSH (C), estradiol (D) and P_4 (E) of quail transferred from long days to short days. Note different scales on y axes.

Table 3.28. Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for ovary weight of female quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

Effect	Ovary weight		
	K-W statistic	**df**	***p***
Time	55.80	8	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
	M-W statistic		
Day 0 vs day 5	2.00		0.000*
Day 0 vs day 12	0.00		0.000*
Day 0 vs day 15	0.00		0.000*
Day 0 vs day 20	0.00		0.000*
Day 0 vs day 25	0.00		0.000*
Day 0 vs day 30	0.00		0.000*
Day 0 vs day 35	0.00		0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	0.00		0.000*
Day 5 vs long day control	0.00		0.000*
Day 12 vs long day control	0.00		0.008*
Day 15 vs long day control	11.00		0.011*
Day 20 vs long day control	13.00		0.200
Day 25 vs long day control	39.00		0.167
Day 30 vs long day control	27.00		0.721
Day 35 vs long day control	35.00		0.321

Table 3.29. Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for ovary weight of female quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

Effect	Ovary weight		
	K-W statistic	**df**	***p***
Time	50.77	8	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
	M-W statistic		
Day 0 vs day 5	34.00		0.605
Day 0 vs day 12	1.00		0.000*
Day 0 vs day 15	0.00		0.000*
Day 0 vs day 20	0.00		0.000*
Day 0 vs day 25	0.00		0.000*
Day 0 vs day 30	0.00		0.000*
Day 0 vs day 35	5.00		0.001*
Quail transferred to short days (days 0 –35) compared to short day controls on day 35			
Day 0 vs short day control	0.00		0.000*
Day 5 vs short day control	0.00		0.000*
Day 12 vs short day control	9.00		0.008*
Day 15 vs short day control	10.00		0.011*
Day 20 vs short day control	22.00		0.200
Day 25 vs short day control	21.00		0.167
Day 30 vs short day control	28.00		0.721
Day 35 vs short day control	25.50		0.321

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.30. Two-way single measures ANOVA for ovary weight on day 35 of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

Effect	Ovary weight		
	F	df	*p*
Time	1.28	2, 47	0.288
Treatment	2.60	1, 47	0.112
Interaction between time and treatment	69.76	2, 47	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	76.14	1, 47	0.000*
Transfer to SD day 35 vs transfer to LD day 35	22.63	1, 47	0.000*
Short day control vs Long day control on day 35	42.55	1, 47	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

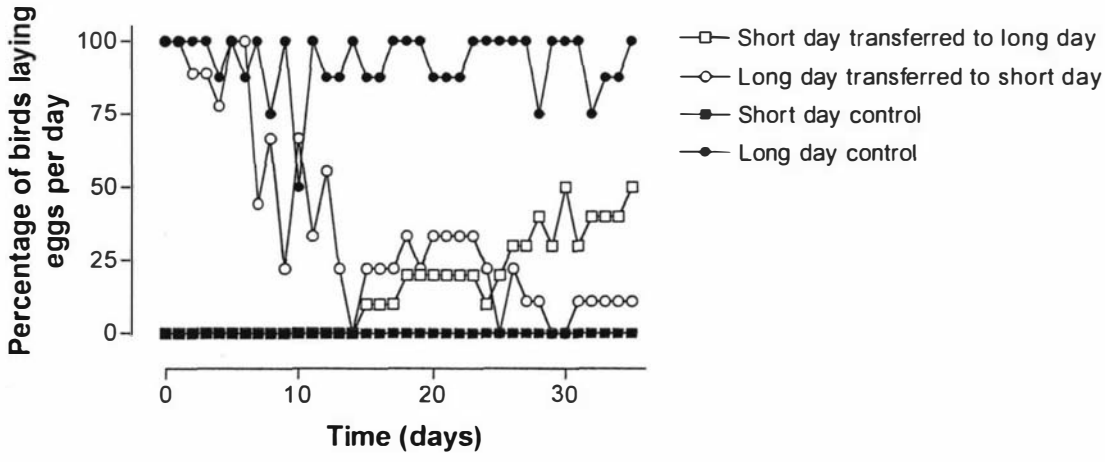

Figure 3.41. Daily egg production of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Table 3.31. Number and size of large yellow follicles present on the ovaries and corresponding ovary weights of quail transferred from short days to long days and quail maintained on long days at 20 °C (long day control) (A) and quail transferred from long days to short days and quail maintained on short days at 10 °C (short day control) (B). Numbers in parentheses denote the number of birds with follicles in the diameter range. Follicles were divided into six classes based on follicle diameter data recorded for the New Zealand strain of Japanese quail (Bennett, 2002).

A

Female quail transferred from short days to long days Number of follicles within specific diameter ranges (mm)							
Day	**1 - 5**	**5 - 10**	**10 - 12**	**12 - 14**	**14 - 16**	**> 16**	**Ovary weight (g)**
0	0	0	0	0	0	0	0.10 ± 0.01
5	0	0	0	0	0	0	0.18 ± 0.01
12	2 (2)	2 (2)	1 (1)	2 (1)	0	0	0.96 ± 0.53
15	3 (3)	8 (5)	3 (3)	2 (2)	1 (1)	3 (2)	2.62 ± 1.14
20	0	4 (2)	1 (1)	3 (3)	0	6 (3)	2.94 ± 1.37
25	5 (5)	8 (6)	2 (2)	4 (4)	4 (4)	15 (7)	6.72 ± 1.48
30	4 (2)	8 (5)	1 (1)	2 (2)	2 (2)	11 (5)	4.97 ± 1.43
35	0	8 (6)	4 (3)	0	6 (4)	10 (5)	6.21 ± 1.17
Female quail maintained on long days							
Long day control	1 (1)	11 (8)	4 (4)	3 (3)	2 (2)	15 (8)	7.69 ± 0.55

B

Female quail transferred from long days to short days Number of follicles within specific diameter ranges (mm)							
Day	**1 - 5**	**5 - 10**	**10 - 12**	**12 - 14**	**14 - 16**	**> 16**	**Ovary weight (g)**
0	0	13 (8)	3 (3)	6 (5)	2 (2)	17 (8)	9.59 ± 0.76
5	4 (4)	10 (8)	3 (3)	5 (5)	3 (3)	16 (8)	8.09 ± 0.77
12	2 (2)	3 (2)	2 (2)	0	2 (2)	2 (2)	1.97 ± 1.07
15	0	0	0	0	0	0	0.45 ± 0.18
20	0	1 (1)	0	1 (1)	0	2 (1)	1.01 ± 0.79
25	0	0	0	0	0	0	0.23 ± 0.03
30	0	0	0	0	0	0	0.18 ± 0.02
35	0	1 (1)	1 (1)	0	1 (1)	2 (1)	1.17 ± 0.98
Female quail maintained on short days							
Short day control	0	0	0	0	0	0	0.17 ± 0.03

3.3.2.4 Oviduct weight

Oviduct growth or regression in quail transferred to long or short days followed the same patterns as ovarian growth and regression. Significant growth occurred after five days and significant regression occurred after 12 days (Figures 3.42 and 3.43; see Tables 3.32 and 3.33 for statistics). Mean oviduct weights of birds transferred to long days were not significantly different from long day controls from day 25, whereas mean ovary weight of birds transferred to short days reached that of short day controls on day 12.

Oviduct weight was not clearly related to LH, FSH, estradiol or P_4 concentrations during photostimulation (Figure 3.44; see Table 3.27 for statistics). Oviduct weight was strongly related to LH, FSH, estradiol and P_4 concentrations during gonadal regression (Figure 3.45; see Table 3.27 for statistics).

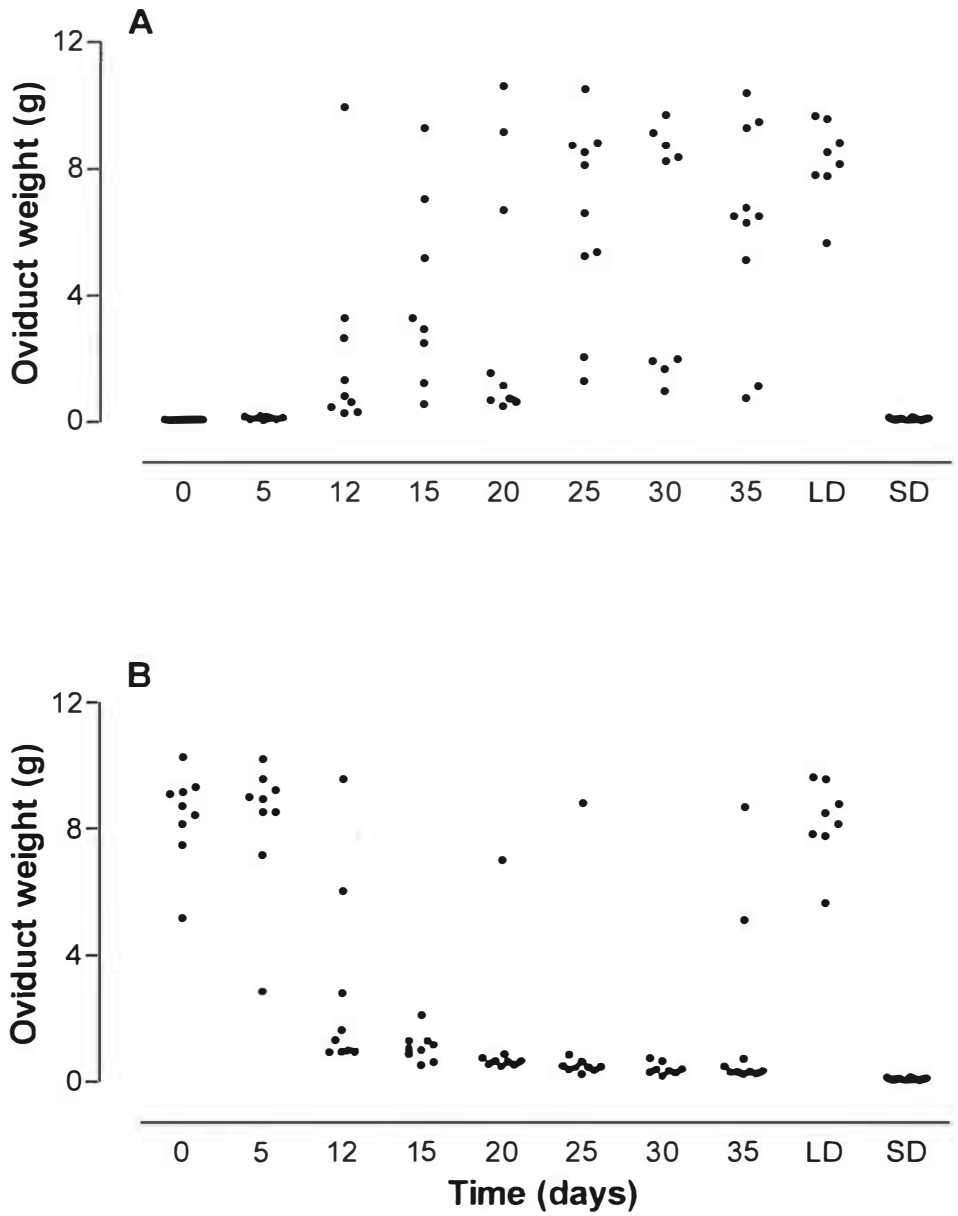

Figure 3.42. Oviduct weights of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

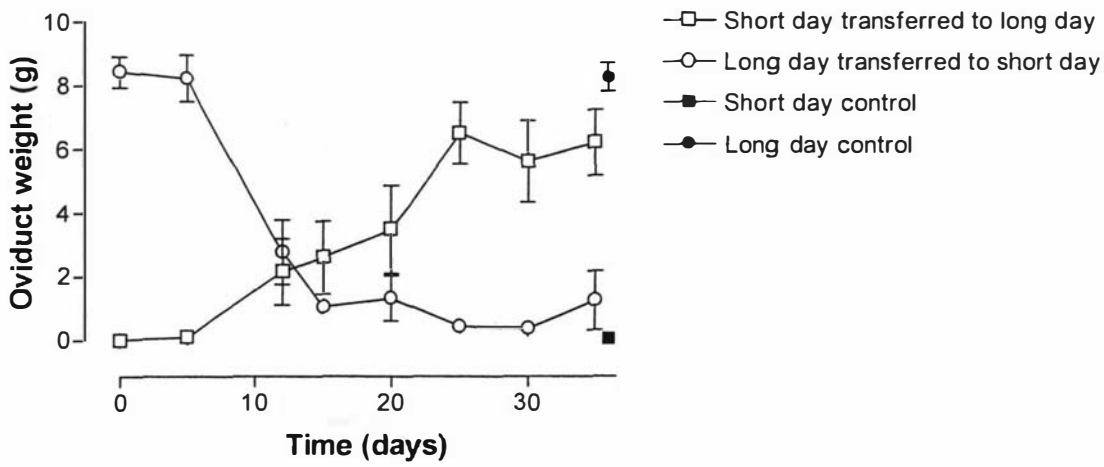

Figure 3.43. Changes in oviduct weight of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 ℃ (short day control) and long days at 20 ℃ (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

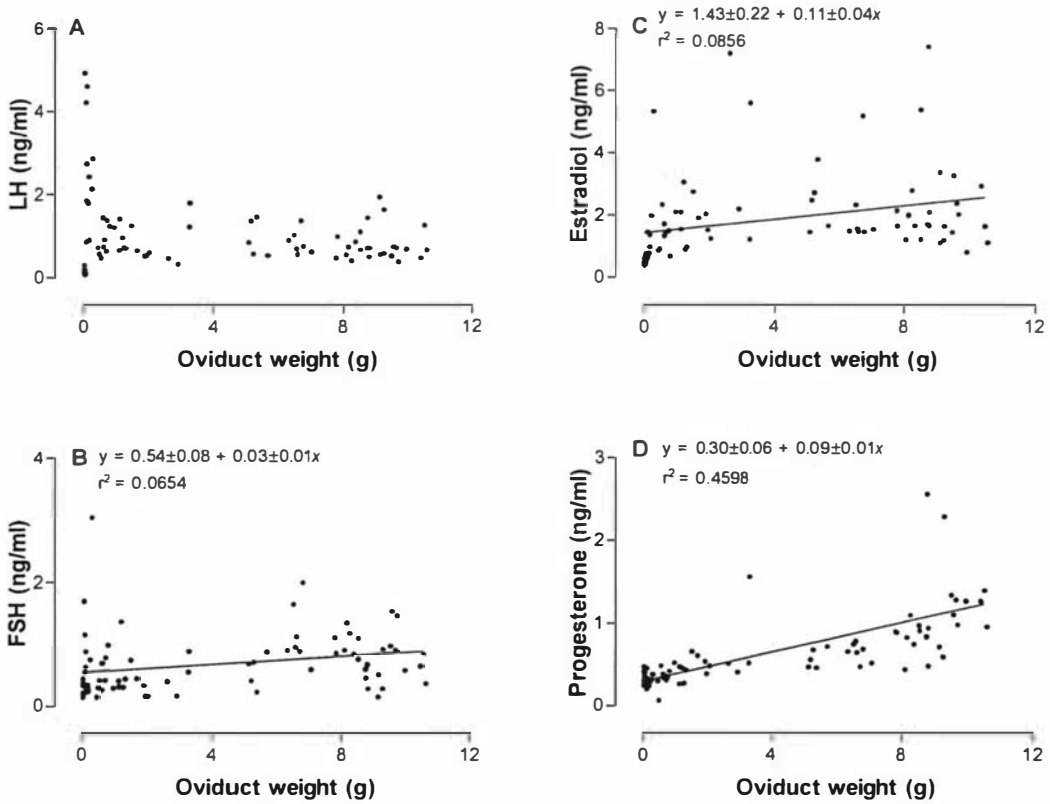

Figure 3.44. Relationships between oviduct weight and LH, FSH, estradiol and progesterone (A-D) of quail transferred from short days to long days for 35 days. Note different scales on y axes.

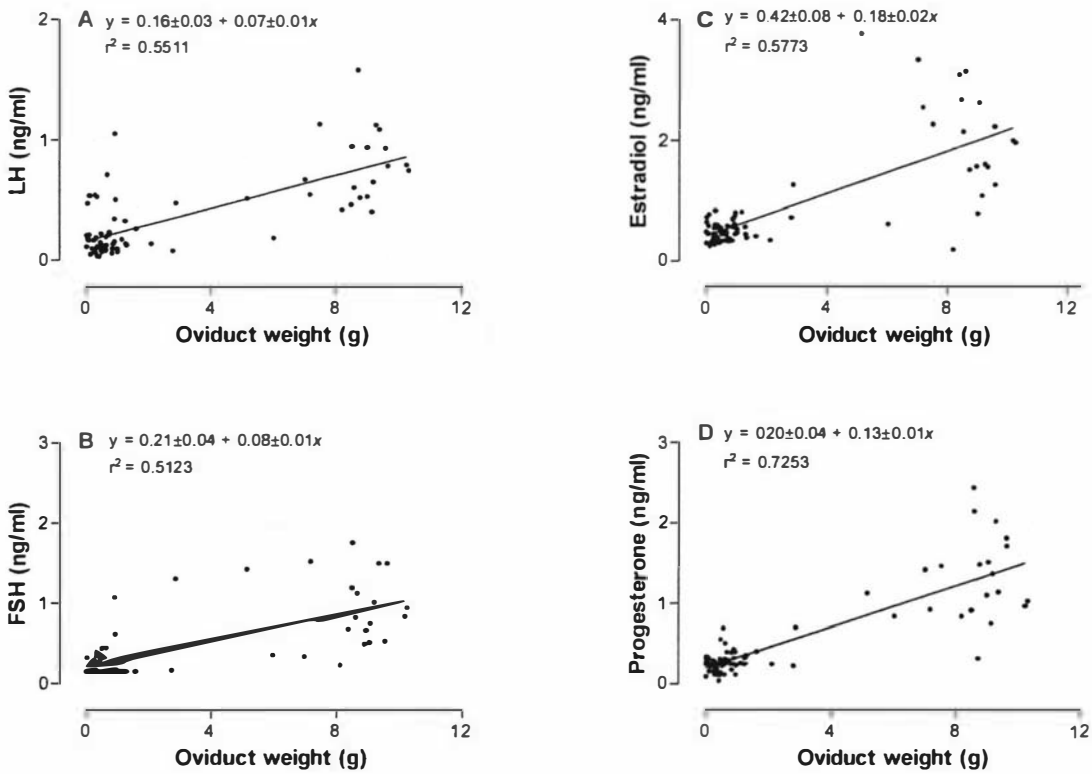

Figure 3.45. Relationships between oviduct weight and LH, FSH, estradiol and progesterone (A-D) of quail transferred from long days to short days for 35 days. Note different scales on y axes.

Table 3.32. Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for oviduct weight of female quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

	Oviduct weight		
Effect	**K-W statistic**	**df**	***p***
Time	51.55	8	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
	M-W statistic		
Day 0 vs day 5	0.00		0.000*
Day 0 vs day 12	0.00		0.000*
Day 0 vs day 15	0.00		0.000*
Day 0 vs day 20	0.00		0.000*
Day 0 vs day 25	0.00		0.000*
Day 0 vs day 30	0.00		0.000*
Day 0 vs day 35	0.00		0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	0.00		0.000*
Day 5 vs long day control	0.00		0.000*
Day 12 vs long day control	0.00		0.000*
Day 15 vs long day control	7.00		0.007*
Day 20 vs long day control	15.00		0.046*
Day 25 vs long day control	28.00		0.315
Day 30 vs long day control	27.00		0.423
Day 35 vs long day control	24.00		0.173

Table 3.33. One way single measures ANOVA for oviduct weight of female quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

	Oviduct weight		
Effect	**F**	**df**	***p***
Time	48.41	8, 78	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5			1.000
Day 0 vs day 12			0.004*
Day 0 vs day 15			0.000*
Day 0 vs day 20			0.000*
Day 0 vs day 25			0.000*
Day 0 vs day 30			0.000*
Day 0 vs day 35			0.000*
Quail transferred to short days (days 0 –35) compared to short day controls on day 35			
Day 0 vs short day control			0.000*
Day 5 vs short day control			0.000*
Day 12 vs short day control			0.066
Day 15 vs short day control			0.964
Day 20 vs short day control			0.873
Day 25 vs short day control			1.000
Day 30 vs short day control			1.000
Day 35 vs short day control			0.899

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.34. Two way single measures ANOVA for oviduct weight on day 35 of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

	Oviduct weight		
Effect	**F**	**df**	***p***
Time	0.31	2, 47	0.734
Treatment	8.29	1, 47	0.006*
Interaction between time and treatment	85.87	2, 47	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	80.75	1, 47	0.000*
Transfer to SD day 35 vs transfer to LD day 35	29.39	1, 47	0.000*
Short day control vs Long day control on day 35	68.05	1, 47	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.5 Luteinising hormone (LH)

LH concentrations in quail transferred to long days increased significantly after five days (Figure 3.46A; see Table 3.35 for statistics) to peak at 2.7 ± 0.51 ng/ml then decreased to reach similar concentrations seen in quail held on long days from day 12 onwards. Quail transferred to short days exhibited a wide range of LH concentrations during the first 12 days (Figure 3.46B). Mean LH had decreased in quail transferred to short days after 15 short days and remained low to day 30 (see Table 3.36 for statistics). On day 35 mean concentrations increased and were not different to concentrations in quail on day 0 or short day controls. One bird on day 35 which had large ovaries had high LH concentrations.

Long day control quail had higher mean LH concentrations than short day control birds on day 35 (Figure 3.47; see Table 3.37 for statistics).

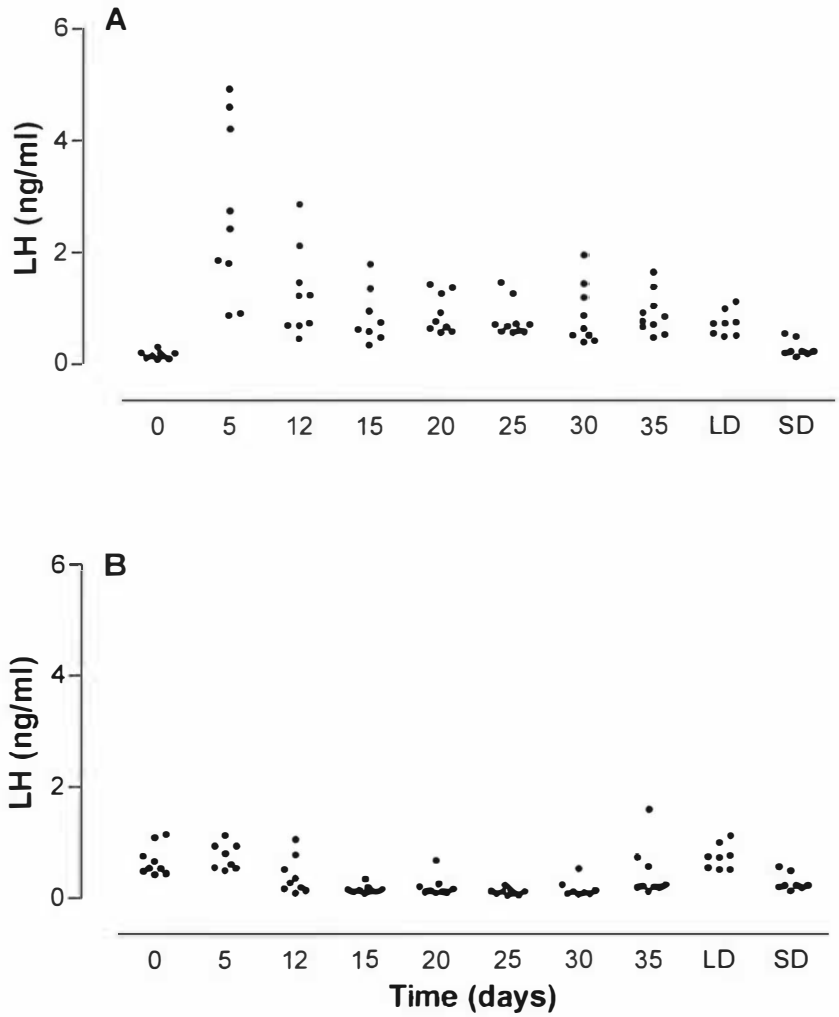

Figure 3.46. LH concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

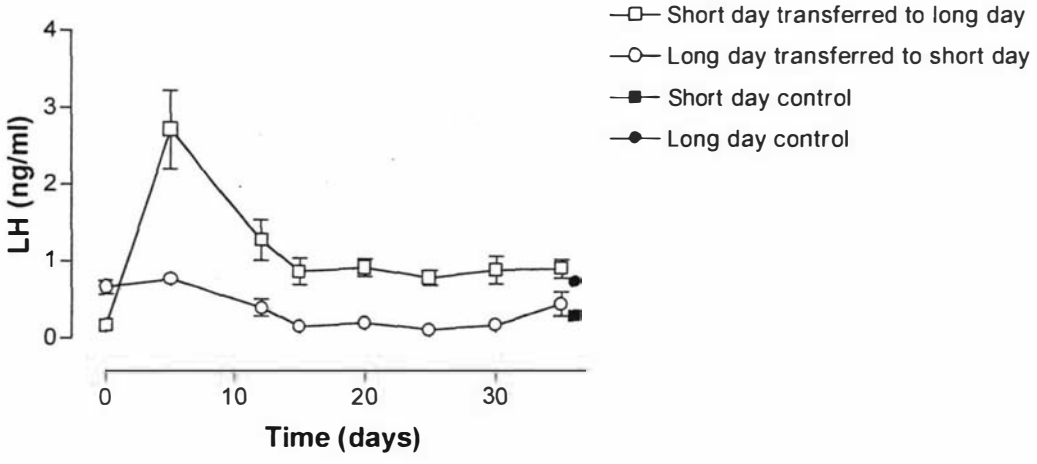

Figure 3.47. Changes in LH concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ● standard error.

Table 3.35. One way single measures ANOVA for LH concentrations of female quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

	LH		
Effect	**F**	**df**	***p***
Time	19.54	8, 80	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5			0.000*
Day 0 vs day 12			0.000*
Day 0 vs day 15			0.614
Day 0 vs day 20			1.000
Day 0 vs day 25			0.994
Day 0 vs day 30			1.000
Day 0 vs day 35			0.997
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control			0.000*
Day 5 vs long day control			0.000*
Day 12 vs long day control			0.000
Day 15 vs long day control			0.614
Day 20 vs long day control			1.000
Day 25 vs long day control			0.994
Day 30 vs long day control			1.000
Day 35 vs long day control			0.997

Table 3.36. One way single measures ANOVA for LH concentrations of female quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

	LH		
Effect	**F**	**df**	***p***
Time	11.94	8, 78	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5			1.000
Day 0 vs day 12			0.219
Day 0 vs day 15			0.000*
Day 0 vs day 20			0.000*
Day 0 vs day 25			0.000*
Day 0 vs day 30			0.000*
Day 0 vs day 35			0.246
Quail transferred to short days (days 0 –35 compared to short day controls on day 35			
Day 0 vs short day control			0.065
Day 5 vs short day control			0.130
Day 12 vs short day control			1.000
Day 15 vs short day control			0.659
Day 20 vs short day control			0.789
Day 25 vs short day control			0.043*
Day 30 vs short day control			0.419
Day 35 vs short day control			0.999

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.37. Two way single measures ANOVA for LH concentrations on day 35 of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

Effect	LH F	df	*p*
Time	3.93	2, 47	0.026*
Treatment	2.66	1, 47	0.110
Interaction between time and treatment	33.60	2, 47	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	33.34	1, 47	0.000*
Transfer to SD day 35 vs transfer to LD day 35	19.07	1, 47	0.000*
Short day control vs Long day control on day 35	17.04	1, 47	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.6 Follicle stimulating hormone (FSH)

FSH concentrations increased five days after transfer to long days but did not reach concentrations similar to those of long day control quail until day 35 (Figure 3.48A; see Table 3.38 for statistics). Birds transferred to short days showed wide ranges of FSH concentrations after five and 12 days, with mean concentrations significantly decreased at 12 days and similar to short day controls from 15 days onwards.

Long day control birds had higher FSH concentrations than short day control birds (Figure 3.49; see Table 3.38 for statistics).

FSH concentrations were strongly correlated with LH during gonadal regression but not gonadal growth (Figure 3.50; see Table 3.27 for statistics).

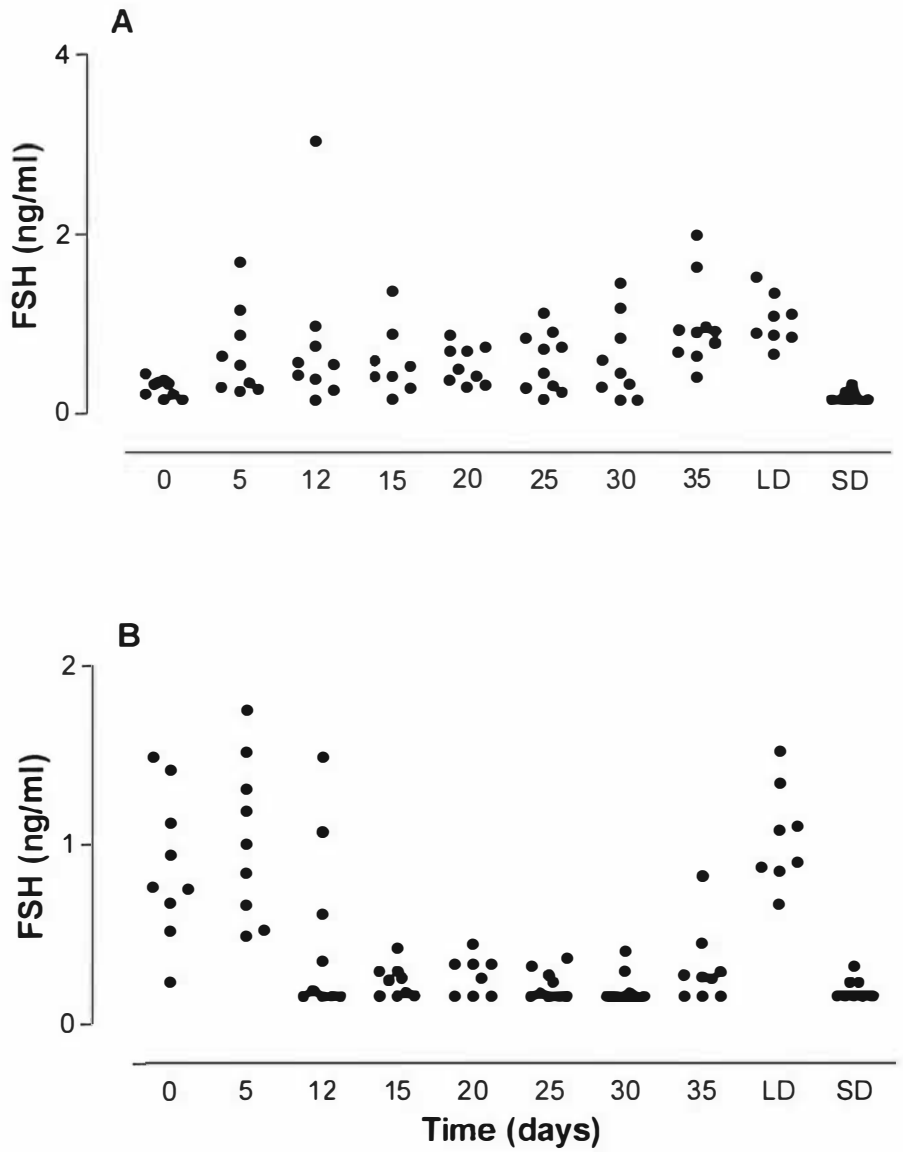

Figure 3.48. FSH concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD). Note different scales on y axes.

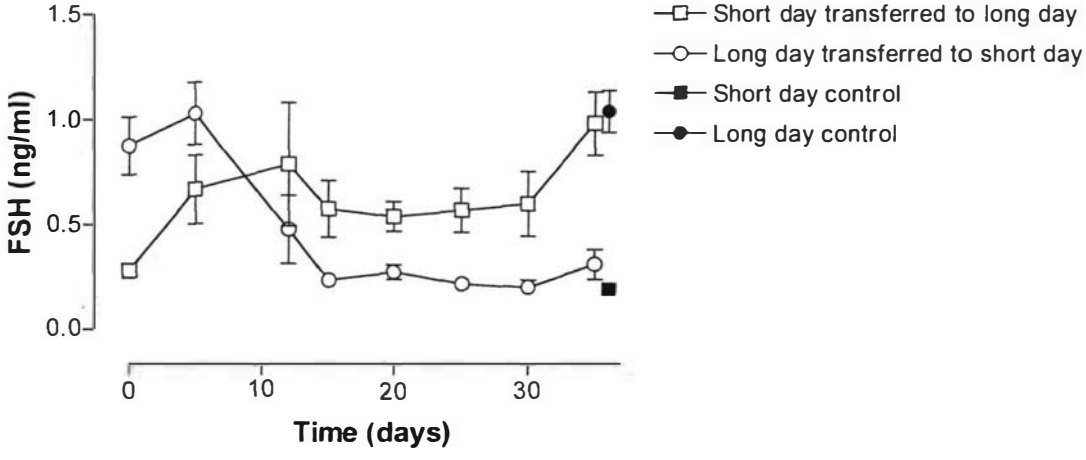

Figure 3.49. Changes in FSH concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

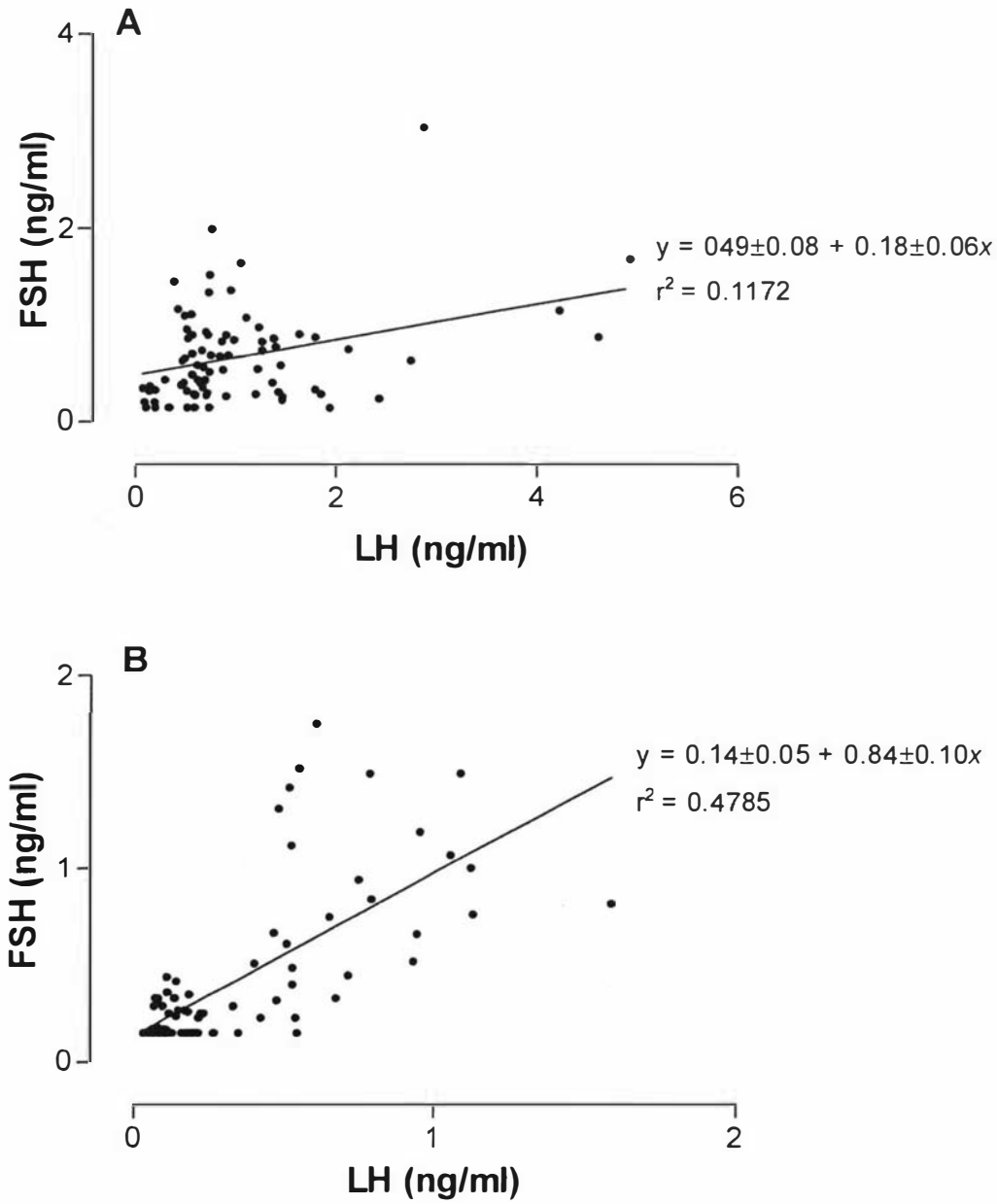

Figure 3.50. Relationships between LH and FSH of quail transferred from short days to long days (A) and quail transferred from long days to short days (B) for 35 days. Note different scales on x and y axes.

Table 3.38. Two way single measures ANOVA for FSH concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

	FSH		
Effect	**F**	**df**	***p***
Time	4.19	8, 142	0.000*
Treatment	37.24	8, 142	0.000*
Interaction between time and treatment	9.91	8, 142	0.000*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	7.15	1, 142	0.008*
Day 0 vs day 12	7.46	1, 142	0.007*
Day 0 vs day 15	4.53	1, 142	0.035*
Day 0 vs day 20	5.75	1, 142	0.018*
Day 0 vs day 25	5.06	1, 142	0.026*
Day 0 vs day 30	4.03	1, 142	0.047*
Day 0 vs day 35	21.69	1, 142	0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	23.42	1, 142	0.000*
Day 5 vs long day control	5.04	1, 142	0.026*
Day 12 vs long day control	4.80	1, 142	0.030*
Day 15 vs long day control	6.94	1, 142	0.009*
Day 20 vs long day control	6.32	1, 142	0.013*
Day 25 vs long day control	7.72	1, 142	0.006*
Day 30 vs long day control	8.36	1, 142	0.004*
Day 35 vs long day control	0.20	1, 142	0.656
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	0.55	1, 142	0.459
Day 0 vs day 12	11.03	1, 142	0.001*
Day 0 vs day 15	21.65	1, 142	0.000*
Day 0 vs day 20	17.06	1, 142	0.000*
Day 0 vs day 25	24.11	1, 142	0.000*
Day 0 vs day 30	25.82	1, 142	0.000*
Day 0 vs day 35	15.75	1, 142	0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	26.56	1, 142	0.000*
Day 5 vs short day control	34.50	1, 142	0.000*
Day 12 vs short day control	3.73	1, 142	0.055
Day 15 vs short day control	0.41	1, 142	0.524
Day 20 vs short day control	1.32	1, 142	0.253
Day 25 vs short day control	0.15	1, 142	0.697
Day 30 vs short day control	0.005	1, 142	0.944
Day 35 vs short day control	1.70	1, 142	0.194
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	16.05	1, 142	0.000*
Transfer to SD day 35 vs transfer to LD day 35	21.33	1, 142	0.000*
Short day control vs Long day control on day 35	35.22	1, 142	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.7 Estradiol

Estradiol concentrations increased after five days of photostimulation (Figure 3.51A; see Table 3.39 for statistics), increased further at 12 days and then remained high and similar to concentrations in long day birds. Estradiol concentrations showed large variation between quail on days 12 to 30.

In most quail transferred to short days mean estradiol concentrations had decreased significantly and reached short day control concentrations at day 12. Concentrations were then low except in two birds with large ovaries in which estradiol remained high (Figure 3.51B; see Table 3.39 for statistics).

Estradiol concentrations of long day control quail were higher than concentrations of short day control quail on day 35 (Figure 3.52). Estradiol concentrations were strongly correlated with LH or FSH during gonadal regression but not growth (Figures 3.53 and 3.54; see Table 3.27 for statistics).

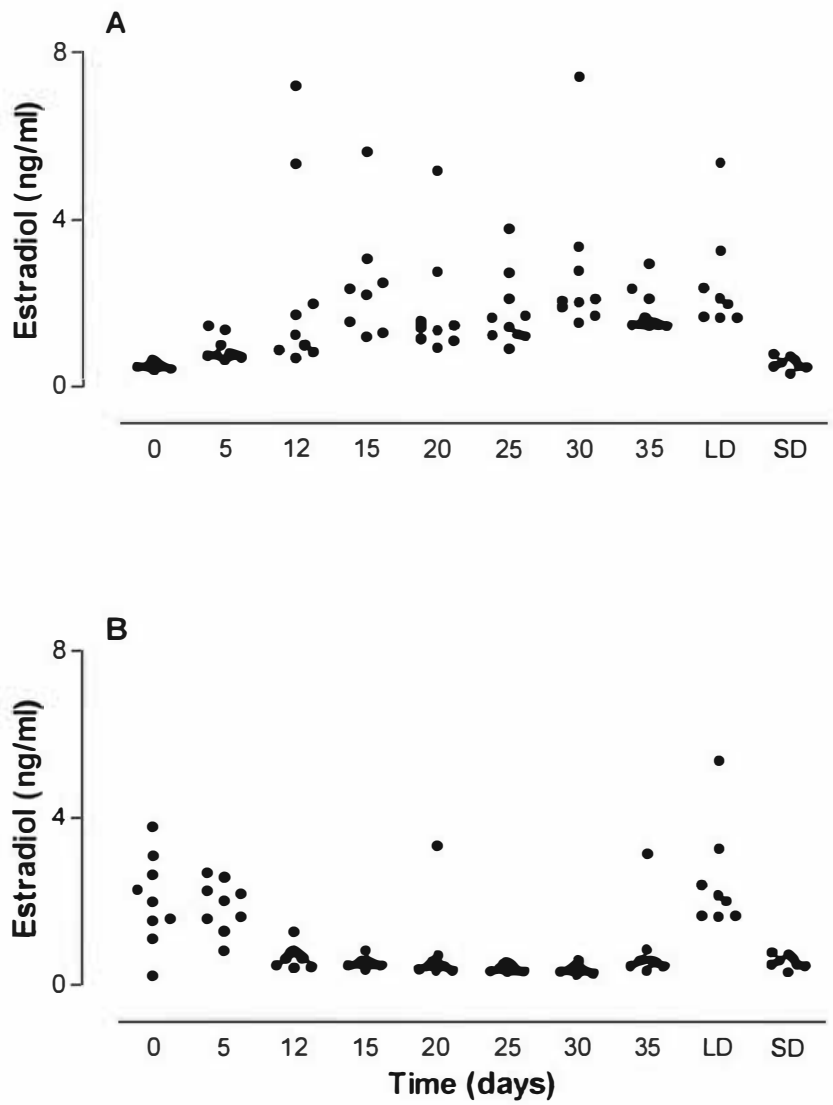

Figure 3.51. Estradiol concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

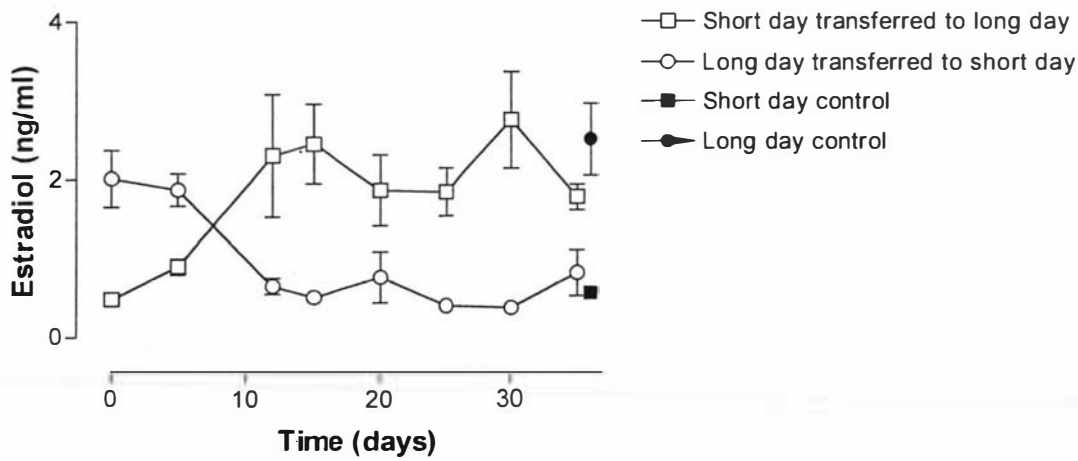

Figure 3.52. Changes in estradiol concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

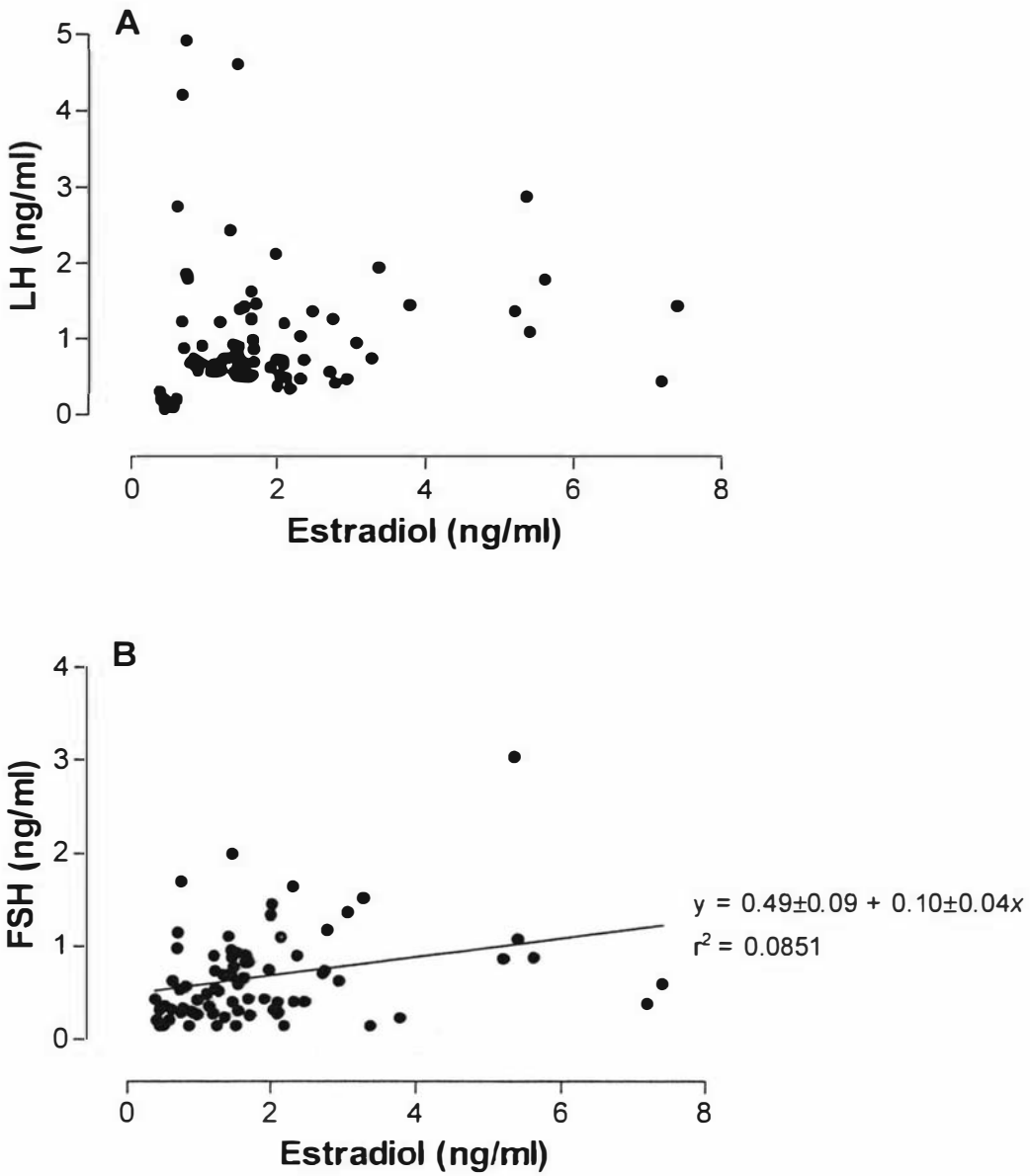

Figure 3.53. Relationships between estradiol and LH (A) and estradiol and FSH (B) of quail transferred from short days to long days for 35 days.

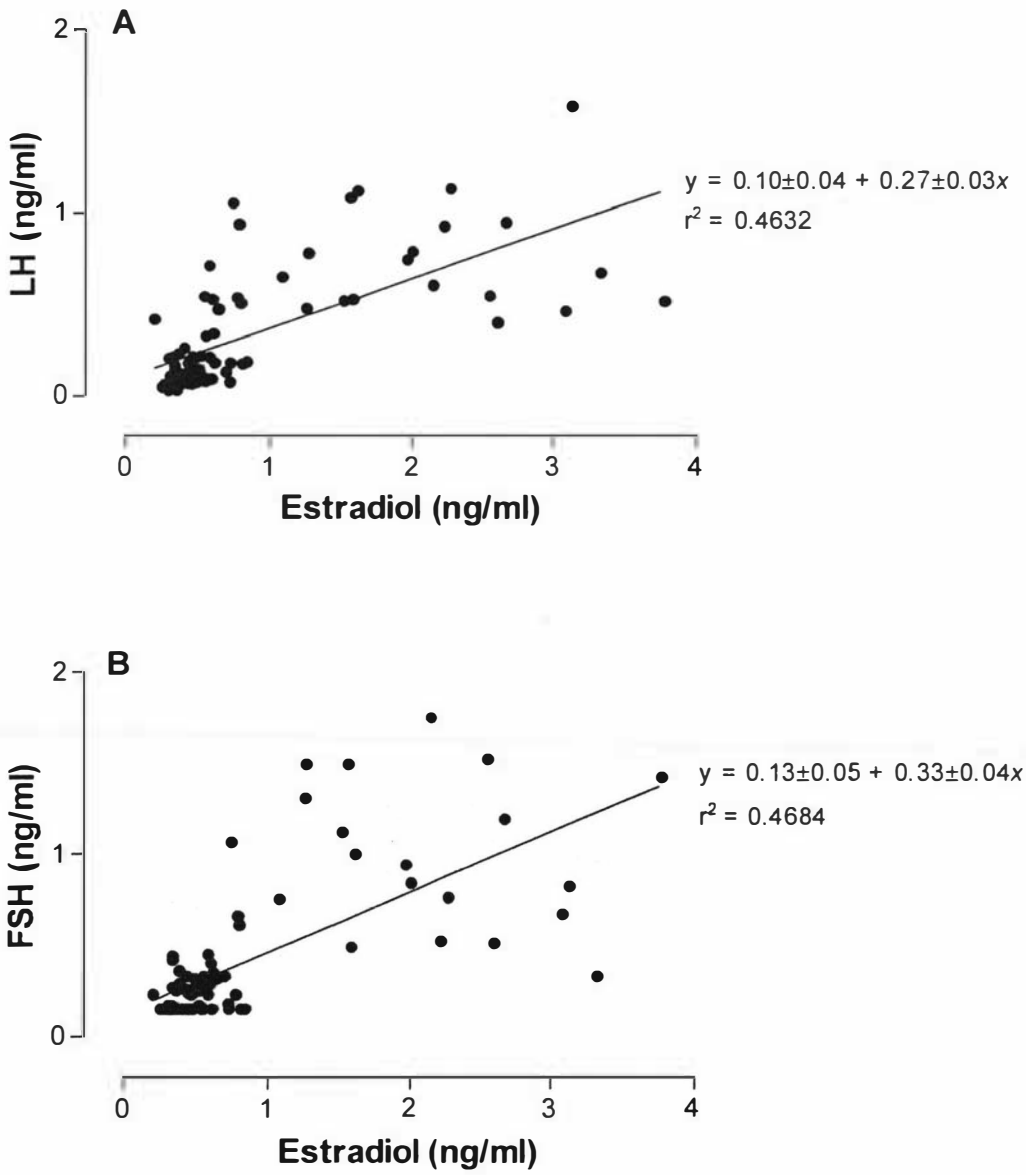

Figure 3.54. Relationships between estradiol and LH (A) and estradiol and FSH (B) of quail transferred from long days to short days for 35 days.

Table 3.39. Two way single measures ANOVA for estradiol concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	Estradiol F	df	*p*
Time	1.22	8, 142	0.292
Treatment	110.66	8, 142	0.000*
Interaction between time and treatment	20.85	8, 142	0.000*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	6.46	1, 142	0.012*
Day 0 vs day 12	27.78	1, 142	0.000*
Day 0 vs day 15	40.74	1, 142	0.000*
Day 0 vs day 20	27.55	1, 142	0.000*
Day 0 vs day 25	30.17	1, 142	0.000*
Day 0 vs day 30	49.81	1, 142	0.000*
Day 0 vs day 35	32.81	1, 142	0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	43.89	1, 142	0.629
Day 5 vs long day control	17.30	1, 142	0.000*
Day 12 vs long day control	2.28	1, 142	0.133
Day 15 vs long day control	0.06	1, 142	0.814
Day 20 vs long day control	2.35	1, 142	0.128
Day 25 vs long day control	2.15	1, 142	0.145
Day 30 vs long day control	0.05	1, 142	0.825
Day 35 vs long day control	1.53	1, 142	0.218
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	0.18	1, 142	0.674
Day 0 vs day 12	16.77	1, 142	0.000*
Day 0 vs day 15	25.27	1, 142	0.000*
Day 0 vs day 20	21.52	1, 142	0.000*
Day 0 vs day 25	36.04	1, 142	0.000*
Day 0 vs day 30	40.04	1, 142	0.000*
Day 0 vs day 35	15.87	1, 142	0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	21.23	1, 142	0.000*
Day 5 vs short day control	25.17	1, 142	0.000*
Day 12 vs short day control	0.40	1, 142	0.526
Day 15 vs short day control	0.07	1, 142	0.788
Day 20 vs short day control	0.01	1, 142	0.914
Day 25 vs short day control	1.48	1, 142	0.226
Day 30 vs short day control	2.79	1, 142	0.097
Day 35 vs short day control	0.55	1, 142	0.459
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	27.55	1, 142	0.000*
Transfer to SD day 35 vs transfer to LD day 35	19.63	1, 142	0.000*
Short day control vs Long day control on day 35	35.61	1, 142	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.8 Progesterone (P_4)

P_4 concentrations increased gradually in quail transferred to long days (Figure 3.55A; see Table 3.40 for statistics) and reached concentrations similar to long day controls at day 25. During this time there was a steady increase in the numbers of large yolk filled follicles in ovaries (Table 3.31).

Quail showed wide variation in P_4 concentrations during the first 12 short days (Figure 3.55B; see Table 3.40 for statistics). During this time there was a substantial decrease in the number of large yolk filled follicles on the ovary. By day 15 P_4 decreased to similar concentrations seen in short day controls.

P_4 concentrations were higher in long day control quail than short day control quail (Figure 3.56; see Table 3.40 for statistics).

P_4 concentrations were not strongly correlated with LH, FSH or estradiol concentrations during gonadal growth (Figure 3.57; see Table 3.27 for statistics) but were during gonadal regression (Figures 3.58 see Table 3.27 for statistics).

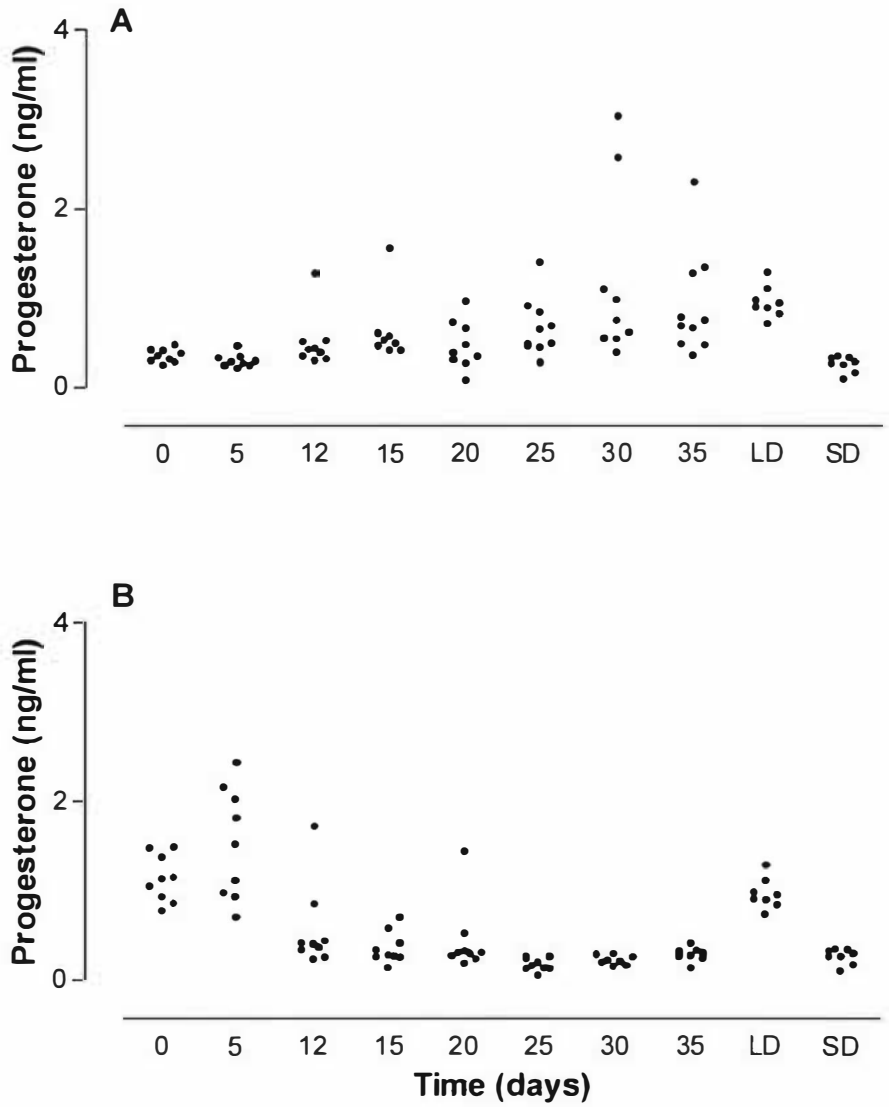

Figure 3.55. Progesterone concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

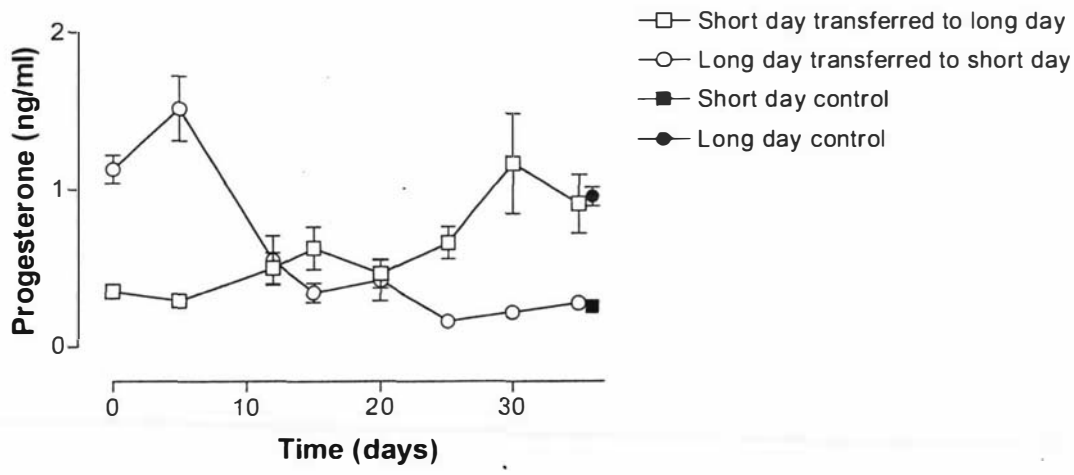

Figure 3.56. Changes in progesterone concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

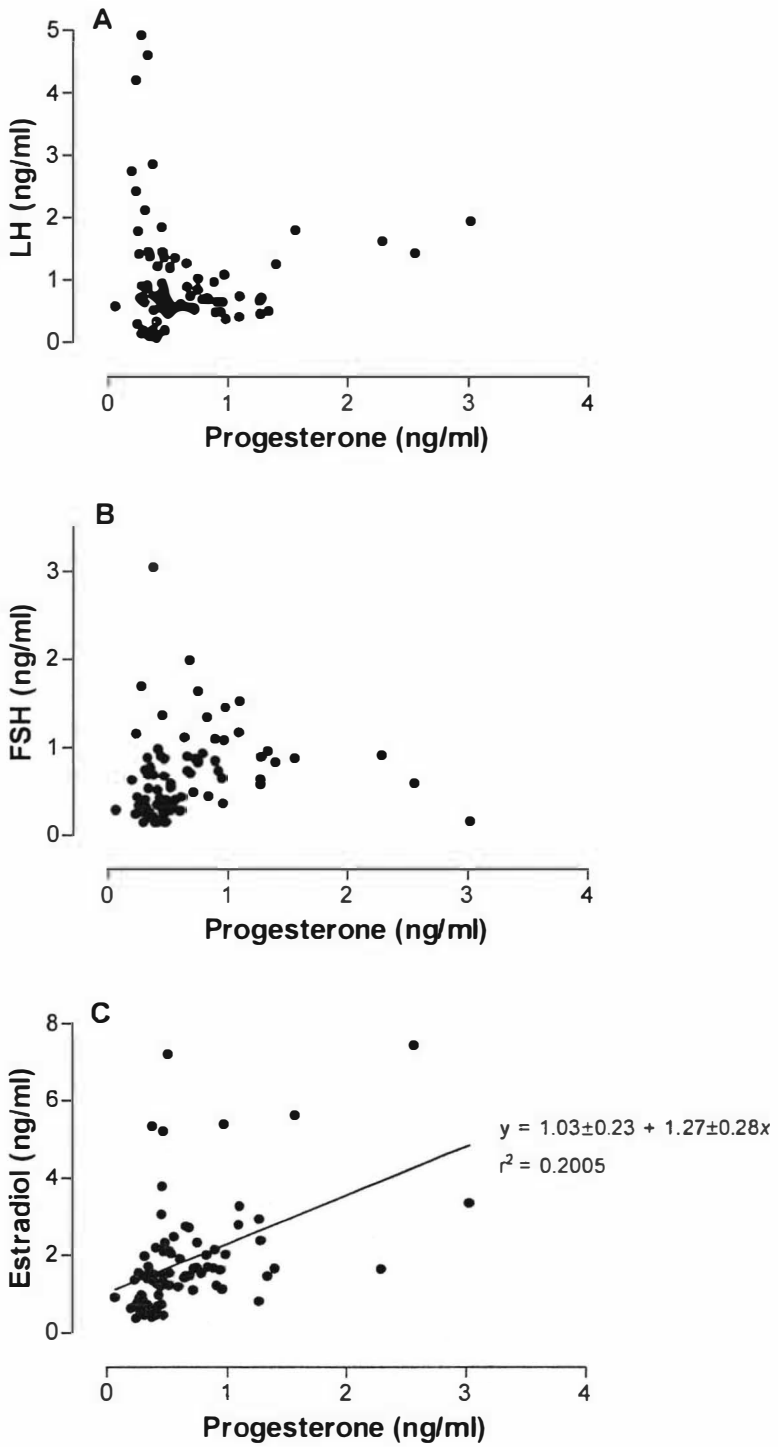

Figure 3.57. Relationships between progesterone and LH (A), FSH (B) and estradiol (C) of quail transferred from short days to long days for 35 days. Note different scales on y axes.

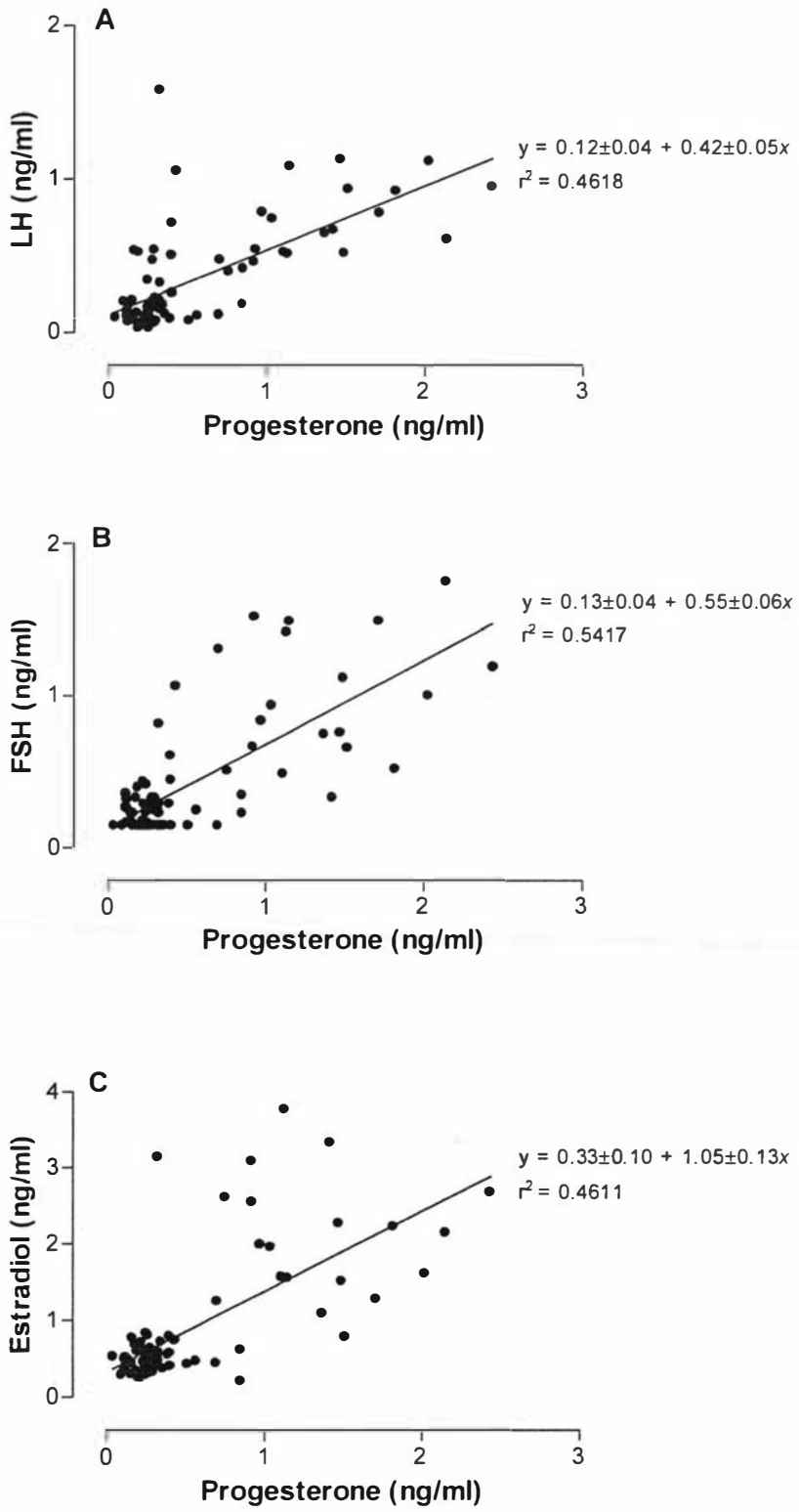

Figure 3.58. Relationships between progesterone and LH (A), FSH (B) and estradiol (C) of quail transferred from long days to short days for 35 days. Note different scales on y axes.

Table 3.40. Two way single measures ANOVA for P_4 concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

	Progesterone		
Effect	**F**	**df**	***p***
Time	4.49	8, 142	0.000*
Treatment	23.64	8, 142	0.000*
Interaction between time and treatment	24.68	8, 142	0.000*
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	0.73	1, 142	0.394
Day 0 vs day 12	1.39	1, 142	0.240
Day 0 vs day 15	4.49	1, 142	0.036*
Day 0 vs day 20	0.17	1, 142	0.678
Day 0 vs day 25	6.39	1, 142	0.013*
Day 0 vs day 30	18.15	1, 142	0.000*
Day 0 vs day 35	13.52	1, 142	0.000*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	18.40	1, 142	0.000*
Day 5 vs long day control	26.20	1, 142	0.000*
Day 12 vs long day control	9.89	1, 142	0.002*
Day 15 vs long day control	4.45	1, 142	0.037*
Day 20 vs long day control	15.10	1, 142	0.000*
Day 25 vs long day control	3.79	1, 142	0.054
Day 30 vs long day control	0.03	1, 142	0.876
Day 35 vs long day control	0.69	1, 142	0.407
Contrasts for quail transferred from long days to short days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	1.14	1, 142	0.287
Day 0 vs day 12	16.01	1, 142	0.000*
Day 0 vs day 15	32.09	1, 142	0.000*
Day 0 vs day 20	26.73	1, 142	0.000*
Day 0 vs day 25	80.65	1, 142	0.000*
Day 0 vs day 30	49.23	1, 142	0.000*
Day 0 vs day 35	39.04	1, 142	0.000*
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	42.89	1, 142	0.000*
Day 5 vs short day control	57.54	1, 142	0.000*
Day 12 vs short day control	7.12	1, 142	0.009*
Day 15 vs short day control	1.11	1, 142	0.294
Day 20 vs short day control	2.35	1, 142	0.127
Day 25 vs short day control	4.68	1, 142	0.032
Day 30 vs short day control	0.21	1, 142	0.651
Day 35 vs short day control	0.24	1, 142	0.627
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	26.04	1, 142	0.000*
Transfer to SD day 35 vs transfer to LD day 35	23.55	1, 142	0.000*
Short day control vs Long day control on day 35	32.75	1, 142	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.9 Prolactin

Mean prolactin concentrations gradually increased in quail transferred to long days (Figure 3.59A; see Table 3.41 for statistics). Concentrations were not significantly different from those of long day control birds from day 12 but were significantly higher than initial values only from day 25. Prolactin varied markedly between birds on all days of long day treatment and in control birds on long days.

Prolactin concentrations decreased in quail transferred to short days after 12 days and then remained low and similar to those of short day control quail (Figure 3.59B; see Table 3.42 for statistics).

Prolactin concentrations were higher in long day compared to short day controls (Figure 3.60; see Table 3.43 for statistics).

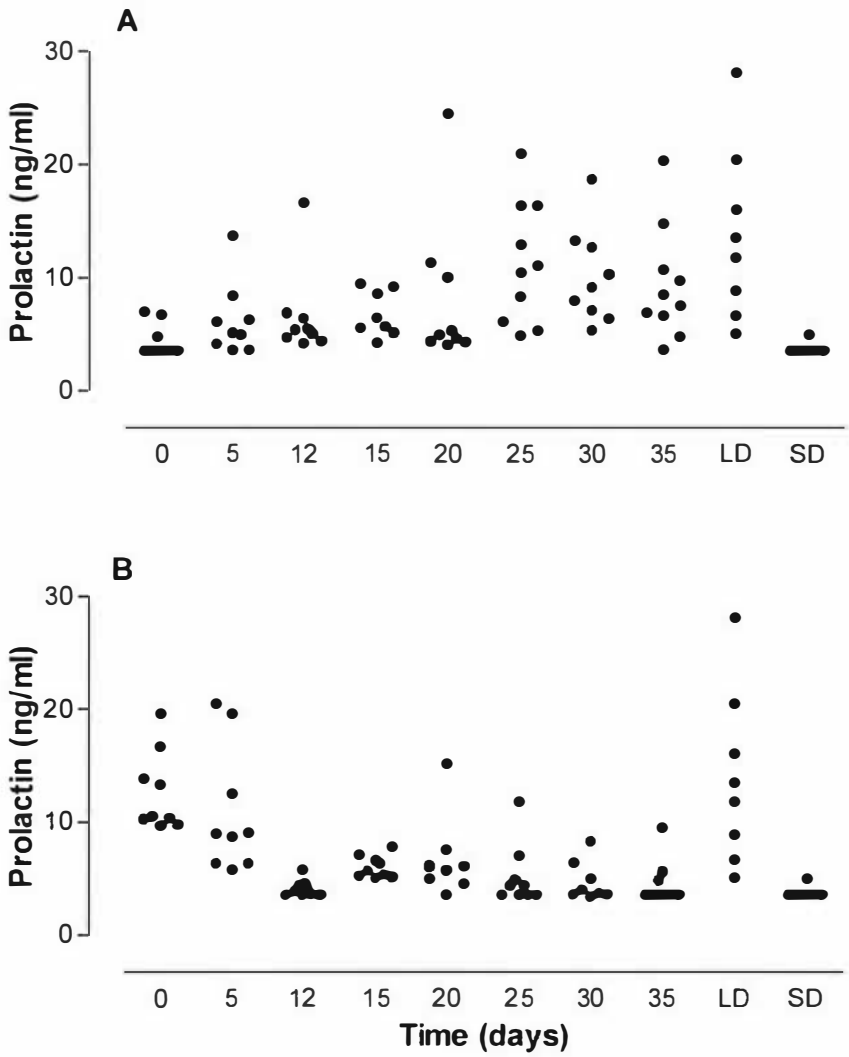

Figure 3.59. Prolactin concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 ºC (LD) and short days at 10 ºC (SD).

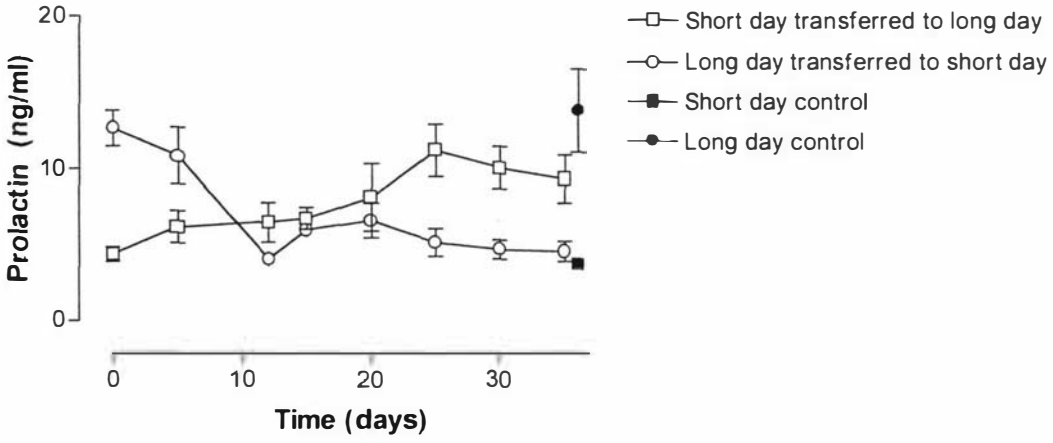

Figure 3.60. Changes in prolactin concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

Table 3.41. One way single measures ANOVA for prolactin concentrations of female quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

ANOVA	Prolactin		
	F	df	*p*
Effect of time	4.46	8, 80	0.000*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5			0.918
Day 0 vs day 12			0.838
Day 0 vs day 15			0.594
Day 0 vs day 20			0.491
Day 0 vs day 25			0.003*
Day 0 vs day 30			0.013*
Day 0 vs day 35			0.053
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control			0.000*
Day 5 vs long day control			0.032*
Day 12 vs long day control			0.054
Day 15 vs long day control			0.188
Day 20 vs long day control			0.193
Day 25 vs long day control			0.996
Day 30 vs long day control			0.974
Day 35 vs long day control			0.753

Table 3.42. One way single measures ANOVA for prolactin concentrations of female quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

ANOVA	Prolactin		
	F	df	*p*
Effect of time	14.77	8, 78	0.000*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5			0.863
Day 0 vs day 12			0.000*
Day 0 vs day 15			0.000*
Day 0 vs day 20			0.000*
Day 0 vs day 25			0.000*
Day 0 vs day 30			0.000*
Day 0 vs day 35			0.000*
Quail transferred to short days (days 0 –35) compared to short day controls on day 35			
Day 0 vs short day control			0.000*
Day 5 vs short day control			0.000*
Day 12 vs short day control			1.000
Day 15 vs short day control			0.064
Day 20 vs short day control			0.049*
Day 25 vs short day control			0.786
Day 30 vs short day control			0.952
Day 35 vs short day control			0.988

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.43. Two way single measures ANOVA for prolactin concentrations on day 35 of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

Effect	Prolactin		
	F	df	*p*
Time	1.07	2, 47	0.350
Treatment	3.78	1, 47	0.058
Interaction between time and treatment	23.12	2, 47	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	18.44	1, 47	0.000*
Transfer to SD day 35 vs transfer to LD day 35	6.41	1, 47	0.015*
Short day control vs Long day control on day 35	24.37	1, 47	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.10 Thyroxine (T4)

There were no significant changes in T4 concentrations in birds transferred from short to long or long to short days (Figures 3.61 and 3.62; see Table 3.44 for statistics). T4 concentrations were not strongly related to LH concentrations during gonadal growth or regression (Figure 3.63; see Table 3.27).

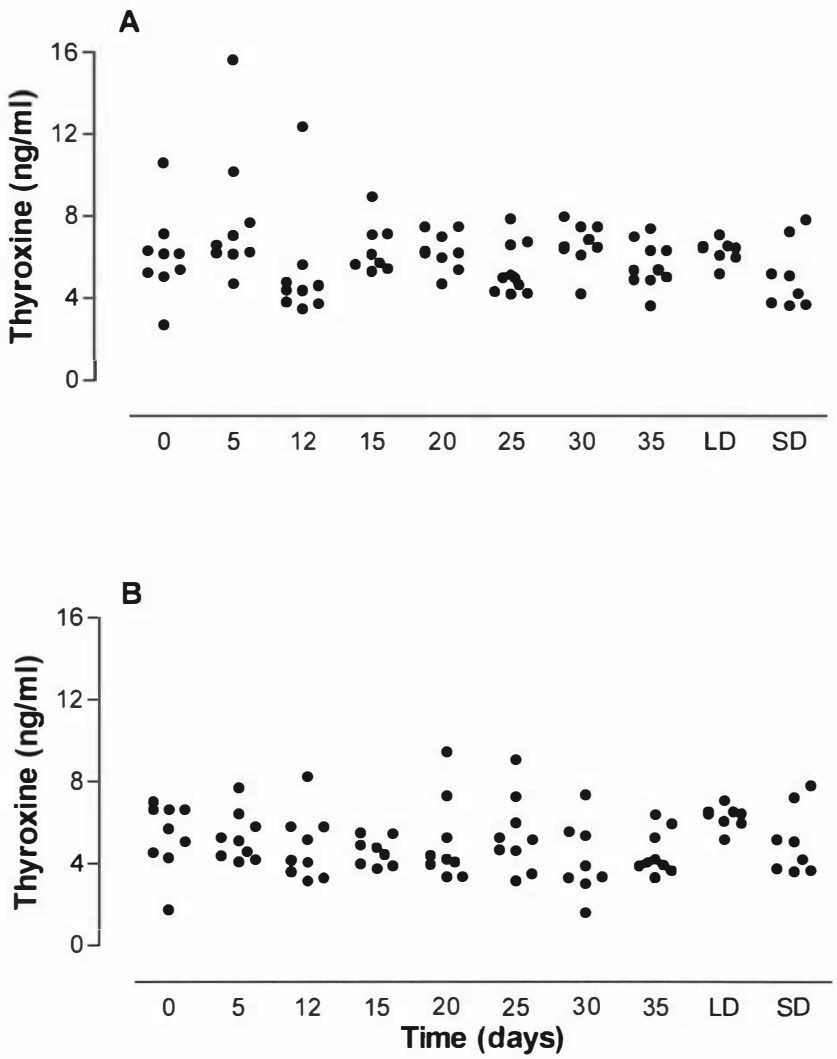

Figure 3.61. Thyroxine concentrations of individual quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

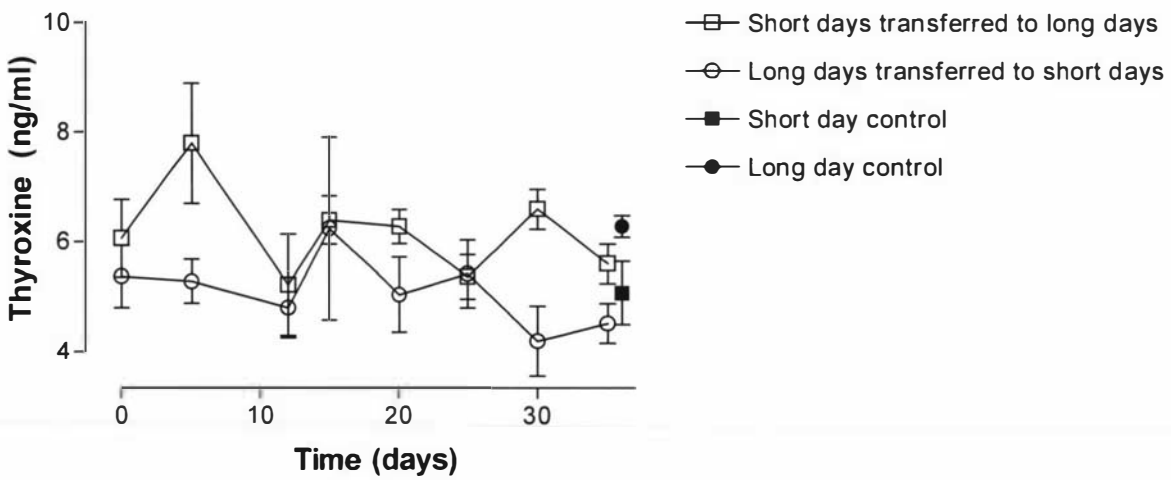

Figure 3.62. Changes in thyroxine concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

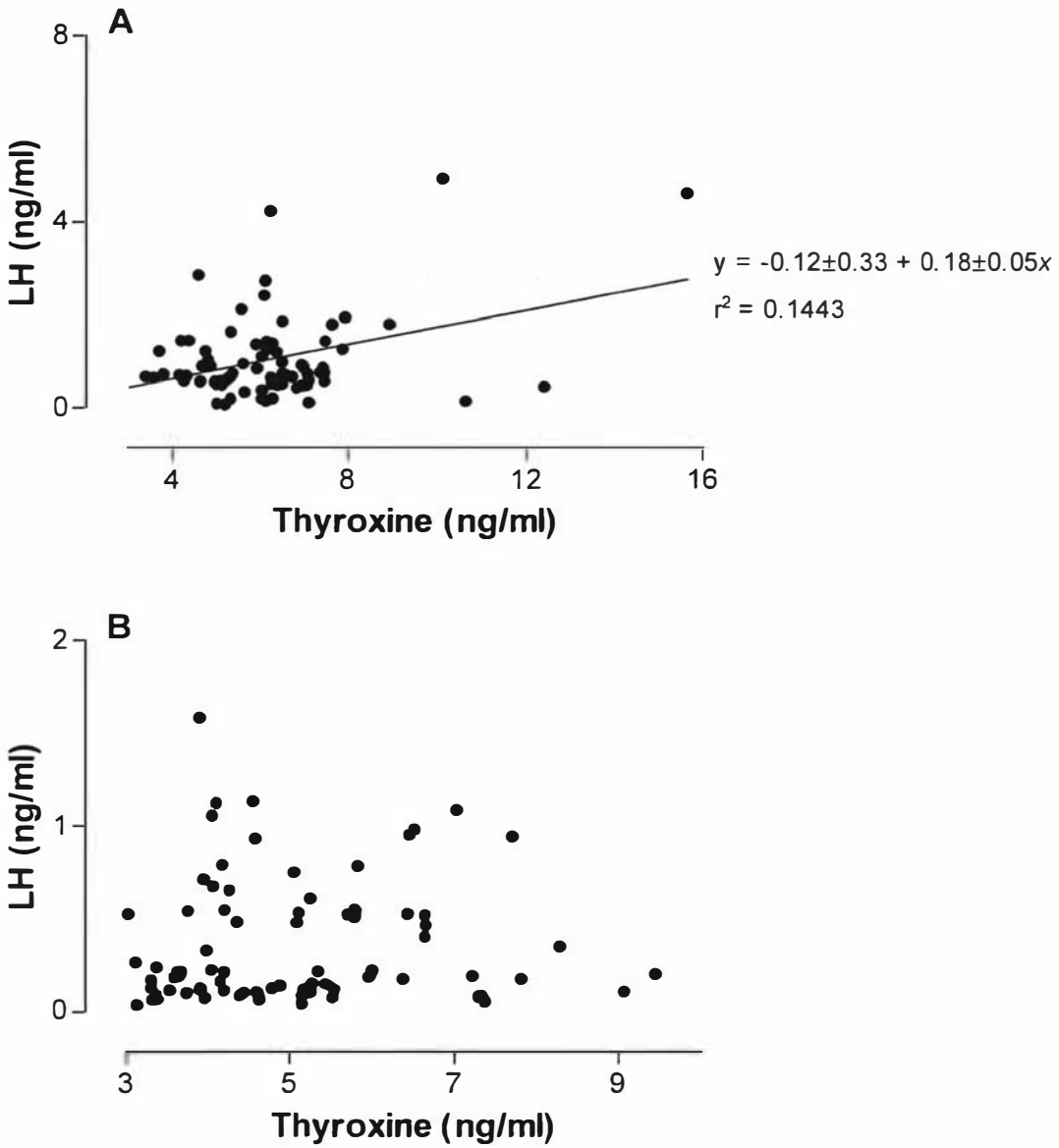

Figure 3.63. Relationships between thyroxine and LH of quail transferred from short days to long days (A) and quail transferred from long days to short days for 35 days. Note different scales on x and y axes.

Table 3.44. Two way single measures ANOVA for thyroxine concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control).

Effect	Thyroxine		
	F	df	*p*
Time	1.29	8, 142	0.253
Treatment	15.85	8, 142	0.000*
Interaction between time and treatment	1.01	8, 142	0.434
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
Day 0 vs day 5	2.58	1, 142	0.110
Day 0 vs day 12	1.27	1, 142	0.260
Day 0 vs day 15	0.33	1, 142	0.570
Day 0 vs day 20	0.26	1, 142	0.610
Day 0 vs day 25	0.33	1, 142	0.560
Day 0 vs day 30	0.66	1, 142	0.420
Day 0 vs day 35	0.07	1, 142	0.790
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	0.29	1, 142	0.590
Day 5 vs long day control	1.04	1, 142	0.310
Day 12 vs long day control	2.66	1, 142	0.110
Day 15 vs long day control	0.001	1, 142	0.970
Day 20 vs long day control	0.002	1, 142	0.970
Day 25 vs long day control	1.23	1, 142	0.270
Day 30 vs long day control	0.06	1, 142	0.800
Day 35 vs long day control	0.66	1, 142	0.420
Contrasts for quail transferred from short days to long days for 35 days			
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	0.04	1, 142	0.843
Day 0 vs day 12	0.34	1, 142	0.563
Day 0 vs day 15	0.17	1, 142	0.680
Day 0 vs day 20	0.17	1, 142	0.680
Day 0 vs day 25	0.03	1, 142	0.856
Day 0 vs day 30	2.82	1, 142	0.095
Day 0 vs day 35	0.71	1, 142	0.400
Quail transferred to short days (days 0 – 35) compared to short day controls on day 35			
Day 0 vs short day control	0.05	1, 142	0.829
Day 5 vs short day control	0.17	1, 142	0.683
Day 12 vs short day control	0.12	1, 142	0.730
Day 15 vs short day control	0.38	1, 142	0.537
Day 20 vs short day control	0.38	1, 142	0.537
Day 25 vs short day control	0.16	1, 142	0.695
Day 30 vs short day control	2.02	1, 142	0.157
Day 35 vs short day control	0.36	1, 142	0.548
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	0.74	1, 142	0.391
Transfer to SD day 35 vs transfer to LD day 35	2.18	1, 142	0.142
Short day control vs Long day control on day 35	2.39	1, 142	0.124

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.11 Triiodothyronine (T3)

T3 concentrations decreased five days after transfer to long days and from day five were similar to concentrations observed in long day control quail on day 35 (Figures 3.64 and 3.65; see Table 3.45 for statistics). T3 concentrations gradually increased in quail transferred to short days with a significant increase observed after five days (Figures 3.64 and 3.65; see Table 3.46 for statistics). However, although T3 was higher in short day than long day quail at the beginning of the experiment, concentrations did not differ between short and long day control groups at the end of the experiment (see Table 3.47 for statistics).

There were no clear relationships between T3 and LH or T3 and T4 concentrations in quail undergoing gonadal growth or regression (Figures 3.66 and 3.67; see Table 3.27 for statistics).

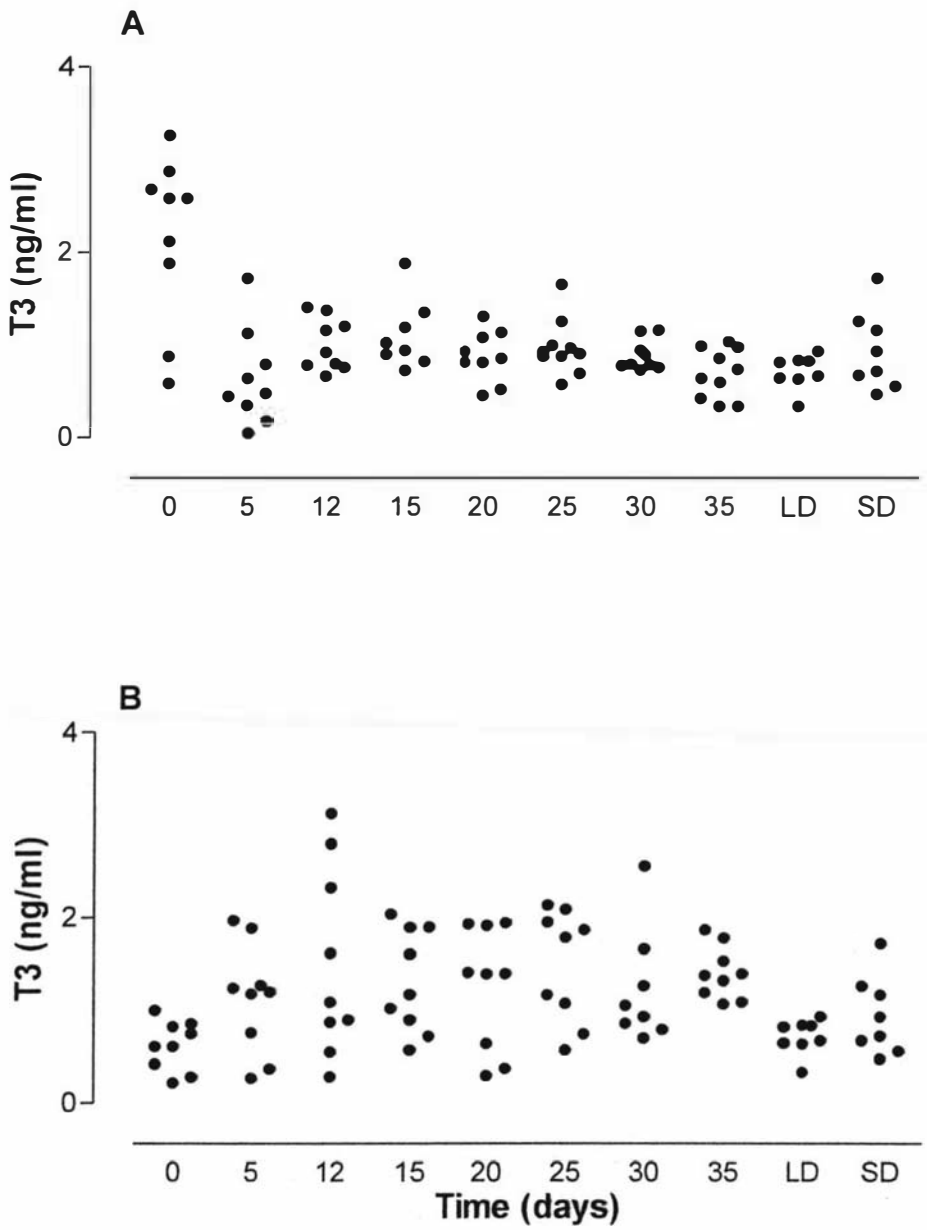

Figure 3.64. T3 concentrations of individual female quail transferred from short days to long days (A), quail transferred from long days to short days (B) and quail held on long days at 20 °C (LD) and short days at 10 °C (SD).

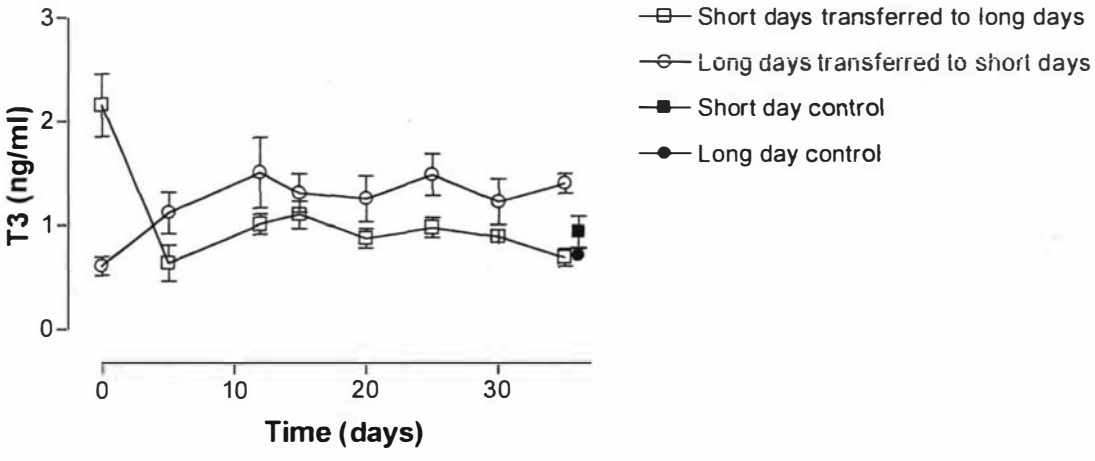

Figure 3.65. Changes in T3 concentrations of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (short day control) and long days at 20 °C (long day control). Results for control groups are shown on day 36 for simplicity. Results are shown as mean ± standard error.

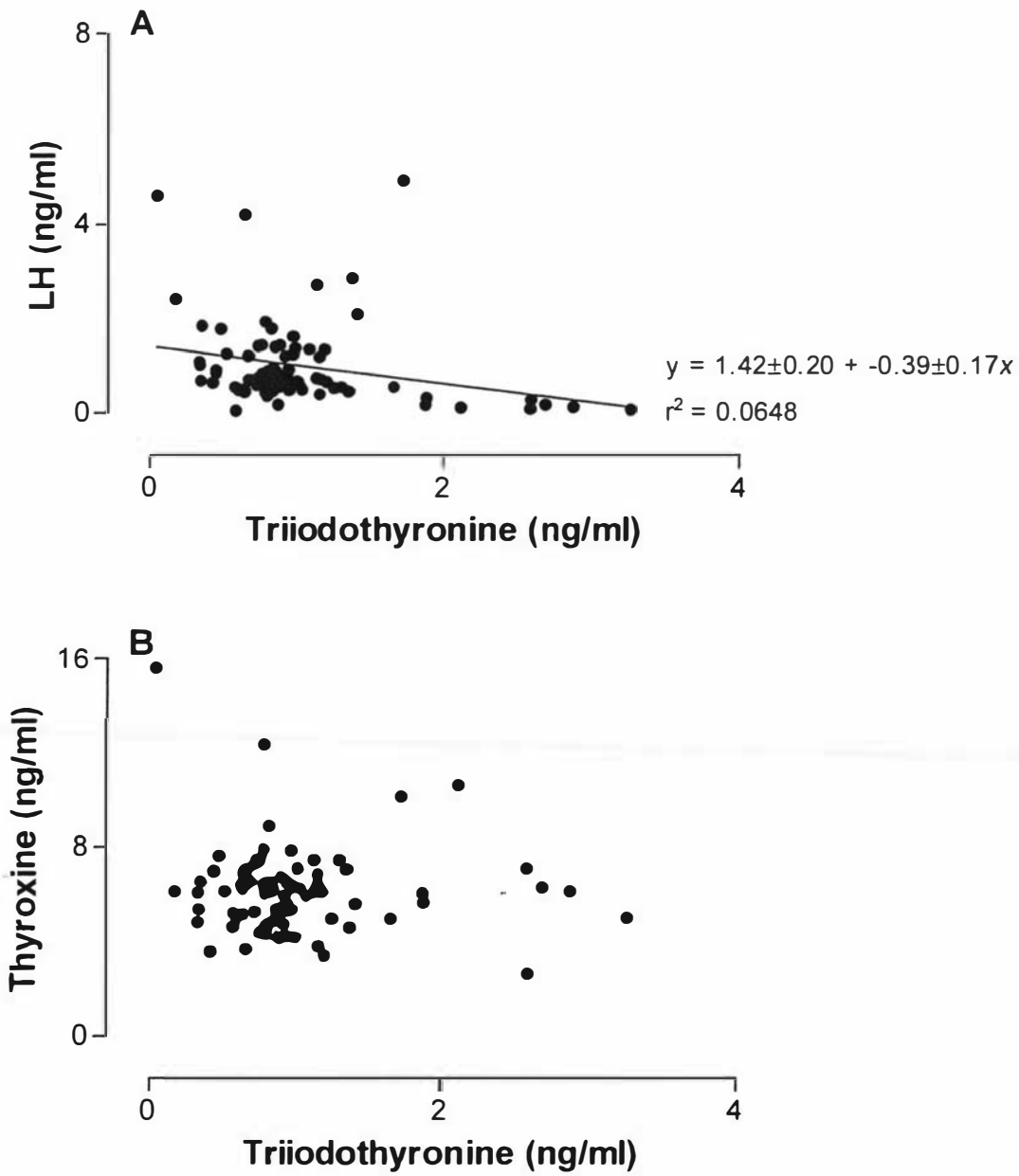

Figure 3.66. Relationships between triiodothyronine and LH (A) and between triiodothyronine and T4 (B) of quail transferred from short days to long days for 35 days. Note different scales on x and y axes.

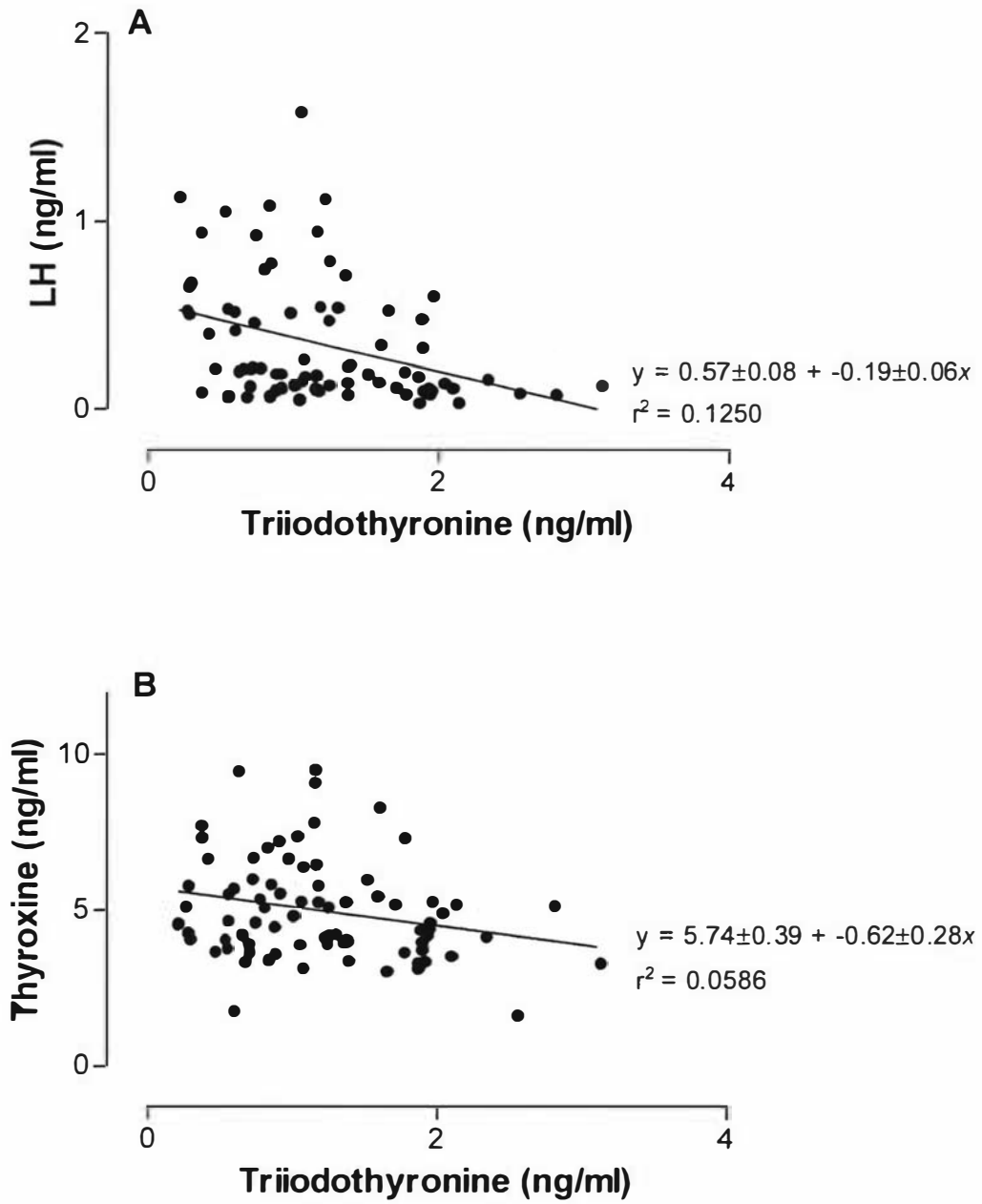

Figure 3.67. Correlation between triiodothyronine and LH (A) and between triiodothyronine and T4 (B) of quail transferred from long days to short days for 35 days. Note different scales on y axes.

Table 3.45. Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for T3 concentrations of female quail transferred from short days to long days and quail held on long days at 20 °C (long day control).

Kruskal-Wallis ANOVA	Triiodothyronine		
	K-W statistic	df	*p*
Effect of time	24.9	8	0.002*
Quail before transfer to long days (day 0) compared to quail transferred to long days (days 5 – 35)			
	M-W statistic		
Day 0 vs day 5	6.0		0.001*
Day 0 vs day 12	14.0		0.019*
Day 0 vs day 15	15.0		0.046*
Day 0 vs day 20	11.0		0.008*
Day 0 vs day 25	17.0		0.022*
Day 0 vs day 30	13.0		0.014*
Day 0 vs day 35	10.0		0.003*
Quail transferred to long days (days 0 – 35) compared to long day controls on day 35			
Day 0 vs long day control	8.0		0.006*
Day 5 vs long day control	26.0		0.370
Day 12 vs long day control	18.0		0.093
Day 15 vs long day control	8.0		0.010*
Day 20 vs long day control	23.0		0.236
Day 25 vs long day control	14.0		0.021*
Day 30 vs long day control	21.0		0.167
Day 35 vs long day control	39.0		0.965

Table 3.46 Kruskal-Wallis (K-W) non-parametric ANOVA and Mann-Whitney (M-W) U test contrasts for T3 concentrations of female quail transferred from long days to short days and quail held on short days at 10 °C (short day control).

ANOVA	Triiodothyronine		
	F	df	*p*
Effect of time	16.0	8	0.042*
Quail before transfer to short days (day 0) compared to quail transferred to short days (days 5 – 35)			
Day 0 vs day 5	18.0		0.050
Day 0 vs day 12	15.0		0.024*
Day 0 vs day 15	11.0		0.008*
Day 0 vs day 20	18.0		0.050
Day 0 vs day 25	10.0		0.006*
Day 0 vs day 30	9.0		0.008*
Day 0 vs day 35	0.0		0.000*
Quail transferred to short days (days 0 –35) compared to short day controls on day 35			
Day 0 vs short day control	21.0		0.167
Day 5 vs short day control	27.0		0.423
Day 12 vs short day control	27.0		0.423
Day 15 vs short day control	21.0		0.167
Day 20 vs short day control	25.0		0.321
Day 25 vs short day control	16.0		0.059
Day 30 vs short day control	22.0		0.328
Day 35 vs short day control	12.0		0.021

NOTE: The first row of each table shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons between days.

Table 3.47. Two way single measures ANOVA for T3 concentrations on day 35 of female quail transferred from short days to long days, quail transferred from long days to short days and quail held on short days at 10 °C (SD - short day control) and long days at 20 °C (LD - long day control).

Effect	Triidothyronine		
	F	df	*p*
Time	1.90	2, 47	0.161
Treatment	0.43	1, 47	0.541
Interaction between time and treatment	25.64	2, 47	0.000*
Contrasts for quail transferred to long or short days on day 0 and day 35			
Transfer to SD day 0 vs transfer to LD day 0	35.98	1, 47	0.000*
Transfer to SD day 35 vs transfer to LD day 35	14.58	1, 47	0.000*
Short day control vs Long day control on day 35	1.12	1, 47	0.294

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons between days.

3.3.2.12 Summary of results for gonadal growth and regression in female Japanese quail

Transfer of female Japanese quail from short days and 10 °C to long days and 20 °C increased LH, FSH, estradiol, ovary and oviduct weights after five days whilst cloacal diameter increased after 12 days and P_4 after 25 long days (Figure 3.68; see Table 3.48). T3 concentrations decreased after five days whilst T4 did not change. Prolactin gradually increased and was significantly elevated after 25 days.

Female Japanese quail transferred from long days and 20 °C to short days and 10 °C had decreased LH, FSH, estradiol, P_4, ovary and oviduct weights after 12 short days and decreased cloacal diameters after 25 days (Figure 3.69; see Table 3.48). T4 did not change whereas T3 increased after five days. Prolactin decreased after 12 days.

Female quail held on long days and 20 °C for eight weeks as long day controls maintained large ovaries and continued to lay eggs whilst birds held on short days and 10 °C did not undergo ovarian growth (Table 3.48). Plasma concentrations of all hormones except T3 remained high in quail held on long days and 20 °C and low in quail held on short days and 10 °C. T3 was low in quail held on long days and high in quail held on short days.

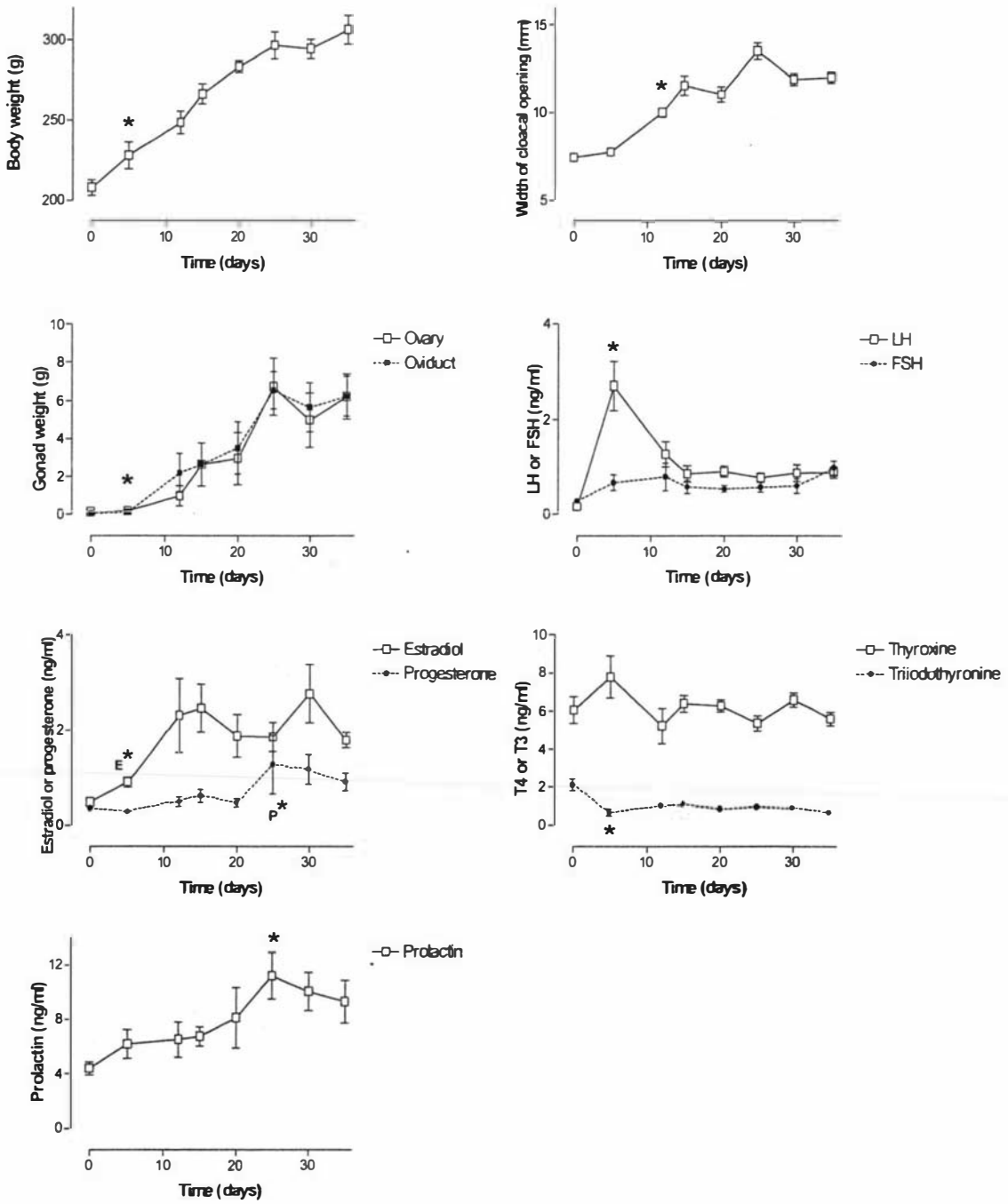

Figure 3.68. Summary graphs of variables measured in female quail transferred from short days to long days for 35 days. The first significant change compared with day 0 is indicated with an asterisk for each variable. Results are shown as mean ± standard error. Note different scales on y axes.

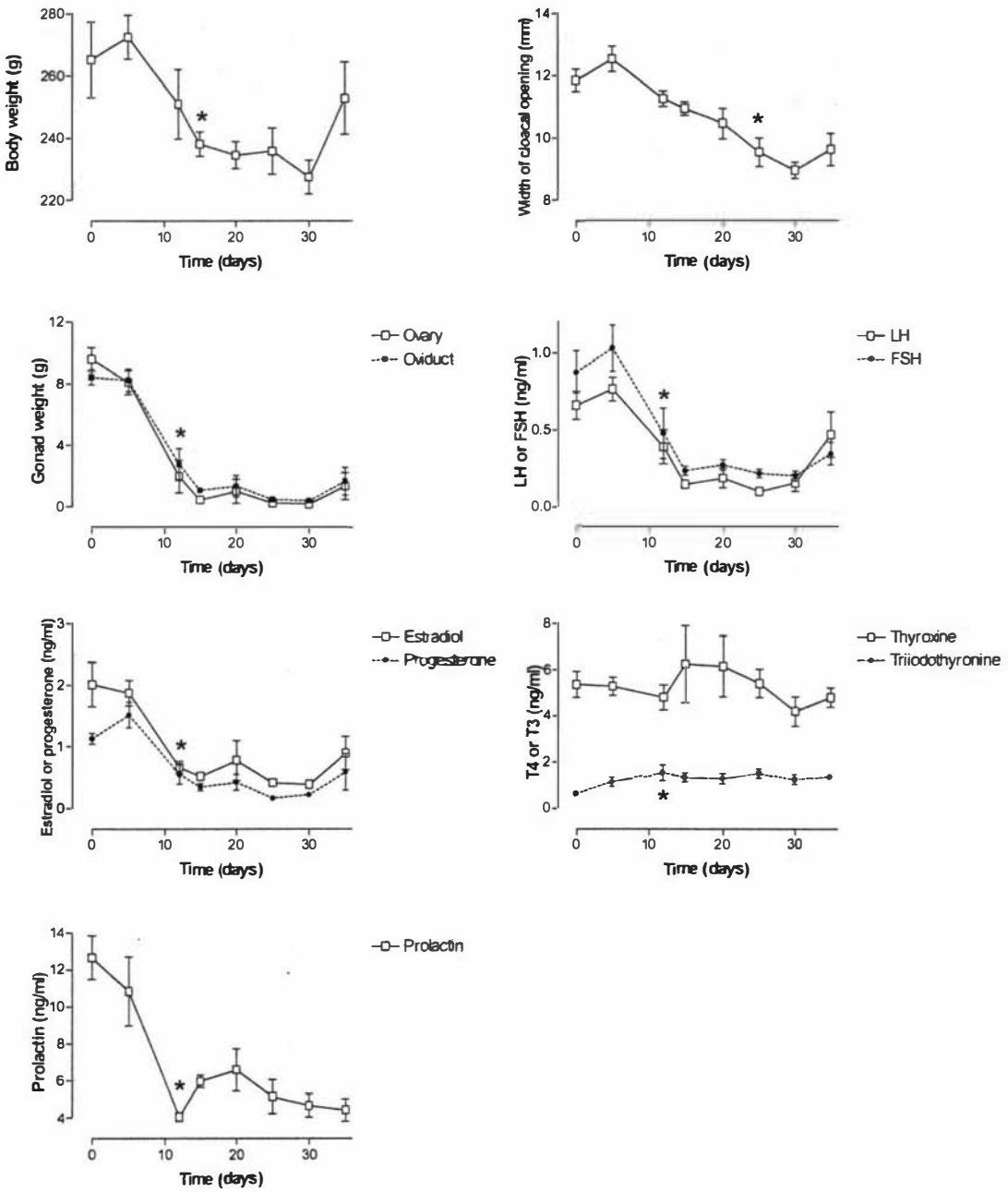

Figure 3.69. Summary graphs of variables measured in female quail transferred from long days to short days for 35 days. Results are shown as mean ± standard error. Note different scales on y axes.

Table 3.48. Summary of the timing of significant changes in variables measured in female quail transferred from short days to long days and in female quail transferred from long days to short days. First significant change from day 0 in each variable is indicated in bold type.

Female quail transferred from short days to long days

Day	Body weight (g)	Cloacal opening (mm)	Ovary weight (g)	Oviduct weight (g)	LH (ng/ml)	FSH (ng/ml)	Estradiol (ng/ml)	P_4 (ng/ml)	PRL (ng/ml)	T4 ng/ml	T3 ng/ml
0	208.3 ± 5.0	7.4 ± 0.1	0.10 ± 0.01	0.03 ± 0.00	0.16 ± 0.02	0.28 ± 0.03	0.48 ± 0.02	0.35 ± 0.02	4.37 ± 0.48	6.06 ± 0.71	2.16 ± 0.30
5	**227.9 ± 8.2**	7.7 ± 0.1	**0.18 ± 0.01**	**0.12 ± 0.01**	**2.70 ± 0.51**	**0.67 ± 0.16**	**0.90 ± 0.10**	0.29 ± 0.02	6.16 ± 1.07	7.80 ± 1.10	**0.64 ± 0.17**
12	248.4 ± 7.1	**10.0 ± 0.3**	0.96 ± 0.53	2.17 ± 1.04	1.27 ± 0.26	0.79 ± 0.29	2.30 ± 0.78	0.50 ± 0.10	6.49 ± 1.30	5.22 ± 0.92	1.01 ± 0.09
15	266.4 ± 6.2	11.6 ± 0.6	2.62 ± 1.14	3.98 ± 0.99	0.86 ± 0.16	0.58 ± 0.12	2.46 ± 0.50	0.63 ± 0.14	6.72 ± 0.67	6.40 ± 0.41	1.10 ± 0.12
20	284.4 ± 3.3	11.0 ± 0.4	2.94 ± 1.37	3.50 ± 1.37	0.90 ± 0.11	0.53 ± 0.07	1.87 ± 0.45	0.46 ± 0.09	8.11 ± 2.23	6.27 ± 0.31	0.88 ± 0.09
25	296.5 ± 8.2	13.5 ± 0.5	6.71 ± 1.48	6.52 ± 0.96	0.77 ± 0.10	0.57 ± 0.10	1.79 ± 0.28	**1.27 ± 0.62**	**11.21 ± 1.71**	5.36 ± 0.40	0.98 ± 0.09
30	294.3 ± 5.9	11.9 ± 0.3	4.97 ± 1.43	5.63 ± 1.28	0.87 ± 0.18	0.60 ± 0.15	2.76 ± 0.61	1.16 ± 0.32	10.06 ± 1.40	6.59 ± 0.37	0.89 ± 0.06
35	306.0 ± 8.9	12.1 ± 0.3	6.21 ± 1.17	6.22 ± 1.03	0.89 ± 0.12	0.98 ± 0.15	1.79 ± 0.16	0.91 ± 0.18	9.30 ± 1.58	5.59 ± 0.36	0.69 ± 0.08
Female quail maintained on long days											
Long day	283.6 ± 8.0	13.8 ± 0.3	7.69 ± 0.55	8.25 ± 0.45	0.72 ± 0.08	1.04 ± 0.10	2.52 ± 0.45	0.85 ± 0.06	13.80 ± 2.72	6.27 ± 0.20	0.71 ± 0.07

Female quail transferred from long days to short days

Day	Body weight (g)	Cloacal opening (mm)	Ovary weight (g)	Oviduct weight (g)	LH (ng/ml)	FSH (ng/ml)	Estradiol (ng/ml)	P_4 (ng/ml)	PRL (ng/ml)	T4 ng/ml	T3 ng/ml
0	265.3 ± 12.2	11.9 ± 0.4	9.59 ± 0.76	8.43 ± 0.49	0.66 ± 0.09	0.88 ± 0.14	2.01 ± 0.36	1.13 ± 0.09	12.67 ± 1.17	5.37 ± 0.57	0.61 ± 0.09
5	272.6 ± 7.0	12.5 ± 0.4	8.09 ± 0.77	8.24 ± 0.73	0.77 ± 0.08	1.03 ± 0.15	1.87 ± 0.21	1.51 ± 0.21	10.85 ± 1.87	5.28 ± 0.40	1.12 ± 0.20
12	247.7 ± 11.9	11.3 ± 0.3	**1.97 ± 1.07**	**2.79 ± 1.01**	**0.39 ± 0.11**	**0.48 ± 0.16**	**0.67 ± 0.09**	**0.55 ± 0.16**	**4.06 ± 0.24**	4.80 ± 0.55	**1.51 ± 0.34**
15	**238.2 ± 3.9**	11.0 ± 0.2	0.45 ± 0.18	1.08 ± 0.16	0.14 ± 0.03	0.23 ± 0.03	0.52 ± 0.04	0.34 ± 0.06	5.97 ± 0.33	6.24 ± 1.67	1.31 ± 0.19
20	234.6 ± 4.4	10.5 ± 0.5	1.01 ± 0.79	1.34 ± 0.71	0.19 ± 0.06	0.27 ± 0.03	0.77 ± 0.32	0.42 ± 0.13	6.59 ± 1.13	6.15 ± 1.33	1.25 ± 0.22
25	235.9 ± 7.4	**9.6 ± 0.5**	0.23 ± 0.03	0.47 ± 0.06	0.10 ± 0.02	0.22 ± 0.03	0.41 ± 0.03	0.16 ± 0.02	5.15 ± 0.91	5.41 ± 0.62	1.49 ± 0.20
30	227.5 ± 5.4	9.0 ± 0.3	0.18 ± 0.02	0.38 ± 0.07	0.16 ± 0.06	0.20 ± 0.03	0.37 ± 0.04	0.22 ± 0.02	4.67 ± 0.63	4.19 ± 0.64	1.22 ± 0.22
35	243.9 ± 8.2	9.6 ± 0.5	1.17 ± 0.98	1.28 ± 0.93	0.43 ± 0.16	0.31 ± 0.07	0.83 ± 0.29	0.60 ± 0.33	4.53 ± 0.67	4.51 ± 036	1.40 ± 0.09
Female quail maintained on short days											
Short day	257.1 ± 10.4	8.3 ± 0.2	0.17 ± 0.02	0.06 ± 0.01	0.26 ± 0.05	0.19 ± 0.02	0.56 ± 0.06	0.25 ± 0.03	3.69 ± 0.19	5.06 ± 0.58	0.94 ± 0.15

3.4 Discussion

This is the first experiment in which the patterns and rates of gonadal growth and regression and changes in gonadal, pituitary and thyroid hormones have been measured together in male or female Japanese quail transferred from short days at 10 °C to long days at 20 °C and quail transferred from long days at 20 °C to short days at 10 °C.

3.4.1 Testicular growth in male Japanese quail

Testicular growth in quail occurs when the seminiferous tubules increase in both diameter, reflecting a thickening of the seminiferous epithelium, and in length (Follett and Maung, 1978). Quail held indoors under an artificial short photoperiod show a biphasic pattern of testicular growth when exposed to an abrupt change to a photoperiod long enough to stimulate rapid gonadal growth (when photoperiod exceeds 11.5 hours of light per day; Nicholls *et al.*, 1973; Gibson *et al.*, 1975; Follett, 1976; Follett and Maung, 1978; Follett *et al.*, 1977, 1981; Follett and Robinson, 1980; Creighton and Follett, 1987; Perera and Follett, 1992). Testicular growth is initiated shortly after the increase in photoperiod with rapid growth over several weeks followed by one or more weeks in which mean testicular weight gradually increases. The rate of testicular growth during the rapid growth period was markedly higher in the present study than in studies with other strains of Japanese quail (Table 3.49).

Table 3.49. Testicular growth rates for male Japanese quail transferred to photoperiods ≥ 16 h light per day.

Author	Year	Strain of Japanese quail	*k*	95 % confidence levels
Follett and Farner	1966	Pullman	0.1000	0.0940 - 0.1060
Follett and Sharp	1969	S7	0.1750	0.1660 - 0.1850
Nicholls et al	1973	S7	0.1340	0.1210 - 0.1460
Follett and Maung	1978	S7	0.1152	0.0926 - 0.1378
Henare	Present study	New Zealand	0.2599	0.2018 - 0.3180

Quail used in New Zealand come from commercial stocks that have been selected for increased body weight for meat production. The mean weight of sexually mature quail is 270 – 300 g whereas quail of the Pullman and S7 strains were less than half this weight. There is a corresponding difference in mean adult testis weight (NZ strain 6 - 7 g compared to Pullman and S7 strains 3 – 4 g) so the approximately doubled testicular

growth rate of the New Zealand strain of Japanese quail is proportional to their greater body and testicular sizes.

The three week period of testicular maturation for the New Zealand strain of quail is similar to that reported by Bacon and Nestor (1975) and one to two weeks shorter than those of other studies (Wilson *et al.,* 1962; Gibson *et al.,* 1975; Nicholls *et al.,* 1973; Follett, 1976; 1978; Gledhill and Follett, 1976; Follett *et al.,* 1977).

The cloacal gland is an androgen dependent structure stimulated by testosterone, androstenedione and 5 α-dihydrotestosterone (Massa *et al.,* 1980). The size of the gland is considered to indicate testis size and hence it has been used as a convenient external measure of gonadal responses to changes in photoperiod (Sachs, 1969; Siopes and Wilson, 1975; Follett and Maung, 1978; Oishi and Konishi, 1983; Robinson and Follett, 1982). Cloacal gland area was strongly correlated with testis weight and testosterone, confirming the utility of gland area for estimation of testis size in the New Zealand strain of quail. There was a moderate correlation between FSH but not LH and testis weight, as found by Follett (1976) and Follett and Milette (1982).

The general pattern of LH secretion in photostimulated birds in this study was similar to previous reports for male and female quail (Nicholls *et al.,* 1973; Gibson *et al.*1975; Follett, 1976; Follett and Maung, 1978; Follett *et al.,* 1977, 1981; Follett and Robinson, 1980; Creighton and Follett, 1987; Perera and Follett, 1992), duck (Balthazart *et al.,* 1977), white crowned sparrow (Follett *et al.,* 1975; Wingfield and Farner 1980; Wingfield *et al.,* 1980), tree sparrow (Wilson and Follett, 1974), willow ptarmigan (Stokkan and Sharp, 1980) and turkey hens (Gooden and Scanes, 1977; El Halawani *et al.,*1984a). In all these species exposure to photoperiods longer than 11.5 hours of light per day cause LH concentrations to increase substantially. In some of these experiments LH concentrations increased and remained stable whilst in others LH rose, declined after the initial increase then increased to earlier peak values. In the present study mean LH concentrations decreased after an initial rise then increased, although not to the earlier peak values. This temporary decline in LH occurred when testosterone concentrations were increasing and may have been due to the initiation of androgen feedback on the hypothalamo-hypophysial-gonadal axis. It has been suggested that hypothalamic sensitivity to testosterone negative feedback decrease as testosterone

increases, thus allowing the maintenance of a constant LH secretion rate (Davies *et al.*, 1976).

LH varied markedly between quail during the first 20 days of photostimulation, as reported in other studies (Nicholls *et al.*, 1973; Gibson *et al.*, 1975; Follett *et al.*, 1977; Follett and Maung, 1978). As in mammals, LH secretion in birds has been shown to be pulsatile and it is possible that sampling in some individuals in the present study occurred between LH pulses. Ottinger (1983) reported episodic variations in peripheral LH at a rate of 6-10 episodes every 12 hours in male Japanese quail and Urbanski (1984) reported large amplitude pulses every 83 minutes (average) in photostimulated castrated males. This however, cannot be the complete explanation for individual variation as some stimulated birds always show a low concentration of LH while in others it is invariably high (Follett *et al.*, 1975).

FSH secretion increased in a similar fashion to LH, with a significant rise seen on day five. However, in contrast to LH, FSH continued to increase to a peak at day 10, declined to day 20 then remained constant. The same pattern of FSH was found by Follett and Maung (1978). FSH concentrations in this study were much lower than those measured by Follett and Maung (1978) due to the higher specificity of the FSH assay used for the New Zealand strain of quail.

It is not known why the decrease in FSH concentrations occurs during photostimulation. The decrease in FSH could be due to increased androgen feedback, as suggested for LH. This proposal is supported by the fact that FSH concentrations in photostimulated castrated male quail do not show this decline (Follett, 1976; Urbanski and Follett, 1982a). The decline in gonadotropin secretion could also be due, at least in part, to an avian inhibin. Recently a dodecapeptide designated gonadotropin-inhibitory hormone (GnIH) was isolated from the Japanese quail brain (Tsutsui *et al.*, 2000). GnIH has been shown to reduce gonadotrophin release from cultured anterior pituitary cells of adult birds (Ubuka *et al.*, 2003). Further investigation is required to clarify the role of GnIH during photo-induced gonadal growth in birds and the possible interaction between GnIH and gonadal steroids during this process.

FSH and LH concentrations were not related during gonadal growth in this study. Gledhill and Follett (1976) reported that both intact and gonadectomized male quail with high FSH levels invariably had high LH levels. The difference between of the two studies may be due to the fact Gledhill and Follett analysed LH and FSH concentrations on one day (day 43 in castrates, day 12 in intact birds) when gonadotrophin concentrations were elevated whereas in this study hormones were analysed across 35 days of photostimulation.

Testosterone increased markedly after the LH and FSH peaks and remained high thereafter, as found by Follett (1976; 1981) and Follett and Maung, (1978) although the magnitude of the testosterone response was more than two times greater in this study. Testosterone concentrations in this study were however similar to those previously measured in the New Zealand strain of Japanese quail (Hawke, 2002; Chua, 2003).

There was considerable variation in testosterone concentrations in birds with large testes. Testosterone concentrations were not strongly related to testis weight in this study or those of Hawke (2002) or Chua (2003), although strong relationships have been found in many species of birds including Japanese quail (Follett and Robinson, 1980; Wingfield *et al.,* 1987). Conversely Wingfield *et al.,* (1987) reported that testosterone concentrations can fluctuate during the breeding cycle whilst the testes remain large. It is possible that low testosterone concentrations in long day males with large testes could result from increased metabolic clearance rates of androgens without a change in hormone secretion as seen in song sparrows by Wingfield (1984) who reported a decline in testosterone concentrations despite high circulating concentrations of LH. Additionally, Ottinger and Follett (1987) reported that plasma testosterone remained elevated for longer after injection in the afternoon than the morning, indicating that the clearance rate for testosterone could change even within one day.

The photoperiodic control of prolactin secretion is generally similar to that in mammals in that it is stimulated by long photoperiods and inhibited by short photoperiods (Dawson *et al.,* 2001). Gonadotropins and prolactin secretion increase immediately after birds are transferred to long days, but the rate of increase of prolactin lags behind that of gonadotropins (Dawson and Goldsmith, 1982; Sharp *et al.,* 1998). In this study

prolactin secretion did not show an overall increase in concentrations during photostimulation.

Thyroid function interacts with gonadal function in birds but the mechanisms underlying these interactions remain unknown. Patterns of thyroid hormone secretion have been described in birds maintained under a variety of environmental conditions. The increase in T4 observed after quail were transferred from short days to long days is consistent with the view that long days directly stimulate the hypothalamo-hypophysial-thyroidal axis (Follett and Riley, 1967; Chandola and Thapliyal, 1973; Sharp and Klandorf, 1981, Klandorf *et al.,* 1982; Decuypere and Kuhn, 1988; Wilson and Reinert, 1995, 1996; Bentley *et al.,* 1997). Treating birds with moderate doses of T4 has been shown to cause similar effects to exposure to long photoperiods (Follett and Nicholls, 1985; 1988; Goldsmith and Nicholls, 1992) while excessive T4 leads to testicular regression (Peczely *et al.,* 1979). In contrast T3 infused into the third ventricle by an osmotic minipump induced testicular growth in Japanese quail maintained on short days whereas T4 infusion had only a minor effect (Yoshimura *et al.,* 2003). The differences between the studies may be due to the site of hormone administration. However there is a high conversion rate of T4 to T3 in birds and it is possible that in the studies by Follett *et al.* (1988) and Goldsmith and Nicholls, (1992) T4 was converted to T3. This however does not explain the results Peczely *et al.* (1979) obtained with a large dose of T4.

Thyroid hormone concentrations are also regulated by additional factors such as food intake (Klandorf *et al.,* 1981). Patterns of food intake are a key factor influencing T4 to T3 conversion and in diurnal patterns of thyroid hormone secretion. As food intake was not measured in the present work it is not possible to determine whether a change in food intake and metabolic rate contributed to the changes seen in thyroid hormone concentrations when quail were transferred to long or short daylengths.

The mechanism responsible for the increase in T4 seen in this study is unknown. In Tree sparrows it has been suggested that the increase in T4 following photostimulation serves to program the subsequent cycle of gonadal growth, regression and moult (Reinert and Wilson, 1996). However Bentley *et al.* (1997) found when they maintained T4 concentrations at subphysiological levels by treating thyroidectomized

starlings with T4 the birds became photorefractory and moulted, thus suggesting that T4 is a permissive factor which only has to be present for the appropriate responses to photoperiod to occur.

The fall in the concentration of T3 after transfer to long daylengths seen in this study is similar to findings in turkeys (Lien and Siopes, 1992; 1993) and quail (Sharp and Klandorf, 1981) exposed to artificial long daylengths. Additionally T3 has been reported to decrease in some birds transferred from low to high temperatures in quail (Klandorf *et al.*, 1981), chickens (May, 1978) and cockerels (Cogburn and Harrison, 1980).

Thyroid hormone concentrations during gonadal growth and regression may be due to changing gonadal steroid concentrations. During photostimulation the rate of increase of T4 in intact quail declines as androgen concentrations increase, raising the possibility that gonadal steroids could affect thyroid activity. This suggestion is supported by the finding that injections of testosterone depress thyroid function in ducks and quail, possibly by inhibiting pituitary secretion of thyroid stimulating hormone (TSH) (Sharp and Klandorf, 1981). However clear relationships between gonadal steroids and thyroid function are not always evident in birds.

3.4.2 Testicular regression in male Japanese quail

Testicular regression in quail is characterised by the accumulation of cellular debris within the lumen of the seminiferous tubules followed by shrinkage of the tubules within the first 10 days of light reduction (Eroschenko and Wilson, 1974). Cessation of spermatogenesis is followed by the complete collapse of the seminiferous tubules, with a simultaneous decline in size of the epithelial cells of the cloacal gland (Sachs, 1967).

In the present study exposure to short days and low temperatures led to a significant decrease in testicular weight and cloacal gland area and the cessation of foam production after 10 short days. Gonadal regression followed a biphasic pattern where testes weight decreased in a log-linear fashion between days 0 - 20 and declined slowly thereafter. Some quail did not undergo full testicular regression whereas regression was complete after 20 short days in the majority of quail. Similar patterns of regression

have been reported in previous studies of quail (Sachs, 1967; Follett and Nicholls, 1984; Delville *et al.,* 1985; Wada *et al.,* 1990). Rates of gonadal regression vary between individual birds and between strains of quail. Some strains have been selected for strong photoperiodic responses with complete gonadal regression after 21 - 32 short days in all birds (Nicholls *et al.,* 1973; Follett *et al.,* 1977; Robinson and Follett, 1982). The New Zealand quail have been selected for increased body weight for meat production and their photoperiodic response is not as strong as that of the Follett quail. Quail used for research in Japan also come from commercial strains that have not been selected for photoperiodic responses. Oishi and Konishi (1983) classified quail according to their cloacal gland response to changing photoperiod. 67 % of birds (type I) did not undergo gonadal or cloacal gland regression on short days. 18 % of birds (type II) exhibited partially regressed cloacal glands when transferred to short days. 15 % (type III) exhibited full cloacal gland growth and regression when exposed to alternating long and short photoperiods respectively.

There was a high correlation between testis weight and cloacal gland area during photoperiod induced regression, in agreement with Sachs (1967) and Siopes and Wilson (1975). There was also a high correlation between paired testis weights and LH and a low correlation between paired testis and FSH.

LH in sexually mature male quail transferred to short days rapidly declined and after 10 short days was similar to concentrations in short day controls. These results are consistent with previously described responses of quail transferred from long to short days (Nicholls *et al.,* 1973; Gledhill and Follett, 1976; Follett *et al.,* 1981; Robinson and Follett, 1982; Urbanski and Follett, 1982) and transferred from long days at high temperatures to short days at low temperatures (Wada *et al.,* 1990; Tsuyoshi and Wada, 1992; Wada, 1993). FSH also decreased rapidly within five days and gradually thereafter.

The persistence of high concentrations of gonadotrophins, particularly LH on transfer to short days is known as "carry-over" and is thought to reflect a delay by the hypothalamo-hypophysial system in reducing GnRH secretion since deafferentiation of the hypothalamus leads to the decrease of circulating LH within one day (Davies and Follett, 1975). In this study the duration of gonadotrophin "carry-over" varied from

individual to individual with at least one bird on each day of sampling having elevated hormone concentrations.

The transfer of male quail from long to short days resulted in a decrease in mean testosterone of more than 50 % after 10 short days and low concentrations in almost all birds after 15 days. These results are in agreement with those reported by Delville *et al.* (1985) who also observed a 50 % decrease in plasma testosterone within one week of mature male quail being transferred to short days. However, testosterone concentrations spontaneously increased after 40 days of short days in that strain of quail with no change in photoperiod length.

Testosterone showed strong relationships with LH, paired testis weight and cloacal gland area but not FSH during gonadal regression. This is consistent with LH being the primary steroidogenic hormone in the male bird, with FSH playing a minor role, if any, in the acute release of testosterone from the mature testis (Maung and Follett, 1978).

Although there were large variations between birds in prolactin concentrations, there was generally a decline of mean prolactin concentrations after transfer to short days. These results are similar to those of Goldsmith and Hall (1980) and Goldsmith and Nicholls (1984a) where prolactin levels decreased in mature male quail and starlings, undergoing short day-induced gonadal regression.

Thyroid hormones are involved in photorefractoriness and gonadal regression in many bird species (Follett and Nicholls, 1984; Goldsmith and Nicholls, 1984b). In Japanese quail short days do not induce gonadal regression in thyroidectomized birds unless they have been treated with exogenous T4 (Follett and Nicholls, 1985) indicating that thyroid hormones are necessary for gonadal regression. In the present study T4 declined then remained relatively constant followed by a gradual increase in T3 concentrations in birds moved from long to short days. The increase in T3 is similar to those of Oishi and Konishi (1978), Tsuyoshi and Wada (1992) and Wada (1993) however these studies also reported no change in T4 in quail on long or short photoperiods with either high or low temperatures. In quail cold temperatures have been shown to decrease the plasma half-life of T4 (McFarland *et al.*, 1966). The decreased T4 half-life is consistent with

increased T4 conversion to T3, which is a key part of the metabolic response to cold in birds (Rudas and Pethes, 1984).

Wada (1993) suggested that in addition to photoperiodic cues, the termination of reproduction in annual cycles of wild birds is also influenced by environmental cues such as temperature. Several studies by Wada and colleagues have shown that short days cause LH concentrations in Japanese quail to decrease but not to concentrations where gonadal regression is induced. The addition of low temperatures and the resulting increase in T3 concentrations cause the complete suppression of LH to non-breeding concentrations and gonadal regression. This effect may be mediated via reduced secretion of GnRH as T4 treatment of starlings results in a decrease in hypothalamic GnRH content (Boulakoud *et al.,* 1991). Several studies have shown a clear inverse relationship between LH and T3 but not LH and T4 (Sharp and Klandorf, 1981; Wada *et al.,* 1990) whereas in the present study there was a weak relationship between LH and T4 but not LH and T3.

Molt refers to the orderly replacement of feathers which generally occurs in female birds at a time of gonadal regression (Johnson, 2000). Little is known about the physiological factors that control molt, although hormones of the pituitary, adrenal and thyroid glands are implicated. In domestic fowl forced molt can be induced by restriction of feed and water, decreased daylength or by administration of prolactin, T4 or large doses of P_4 (Johnson, 2000). Molt associated with the end of breeding in seasonal birds has been associated with high prolactin concentrations in some studies (Dawson and Goldsmith, 1982; Lien and Siopes, 1993; Boswell *et al.,* 1996). It also coincided with changes in plasma thyroid hormone levels (McNabb, 2000). In the present study molt was first detected in male quail transferred to short days on day 14 when paired testis weight and gonadotrophin, testosterone, prolactin and T4 concentrations had significantly decreased and T3 increased.

3.4.3 Ovarian growth in female Japanese quail

In general little attention has been given to the photoperiodic control of ovarian development in birds as ovarian weights do not appear to increase with the same quantitative uniformity as do testicular weights (Follett and Farner, 1966) and the onset

of sexual maturity is reported to be slower in females compared to males (Tanaka *et al.*, 1964). Ovarian growth involves increases in the numbers and sizes of follicles. After five long days small white follicles could be distinguished with yolk filled follicles observed after 12 days and large yolky follicles after 15 days, as found by Davies (1980) and Sharp and Klandorf (1981). Although there were two distinct classes of ovary weights observed during ovarian growth or regression, relationships between ovary weights and the other measured variables were determined across the full range of ovary weights. The increase in the weight of the oviduct in photostimulated quail closely parallelled the increase in ovarian weight, as seen in female quail raised on 16 L:8 D (Brain *et al.*, 1988). The diameter of the cloacal opening showed strong relationships with ovary and oviduct weights, confirming the value of this measure as a useful external indicator of ovarian and oviductal development in quail (Bennett, 2002).

Quail began laying eggs after 15 long days with 22 % of birds laying after 18 long days. These results are similar to those of Gibson *et al.* (1975) who reported that Japanese quail transferred to 20L:4D laid eggs after 12-18 days of photostimulation. Ovarian growth rate in the current study was greater than that reported by Follett and Farner (1966). As discussed for the male quail, the body and ovarian weights of the female Japanese quail of the New Zealand strain is approximately twice that of the S7 (United Kingdom) strain, therefore the greater ovarian growth rate is proportional to the greater body and ovarian weights.

LH rose after five days in female Japanese quail transferred to long days then declined over the next week to concentrations similar to those of long day controls. Similar patterns of LH have been reported in female and male Japanese quail (Nicholls *et al.*, 1973; Gibson *et al.*, 1975), layer strains of domestic pullets (Lewis *et al.*, 1998) and other birds (Sharp, 1975; Williams and Sharp, 1977; Lewis *et al.*, 1998). LH concentrations continued to increase in ovariectomized quail moved to long days (Gibson *et al.*, 1975; Sharp and Klandorf, 1981), so the observed LH decline when ovarian steroids were increasing, could be due to feedback on the hypothalamo-hypophysial system. Elevated estradiol concentrations inhibit LH secretion (Davies and Follett, 1980) whilst P_4 concentrations can both stimulate or inhibit LH secretion depending upon the stage of the ovulatory cycle (Etches, 1990). Additionally the recently discovered avian inhibin GnIH may play an important role in the changes

which occur in gonadotrophin secretion as previously discussed for male quail transferred to long days.

Individual variation in LH levels was greater during the beginning of photostimulation than later, as seen in the male quail transferred to long days. As in male quail, LH secretion in females has been shown to be pulsatile with episodic variations in peripheral LH reported to be at a rate of 4 – 8 pulses per 24 hours (Gledhill and Follett, 1976). It is possible sampling occurred during such pulses in some birds. It has been reported that on exposure to artificial long daylengths higher concentrations of LH are measured in intact male than female birds in several species including quail (Nicholls *et al.,* 1973; Gibson *et al.,* 1975; Davies, 1976; Urbanski and Follett, 1982b) and chickens (Sharp, 1975). Davies (1976) and Gibson *et al.* (1975) suggested that the higher LH concentrations in male quail were due to sex differences in sex steroid feedback as LH was similar in gonadectomized male and female quail exposed to long days for the first time. However Urbanski and Follett (1982a) showed that in quail that had been through a breeding cycle and then gonadectomized, photostimulation resulted in higher LH concentrations in males compared to females, as seen in intact birds. The contrasting findings may be due to the timing of gonadectomy in relation to the onset of photostimulation or the fact that quail studied by Urbanski had previously been exposed to both high pituitary and plasma gonadotrophin concentrations.

FSH increased gradually during photostimulation, as seen in female White crowned sparrows (Wingfield *et al.,* 1997). Unlike the decrease in FSH in males as the testes grew, FSH in female quail remained high during gonadal growth

It is highly likely that the ovaries are the main source of circulating steroid hormones in female Japanese quail. *In vitro* studies have shown that the small prehierarchal yolk filled follicles produce estrogens together with smaller amounts of P_4 and T, particularly when stimulated by FSH (Kumagai and Homma, 1974; Onagbesan and Peddie, 1988). It is therefore not surprising to find increasing estradiol concentrations in maturing quail. However Peczely *et al.* (1980) reported that photostimulation of ovariectomized Japanese quail increased plasma concentrations of sex steroids thought to be products of the adrenal gland.

The general pattern of P_4 increase seen during photostimulation in female quail was similar to that seen in chickens where P_4 rose substantially during the week before the onset of lay and reached variable high concentrations in mature birds (Williams and Sharp, 1977). In the present study P_4 concentrations gradually increased during photostimulation with maximum concentrations observed when there were many large ovarian follicles with diameters greater than 14 mm. These findings are generally similar to those reported by Brain *et al.*, (1988) who measured maximal progesterone values when ovarian follicles were greater than 12 mm. *In vitro* studies show that the cells of the largest yolk filled follicles secrete the majority of P_4 seen in the peripheral circulation of birds, particularly when stimulated by LH (Mori *et al.*, 1984; Assem *et al.*, 1985; Onagbesan and Peddie, 1988).

Although prolactin secretion generally increases with long daylengths and decreases with short daylengths, the role of prolactin in sexual development is not clear in birds. Treatment with exogenous prolactin has resulted in stimulation or inhibition of gonadal maturation in different species and experiments (for review see de Vlaming, 1979). The gradual increase in prolactin concentrations associated with photostimulation in the present experiment was similar to reports in fowl (Scanes *et al.*, 1976) and turkey hens (Scanes *et al.*, 1979; Burke and Dennison, 1980; Gahali *et al.*, 2001) and in male starlings where a prolactin increase lagged behind the increases in gonadotrophins (Dawson and Goldsmith, 1983). Prolactin levels in the present study were higher in laying than non laying quail. This result agrees with findings in Japanese quail (Goldsmith and Hall, 1980), turkey hens (Scanes *et al.*, 1979) and bantam fowl (Sharp *et al.*, 1979). The higher prolactin concentrations in laying quail in the present study were not due to the presence of an accumulating clutch of eggs as seen in birds able to incubate (Lea *et al.*, 1981) as eggs were removed every day. The high prolactin concentrations may reflect a potentiating effect of ovarian produced estrogens on the secretion of prolactin from the adenohypophysis (El Halawani *et al.*, 1983; Hall and Chadwick, 1984).

Female birds transferred to photostimulatory daylengths generally show an increase in T4 which is subsequently depressed when the secretion of the gonadal steroids are increasing (Follett and Riley, 1967; Peczely, 1976; Scanes *et al.*, 1979; Peczely *et al.*, 1980; Sharp and Klandorf, 1981; Wingfield *et al.*, 1997) whereas in the present study

there was no clear pattern of T4 secretion in females. In the absence of gonads, T4 concentrations have been reported to increase after photostimulation and decrease after transfer to short days closely following the pattern of LH secretion (Sharp and Klandorf, 1981). This pattern of secretion suggests the changes in T4 secretion may be dependent upon daylength-induced changes in the secretion of TRH and/or TSH.

The decline in the concentration of T3 after transfer to long days seen in females in this study was similar to the findings in male quail in this study and to a previous study in female quail (Sharp and Klandorf, 1981). The decrease in T3 concentrations after five days of photostimulation occurred when gonadotrophin levels were high and ovarian growth was occurring.

3.4.4 Ovarian regression in female Japanese quail

There have been limited studies in which changes in ovary size have been described in female quail moved from long to short days. In the present study transfer to short days and low temperatures led to almost complete ovarian and oviductal regression after 12 days in the great majority of quail. A similar time course for ovarian regression was described by Sharp and Klandorf (1981) for quail transferred to 6L: 18D. Egg laying ceased in the majority of quail within three weeks. The rate of decline in egg production was similar to that seen in Japanese quail by Gibson *et al.* (1975), somewhat slower than those observed in previous studies where egg laying stopped after approximately one to two weeks of short daylengths (Wilson *et al.*, 1971; Bacon *et al.*, 1973; Stein and Bacon, 1976; Urbanski and Follett, 1982b) and faster than that in quail transferred to 8 L:16 D observed by Goldsmith and Hall (1980) where birds ceased laying after 50 short days. Complete gonadal regression occurred in virtually all quail within four weeks, although a few birds were still laying eggs after 35 days. These findings agree with histological and morphological studies by Eroschenko and Wilson (1974) and Davies (1980).

LH and FSH remained constant for five days, declined steadily then remained relatively constant after 12 short days. These patterns were similar to those in males and those reported previously for female Japanese quail (Gibson *et al.*, 1975; Davies, 1980; Sharp and Klandorf, 1981) and layer strains of domestic pullets (Lewis *et al.*, 1998). The

persistence of the high concentrations of LH and FSH in female birds for five days after transfer to short days is similar to the pattern seen in male Japanese quail and supports the notion that there is a delay in the reduction of long day induced GnRH synthesis and/or secretion. The time course for reductions in LH and FSH was very similar to those for ovary weight and ovarian steroid concentrations.

Prolactin concentrations were generally low after 12 or more short days, as seen in male Japanese quail in the present study. These results are consistent with a decrease in pituitary prolactin content during gonadal regression in Japanese quail transferred to short daily photoperiods (Goldsmith and Hall, 1980).

There was no clear pattern of T4 secretion in female quail transferred to short days whereas T4 decreased after five days in male quail and T3 increased in both sexes.

In female quail transferred to short days molt was first detected on day 11. Ovarian steroids and prolactin concentrations were lower in molting non-laying females compared to laying females. These findings are in agreement with those seen in domestic and turkey hens (Scanes *et al.*, 1979; Proudman and Opel, 1981). Decreases in estrogens are thought to be important in the initiation of molt and an increase in the thyroid hormone/estrogen ratio has been suggested to be important in new feather formation (for review see Decuypere and Verheyen, 1986). The increase in T3 concentrations and decrease in estradiol in this study support this suggestion.

3.5 Conclusion

This is the first study in which gonadal and endocrine changes have been comprehensively described for Japanese quail of both sexes during photoperiodically induced gonadal growth and regression. These results will be valuable for future studies of the photoperiodic control of reproduction in quail. They also provide crucial information on gonadal growth to serve as a reference for studies in which quail will be treated with GnRH with the aim of stimulating gonadal growth.

4 Effect of a single GnRH injection on LH concentrations and effect of daily GnRH injections on LH concentrations and ovarian development

4.1 Introduction

Gonadotropin releasing hormone (GnRH) is used in human reproduction programmes as well as in the treatment of reproduction disorders and the control of reproduction in farm animals. As previously discussed in chapter one the avian hypothalamus contains two forms of GnRH, cGnRH-I and cGnRH-II. Studies in several avian species have demonstrated that both forms stimulate the release of LH and FSH (Millar and King, 1984; Chou *et al.*, 1985; Dunn *et al.*, 1986; Millar *et al.*, 1986; Sharp *et al.*, 1986; 1987; Wilson *et al.*, 1989), with the gonadotropin response to exogenous GnRH administration being both rapid and short-lived.

During the last 20 years efforts focussed on increasing the GnRH-induced gonadotropin response have resulted in the development of superactive analogues. These analogues are based on the conformation of mGnRH, cGnRH-I or cGnRH-II and are designed to induce a more potent stimulatory action than the original GnRH peptide. Several analogues that incorporate combinations of amino acids in specific positions, particularly the incorporation of a D-amino acid in position six with various substitutions in positions 7 – 10, have been investigated in birds (Delobelle *et al.*, 1995). The increased activity of some of these analogues suggests a potential and promising application for use in the financially important domestic poultry industry.

Another potential method to prolong GnRH-induced gonadotropin secretion is to dissolve the GnRH in a polymer that possesses properties that allow it to bind and stabilise molecules and thus prolong their clearance rate when injected *in vivo*. One

such polymer is polyvinylpyrrolidone (PVP), which is a synthetic organic polymer that is soluble in water and organic solvents. PVP has been used in cattle and ewes to prolong the action of exogenous FSH in order to induce superovulation with a single injection (Takedomi *et al.*, 1993; Yamamoto *et al.*, 1993; Satoh *et al.*, 1996; Sugano and Shinogi, 1999; D'Alessandro *et al.*, 2001). Previous programmes used to induce superovulation in cattle and ewes used multiple injection regimes that were both economically disadvantageous and stressful for the animals. In birds, the effectiveness of either analogues or GnRH dissolved in PVP for the stimulation of enhanced LH secretion and ovarian development has not been fully investigated and knowledge is limited.

The aims of the current experiments were to determine the LH response of Japanese quail to different forms of GnRH, and to determine whether daily injections of a GnRH solution dissolved in saline or PVP would increase baseline LH and FSH concentrations resulting in the induction of ovarian development.

4.2 Materials and Methods

4.2.1 Animals and housing

Seven week old sexually mature female Japanese quail (*Coturnix coturnix japonica*) with wildtype plumage were purchased from a commercial source (Rangitikei Game Birds, Bulls, New Zealand). Before the start of the experiments quail were identified and housed as described in section 3.2.1.

Birds were held under a long day photoperiod until they reached eight weeks of age. This treatment allowed egg production to be recorded daily and to ensure that all birds were sexually mature. At eight weeks of age all birds were transferred to a short day photoperiod for five weeks. This treatment caused the regression of the reproductive system. Sexually regressed birds were considered to be those that had not laid eggs for more than 10 days and had cloacal diameters less than 9.0 mm.

4.2.2 Experimental Design

Experiments one and two were conducted concurrently.

4.2.2.1 Experiment one: Acute LH response to a single subcutaneous cGnRH-I, cGnRH-II or buserelin injection in sexually regressed female Japanese quail

Fourteen week old female Japanese quail were divided into 15 groups. These groups received either a single subcutaneous injection of saline or a GnRH solution as shown in Table 4.1. Data from the literature were used to choose GnRH doses used in these studies.

Table 4.1. Experimental groups for experiment one.

Group number	n	Type of GnRH	Solution concentration (µg/kg)	Dose per injection (µg/bird)
1	6	Saline	-	-
2	8	Buserelin	0.5	0.125
3	8	Buserelin	1.0	0.250
4	8	Buserelin	2.5	0.625
5	8	Buserelin	4.0	1.000
6	8	cGnRH-I	0.25	0.063
7	8	cGnRH-I	0.5	0.125
8	8	cGnRH-I	1.0	0.250
9	8	cGnRH-I	2.5	0.625
10	8	cGnRH-I	5.0	1.250
11	8	cGnRH-II	0.25	0.063
12	8	cGnRH-II	0.5	0.125
13	8	cGnRH-II	1.0	0.250
14	8	cGnRH-II	2.5	0.625
15	8	cGnRH-II	5.0	1.250

Blood samples were collected immediately before the injection (time 0 min) and then 5, 10 and 20 minutes after the injection. Plasma concentrations of LH were measured in all blood samples.

4.2.2.2 Experiment two: Acute LH response to a single subcutaneous D-Lys^{6}Trp^{7}Tyr^8-GnRH injection in sexually regressed female Japanese quail

Fourteen week old female Japanese quail were divided into five groups. These groups received either a single subcutaneous injection of saline or a GnRH solution as shown in Table 4.2.

Table 4.2. Experimental groups for experiment two.

Group number	n	Type of GnRH	Solution concentration (µg/kg)	Dose per injection (µg/bird)
1	6	Saline	-	-
2	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.2	0.060
3	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH	1.0	0.250
4	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.5	0.625
5	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH	5.0	1.250

Blood samples were collected immediately before the injection (time 0 min) and then 5, 10 and 20 minutes after the injection. Plasma concentrations of LH were measured in all blood samples.

4.2.2.3 Experiment three: Daily injections of cGnRH-II or D-Lys^{6}Trp^{7}Tyr^8-GnRH injection in either saline or polyvinylpyrrolidone (PVP) for seven days in sexually regressed female Japanese quail

Fourteen week old female Japanese quail were divided into 20 groups. 18 groups received either subcutaneous injections of saline, PVP or a GnRH solution each day for seven days as shown in Table 4.3. The results from experiments one and two were used to choose GnRH peptides and doses for this experiment.

Table 4.3. Experimental groups for experiment three.

Group number	n	Type of GnRH	Solution concentration (µg/kg)	Dose per injection (µg/bird)
1	6	Euthanased on day 0	-	-
2	6	Saline	-	-
3	6	PVP	-	-
4	8	cGnRH-II in saline	0.5	0.125
5	8	cGnRH-II in saline	1.0	0.250
6	8	cGnRH-II in saline	2.5	0.625
7	8	cGnRH-II in saline	5.0	1.250
8	8	cGnRH-II in PVP	0.5	0.125
9	8	cGnRH-II in PVP	1.0	0.250
10	8	CGnRH-I in PVP	2.5	0.6250
11	8	cGnRH-II in PVP	5.0	1.250
12	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in saline	0.2	0.060
13	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in saline	1.0	0.250
14	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in saline	2.5	0.625
15	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in saline	5.0	1.250
16	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in PVP	0.2	0.060
17	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in PVP	1.0	0.250
18	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in PVP	2.5	0.625
19	8	D-Lys^{6}Trp^{7}Tyr^8-GnRH in PVP	5.0	1.250
20	6	Transferred to 16L:8D on day 0	-	-

Group one was euthanased at the start of the treatment period to determine the size of the ovary and oviduct prior to treatment. Group 20 was transferred to a 16L:8D photoperiod at 20 ºC on the first day of the treatment period. The amount of oviductal and ovarian development stimulated by this long day group provided a target for the GnRH treatments to achieve. Blood samples were collected and ovaries and oviducts weighed and recorded at the end of the experiment, 24 hours after the last injection. Plasma concentrations of LH were measured in all blood samples.

All experimental procedures were approved by the Massey University Animal Ethics Committee.

4.2.3 Hormone preparation and administration

The GnRH solutions were prepared the day before the experiment and kept in glass flasks at 4 ºC overnight. Injections were administered subcutaneously on the abdomen using a 1 ml insulin syringe and a 27 gauge, ½ inch needle.

4.2.3.1 cGnRH-I preparation and administration

0.5 mg of cGnRH-I (Peninsula Laboratories Inc., California, USA) was dissolved in 25 ml of 0.9 % sterilised, non-pyrogenic saline (Bomac, New Zealand) producing a 25 μg/ml cGnRH-I stock solution. Solutions were made from the stock solution by dilution with saline where necessary, producing doses of 0.25, 0.5, 1, 2.5 or 5 μg/kg body weight in a 200 μl volume injection.

4.2.3.2 cGnRH-II preparation and administration

0.5 mg of cGnRH-II (Peninsula Laboratories Inc., California, USA) was dissolved in 25 ml of 0.9 % sterilised, non-pyrogenic saline producing a 25 μg/ml cGnRH-I stock solution. Solutions were made from the stock solution by dilution with saline or 10 % PVP solution where necessary, producing doses of 0.25, 0.5, 1, 2.5 or 5 μg/kg body weight in a 200 μl volume injection.

4.2.3.3 Buserelin preparation and administration

4 μg/ml buserelin stock solution (Receptal; Intervet, Auckland, New Zealand) was used. Solutions were made from the stock solution by dilution with 0.9 % sterilised, non-pyrogenic saline where necessary, producing doses of 0.5 – 4.0 μg/kg body weight in a 200 μl volume injection.

4.2.3.4 D-Lys^{6}Trp^{7}Tyr^8-GnRH preparation and administration

2.0 mg of D-Lys^{6}Trp^{7}Tyr^8-GnRH (gift from Dr R Millar, Cape Town, South Africa) was dissolved in 100 ml of 0.9 % sterilised, non-pyrogenic saline producing a 20 μg/ml analogue stock solution. Solutions were made from the stock solution by dilution with saline or 10 % PVP solution where necessary, producing doses of 0.2, 1, 2.5 or 5 μg/kg body weight in a 200 μl volume injection.

4.2.4 Data collection

4.2.4.1 Experiments one and two

Blood samples were collected by venipuncture of the brachial vein in the wing with a 27 gauge needle, with 200 μl of blood collected into heparinized capillary tubes. All samples were collected within two minutes from the time the bird was removed from the cage. The blood was stored on ice until centrifugation at 2 000 g for 15 minutes at 4 °C (Heraeus Christ 5000S refrigerated centrifuge). The plasma was removed and stored in 1.5 ml polypropylene Eppendorf tubes and frozen at – 20 °C until assay.

4.2.4.2 Experiment three

Regular measurements (every two – three days) of cloacal opening diameter were made during the experiment as an indicator of the development of the oviduct. Birds were euthanased by stunning followed by decapitation. Trunk blood was collected into heparinized 10 ml polypropylene centrifuge tubes and kept on ice until centrifugation. Samples were centrifuged and stored as described in 4.2.4.1. Ovaries and oviducts were removed and weighed as described in section 3.2.3.2.

4.2.5 Radioimmunoassays

Luteinising hormone (LH) concentrations in plasma samples were determined by radioimmunoassay. Plasma samples were thawed and spun at 14 000 g for five minutes (IEC Micromax ventilated microcentrifuge OM3590) in a 1.5 ml Eppendorf tube to separate lipid from plasma before assay. Clear plasma was removed from underneath the lipid using a glass Hamilton syringe.

Hormone concentrations for samples that measured below the sensitivity of each assay were assigned a value equal to the sensitivity of that assay.

4.2.5.1 Luteinising hormone (LH)

Luteinising hormone concentrations in quail plasma were measured using a double-antibody radioimmunoassay that has previously been used in domestic chickens and fowl (Sharp *et al.*, 1987; 1990). Purified chicken LH (PRC-AE1-s-1; donation from Dr R Talbot) was used to establish this assay in the Massey University laboratory. Iodination was performed by Miss J Candy, (Radioimmunoassay Laboratory, Institute of Veterinary, Animal and Biomedical Sciences, Massey University) following the methodology of (Sharp *et al.*, 1987). Samples were analysed in triplicate in a single assay following the methodology outlined in section 3.2.4.1.

Validations for this assay were determined in this laboratory. The sensitivity of the radioimmunoassay for LH was determined as the hormone concentration at the mean minus two standard deviations from the percentage bound for the zero hormone tubes. The sensitivity of the assay was 0.2 ng/ml (n = 4 assays) of LH in the plasma. Serial dilutions of quail plasma in LH assay buffer (0.5 M phosphate buffer, pH 7.5) were parallel to the LH standard curve (n = 3; Figure 4.1). Recovery of LH added to quail plasma was 89 ± 3.3 % and 94 ± 4.2 %. Solutions of LH in assay buffer at concentrations that gave approximately 20, 50 and 80 % binding on the standard curve were used as high, medium and low quality controls. Intra-assay coefficients of variation for these three samples were 22.04, 14.9 and 9.9 %.

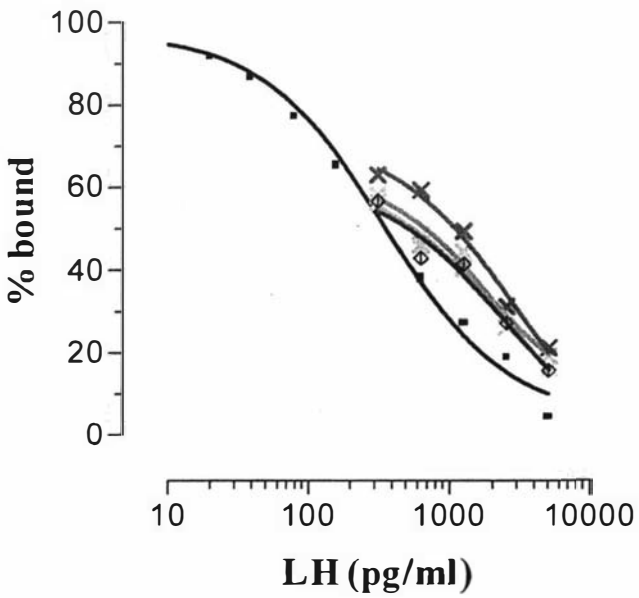

Figure 4.1. Parallelism demonstrated for LH concentrations in Japanese quail plasma samples. The curve with filled squares is the standard curve and all other curves are samples.

4.2.6 Statistics

The experiments were arranged as randomised block designs with treatment (type of GnRH) and dose as the main effects. Birds were placed at random, in cages on two racks consisting of four tiers of 10 cages per tier in two rooms. Data were transformed to logarithms to reduce heteroscedascity where necessary. All variables were tested for homogeneity of variance using the Levenes test. Repeated measures two way ANOVA with time and treatment as the grouping factors were used to compare groups in experiment one and experiment two. Comparisons between times within each treatment and between treatments for each time were examined with Tukey post hoc pairwise repeated measures contrasts.

In experiment three repeated measures two way ANOVA with time and treatment as the grouping factors was used to compare the width of the cloacal opening between groups. Comparisons between times within each treatment and between treatments for each time were examined with post hoc repeated measures contrasts. All other variables were analysed using single measures two way ANOVA followed by Tukeys post hoc pairwise comparisons to determine differences between groups.

A value of $p < 0.05$ was taken as statistically significant and indicated by an asterisk (*). ANOVAs followed by post hoc tests and the non-parametric equivalents were performed using Systat Version 8 (SPSS Inc., 1988). Data are presented as individual (raw data) or as means ● standard error (group data) using GraphPad Prism Version 3.0 (GraphPad Software Inc., 1999).

4.3 Results

4.3.1 Experiment one: Acute LH responses to exogenous GnRH administration

4.3.1.1 Acute LH response to a single subcutaneous cGnRH-I injection

LH concentrations were similar in all groups at the start of the experiment (Figures 4.2 and 4.3; see Table 4.4 for statistics). LH concentrations did not change after injections of saline, 0.25 or 0.5 μg cGnRH-I/kg. Mean LH concentrations increased from pre-treatment values five minutes after injection of 1, 2.5 or 5 μg/kg, remained elevated

after 10 minutes then declined to concentrations not different to pre-treatment LH concentrations at 20 minutes.

There was a clear dose response relationship for the stimulation of LH by cGnRH-I. The LH response to 5 μg cGnRH-I/kg was greater than the response to 1 μg cGnRH-I/kg at 5, 10 and 20 minutes (see Table 4.4 for statistics for these contrasts) although the response to 5 μg cGnRH-I/kg was significantly greater than the response to 2.5 μg cGnRH-I/kg only at 20 minutes.

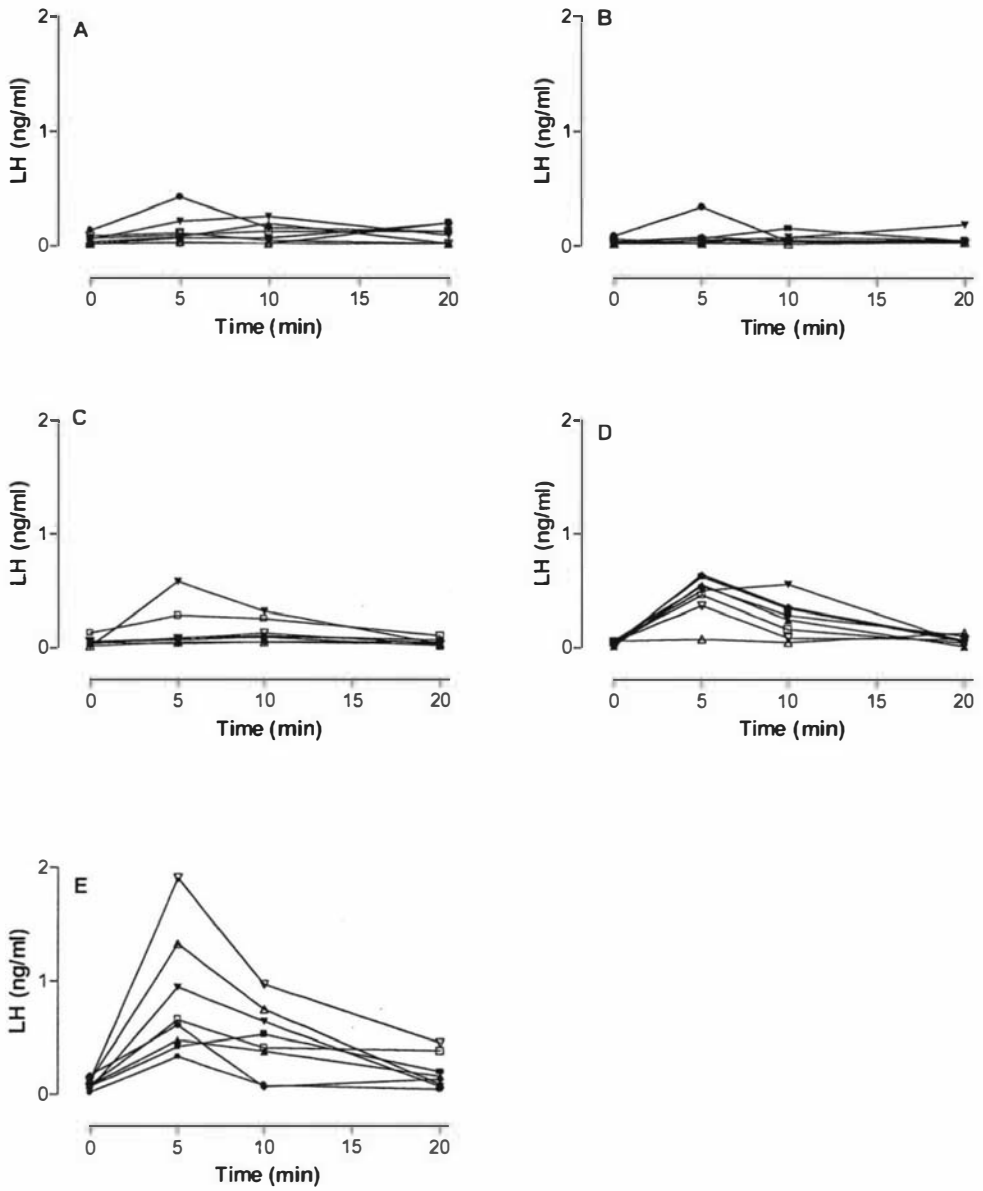

Figure 4.2. LH concentrations in individual female quail treated with 0.25 (A), 0.5 (B), 1 (C), 2.5 (D) or 5 (E) μg cGnRH-I/kg body weight.

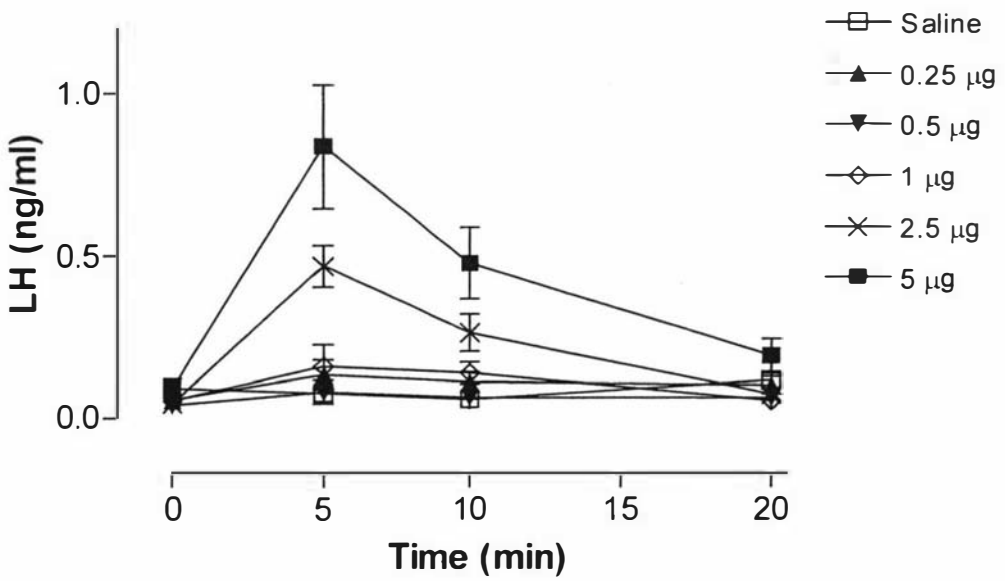

Figure 4.3. Mean LH concentrations in female quail treated with saline or 0.25, 0.5, 1, 2.5 or 5 μg cGnRH-I/kg body weight. Results are shown as mean ± standard error.

Table 4.4. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline and 0.25, 0.5, 1, 2.5 and 5 μg cGnRH-I/kg body weight.

Effect		LH F	LH df	LH *p*
Treatment		8.141	5,40	0.000*
Time		27.487	3,120	0.000*
Interaction of treatment and time		4.472	15,120	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	0.001	1,40	0.981
	0.25 μg	6.595	1,40	0.014*
	0.5 μg	2.210	1,40	0.145
	1 μg	15.740	1,40	0.000*
	2.5 μg	52.822	1,40	0.000*
	5 μg	59.142	1,40	0.006*
0 vs 10 min	Saline	1.161	1,40	0.188
	0.25 μg	3.056	1,40	0.088
	0.5 μg	2.649	1,40	0.111
	1 μg	12.836	1,40	0.001*
	2.5 μg	21.302	1,40	0.000*
	5 μg	22.043	1,40	0.000*
0 vs 20 min	Saline	1.609	1,40	0.112
	0.25 μg	1.920	1,40	0.174
	0.5 μg	2.259	1,40	0.141
	1 μg	0.045	1,40	0.833
	2.5 μg	1.894	1,40	0.176
	5 μg	4.040	1,40	0.051

Table 4.4 cont. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline and 0.25, 0.5, 1, 2.5 and 5 μg cGnRH-I/kg body weight.

Comparisons between treatments for each time		LH F	LH df	LH *p*
0 min	Saline vs 0.25 μg	1.306	1,40	0.260
	Saline vs 0.5 μg	3.268	1,40	0.078
	Saline vs 1 μg	1.469	1,40	0.233
	Saline vs 2.5 μg	1.736	1,40	0.195
	Saline vs 5 μg	0.171	1,40	0.681
5 min	Saline vs 0.25 μg	0.510	1,40	0.479
	Saline vs 0.5 μg	0.318	1,40	0.576
	Saline vs 1 μg	2.446	1,40	0.126
	Saline vs 2.5 μg	14.409	1,40	0.000*
	Saline vs 5 μg	28.119	1,40	0.000*
	0.25 vs 0.5 μg	1.976	1,40	0.168
	0.25 vs 1 μg	0.842	1,40	0.364
	0.25 vs 2.5 μg	11.125	1,40	0.002*
	0.25 vs 5 μg	24.565	1,40	0.000*
	0.5 vs 1 μg	5.523	1,40	0.024*
	0.5 vs 2.5 μg	22.856	1,40	0.000*
	0.5 vs 5 μg	42.325	1,40	0.000*
	1 vs 2.5 μg	5.996	1,40	0.019*
	1 vs 5 μg	16.310	1,40	0.000*
	2.5 vs 5 μg	2.111	1,40	0.154
10 min	Saline vs 0.25 μg	1.347	1,40	0.253
	Saline vs 0.5 μg	0.287	1,40	0.595
	Saline vs 1 μg	5.660	1,40	0.022*
	Saline vs 2.5 μg	9.644	1,40	0.003*
	Saline vs 5 μg	19.714	1,40	0.000*
	0.25 vs 0.5 μg	0.509	1,40	0.480
	0.25 vs 1 μg	1.720	1,40	0.197
	0.25 vs 2.5 μg	4.506	1,40	0.004*
	0.25 vs 5 μg	12.515	1,40	0.001*
	0.5 vs 1 μg	4.255	1,40	0.046*
	0.5 vs 2.5 μg	8.244	1,40	0.007*
	0.5 vs 5 μg	18.952	1,40	0.000*
	1 vs 2.5 μg	0.732	1,40	0.397
	1 vs 5 μg	4.956	1,40	0.032*
	2.5 vs 5 μg	1.677	1,40	0.203
20 min	Saline vs 0.25 μg	1.282	1,40	0.264
	Saline vs 0.5 μg	3.003	1,40	0.091
	Saline vs 1 μg	4.452	1,40	0.041*
	Saline vs 2.5 μg	1.516	1,40	0.225
	Saline vs 5 μg	0.658	1,40	0.422
	0.25 vs 0.5 μg	0.386	1,40	0.538
	0.25 vs 1 μg	1.115	1,40	0.297
	0.25 vs 2.5 μg	0.020	1,40	0.888
	0.25 vs 5 μg	4.405	1,40	0.042*
	0.5 vs 1 μg	0.217	1,40	0.644
	0.5 vs 2.5 μg	0.205	1,40	0.653
	0.5 vs 5 μg	7.733	1,40	0.008*
	1 vs 2.5 μg	0.771	1,40	0.385
	1 vs 5 μg	9.953	1,40	0.003*
	2.5 vs 5 μg	4.708	1,40	0.036*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.1.2 Acute response to a single subcutaneous cGnRH-II injection

LH concentrations did not differ between groups before injection and showed a dose response relationship to injections of cGnRH-II (Figures 4.4 and 4.5; see Table 4.5 for statistics). There was no LH response to 0.25 μg cGnRH-II/kg. LH concentrations in response to 0.5 – 5 μg cGnRH-II/kg increased to broad peaks five and 10 minutes after injection then declined at 20 minutes to concentrations that were still elevated above initial concentrations. LH responses at five and 10 minutes increased with increasing dose of cGnRH-II (see Table 4.5 for statistics for contrasts between doses), whilst at 20 minutes LH was significantly greater than saline treated birds in birds that received 2.5 or 5 μg cGnRH-II/kg.

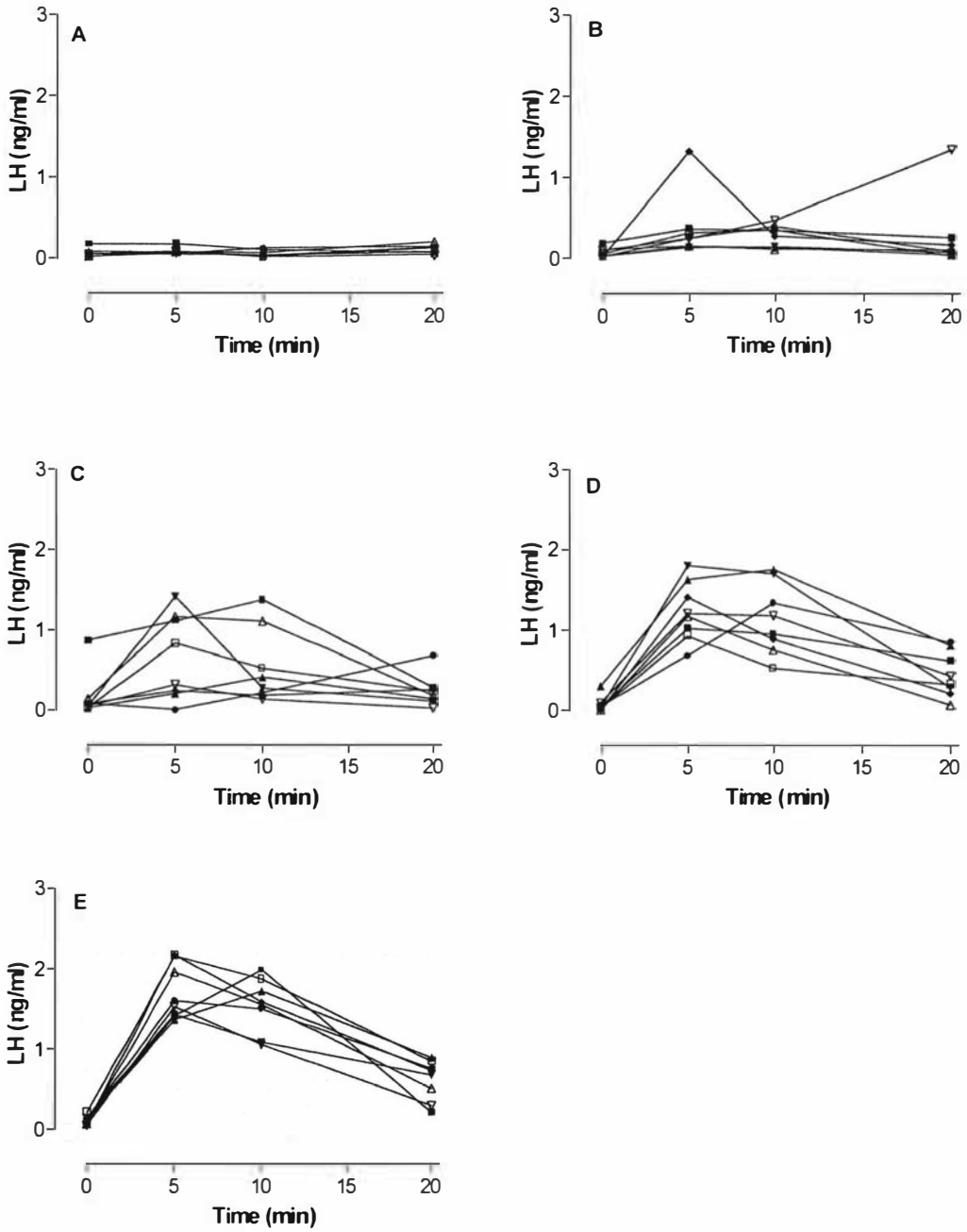

Figure 4.4. LH concentrations in individual female quail treated with 0.25 (A), 0.5 (B), 1 (C), 2.5 (D) or 5 μg (E) cGnRH-II /kg body weight.

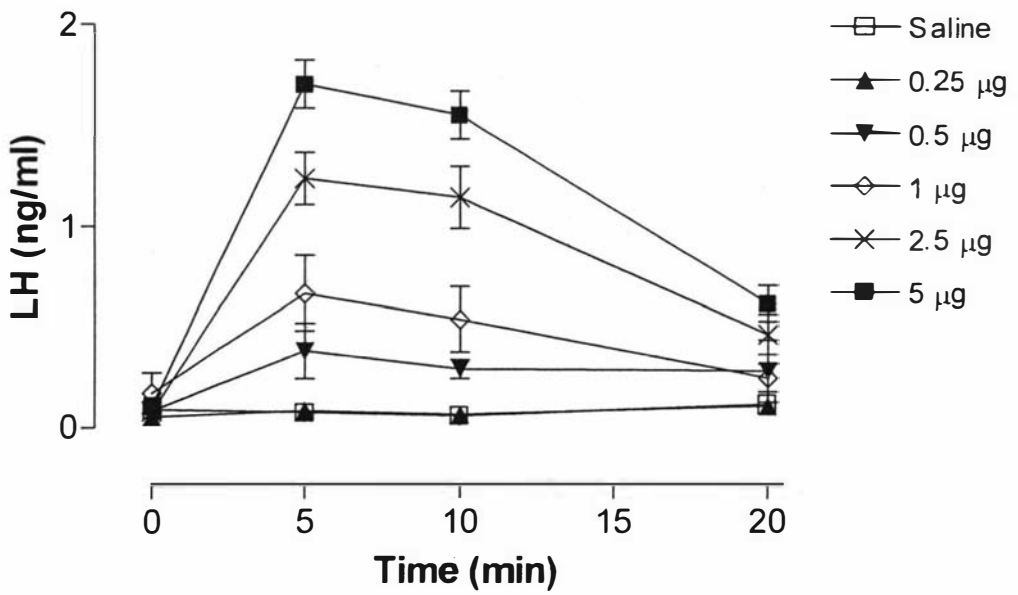

Figure 4.5. Mean LH concentrations in female quail treated with saline or 0.25, 0.5, 1, 2.5 or 5 μg cGnRH-II/kg of body weight. Results are shown as mean ± standard error.

Table 4.5. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline and 0.25, 0.5, 1, 2.5 and 5 µg cGnRH-II/kg body weight.

		LH		
Effect		**F**	**df**	***p***
Treatment		26.358	5,40	0.000*
Time		59.172	3,120	0.000*
Interaction of treatment and time		7.380	15,120	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	0.000	1,40	0.985
	0.25 µg	3.584	1,40	0.066
	0.5 µg	18.087	1,40	0.000*
	1 µg	18.631	1,40	0.000*
	2.5 µg	82.306	1,40	0.000*
	5 µg	70.869	1,40	0.000*
0 vs 10 min	Saline	1.642	1,40	0.207
	0.25 µg	1.031	1,40	0.316
	0.5 µg	25.335	1,40	0.000*
	1 µg	31.171	1,40	0.000*
	2.5 µg	124.212	1,40	0.000*
	5 µg	107.071	1,40	0.000*
0 vs 20 min	Saline	1.580	1,40	0.216
	0.25 µg	7.290	1,40	0.010*
	0.5 µg	6.998	1,40	0.012*
	1 µg	5.831	1,40	0.020*
	2.5 µg	39.734	1,40	0.000*
	5 µg	7.290	1,40	0.001*

Table 4.5 cont. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline and 0.25, 0.5, 1, 2.5 and 5/kg μg cGnRH-II.

Comparisons between treatments for each time		LH F	LH df	LH p
0 min	Saline vs 0.25 μg	1.291	1,40	0.263
	Saline vs 0.5 μg	0.001	1,40	0.977
	Saline vs 1 μg	0.440	1,40	0.511
	Saline vs 2.5 μg	0.208	1,40	0.651
	Saline vs 5 μg	0.704	1,40	0.406
5 min	Saline vs 0.25 μg	0.121	1,40	0.730
	Saline vs 0.5 μg	13.642	1,40	0.001*
	Saline vs 1 μg	19.878	1,40	0.000*
	Saline vs 2.5 μg	53.622	1,40	0.000*
	Saline vs 5 μg	67.340	1,40	0.000*
	0.25 vs 0.5 μg	13.062	1,40	0.001*
	0.25 vs 1 μg	19.717	1,40	0.000*
	0.25 vs 2.5 μg	56.762	1,40	0.000*
	0.25 vs 5 μg	72.051	1,40	0.000*
	0.5 vs 1 μg	0.683	1,40	0.414
	0.5 vs 2.5 μg	15.366	1,40	0.000*
	0.5 vs 5 μg	23.757	1,40	0.000*
	1 vs 2.5 μg	9.571	1,40	0.004*
	1 vs 5 μg	16.385	1,40	0.000*
	2.5 vs 5 μg	0.911	1,40	0.346
10 min	Saline vs 0.25 μg	0.258	1,40	0.614
	Saline vs 0.5 μg	28.573	1,40	0.000*
	Saline vs 1 μg	44.156	1,40	0.000*
	Saline vs 2.5 μg	93.593	1,40	0.000*
	Saline vs 5 μg	115.845	1,40	0.000*
	0.25 vs 0.5 μg	27.302	1,40	0.000*
	0.25 vs 1 μg	43.942	1,40	0.000*
	0.25 vs 2.5 μg	98.029	1,40	0.000*
	0.25 vs 5 μg	122.699	1,40	0.000*
	0.5 vs 1 μg	1.971	1,40	0.168
	0.5 vs 2.5 μg	21.863	1,40	0.000*
	0.5 vs 5 μg	34.244	1,40	0.000*
	1 vs 2.5 μg	10.706	1,40	0.002*
	1 vs 5 μg	19.786	1,40	0.000*
	2.5 vs 5 μg	1.383	1,40	0.247
20 min	Saline vs 0.25 μg	0.085	1,40	0.722
	Saline vs 0.5 μg	0.841	1,40	0.365
	Saline vs 1 μg	2.035	1,40	0.161
	Saline vs 2.5 μg	9.688	1,40	0.003*
	Saline vs 5 μg	16.811	1,40	0.000*
	0.25 vs 0.5 μg	1.705	1,40	0.199
	0.25 vs 1 μg	3.445	1,40	0.071
	0.25 vs 2.5 μg	13.522	1,40	0.001*
	0.25 vs 5 μg	22.504	1,40	0.000*
	0.5 vs 1 μg	0.303	1,40	0.585
	0.5 vs 2.5 μg	5.623	1,40	0.023*
	0.5 vs 5 μg	11.819	1,40	0.001*
	1 vs 2.5 μg	3.317	1,40	0.076
	1 vs 5 μg	8.339	1,40	0.006*
	2.5 vs 5 μg	1.138	1,40	0.293

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.1.3 Acute LH response to a single subcutaneous buserelin injection

LH concentrations were low in all groups before injection and showed a dose response relationship to injections of buserelin (Figures 4.6 and 4.7; see Table 4.6 for statistics). LH concentrations in response to 0.5 μg buserelin/kg increased to a broad peak five and 10 minutes after injection then declined at 20 minutes to concentrations that were similar to initial concentrations. LH concentrations in response to 1 – 4 μg buserelin/kg increased to broad peaks five and 10 minutes after injection then declined at 20 minutes to concentrations that were elevated above initial concentrations. LH responses at five and 10 minutes increased with increasing doses of buserelin (see Table 4.6 for statistics for contrasts between doses) whilst at 20 minutes LH was significantly greater than saline treated birds in birds that received 2.5 or 4 μg buserelin/kg.

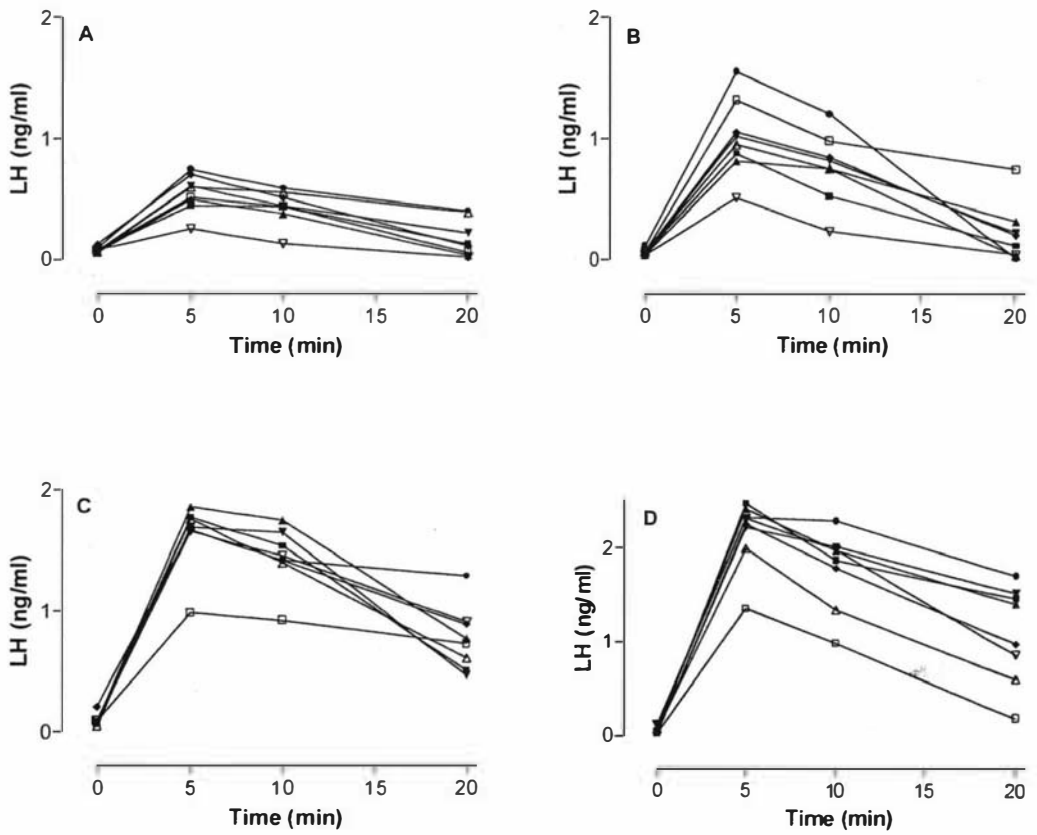

Figure 4.6. LH concentrations in individual female quail treated with 0.5 (A), 1 (B), 2.5 (C) or 4 (D) μg buserelin/kg body weight. Note different scales on y axes.

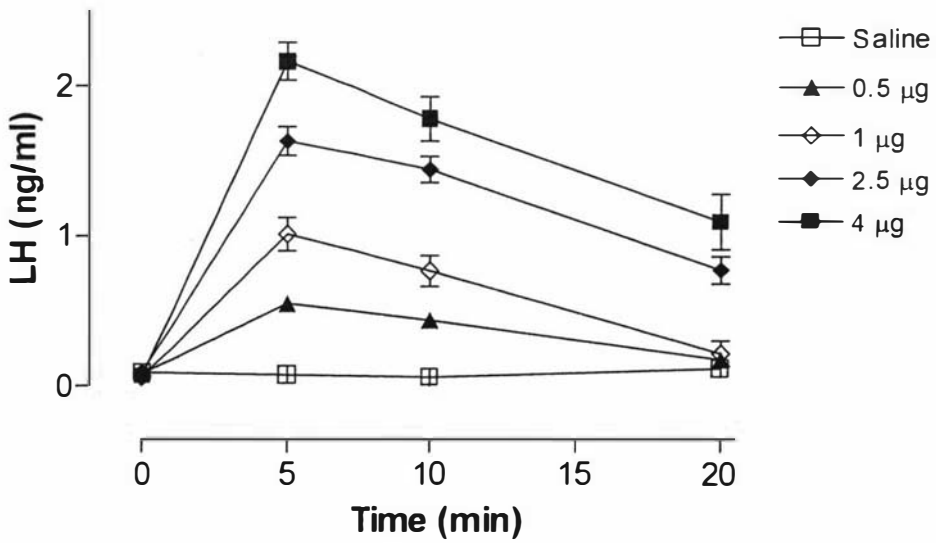

Figure 4.7. Mean LH concentrations in female quail treated with saline or 0.5, 1, 0.25 or 4 µg buserelin/kg body weight. Results are shown as mean ± standard error.

Table 4.6. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline or 0.5, 1, 2.5 or 4 µg buserelin/kg body weight.

Effect		LH F	LH df	LH p
Treatment		34.95	4,32	0.000*
Time		168.287	3,96	0.000*
Interaction of treatment and time		17.403	12,96	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	0.002	1,32	0.966
	Buserelin 0.5 µg	148.272	1,32	0.000*
	Buserelin 1 µg	345.523	1,32	0.000*
	Buserelin 2.5 µg	359.813	1,32	0.000*
	Buserelin 4.0 µg	547.984	1,32	0.000*
0 vs 10 min	Saline	4.506	1,32	0.420
	Buserelin 0.5 µg	104.898	1,32	0.000*
	Buserelin 1 µg	237.143	1,32	0.000*
	Buserelin 2.5 µg	286.425	1,32	0.000*
	Buserelin 4.0 µg	424.553	1,32	0.000*
0 vs 20 min	Saline	1.371	1,32	0.250
	Buserelin 0.5 µg	2.705	1,32	0.110
	Buserelin 1 µg	4.845	1,32	0.035*
	Buserelin 2.5 µg	37.475	1,32	0.000*
	Buserelin 4.0 µg	67.689	1,32	0.000*

Table 4.6 cont. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline or 0.5, 1, 2.5 or 4 μg buserelin/kg body weight.

Comparisons between treatments for each time				
0 min	Saline vs buserelin 0.5 μg	0.491	1,32	0.488
	Saline vs buserelin 1.0 μg	0.212	1,32	0.648
	Saline vs buserelin 2.5 μg	0.614	1,32	0.439
	Saline vs buserelin 4.0 μg	0.040	1,32	0.843
5 min	Saline vs buserelin 0.5 μg	131.635	1,32	0.000*
	Saline vs buserelin 1.0 μg	214.080	1,32	0.000*
	Saline vs buserelin 2.5 μg	282.742	1,32	0.000*
	Saline vs buserelin 4.0 μg	361.327	1,32	0.000*
	Buserelin 0.5 vs buserelin 1.0 μg	8.612	1,32	0.006*
	Buserelin 0.5 vs buserelin 2.5 μg	27.175	1,32	0.000*
	Buserelin 0.5 vs buserelin 5 μg	56.281	1,32	0.000*
	Buserelin 1 vs buserelin 2.5 μg	5.562	1,32	0.025*
	Buserelin 1 vs buserelin 5 μg	22.353	1,32	0.000*
	Buserelin 2.5 vs buserelin 5 μg	5.614	1,32	0.024*
10 min	Saline vs buserelin 0.5 μg	83.589	1,32	0.000*
	Saline vs buserelin 1.0 μg	120.737	1,32	0.000*
	Saline vs buserelin 2.5 μg	177.762	1,32	0.000*
	Saline vs buserelin 4.0 μg	213.897	1,32	0.000*
	Buserelin 0.5 vs buserelin 1.0 μg	2.683	1,32	0.111
	Buserelin 0.5 vs buserelin 2.5 μg	16.684	1,32	0.000*
	Buserelin 0.5 vs buserelin 5 μg	29.520	1,32	0.000*
	Buserelin 1 vs buserelin 2.5 μg	6.414	1,32	0.016*
	Buserelin 1 vs buserelin 5 μg	15.434	1,32	0.000*
	Buserelin 2.5 vs buserelin 5 μg	1.949	1,32	0.172
20 min	Saline vs buserelin 0.5 μg	0.503	1,32	0.483
	Saline vs buserelin 1.0 μg	0.112	1,32	0.741
	Saline vs buserelin 2.5 μg	15.357	1,32	0.000*
	Saline vs buserelin 4.0 μg	23.456	1,32	0.000*
	Buserelin 0.5 vs buserelin 1.0 μg	0.172	1,32	0.681
	Buserelin 0.5 vs buserelin 2.5 μg	11.065	1,32	0.002*
	Buserelin 0.5 vs buserelin 5 μg	18.413	1,32	0.000*
	Buserelin 1 vs buserelin 2.5 μg	14.992	1,32	0.001*
	Buserelin 1 vs buserelin 5 μg	23.721	1,32	0.000*
	Buserelin 2.5 vs buserelin 5 μg	0.997	1,32	0.326

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.2 Experiment two: Acute LH responses to exogenous GnRH administration

4.3.2.1 Acute LH response to a single subcutaneous D-Lys^{6}Trp^{7}Tyr^8-GnRH injection

LH concentrations did not differ between groups before injection and showed a dose response relationship to injections of 1 - 5μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg (Figures 4.8 and 4.9; see Table 4.7 for statistics). There was a small but significant increase in LH in birds injected with 0.2 μg between 0 and five minutes. LH concentrations then declined in this group to concentrations lower than those in saline treated birds at 20 minutes. LH concentrations in response to 1 – 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg increased to broad peaks five and 10 minutes after injection then declined at 20 minutes to concentrations that were still elevated above initial concentrations. LH responses at five and 10 minutes increased with increasing doses of D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg (see Table 4.7 for statistics for contrasts between doses), whilst at 20 minutes LH was significantly greater than saline treated birds in birds that received 1 – 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg.

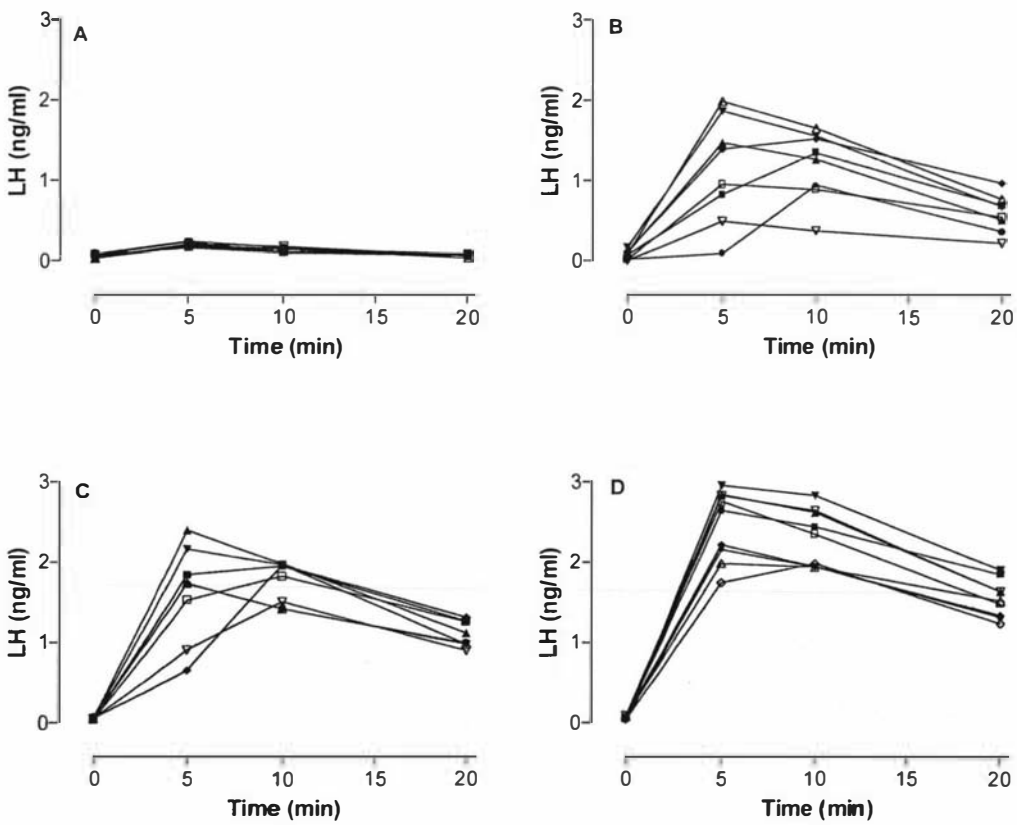

Figure 4.8. LH concentrations in individual female quail treated with 0. 2 (A), 1 (B), 2.5 (C) or 5 (D) μg D-Lys^{6} Trp^{7}Tyr^{8}-GnRH/kg body weight.

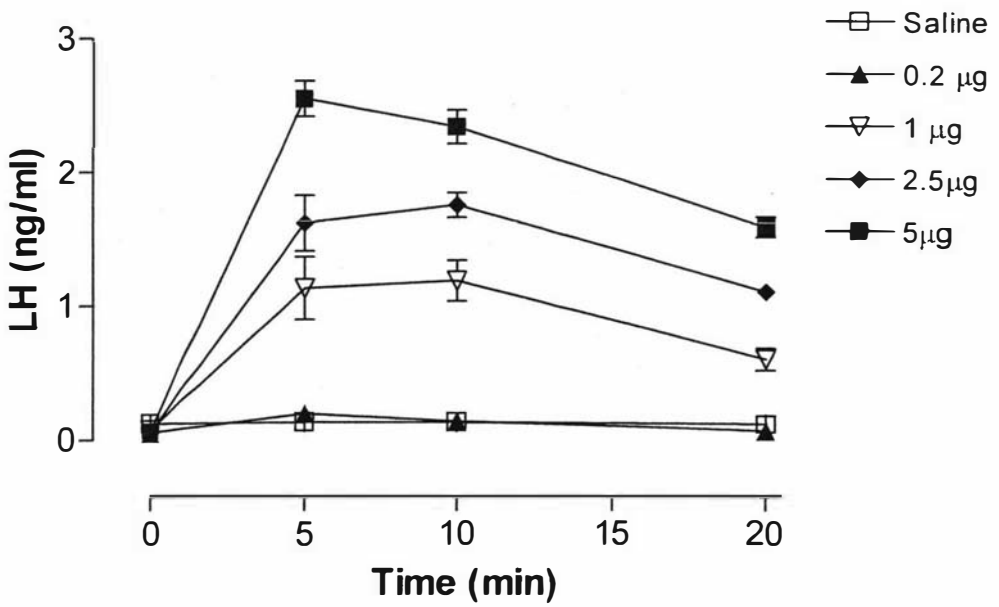

Figure 4.9. Mean LH concentrations in female quail treated with saline or 0.2, 1, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg body weight. Results are shown as mean ± standard error.

Table 4.7. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline and 0.2, 1, 2.5 and 5 µg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight.

Effect		LH F	df	*p*
Treatment		45.963	4,33	0.000*
Time		302.750	3,99	0.000*
Interaction of treatment and time		27.320	12,99	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	1.244	1,33	0.273
	0.2 µg	31.041	1,33	0.000*
	1 µg	150.984	1,33	0.000*
	2.5 µg	198.719	1,33	0.000*
	5 µg	227.456	1,33	0.000*
0 vs 10 min	Saline	1.309	1,33	0.261
	0.2 µg	18.879	1,33	0.000*
	1 µg	197.238	1,33	0.000*
	2.5 µg	232.915	1,33	0.000*
	5 µg	241.147	1,33	0.000*
0 vs 20 min	Saline	0.248	1,33	0.622
	0.2 µg	1.582	1,33	0.217
	1 µg	122.584	1,33	0.000*
	2.5 µg	175.359	1,33	0.000*
	5 µg	191.773	1,33	0.000*

Table 4.7 cont. Two way repeated measures ANOVA for LH concentrations in female quail treated with saline and 0.2, 1, 2.5 and 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg of body weight.

Comparisons between treatments for each time		LH F	df	*p*
0 min	Saline vs 0.2 μg	1.928	1,33	0.174
	Saline vs 1 μg	3.136	1,33	0.086
	Saline vs 2.5 μg	1.835	1,33	0.185
	Saline vs 5 μg	0.705	1,33	0.407
5 min	Saline vs 0.2 μg	2.540	1,33	0.127
	Saline vs 1 μg	46.208	1,33	0.000*
	Saline vs 2.5 μg	81.172	1,33	0.000*
	Saline vs 5 μg	113.629	1,33	0.000*
	0.2 vs 1 μg	31.925	1,33	0.000*
	0.2 vs 2.5 μg	64.630	1,33	0.000*
	0.2 vs 5 μg	96.465	1,33	0.000*
	1 vs 2.5 μg	5.708	1,33	0.023*
	1 vs 5 μg	17.401	1,33	0.000*
	2.5 vs 5 μg	3.177	1,33	0.084*
10 min	Saline vs 0.2 μg	0.346	1,33	0.560
	Saline vs 1 μg	179.474	1,33	0.002*
	Saline vs 2.5 μg	264.052	1,33	0.000*
	Saline vs 5 μg	324.457	1,33	0.000*
	0.2 vs 1 μg	191.401	1,33	0.000*
	0.2 vs 2.5 μg	286.159	1,33	0.000*
	0.2 vs 5 μg	354.213	1,33	0.000*
	1 vs 2.5 μg	9.495	1,33	0.004*
	1 vs 5 μg	24.858	1,33	0.000*
	2.5 vs 5 μg	3.626	1,33	0.066
20 min	Saline vs 0.2 μg	6.500	1,33	0.016*
	Saline vs 1 μg	90.307	1,33	0.000*
	Saline vs 2.5 μg	179.949	1,33	0.000*
	Saline vs 5 μg	240.211	1,33	0.001*
	0.2 vs 1 μg	169.471	1,33	0.000*
	0.2 vs 2.5 μg	297.321	1,33	0.000*
	0.2 vs 5 μg	380.026	1,33	0.000*
	1 vs 2.5 μg	17.850	1,33	0.000*
	1 vs 5 μg	41.941	1,33	0.000*
	2.5 vs 5 μg	5.068	1,33	0.031*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.3 Comparisons of LH responses to different forms of GnRH

There were no significant differences between the effects of aline injections on LH secretion in the control groups in experiment one and experiment two (see table 4.9 for statistics). Therefore data from experiments one and two were statistically analysed to compare the LH-inducing abilities of the different GnRH forms tested.

4.3.3.1 Comparisons of LH responses to 0.5 μg GnRH/kg

There were significant differences between the abilities of the different GnRH forms to induce LH secretion (Figure 4.10; see Table 4.8 for statistics). The maximum increase in LH concentrations after an injection of 0.5 μg cGnRH-II or buserelin/kg did not different between the two groups and was significantly greater than that induced by the same dose of cGnRH-I. At 20 minutes LH concentrations had declined in birds injected with buserelin and remained elevated in birds injected cGnRH-II.

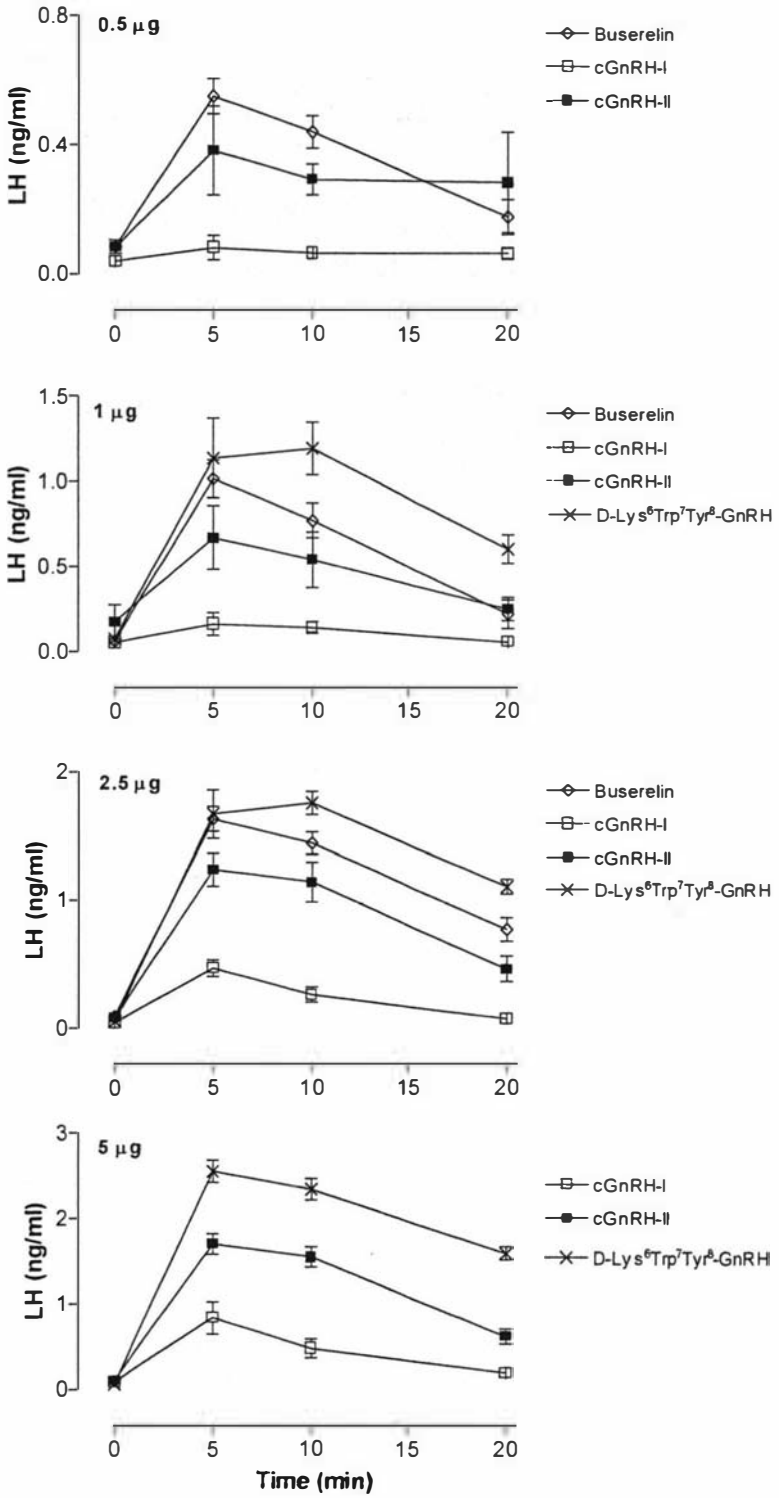

Figure 4.10. Mean LH concentrations in female quail treated with either buserelin, cGnRH-I, cGnRH-II or D-Lys^6 Trp^{7}Tyr^8-GnRH at 0.5 µg, 1 µg, 2.5 µg or 5 µg/kg. Results are shown as mean ± standard error. Note different scales on y axes.

Table 4.8. Two way repeated measures ANOVA for LH concentrations in female quail treated with 0.5 μg cGnRH-I, cGnRH-II or buserelin/kg body weight.

Effect		LH F	LH df	LH *p*
Treatment		24.914	2,21	0.000*
Time		23.518	3,63	0.000*
Interaction of treatment and time		3.811	6,63	0.003*
Comparisons between treatments for each time				
0 min	cGnRH-I vs cGnRH-II	7.271	1,21	0.014*
	cGnRH-I vs buserelin	11.600	1,21	0.003*
	cGnRH-II vs buserelin	0.503	1,21	0.486
5 min	cGnRH-I vs cGnRH-II	24.460	1,21	0.000*
	cGnRH-I vs buserelin	44.441	1,21	0.000*
	cGnRH-II vs buserelin	2.961	1,21	0.100
10 min	cGnRH-I vs cGnRH-II	33.481	1,21	0.000*
	cGnRH-I vs buserelin	55.158	1,21	0.000*
	cGnRH-II vs buserelin	2.692	1,21	0.116
20 min	cGnRH-I vs cGnRH-II	5.643	1,21	0.027*
	cGnRH-I vs buserelin	3.206	1,21	0.088
	cGnRH-II vs buserelin	0.342	1,21	0.565

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.3.2 Comparisons of LH responses to 1 μg GnRH/kg

There were significant differences between GnRH-induced LH responses in female quail injected with 1 μg/kg (Figure 4.10; see Table 4.9 for statistics). Mean LH concentrations increased from 0 to five minutes for all GnRH treatments. The LH response in birds injected with cGnRH-I was significantly less than that of birds treated with the other GnRH-solutions. Mean LH concentrations in birds injected with cGnRH-II and buserelin were not different across the sampling period. The LH response of birds injected with D-Lys^{6}Trp^{7}Tyr^{8}-GnRH was greater than the LH responses of birds in other groups at 20 minutes.

Table 4.9. Two way repeated measures ANOVA for LH concentrations in female quail treated with 1 μg cGnRH-I, cGnRH-II, buserelin or D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg body weight.

Effect		LH F	df	p
Treatment		11.441	3,28	0.000*
Time		59.158	3,84	0.000*
Interaction of treatment and time		5.428	9,84	0.000*
Comparisons between treatments for each time				
0 min	cGnRH-I vs cGnRH-II	2.106	1,28	0.158
	cGnRH-I vs buserelin	0.350	1,28	0.559
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.021	1,28	0.886
	cGnRH-II vs buserelin	0.739	1,28	0.397
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.546	1,28	0.122
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.542	1,28	0.468
5 min	cGnRH-I vs cGnRH-II	6.983	1,28	0.013*
	cGnRH-I vs buserelin	20.043	1,28	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	17.778	1,28	0.000*
	cGnRH-II vs buserelin	3.363	1,28	0.077
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.475	1,28	0.127
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.068	1,28	0.796
10 min	cGnRH-I vs cGnRH-II	15.550	1,28	0.000*
	cGnRH-I vs buserelin	33.202	1,28	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	51.567	1,28	0.000*
	cGnRH-II vs buserelin	3.308	1,28	0.008*
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	10.483	1,28	0.003*
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.013	1,28	0.167
20 min	cGnRH-I vs cGnRH-II	10.863	1,28	0.003*
	cGnRH-I vs buserelin	5.117	1,28	0.032*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	34.603	1,28	0.000*
	cGnRH-II vs buserelin	1.069	1,28	0.310
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	6.690	1,28	0.015*
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	13.107	1,28	0.001*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.3.3 Comparisons of LH responses to 2.5 μg GnRH/kg

LH responses in birds injected with 2.5 μg GnRH/kg solutions were similar to those in birds injected with 1 μg GnRH/kg (Figure 4.10; see Table 4.10 for statistics). Mean LH concentrations increased in all groups and were significantly less in birds injected with cGnRH-I compared with birds in all other groups. cGnRH-II and buserelin treatment resulted in a similar LH response over the first 15 minutes, whereas buserelin-induced LH responses were higher than those of cGnRH-II to 20 minutes. The LH induced response in birds injected with D-Lys^{6}Trp^{7}Tyr^8-GnRH was significantly greater than that of cGnRH-II but not different to buserelin induced LH concentrations.

Table 4.10. Two way repeated measures ANOVA for LH concentrations in female quail treated with 2.5 μg cGnRH-I, cGnRH-II, buserelin or D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg body weight.

Effect		LH F	df	*p*
Treatment		11.441	3,28	0.000*
Time		59.158	3,84	0.000*
Interaction of treatment and time		5.428	9,84	0.000*
Comparisons between treatments for each time				
0 min	cGnRH-I vs cGnRH-II	1.033	1,28	0.318
	cGnRH-I vs buserelin	6.742	1,28	0.015*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.434	1,28	0.515
	cGnRH-II vs buserelin	2.496	1,28	0.125
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.128	1,28	0.723
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	3.755	1,28	0.063
5 min	cGnRH-I vs cGnRH-II	24.755	1,28	0.000*
	cGnRH-I vs buserelin	40.874	1,28	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	40.054	1,28	0.000*
	cGnRH-II vs buserelin	2.010	1,28	0.167
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	1.832	1,28	0.187
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.004	1,28	0.949
10 min	cGnRH-I vs cGnRH-II	49.887	1,28	0.000*
	cGnRH-I vs buserelin	69.437	1,28	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	84.863	1,28	0.000*
	cGnRH-II vs buserelin	1.613	1,28	0.215
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	4.619	1,28	0.040*
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.773	1,28	0.387
20 min	cGnRH-I vs cGnRH-II	45.660	1,28	0.000*
	cGnRH-I vs buserelin	87.520	1,28	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	118.607	1,28	0.000*
	cGnRH-II vs buserelin	6.749	1,28	0.015*
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	17.085	1,28	0.000*
	Buserelin vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.357	1,28	0.136

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.3.4 Comparisons of LH responses to 5 μg GnRH/kg

There were significant differences between GnRH treatments at 5 μg/kg (Figure 4.10; see Table 4.11 for statistics). The increases in LH concentrations after an injection of 5 μg cGnRH-II or D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg were significantly greater than those after the same dose of cGnRH-I across the sampling period. D-Lys^{6}Trp^{7}Tyr^8-GnRH induced LH concentrations were significantly greater than those in response to cGnRH-II.

Table 4.11. Two way repeated measures ANOVA for LH concentrations in female quail treated with 5 μg cGnRH-I, cGnRH-II or D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg body weight.

Effect		LH F	df	*p*
Treatment		25.788	2,21	0.000*
Time		254.394	3,63	0.000*
Interaction of treatment and time		16.494	6,63	0.000*
Comparisons between treatments for each time				
0 min	cGnRH-I vs cGnRH-II	0.750	1,21	0.396
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.795	1,21	0.383
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	3.091	1,21	0.093
5 min	cGnRH-I vs cGnRH-II	21.349	1,21	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	46.849	1,21	0.000*
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	4.947	1,21	0.037*
10 min	cGnRH-I vs cGnRH-II	23.818	1,21	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	39.513	1,21	0.000*
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	4.976	1,21	0.017*
20 min	cGnRH-I vs cGnRH-II	22.794	1,21	0.000*
	cGnRH-I vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	71.299	1,21	0.000*
	cGnRH-II vs D-Lys^{6}Trp^{7}Tyr^8-GnRH	13.466	1,21	0.001*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

4.3.4 Experiment 3: Daily injections of either cGnRH-II or D-Lys^{6}Trp^{7}Tyr^8-GnRH injection in either saline or PVP for 7 days

Daily subcutaneous injections of 0.5 - 5 μg cGnRH-II/kg or 0.2 – 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg in saline or PVP for seven days did not induce follicular development in sexually regressed female Japanese quail. The width of the cloacal opening did not change in birds injected with saline or PVP but increased in birds transferred to long days (Figure 4.11; see Table 4.12 for statistics). On day eight birds transferred to long days had undergone gonadal growth and had higher LH concentrations compared with birds treated with saline or PVP (Figure 4.12; see Table 4.13 for statistics).

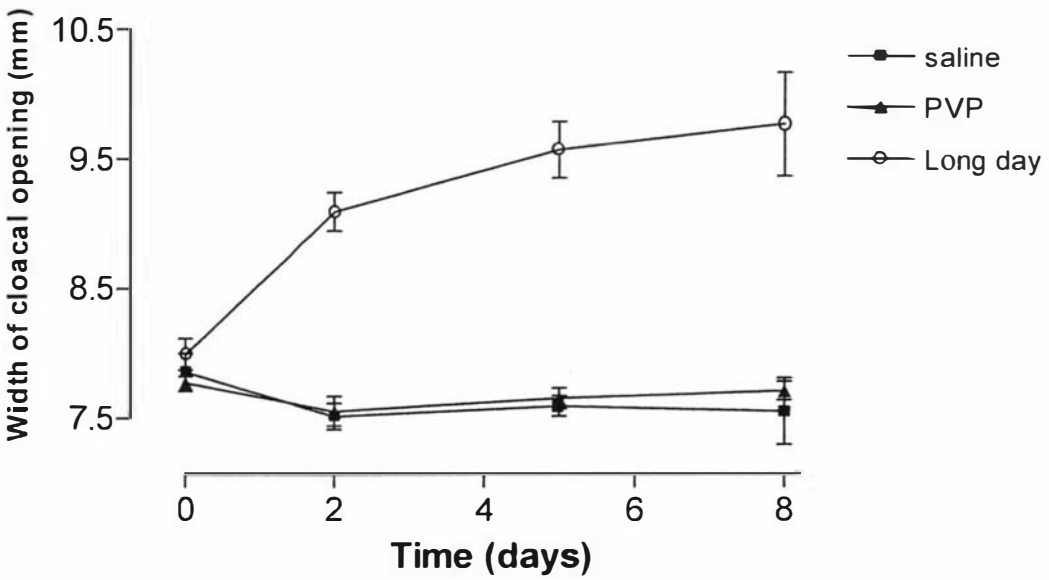

Figure 4.11. Mean width of cloacal opening in female quail injected daily with saline or PVP, or transferred to a long day photoperiod (16L:8D) on day 0. Results are shown as mean ± standard error.

Table 4.12. Two way repeated measures ANOVA for cloacal diameter in female quail injected daily with saline, PVP or transferred to long days for seven days.

		Width of cloacal opening		
Effect		**F**	**df**	***p***
Treatment		60.120	2,11	0.000*
Time		4.744	3,33	0.007*
Interaction of treatment and time		9.634	6,33	0.000*
Comparisons between treatments for each time				
Day 0	Saline vs PVP	0.231	1,11	0.640
	Saline vs Long day	0.727	1,11	0.412
	PVP vs Long day	1.706	1,11	0.218
Day 2	Saline vs PVP	0.064	1,11	0.806
	Saline vs Long day	76.167	1,11	0.000*
	PVP vs Long day	72.074	1,11	0.000*
Day 5	Saline vs PVP	0.159	1,11	0.698
	Saline vs Long day	121.451	1,11	0.000*
	PVP vs Long day	113.316	1,11	0.000*
Day 8	Saline vs PVP	0.307	1,11	0.591
	Saline vs Long day	33.389	1,11	0.000*
	PVP vs Long day	27.628	1,11	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

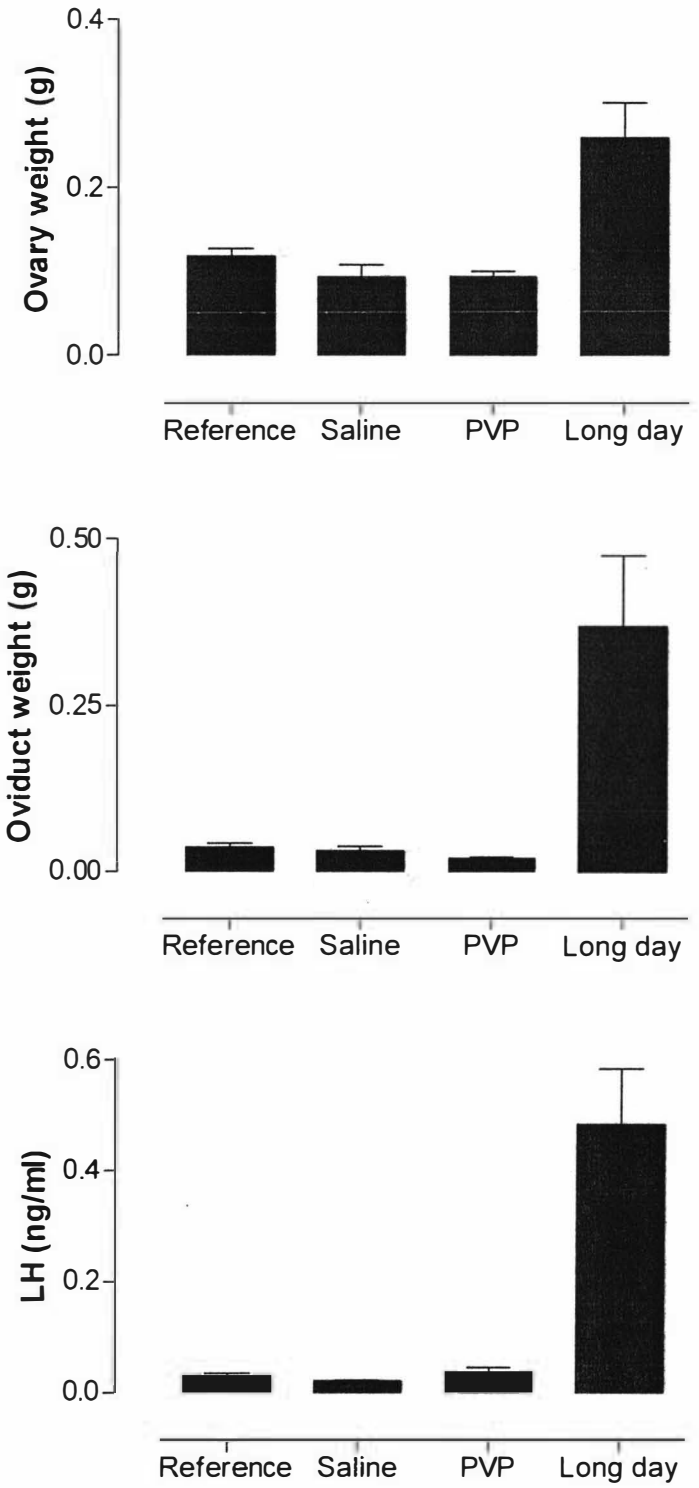

Figure 4.12. Mean ovary weights, oviduct weights and LH concentrations in female quail euthanased at the start of the experiment (reference), injected daily with saline or PVP or transferred to long daylengths for seven days. Results are shown as mean ± standard error.

Table 4.13. Single measures ANOVA for ovary weights (A), oviduct weights (B) and LH concentrations (C) in female quail before treatment (reference), quail injected daily with saline, PVP or transferred to long days for seven days.

A	Ovary weight		
Effect	**F**	**df**	***p***
Group	14.296	3,15	0.000*
Reference vs saline			0.712
Reference vs PVP			1.000
Reference vs long day			0.004*
Saline vs PVP			1.000
Saline vs long day			0.000*
PVP vs long day			0.000*

B	Oviduct weight		
Effect	**F**	**df**	***p***
Group	27.587	3,15	0.000*
Reference vs saline			1.000
Reference vs PVP			0.559
Reference vs long day			0.000*
Saline vs PVP			1.000
Saline vs long day			0.000*
PVP vs long day			0.000*

C	LH		
Effect	**F**	**df**	***p***
Group	53.215	3,15	0.000*
Reference vs saline			1.000
Reference vs PVP			1.000
Reference vs long day			0.000*
Saline vs PVP			0.733
Saline vs long day			0.000*
PVP vs long day			0.000*

NOTE: The first row of each table shows the result of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

4.3.4.1 Daily injections of cGnRH-II in saline or PVP

The width of the cloacal opening in birds injected with cGnRH-II in saline or PVP varied but remained narrow in the treatment groups (Figure 4.13; see Table 4.14 and 4.15 for statistics). The mean width of the cloacal opening of birds transferred to long days was significantly wider than those of cGnRH-II treated birds from day two.

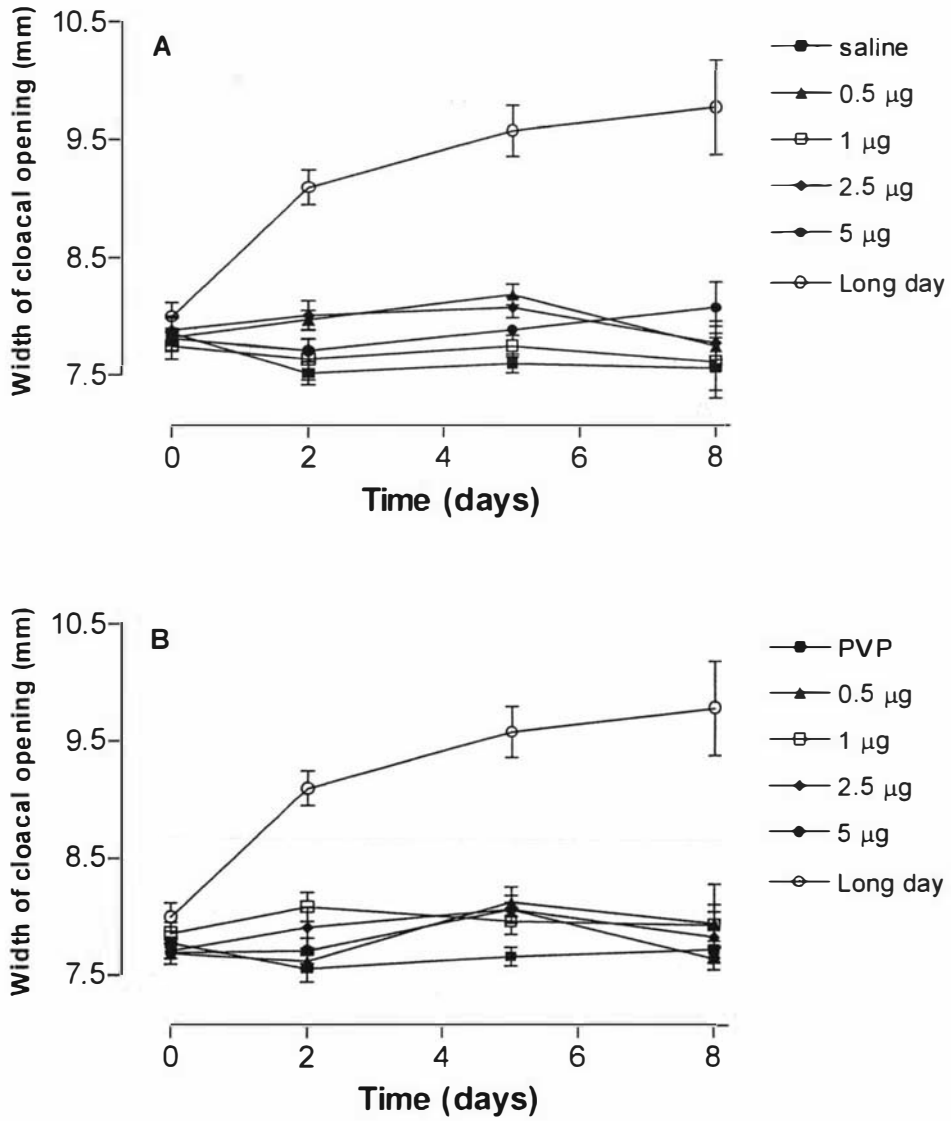

Figure 4.13. Mean width of cloacal opening in female quail treated daily with 0.5 µg, 1 µg, 2.5 µg or 5 µg cGnRH-II/kg body weight dissolved in saline (A) or PVP (B) for seven days. The mean width of cloacal opening of birds transferred to long days is also shown for comparison with treated birds. Results are shown as mean ± standard error.

Table 4.14. Two way repeated measures ANOVA for cloacal diameter in female quail injected daily with 0.5, 1, 2.5, 5 μg cGnRH-II /kg body weight in saline and birds transferred to long days for seven days.

		Width of cloacal opening		
Effect		**F**	**df**	***p***
Treatment		18.143	5,35	0.000*
Time		4.548	3,105	0.005*
Interaction of treatment and time		3.796	15,105	0.000*
Comparisons between treatments for each time				
Day 0	Saline vs 0.5 μg cGnRH-II	0.045	1,35	0.834
	Saline vs I μg cGnRH-II	0.546	1,35	0.465
	Saline vs 2.5 μg cGnRH-II	0.036	1,35	0.850
	Saline vs 5 μg cGnRH-II	0.083	1,35	0.775
	Saline vs long day	0.640	1,35	0.429
	Long day vs 0.5 μg cGnRH-II	1.151	1,35	0.291
	Long day vs I μg cGnRH-II	2.447	1,35	0.127
	Long day vs 2.5 μg cGnRH-II	0.489	1,35	0.489
	Long day vs 5 μg cGnRH-II	1.309	1,35	0.260
Day 2	Saline vs 0.5 μg cGnRH-II	5.713	1,35	0.022*
	Saline vs I μg cGnRH-II	0.324	1,35	0.573
	Saline vs 2.5 μg cGnRH-II	6.558	1,35	0.015*
	Saline vs 5 μg cGnRH-II	1.042	1,35	0.314
	Saline vs long day	43.467	1,35	0.000*
	Long day vs 0.5 μg cGnRH-II	24.971	1,35	0.000*
	Long day vs I μg cGnRH-II	44.782	1,35	0.000*
	Long day vs 2.5 μg cGnRH-II	23.407	1,35	0.000*
	Long day vs 5 μg cGnRH-II	39.338	1,35	0.000*
Day 5	Saline vs 0.5 μg cGnRH-II	19.090	1,35	0.000*
	Saline vs I μg cGnRH-II	1.287	1,35	0.264
	Saline vs 2.5 μg cGnRH-II	12.648	1,35	0.001*
	Saline vs 5 μg cGnRH-II	4.795	1,35	0.035*
	Saline vs long day	132.779	1,35	0.000*
	Long day vs 0.5 μg cGnRH-II	73.193	1,35	0.000*
	Long day vs I μg cGnRH-II	133.787	1,35	0.000*
	Long day vs 2.5 μg cGnRH-II	86.712	1,35	0.000*
	Long day vs 5 μg cGnRH-II	112.027	1,35	0.000*
Day 8	Saline vs 0.5 μg cGnRH-II	0.301	1,35	0.587
	Saline vs I μg cGnRH-II	0.014	1,35	0.906
	Saline vs 2.5 μg cGnRH-II	0.425	1,35	0.519
	Saline vs 5 μg cGnRH-II	2.132	1,35	0.153
	Saline vs long day	23.553	1,35	0.000*
	Long day vs 0.5 μg cGnRH-II	23.095	1,35	0.000*
	Long day vs I μg cGnRH-II	27.097	1,35	0.000*
	Long day vs 2.5 μg cGnRH-II	22.178	1,35	0.000*
	Long day vs 5 μg cGnRH-II	15.658	1,35	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

Table 4.15. Two way repeated measures ANOVA for cloacal diameter in female quail injected daily with 0.5, 1, 2.5, 5 μg cGnRH-II /kg body weight in PVP and birds transferred to long days for seven days.

Effect		Width of cloacal opening F	df	*p*
Treatment		16.157	5,35	0.000*
Time		9.859	3,105	0.000*
Interaction of treatment and time		3.320	15,105	0.000*
Comparisons between treatments for each time				
Day 0	PVP vs 0.5 μg cGnRH-II	0.502	1,35	0.483
	PVP vs 1 μg cGnRH-II	0.340	1,35	0.564
	PVP vs 2.5 μg cGnRH-II	0.261	1,35	0.613
	PVP vs 5 μg cGnRH-II	0.391	1,35	0.536
	PVP vs long day	1.817	1,35	0.186
	Long day vs 0.5 μg cGnRH-II	4.563	1,35	0.040*
	Long day vs 1 μg cGnRH-II	0.873	1,35	0.357
	Long day vs 2.5 μg cGnRH-II	3.812	1,35	0.059
	Long day vs 5 μg cGnRH-II	4.237	1,35	0.047*
Day 2	PVP vs 0.5 μg cGnRH-II	0.127	1,35	0.724
	PVP vs 1 μg cGnRH-II	7.443	1,35	0.010*
	PVP vs 2.5 μg cGnRH-II	3.241	1,35	0.080
	PVP vs 5 μg cGnRH-II	0.644	1,35	0.428
	PVP vs long day	41.055	1,35	0.000*
	Long day vs 0.5 μg cGnRH-II	44.720	1,35	0.000*
	Long day vs 1 μg cGnRH-II	20.062	1,35	0.000*
	Long day vs 2.5 μg cGnRH-II	28.549	1,35	0.000*
	Long day vs 5 μg cGnRH-II	39.337	1,35	0.000*
Day 5	PVP vs 0.5 μg cGnRH-II	7.404	1,35	0.010*
	PVP vs 1 μg cGnRH-II	3.189	1,35	0.083
	PVP vs 2.5 μg cGnRH-II	5.598	1,35	0.024*
	PVP vs 5 μg cGnRH-II	5.720	1,35	0.022*
	PVP vs long day	77.994	1,35	0.000*
	Long day vs 0.5 μg cGnRH-II	50.997	1,35	0.000*
	Long day vs 1 μg cGnRH-II	64.188	1,35	0.000*
	Long day vs 2.5 μg cGnRH-II	55.826	1,35	0.000*
	Long day vs 5 μg cGnRH-II	55.469	1,35	0.000*
Day 8	PVP vs 0.5 μg cGnRH-II	0.248	1,35	0.622
	PVP vs 1 μg cGnRH-II	0.317	1,35	0.577
	PVP vs 2.5 μg cGnRH-II	0.064	1,35	0.802
	PVP vs 5 μg cGnRH-II	0.065	1,35	0.801
	PVP vs long day	20.725	1,35	0.000*
	Long day vs 0.5 μg cGnRH-II	20.462	1,35	0.000*
	Long day vs 1 μg cGnRH-II	19.915	1,35	0.000*
	Long day vs 2.5 μg cGnRH-II	22.581	1,35	0.000*
	Long day vs 5 μg cGnRH-II	27.290	1,35	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

There was no effect of cGnRH-II in saline on ovary or oviduct weight or on plasma LH measured 24 hours after the last injection with, mean values of all these variables less than those of birds transferred to long days (Figure 4.14; see Table 4.16, 4.17 and 4.18 for statistics).

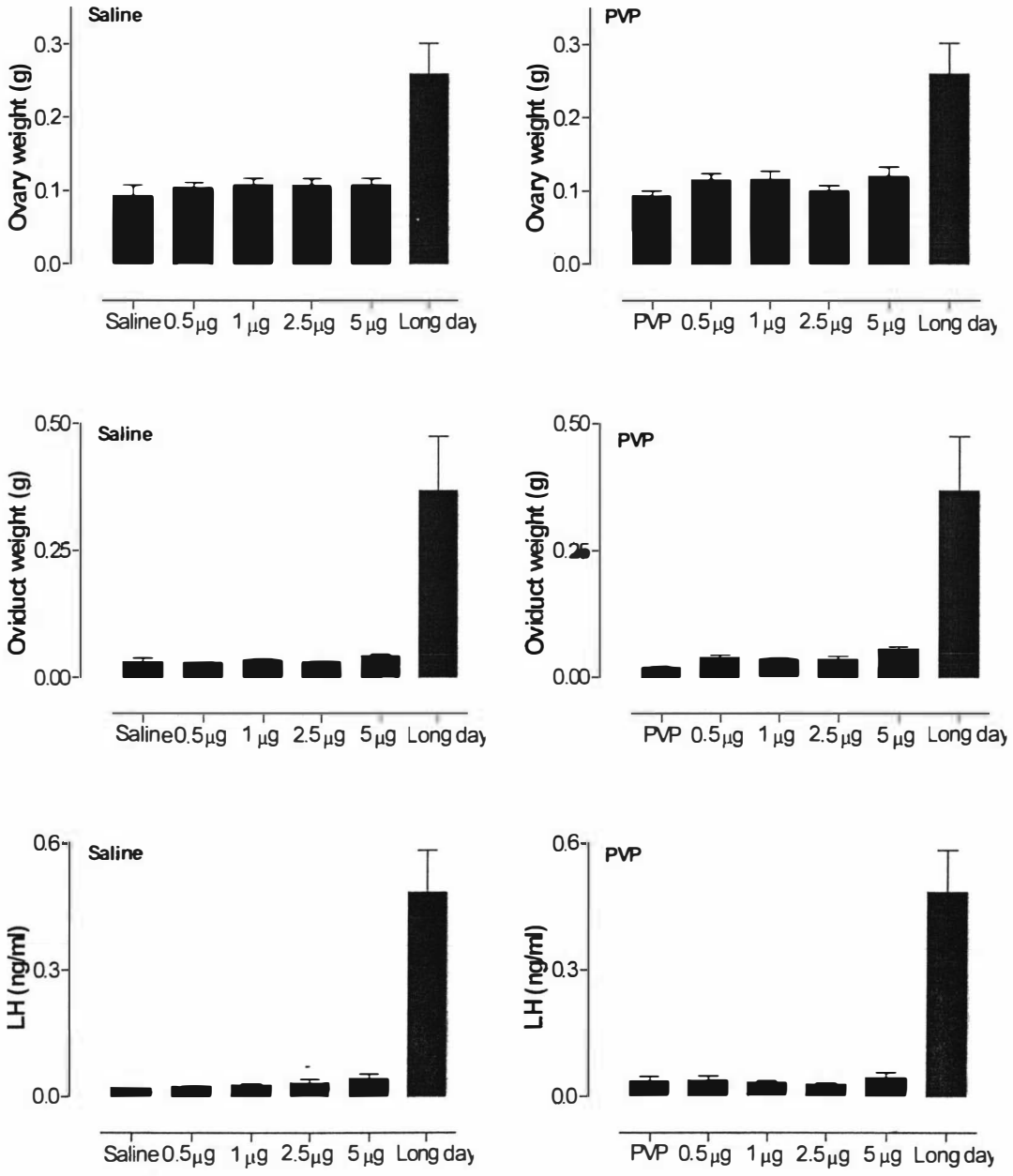

Figure 4.14. Mean ovary weights, oviduct weights and LH concentrations in female quail treated daily with 0.5, 1, 2.5 or 5 µg cGnRH-II/kg dissolved in saline or PVP for seven days or transferred to long days. Results are shown as mean ± standard error. Note different scales on y axes.

Table 4.16. One way ANOVA for ovary weights of female quail injected daily with 0.5, 1, 2.5, 5 μg cGnRH-II /kg or 0.5, 1, 2.5, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg in saline and birds transferred to long days for seven days.

	Ovary weight		
Effect	**F**	**df**	***p***
Group	6.340	9,63	0.000*
Comparisons between cGnRH-II groups			
Saline vs 0.5 μg cGnRH-II/kg			0.981
Saline vs 1 μg cGnRH-II/kg			0.965
Saline vs 2.5 μg cGnRH-II/kg			0.957
Saline vs 5 μg cGnRH-II/kg			0.935
Long day vs 0.5 μg cGnRH-II/kg			0.000*
Long day vs 1 μg cGnRH-II/kg			0.000*
Long day vs 2.5μg cGnRH-II/kg			0.000*
Long day vs 5 μg cGnRH-II/kg			0.000*
Comparisons between D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
Saline vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.993
Saline vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.998
Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.189
Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			1.000
Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 2.5μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Comparisons between cGnRH-II and D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
1 μg cGnRH-II vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000
2.5 μg cGnRH-II vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.817
5 μg cGnRH-II vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.999

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

Table 4.17. One way ANOVA for oviduct weights of female quail injected daily with 0.5, 1, 3, 5 μg cGnRH-II /kg or 0.5, 1, 2.5, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg in saline and birds transferred to long days for seven days.

	Oviduct weight		
Effect	**F**	**df**	***p***
Group	26.521	9,63	0.000*
Comparisons between cGnRH-II groups			
Saline vs 0.5 μg cGnRH-II/kg			1.000
Saline vs 1 μg cGnRH-II/kg			0.930
Saline vs 2.5 μg cGnRH-II/kg			0.997
Saline vs 5 μg cGnRH-II/kg			0.197
Long day vs 0.5 μg cGnRH-II/kg			0.000*
Long day vs 1 μg cGnRH-II/kg			0.000*
Long day vs 2.5μg cGnRH-II/kg			0.000*
Long day vs 5 μg cGnRH-II/kg			0.000*
Comparisons between D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
Saline vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.938
Saline vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.884
Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.085
Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.384
Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 2.5μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Comparisons between cGnRH-II and D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
1 μg cGnRH-II vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000
2.5 μg cGnRH-II vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.280
5 μg cGnRH-II vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

Table 4.18. One way ANOVA for LH concentrations of female quail injected daily with 0.5, 1, 2.5, 5 μg cGnRH-II /kg or 0.5, 1, 2.5, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg in saline and birds transferred to long days for seven days.

Effect	F	LH df	*p*
Group	17.448	9,63	0.000*
Comparisons between cGnRH-II groups			
Saline vs 0.5 μg cGnRH-II/kg			1.000
Saline vs 1 μg cGnRH-II/kg			0.995
Saline vs 2.5 μg cGnRH-II/kg			0.981
Saline vs 5 μg cGnRH-II/kg			0.472
Long day vs 0.5 μg cGnRH-II/kg			0.000*
Long day vs 1 μg cGnRH-II/kg			0.000*
Long day vs 2.5μg cGnRH-II/kg			0.000*
Long day vs 5 μg cGnRH-II/kg			0.000*
Comparisons between D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
Saline vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.996
Saline vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.972
Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.938
Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.963
Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 2.5μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Comparisons between cGnRH-II and D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
1 μg cGnRH-II vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000
2.5 μg cGnRH-II vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000
5 μg cGnRH-II vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.984

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

cGnRH-II dissolved in PVP also did not affect ovary or oviduct weights or LH concentrations which were all less than those of birds transferred to long days (Figure 4.14; see Table 4.19, 4.20 and 4.21 for statistics). A significant difference in oviduct weight between birds treated with PVP or 5 μg cGnRH-II/kg can be ascribed to the low oviduct weights of the PVP group compared with the saline only treated birds.

Table 4.19. One way ANOVA for ovary weights of female quail injected daily with 0.5, 1, 2.5, 5 μg cGnRH-II /kg or 0.5, 1, 3, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg in PVP and birds transferred to long days for seven days.

	Ovary weight		
Effect	**F**	**df**	***p***
Group	4.860	9,63	0.000*
Comparisons between cGnRH-II groups			
PVP vs 0.5 μg cGnRH-II/kg			0.954
PVP vs 1 μg cGnRH-II/kg			0.943
PVP vs 2.5 μg cGnRH-II/kg			1.000
PVP vs 5 μg cGnRH-II/kg			0.885
Long day vs 0.5 μg cGnRH-II/kg			0.000*
Long day vs 1 μg cGnRH-II/kg			0.000*
Long day vs 2.5μg cGnRH-II/kg			0.000*
Long day vs 5 μg cGnRH-II/kg			0.000*
Comparisons between D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
PVP vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			1.000
PVP vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.760
PVP vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.993
PVP vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.836
Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.001*
Long day vs 2.5μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.001*
Comparisons between cGnRH-II and D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
1 μg cGnRH-II vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000
2.5 μg cGnRH-II vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000
5 μg cGnRH-II vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

Table 4.20. One way ANOVA for oviduct weights of female quail injected daily with 0.5, 1, 2.5, 5 μg cGnRH-II /kg or 0.5, 1, 3, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg in PVP and in birds transferred to long days for seven days.

	Oviduct weight		
Effect	**F**	**df**	***p***
Group	25.667	9,63	0.000*
Comparisons between cGnRH-II groups			
PVP vs 0.5 μg cGnRH-II/kg			0.009*
PVP vs 1 μg cGnRH-II/kg			0.066
PVP vs 2.5 μg cGnRH-II/kg			0.088
PVP vs 5 μg cGnRH-II/kg			0.000*
Long day vs 0.5 μg cGnRH-II/kg			0.000*
Long day vs 1 μg cGnRH-II/kg			0.000*
Long day vs 2.5μg cGnRH-II/kg			0.000*
Long day vs 5 μg cGnRH-II/kg			0.000*
Comparisons between g D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
PVP vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.079
PVP vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
PVP vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
PVP vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.001*
Long day vs 2.5μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.001*
Comparisons between cGnRH-II and D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
1 μg cGnRH-II vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.534
2.5 μg cGnRH-II vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.485
5 μg cGnRH-II vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.941

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

Table 4.21. One way ANOVA for LH concentrations of female quail injected daily with 0.5, 1, 2.5, 5 μg cGnRH-II /kg or 0.5, 1, 3, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg in PVP and birds transferred to long days for seven days.

		LH	
Effect	**F**	**df**	***p***
Group	13.924	9,63	0.000*
Comparisons between cGnRH-II groups			
PVP vs 0.5 μg cGnRH-II/kg			1.000
PVP vs 1 μg cGnRH-II/kg			1.000
PVP vs 2.5 μg cGnRH-II/kg			0.998
PVP vs 5 μg cGnRH-II/kg			1.000
Long day vs 0.5 μg cGnRH-II/kg			0.000*
Long day vs 1 μg cGnRH-II/kg			0.000*
Long day vs 2.5μg cGnRH-II/kg			0.000*
Long day vs 5 μg cGnRH-II/kg			0.000*
Comparisons between D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
PVP vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.817
PVP vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.977
PVP vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.996
PVP vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			1.000
Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.001*
Long day vs 2.5μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.000*
Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg			0.001*
Comparisons between cGnRH-II and D-Lys^{6}Trp^{7}Tyr^8-GnRH groups			
1 μg cGnRH-II vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.992
2.5 μg cGnRH-II vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			0.628
5 μg cGnRH-II vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH			1.000

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

4.3.4.2 Daily injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH in saline or PVP

The width of the cloacal opening in birds injected with D-Lys^{6}Trp^{7}Tyr^8-GnRH in saline or PVP varied but remained narrow in the treatment groups (Figure 4.15; see Tables 4.22 and 4.23 for statistics). The mean width of the cloacal opening of birds transferred to long days was significantly wider than those of D-Lys^{6}Trp^{7}Tyr^8-GnRH treated birds from day two.

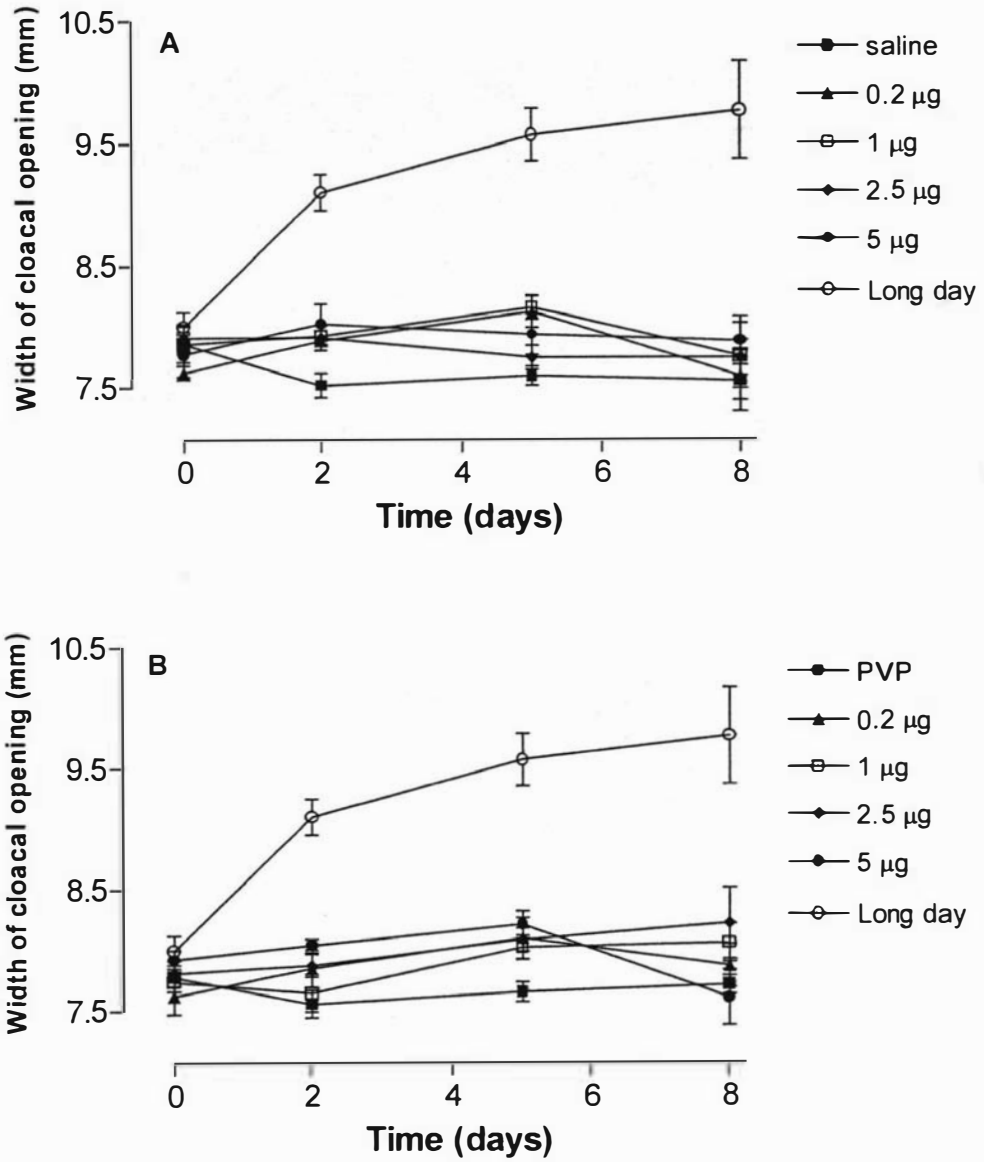

Figure 4.15. Mean width of cloacal opening in female quail treated daily with 0.2 µg, 1 µg, 2.5 µg or 5 µg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg body weight dissolved in saline (A) or PVP (B) for 7 days. The mean width of cloacal opening of birds transferred to long days is also shown for comparison with treated birds. Results are shown as mean ± standard error.

Table 4.22. Two way repeated measures ANOVA for cloacal diameter in female quail injected daily with 0.2, 1, 2.5, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg body weight in saline and birds transferred to long days for seven days.

Effect		Width of cloacal opening F	df	*p*
Treatment		17.646	5,35	0.000*
Time		4.537	3,105	0.005*
Interaction of treatment and time		3.308	15,105	0.000*
Comparisons between treatments for each time				
Day 0	Saline vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	1.559	1,35	0.220
	Saline vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.001	1,35	0.022*
	Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.079	1,35	0.781
	Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.285	1,35	0.615
	Saline vs long day	0.411	1,35	0.526
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	3.476	1,35	0.071
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.457	1,35	0.504
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.194	1,35	0.662
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	1.380	1,35	0.248
Day 2	Saline vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	4.735	1,35	0.036*
	Saline vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	5.718	1,35	0.022*
	Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	5.339	1,35	0.027*
	Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	8.490	1,35	0.006*
	Saline vs long day	54.617	1,35	0.000*
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	36.845	1,35	0.000*
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	34.452	1,35	0.000*
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	35.339	1,35	0.000*
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	28.979	1,35	0.000*
Day 5	Saline vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	7.427	1,35	0.010*
	Saline vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	8.585	1,35	0.006*
	Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.626	1,35	0.434
	Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	3.019	1,35	0.091
	Saline vs long day	65.132	1,35	0.000*
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	39.736	1,35	0.000*
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	37.370	1,35	0.000*
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	65.681	1,35	0.000*
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	52.175	1,35	0.000*
Day 8	Saline vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.014	1,35	0.905
	Saline vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.307	1,35	0.583
	Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.344	1,35	0.561
	Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.921	1,35	0.344
	Saline vs long day	24.248	1,35	0.000*
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	27.907	1,35	0.000*
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	23.797	1,35	0.000*
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	23.503	1,35	0.000*
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	20.255	1,35	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

Table 4.23. Two way repeated measures ANOVA for cloacal diameter in female quail injected daily with 0.2, 1, 2.5, 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight in PVP and birds transferred to long days for seven days.

		Width of cloacal opening		
Effect		**F**	**df**	***p***
Treatment		10.912	5,35	0.000*
Time		14.330	3,105	0.000*
Interaction of treatment and time		4.850	15,105	0.000*
Comparisons between treatments for each time				
Day 0	PVP vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	1.062	1,35	0.310
	PVP vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.075	1,35	0.786
	PVP vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.030	1,35	0.863
	PVP vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.766	1,35	0.387
	PVP vs long day	1.269	1,35	0.268
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	4.810	1,35	0.035*
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.218	1,35	0.145
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	1.150	1,35	0.291
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.176	1,35	0.678
Day 2	PVP vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.279	1,35	0.140
	PVP vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.200	1,35	0.657
	PVP vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	2.810	1,35	0.103
	PVP vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	6.395	1,35	0.016*
	PVP vs long day	41.939	1,35	0.000*
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	32.361	1,35	0.000*
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	44.593	1,35	0.000*
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	30.621	1,35	0.000*
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	22.467	1,35	0.000*
Day 5	PVP vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	5.282	1,35	0.028*
	PVP vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	3.804	1,35	0.059
	PVP vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	5.203	1,35	0.029*
	PVP vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	8.526	1,35	0.006*
	PVP vs long day	63.672	1,35	0.000*
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	43.580	1,35	0.000*
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	47.960	1,35	0.000*
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	43.792	1,35	0.000*
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	36.273	1,35	0.000*
Day 8	PVP vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.184	1,35	0.670
	PVP vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.991	1,35	0.326
	PVP vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	1.950	1,35	0.171
	PVP vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	0.161	1,35	0.690
	PVP vs long day	21.866	1,35	0.000*
	Long day vs 0.2 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	22.305	1,35	0.000*
	Long day vs 1 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	17.604	1,35	0.000*
	Long day vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	14.611	1,35	0.001*
	Long day vs 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH	30.209	1,35	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

Ovarian development did not occur in birds injected with D-Lys^{6}Trp^{7}Tyr^{8}-GnRH in saline or PVP and LH concentrations did not increase (Figure 4.16; see Tables 4.16, 4.17 and 4.18 for statistics). The apparent effects of D-Lys^{6}Trp^{7}Tyr^{8}-GnRH on oviduct weight were due to the low oviduct weight of the PVP only group.

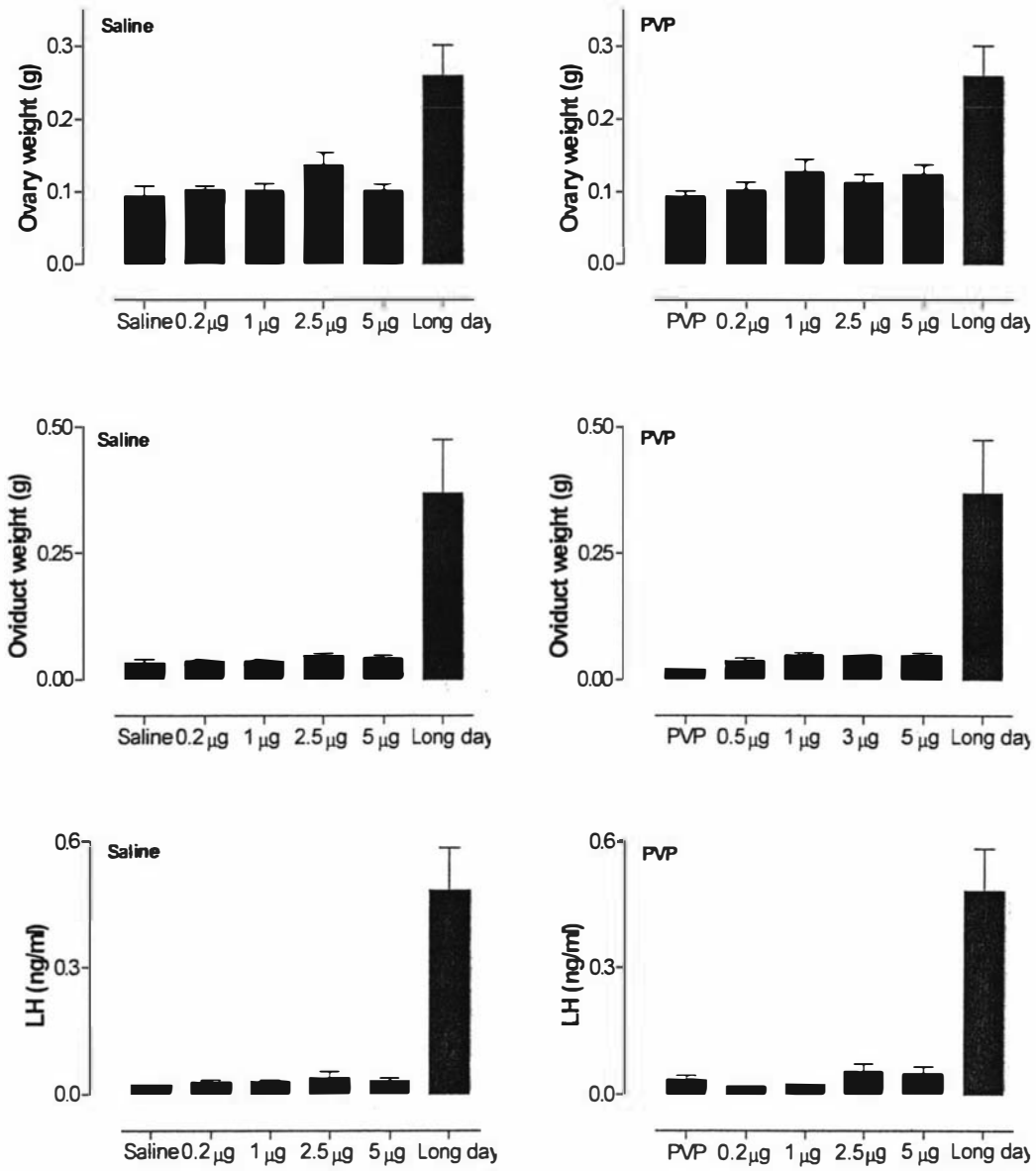

Figure 4.16. Mean ovary weights, oviduct weights and LH concentrations in female quail treated daily with 0.2, 1, 2.5 or 5 µg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg dissolved in saline or PVP for seven days or transferred to long days. Results are shown as mean ● standard error. Note different scales on y axes.

4.4 Discussion

4.4.1 Acute LH response to a single subcutaneous GnRH injection

Administration of cGnRH-I, cGnRH-II, buserelin or D-Lys^{6}Trp^{7}Tyr^8-GnRH by subcutaneous injection stimulated the secretion of LH in sexually regressed female Japanese quail. Thus indicating that the pituitary gland of sexually regressed birds contains a releasable pool of LH that can be rapidly secreted when stimulated with a sufficiently large GnRH stimulus. The results of the present study indicate that cGnRH-II is more potent (approximately double) than cGnRH-I in releasing LH *in vivo* in sexually regressed female quail. Similar results have been reported in sexually immature and mature hens (Dunn *et al.*, 1986; Sharp *et al.*, 1986; 1987; Wilson *et al.*, 1989) and for chicken and quail pituitaries *in vitro* (Millar and King, 1984; Chou *et al.*, 1985; Millar *et al.*, 1986). However, cGnRH-I and cGnRH-II have also been found to be equipotent in male quail and cockerels *in vivo* and *in vitro* (Hattori and Ishii, 1984; Chou *et al.*, 1985; Hattori *et al.*, 1986a). The discrepancies in these studies may be related to the purity of the GnRHs used, the timing of the collection of blood samples (*in vivo*) and incubation periods (*in vitro*). The weight of evidence indicates that cGnRH-II is the more active form in birds for LH release when administered exogenously, although cGnRH-I is the major regulator of gonadotropin secretion.

The greater potency of cGnRH-II is not due to a greater biological half-life. In domestic fowl the half-lives of cGnRH-I and cGnRH-II in males and females are approximately three minutes (Sharp *et al.*, 1987). The greater potency of cGnRH-II is most likely due to a greater affinity for the avian pituitary GnRH receptor (King *et al.*, 1988). A study of superactive GnRH analogues in birds found that GnRH molecules that combine the presence of Trp^{7}Tyr^8, as in cGnRH-II, have increased receptor binding and therefore increased LH/FSH activity (Delobelle *et al.*, 1995). Another mechanism which may explain the greater potency of cGnRH-II compared with cGnRH-I involves the degrading activities of both peptides. The degradation of cGnRH-I involves enzymatic cleaving to produce cGnRH-I$^{1\text{-}9}$ followed by cleaving between the Tyr^5-Gly^6 amino bond (discussed in chapter one). The difference in the activity between cGnRH-I and cGnRH-II may be due to the His^5-Gly^6 bond of cGnRH-II being less readily attacked by the degrading enzymes (Chou *et al.*, 1985). The physiological significance of the differences in the LH releasing abilities of cGnRH-I and cGnRH-II remains uncertain,

although it appears that cGnRH-II is not released into the pituitary portal blood in physiologically significant amounts.

Mammalian GnRH (mGnRH) and analogues of mGnRH have been shown to be effective in stimulating LH release in quail (Davies and Bicknell, 1976; Follett, and Davies, 1979) and hens (Bonney *et al.,* 1974; Wilson and Sharp, 1975c; Williams and Sharp, 1978; Johnson and van Tienhoven, 1981; Sterling and Sharp, 1984; Millar *et al.,* 1986; Sharp *et al.,* 1986; 1987). Buserelin is a classical superactive mGnRH analogue that is used clinically in mammals. Buserelin combines a D-amino acid in position six with the substitution of Gly-NH_2^{10} with ethylamide. Ethylamide is claimed to reduce degradation and metabolic clearance because it is more resistant to enzymatic degradation (Marks and Stern, 1974).

In mammals, buserelin is five to 100 fold more potent than mGnRH (Sandow, 1981; 1982). In the current study doses of 0.5 – 2.5 μg buserelin/kg induced LH responses similar to those in birds given cGnRH-II. These responses were approximately five times greater than LH responses to cGnRH-I. These results are similar to those reported in cockerels where 1 μg buserelin/kg was twice as potent as cGnRH-I (Sterling and Sharp, 1984). However in laying hens injection of 1 μg buserelin/kg produced a less significant LH response and had no effect on egg production (Sterling and Sharp, 1984). It is likely that the reduced responsiveness of the anterior pituitary gland to buserelin in the laying hen relative to the response in the cockerel and sexually regressed female quail (current study) is related to the high concentrations of ovarian steroids produced by the fully developed ovary which may affect the interaction of GnRH and its receptor. In the current study the highest doses of buserelin (2.5 – 4 μg) caused sustained LH secretion. Similar results were reported in incubating bantams injected three times in a 24 hour period with approximately 14 μg buserelin/kg per injection (Sharp *et al.,* 1986).

Administration of the cGnRH-II analogue, D-$Lys^6Trp^7Tyr^8$-GnRH by subcutaneous injection caused the greatest and longest LH responses in the current study. These results were similar to those reported in laying hens (Delobelle *et al.,* 1995) and confirm that the incorporation of a basic D-amino acid in position six combined with the presence of Trp^7Tyr^8 results in enhanced and prolonged LH release in birds. Trp^7Tyr^8-GnRH has previously been shown to be highly active in releasing LH *in vitro* without

the addition of a D-amino acid in position six of the peptide (Millar *et al.*, 1989). The substitution of Gly^6 in mGnRH with a D-amino acid increased GnRH activity by up to 10-fold in mammalian systems (Millar *et al.*, 1989). This increased activity was due to decreased degradation by endopeptidases, decreased metabolic clearance and increased binding affinity resulting from conformational constraint of a β-turn in the molecule that is needed for its biologically active conformation (Millar *et al.*, 1989). This conformational modification on its own only slightly enhances GnRH activity in chickens (Hasegawa *et al.*, 1984; Millar *et al.*, 1986; 1989). It appears that in non mammalian vertebrates the pituitary GnRH receptors are less discriminatory than mammalian GnRH receptors. However analogues in which D-amino acids in position six are combined with Trp^7 and Tyr^8 stimulate enhanced and prolonged LH secretion (Delobelle *et al.*, 1995). This combination is likely to increase the binding of GnRH to its receptor.

4.4.2 Effect of a single daily injection of GnRH

Daily injections of mGnRH and GnRH analogues can induce follicular growth and ovulation in seasonally anoestrous mammals. Pregnant mare serum gonadotropin (PMSG) has been used in our laboratory to stimulate ovarian growth in quail (Bennett, 2002), but the results were variable and often included overstimulation of the ovary. Potential advantages of GnRH treatment include the stimulation of homologous gonadotropin secretion from the pituitary gland and little risk of overstimulation of the ovary. cGnRH-II and synthetic versions of GnRH prolonged LH secretion more than cGnRH-I in quail, so it was considered worthwhile to determine whether daily injections, the simplest method of treatment, could stimulate ovarian growth. cGnRH-II and D-$Lys^6Trp^7Tyr^8$-GnRH were also dissolved in PVP to prolong their actions.

Seven days of treatment with cGnRH-II or D-$Lys^6Trp^7Tyr^8$-GnRH, with or without PVP did not stimulate ovarian growth in adult female quail with regressed ovaries. This experiment with mature birds differed from previous studies that used sexually immature birds. Single or twice daily injections of mGnRH, porcine GnRH or cGnRH-I for six – 10 days failed to induce ovarian development in hens (Reeves *et al.*, 1973), turkey hens (Burke *et al.*, 1977) or Japanese quail transferred to long days (16L:8D) during treatment (Zeman *et al.*, 1991) However in the latter study administration of cGnRH-I analogue, D-Tle^6 GnRH, did induce accelerated sexual development and egg

laying in Japanese quail transferred to long days at the start of treatment (Zeman *et al.*, 1991). It is possible that in the current and previous studies the GnRH-induced LH response to each injection was not long enough to stimulate ovarian growth.

It would have been informative to measure the LH responses to GnRH injections on successive days during the seven day treatment period. Repeated blood sampling of quail on successive days was not possible for ethical reasons associated with multiple blood sampling. If such measurements had been possible they would have revealed whether the magnitude and duration of LH responses was maintained or whether the responses declined with repeated treatment. It is possible that higher doses of GnRH that initially stimulated the greatest LH responses could subsequently have caused down regulation of GnRH receptors or depletion of pituitary LH leading to reductions in LH responses.

There have been two studies in which LH responses to GnRH were measured several times during daily GnRH treatment. In male redwinged blackbirds the GnRH induced LH response to 10 μg D-Trp^{6}Pro^9-N ethyl-amide GnRH declined after six and 14 days of daily injections (Lacombe *et al.*, 1992) suggesting that the pituitary gland had become desensitised to GnRH administration. Desensitisation of the pituitary gland in birds can be attributed to several mechanisms including the down regulation of GnRH receptors and depletion of releasable gonadotropin pools (King *et al.*, 1986). In contrast to the redwinged blackbirds, Sterling and Sharp (1984) reported similar sustained buserelin induced LH responses on days one and 12 in laying hens injected daily with either one or 10 μg buserelin/kg body weight.

Other studies have reported conflicting results from GnRH treatment of laying hens. Tilbrook *et al.* (1992) did not achieve complete desensitisation of the pituitary or cessation of egg production in hens treated daily with an extremely high dose (200 μg/kg) GnRH agonist (D-Trp^{6}Pro^{9}N-ethyl-amide GnRH), whilst cGnRH-II injected daily has caused ovarian regression (Sharp *et al.*, 1986) and complete cessation of egg production (Dickerman and Bahr, 1989).

4.5 Conclusion

cGnRH-I, cGnRH-II, buserelin and D-Lys^{6}Trp^{7}Tyr^{8}-GnRH injections induced secretion of LH but daily injections did not induce ovarian development in sexually regressed female Japanese quail. The absence of ovarian development could have been due to insufficient stimulation of pituitary gonadotropin secretion, or to desensitisation of the pituitary gland to GnRH stimulation. Unlike mammals where a single daily injection of GnRH can induce follicular growth and ovulation in some seasonally anoestrous animals, single daily injections of natural cGnRH or synthetic GnRH analogues may not be suitable for the induction of ovarian growth in birds with regressed ovaries. Further studies, described in the following chapters were therefore conducted in which quail were treated with repeated injections during a 24 hour period or continuous delivery of GnRH in osmotic pumps.

5 Stimulation of LH secretion by repeated D-Lys^{6}Trp^{7}Tyr^8-GnRH injections

5.1 Introduction

Repeated injections of mGnRH and GnRH analogues can induce follicular growth and ovulation in seasonally anoestrous mammals and in humans with reproductive disorders (Evans and Irvine, 1979; Riley *et al.*, 1981; McLeod *et al.*, 1982a; 1982b; McNeilly *et al.*, 1982 Walters *et al.*, 1982; Turner and Irvine, 1991). In the previous chapter it was found that single daily injections of various forms of GnRH for seven days did not stimulate increased baseline concentrations of LH or ovarian growth in Japanese quail. It was considered worthwhile to next determine the acute LH responses to repeated subcutaneous injections of GnRH.

The welfare of birds in procedures where multiple injections and serial blood sampling are employed is an important factor when designing such experiments. It was therefore important to determine the short-term LH responses to repeated GnRH injections before considering undertaking such an experiment over a longer period of time. Whilst the advantages of using injections include the ability to control the timing and dose of the hormone to be administered and injections are less invasive than surgery, there are disadvantages associated with multiple injection treatments. The administration of repeated injections followed by serial blood sample collection requires regular capture and handling of birds. Experiments in our laboratory have shown that Japanese quail do not initiate a corticosterone response when handled for less than two minutes which is sufficient time to administer a single injection and/or collect a blood sample (Boyd, 2000; Chua, 2003). However, repeated injections and serial blood sampling over a prolonged period might result in elevated corticosterone concentrations. Constantly elevated plasma corticosterone concentrations caused by continuous infusion resulted in

decreased LH concentrations in female birds (Etches *et al.,* 1984; Petitte and Etches, 1988; 1989) and decreased the pituitary's response to exogenous GnRH in male quail (Connolly and Callard, 1987). Additionally the increased risks of infection associated with multiple injections and sampling are important factors to be assessed.

Repeated injections of GnRH during a 24-hour period in birds have induced LH responses leading to ovarian development in some studies (Minoia *et al.,* 1984) but not others (Burke *et al.,* 1977; Sharp *et al.,* 1986). The aims of the current experiment were to determine the LH responses of Japanese quail to injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH at six-hour intervals over a 24-hour period. In the study reported in the previous chapter a single injection of D-Lys^{6}Trp^{7}Tyr^8-GnRH produced consistent and prolonged LH responses in sexually regressed female quail, so D-Lys^{6}Trp^{7}Tyr^8-GnRH was used in this study. The experiment was designed to determine whether the LH response to GnRH was maintained or diminished with repeated injections over 24 hours. It was intended that a further experiment be conducted in which multiple injections were given over a week or more to determine if this treatment regime would stimulate ovarian growth. Practical constraints meant that this second experiment would not be performed.

5.2 Materials and Methods

5.2.1 Animals and housing

Seven week old sexually mature female Japanese quail (*Coturnix coturnix japonica*) with wildtype plumage were purchased from a commercial source (Canter Valley Farms, Christchurch, New Zealand). The birds were reared under a long day photoperiod (15 hours light: 9 hours dark) at ambient temperatures (20 - 25°C). Before the start of the experiments quail were identified and housed as described in section 3.2.1.

Birds were held under a long day photoperiod until they reached nine weeks of age. This treatment allowed the quail to be maintained with sexually active reproductive systems and daily egg laying to be recorded for each bird. At nine weeks of age the birds were transferred to a short day photoperiod at 10 ± 2 °C. This treatment caused regression of the reproductive system. Body weight and cloacal diameter measurements were measured weekly and egg laying continued to be recorded daily. Sexually

regressed birds were determined to be those that had not laid eggs for more than 10 days and had cloacal diameters less than 9.0 mm. The experiment began after six weeks of short days and only sexually regressed birds were included in the experiment.

5.2.2 Experimental Design

Fifteen week old female Japanese quail were divided into four groups of six birds and eight groups of eight birds (Table 5.1).

Table 5.1. Experimental groups

Group	Injection solution	Time injection administered	Time serial blood sample collection started
1	Saline	0800 h	0800 h
2	Saline	0800, 1400 h	1400 h
3	Saline	0800, 1400, 2000 h	2000 h
4	Saline	0800, 1400, 2000, 0200 h	0200 h
5	2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800 h	0800 h
6	2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800, 1400 h	1400 h
7	2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800, 1400, 2000 h	2000 h
8	2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800, 1400, 2000, 0200 h	0200 h
9	5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800 h	0800 h
10	5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800, 1400 h	1400 h
11	5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800, 1400, 2000 h	2000 h
12	5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg	0800, 1400, 2000, 0200 h	0200 h

The groups of six birds received either one, two, three or four subcutaneous injections of a 0.9 % saline solution during a 24-hour period. The groups of eight birds received either one, two, three or four subcutaneous injections of 2.5 or 5 μg cGnRH-II analogue solution during a 24-hour period. Serial blood samples were collected from groups 1, 5 and 9 beginning at 0800 h, from groups 2, 6 and 10 beginning at 1400 h, from groups 3, 7 and 11 at 2000 h and from groups 4, 8 and 12 at 0200 hours. Each series of blood samples consisted of a blood sample taken immediately before the injection (time 0) followed by samples taken 5, 10 and 20 minutes after the injection. Blood samples were collected in this manner to minimise handling effects on the endogenous hormone rhythms in the birds and welfare issues associated with multiple sampling collection. A final blood sample was collected 24 hours after the start of the first series of samples. Plasma concentrations of LH were measured in all blood samples.

All experimental procedures were approved by the Massey University Animal Ethics Committee.

5.2.3 Hormone preparation and administration

Solutions were made from a 20 μg/ml D-Lys^{6}Trp^{7}Tyr^8-GnRH stock solution by dilution with 0.9 % saline where necessary, to give doses of 2.5 or 5 μg/kg body weight in a 200 μl volume injection. The GnRH solutions were prepared the day before the experiment and kept at 4 °C overnight in glass flasks. Injections were administered subcutaneously on the abdomen using a 1 ml insulin syringe and a 27 gauge, ½ inch needle.

5.2.4 Data collection

Blood samples were collected by puncture of the ulnar vein with a 27 gauge needle and approximately 150 μl of blood was collected into heparinized capillary tubes. Blood was expelled into heparinized 1 ml tubes and kept on ice until centrifugation. Samples were centrifuged at 1 900 g for 15 minutes (Beckman TJ-6 centrifuge). Plasma was removed with a glass Hamilton syringe, stored in 1.5 ml polypropylene Eppendorf tubes and frozen at – 20 °C until assay.

5.2.5 LH radioimmunoassays

LH concentrations were determined by radioimmunoassay using the methodology outlined in section 4.2.5.1.

5.2.6 Statistics

The experiment was arranged as a randomised block design with a factorial arrangement of treatment (saline or GnRH) and time of injection as the main effects. Birds were placed at random, in cages on two racks consisting of four tiers of 10 cages per tier and one rack consisting of one tier of 10 cages. Data were transformed to logarithms to reduce heteroscedascity where necessary. All variables were tested for homogeneity of variance using the Levene's test. Repeated measures two way ANOVA with time and treatment as the grouping factors was used to compare groups. Comparisons between times within each treatment and between treatments for each time were examined with post-hoc repeated measures contrasts. A value of $p < 0.05$ was taken as statistically significant and indicated by an asterisk (*).

ANOVAs followed by post hoc tests and the non-parametric equivalents were performed using Systat Version 8 (SPSS Inc., 1988). Data are presented as individual

points (raw data) or as means ± standard error (group data) using GraphPad Prism Version 3.0 (GraphPad Software Inc., 1999).

5.3 Results

5.3.1 LH response to the first injection of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8 – GnRH

LH concentrations were similar in all groups at the start of the experiment (Figures 5.1 and 5.2; see Table 5.2 for statistics). LH concentrations did not change after an injection of saline from 0 to 10 minutes then increased slightly at 20 minutes. Mean LH concentrations increased from pre-treatment values five minutes after injection of 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH, remained elevated after 10 minutes then declined at 20 minutes to concentrations that were still elevated above pre-treatment values. The LH responses at five and 10 minutes but not 20 minutes were greater for the 5 μg than the 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH.

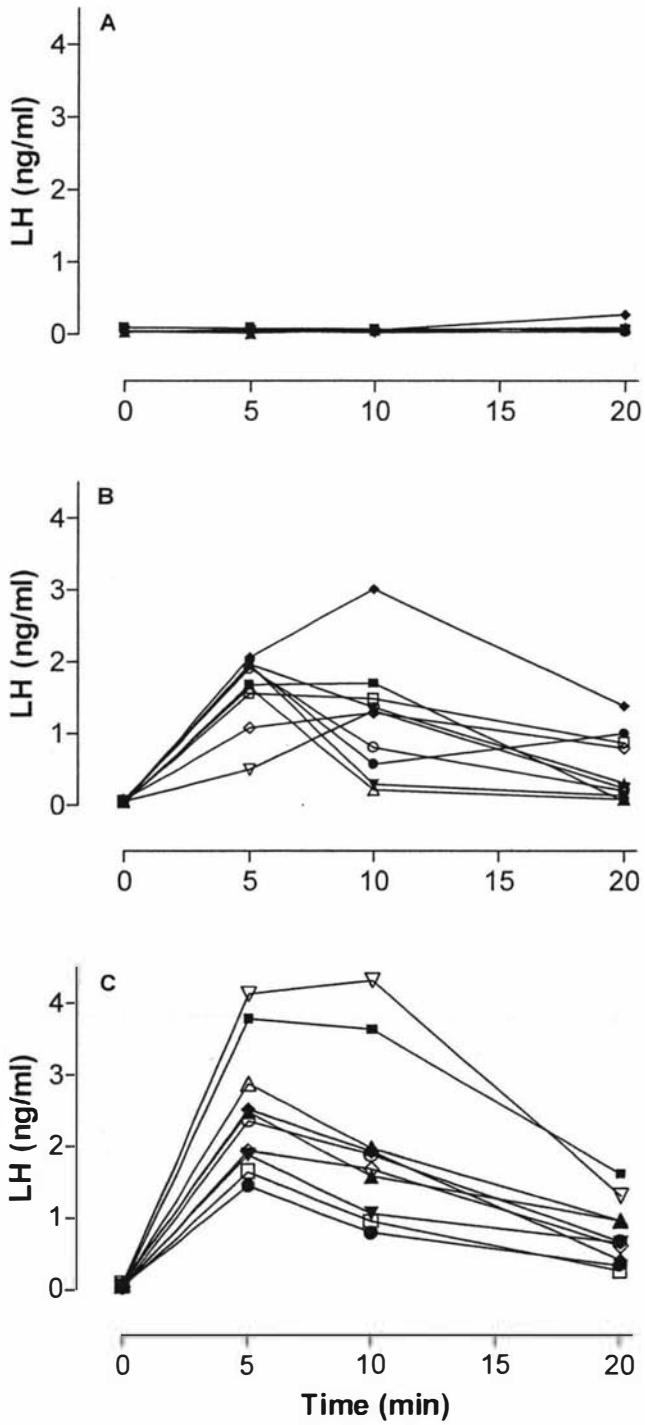

Figure 5.1. Individual LH concentrations in birds after a single injection of saline (A), 2.5 (B) or 5 (C) μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg.

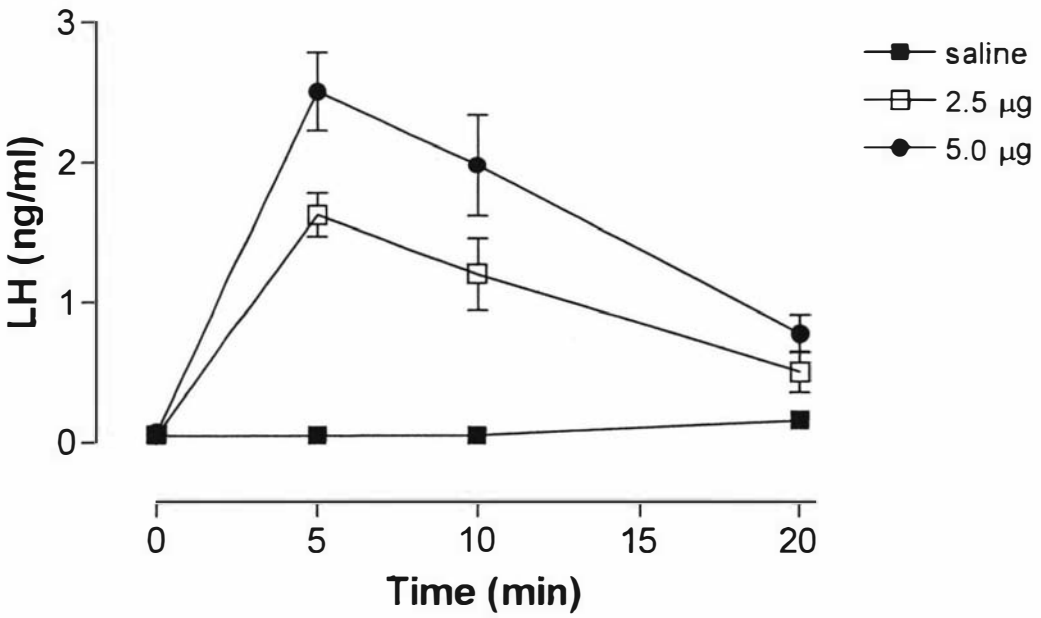

Figure 5.2. Mean LH concentrations in birds after a single injection of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg. Results are shown as mean ± standard error.

Table 5.2. Two way repeat measures ANOVA for LH concentrations in female quail after a single injection of saline or 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8 –GnRH /kg body weight.

Effect		LH F	LH df	LH *p*
Treatment		62.176	2,22	0.000*
Time		81.259	3,66	0.000*
Interaction of treatment and time		18.929	6,66	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	0.047	1,22	0.831
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8 –GnRH	461.813	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8 –GnRH	529.653	1,22	0.000*
0 vs 10 min	Saline	0.006	1,22	0.938
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8 –GnRH	162.310	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8 –GnRH	212.683	1,22	0.000*
0 vs 20 min	Saline	6.144	1,22	0.021*
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8 –GnRH	40.099	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8 –GnRH	70.140	1,22	0.000*
Comparisons between treatments for each time				
0 min	Saline vs 2.5 μg	1.290	1,22	0.268
	Saline vs 5 μg	5.454	1,22	0.029*
	2.5 vs 5 μg	2.159	1,22	0.156
5 min	Saline vs 2.5 μg	215.002	1,22	0.000*
	Saline vs 5 μg	272.226	1,22	0.000*
	2.5 vs 5 μg	5.058	1,22	0.035*
10 min	Saline vs 2.5 μg	66.817	1,22	0.000*
	Saline vs 5 μg	97.276	1,22	0.000*
	2.5 vs 5 μg	4.278	1,22	0.051
20 min	Saline vs 2.5 μg	4.034	1,22	0.057
	Saline vs 5 μg	12.938	1,22	0.002*
	2.5 vs 5 μg	3.785	1,22	0.065

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

5.3.2 LH response to the second injection of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8 – GnRH

LH concentrations did not differ between groups before the second injection of saline or D-Lys^{6}Trp^{7}Tyr^8-GnRH (Figures 5.3 and 5.4; see Table 5.3 for statistics). LH concentrations in response to saline declined from 0 to 10 minutes followed by an increase to concentrations not different to pre-treatment values. LH concentrations in response to 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg increased to broad peaks five and 10 minutes after injection then declined at 20 minutes to concentrations that were still elevated above initial concentrations. D-Lys^{6}Trp^{7}Tyr^8-GnRH induced LH responses at five and 10 minutes which did not differ between the 2.5 and 5 μg doses but were greater at 20 minutes for the 5 μg dose.

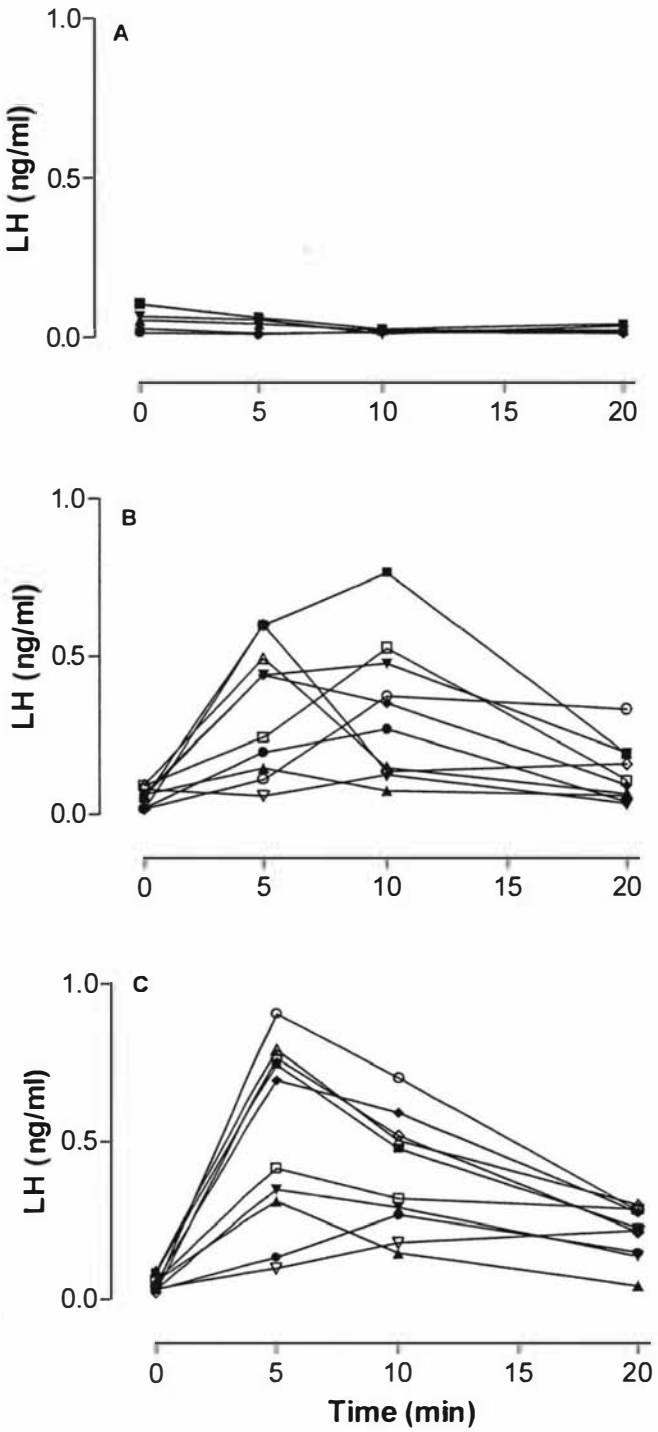

Figure 5.3. Individual LH concentrations in birds after a second injection of saline (A), 2.5 (B) or 5 (C) μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg given six hours after the first injection.

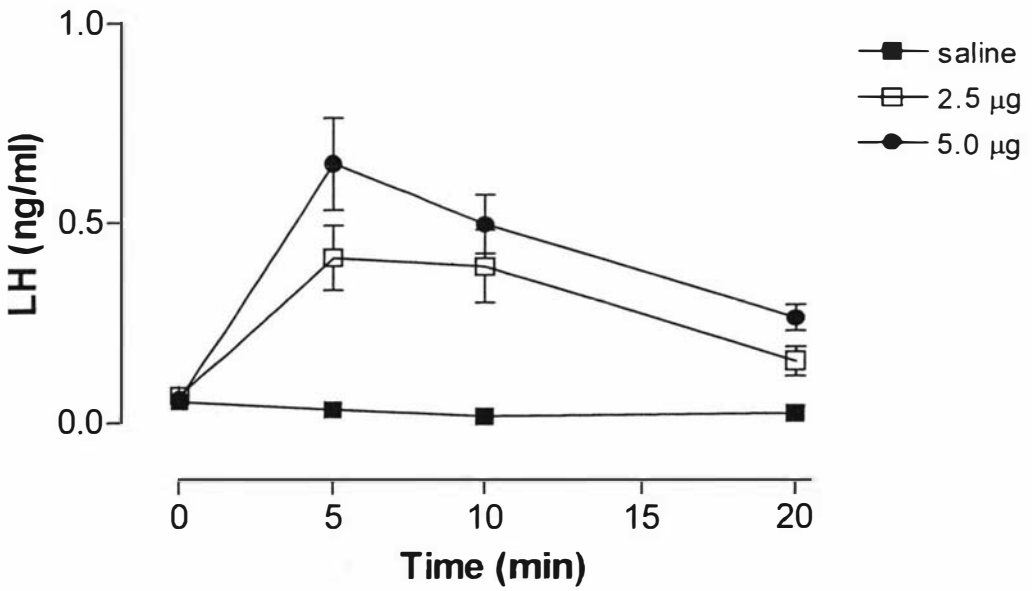

Figure 5.4. Mean LH concentrations in birds after a second injection of saline, 2.5 or 5 µg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg given six hours after the first injection. Results are shown as mean ± standard error.

Table 5.3. Two way repeat measures ANOVA for LH concentrations in female quail after two subcutaneous injections (one injection every six hours) of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight.

Effect		LH F	LH df	LH *p*
Treatment		33.551	2,22	0.000*
Time		17.428	3,66	0.000*
Interaction of treatment and time		9.658	6,66	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	1.402	1,22	0.249
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	42.448	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	70.831	1,22	0.000*
0 vs 10 min	Saline	4.474	1,22	0.046*
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	26.080	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	44..922	1,22	0.000*
0 vs 20 min	Saline	2.236	1,22	0.149
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	6.702	1,22	0.017*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	24.945	1,22	0.000*
Comparisons between treatments for each time				
0 min	Saline vs 2.5 μg	0.742	1,22	0.398
	Saline vs 5 μg	0.586	1,22	0.452
	2.5 vs 5 μg	0.014	1,22	0.908
5 min	Saline vs 2.5 μg	30.929	1,22	0.000*
	Saline vs 5 μg	43.868	1,22	0.000*
	2.5 vs 5 μg	1.692	1,22	0.207
10 min	Saline vs 2.5 μg	49.16	1,22	0.000*
	Saline vs 5 μg	67.006	1,22	0.000*
	2.5 vs 5 μg	2.005	1,22	0.171
20 min	Saline vs 2.5 μg	21.528	1,22	0.000*
	Saline vs 5 μg	42.195	1,22	0.000*
	2.5 vs 5 μg	5.167	1,22	0.033*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

5.3.3 LH response to the third injection of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8 – GnRH

LH concentrations did not differ before the third injection between groups injected with saline or 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg and were significantly higher in birds injected with 5 μg (Figures 5.5 and 5.6; see Table 5.4 for statistics). There was no LH response to saline injections. LH concentrations in response to 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg increased to broad peaks five and 10 minutes after injection then declined at 20 minutes. There was a clear dose response relationship for the stimulation of LH after a third injection of D-Lys^{6}Trp^{7}Tyr^8-GnRH. The LH response to 5 μg was greater than the response to 2.5 μg at five, 10 and 20 minutes. At 20 minutes LH concentrations of 5 but not 2.5 μg-treated birds were elevated above those of saline treated birds.

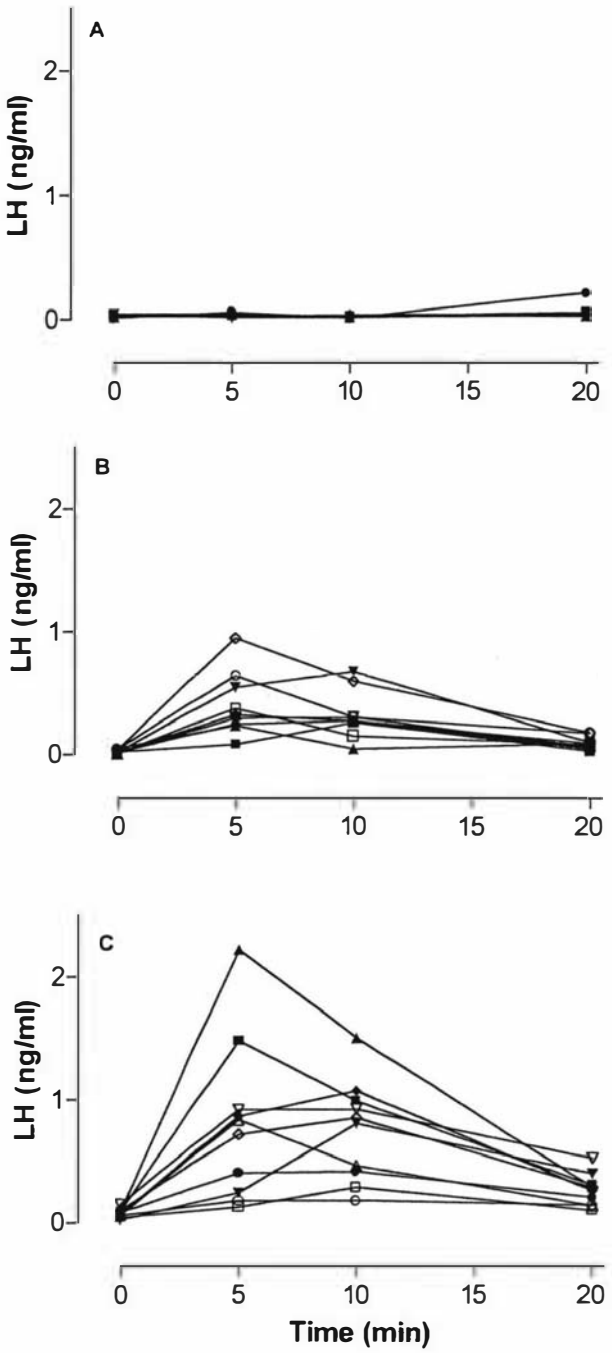

Figure 5.5. Individual LH concentrations in birds after a third injection of saline (A), 2.5 (B) or 5 (C) μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg given six hours after the second injection and 12 hours after the first injection.

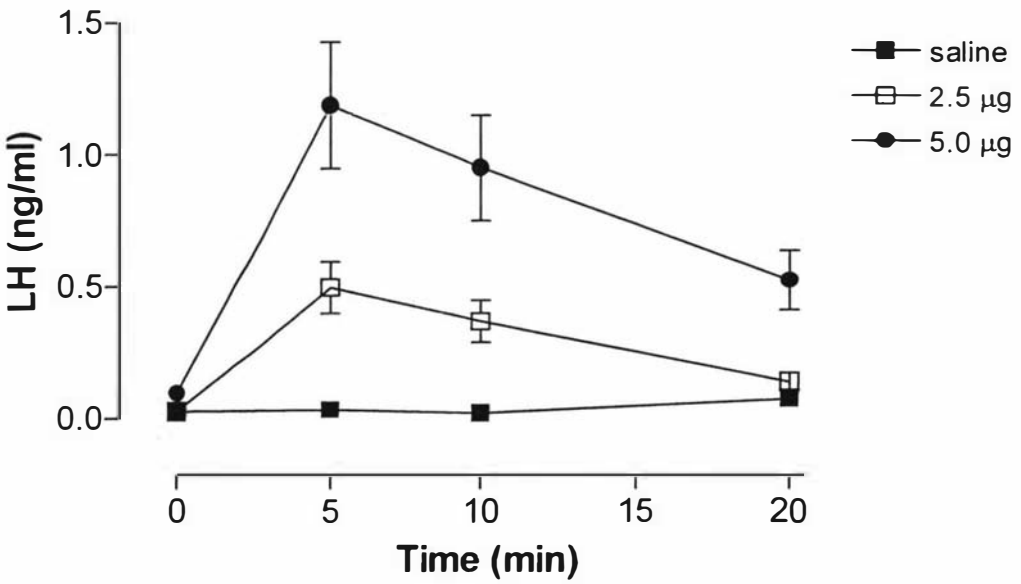

Figure 5.6. Mean LH concentrations in birds after a third injection of saline, 2.5 or 5 µg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg given 6 hours after the second injection and 12 hours after the first injection. Results are shown as mean ±standard error.

Table 5.4. Two way repeat measures ANOVA for LH concentrations in female quail after three subcutaneous injections (one injection every six hours) of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight.

Effect		LH F	df	*p*
Treatment		50.584	2,22	0.000*
Time		50.031	3,66	0.000*
Interaction of treatment and time		11.680	6,66	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	0.404	1,22	0.531
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	133.731	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	93.052	1,22	0.000*
0 vs 10 min	Saline	0.602	1,22	0.446
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	107.101	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	79.127	1,22	0.000*
0 vs 20 min	Saline	4.294	1,22	0.050
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	32.182	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	31.129	1,22	0.000*
Comparisons between treatments for each time				
0 min	Saline vs 2.5 μg	0.116	1,22	0.737
	Saline vs 5 μg	14.325	1,22	0.001*
	2.5 vs 5 μg	25.521	1,22	0.000*
5 min	Saline vs 2.5 μg	55.900	1,22	0.000*
	Saline vs 5 μg	100.689	1,22	0.000*
	2.5 vs 5 μg	9.813	1,22	0.005*
10 min	Saline vs 2.5 μg	45.593	1,22	0.000*
	Saline vs 5 μg	86.188	1,22	0.000*
	2.5 vs 5 μg	9.612	1,22	0.005*
20 min	Saline vs 2.5 μg	2.898	1,22	0.103
	Saline vs 5 μg	26.383	1,22	0.000*
	2.5 vs 5 μg	17.690	1,22	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

5.3.4 LH response to the fourth injection of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH

LH concentrations did not differ before the fourth injection between groups injected with saline or 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg whereas LH concentrations were significantly higher in birds injected with 5 μg (Figures 5.7 and 5.8; see Table 5.5 for statistics). There was no LH response to saline injections. LH concentrations in response to 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH increased to broad peaks five and 10 minutes after injection then declined at 20 minutes to concentrations that were still elevated above initial concentrations. D-Lys^{6}Trp^{7}Tyr^8-GnRH-induced LH responses of birds injected with 2.5 or 5 μg did not differ significantly.

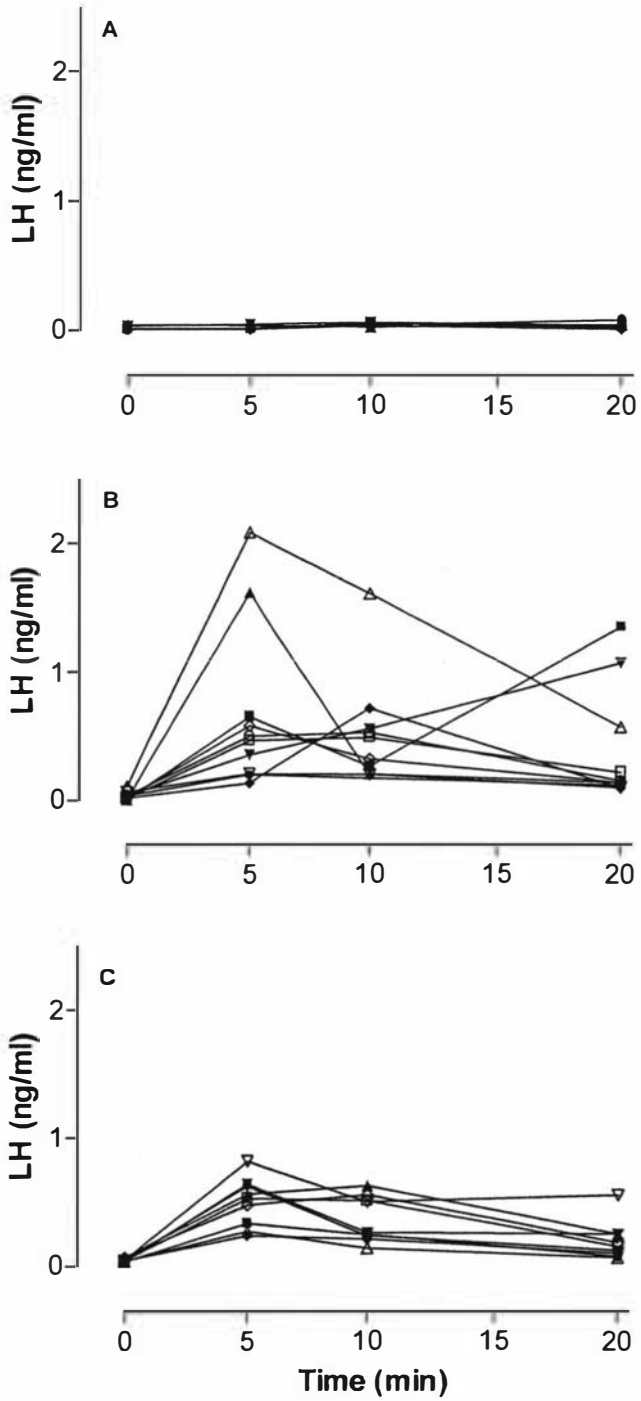

Figure 5.7. Individual LH concentrations in birds after a fourth injection of saline (A), 2.5 (B) or 5 (C) μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg given six hours after the third injection, 12 hours after the second injection and 18 hours after the first injection.

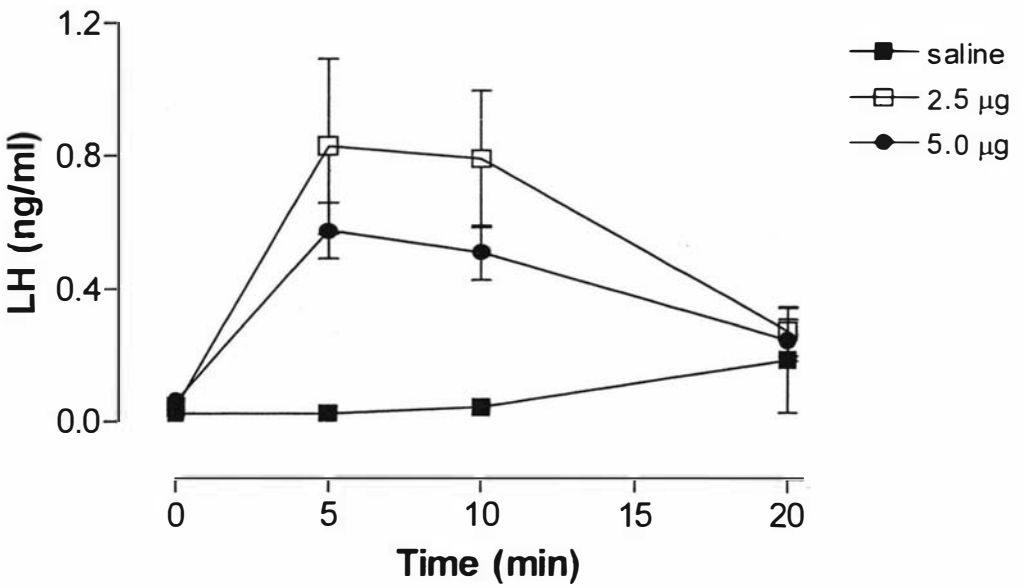

Figure 5.8. Mean LH concentrations in birds after a fourth injection of saline, 2.5 or 5 µg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg given six hours after the third injection, 12 hours after the second injection and 18 hours after the first injection. Results are shown as mean ±standard error.

Table 5.5. Two way repeat measures ANOVA for LH concentrations in female quail after four subcutaneous injections (one injection every six hours) of saline, 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight.

Effect		LH F	LH df	LH *p*
Treatment		22.731	2,22	0.000*
Time		45.824	3,66	0.000*
Interaction of treatment and time		7.721	6,66	0.000*
Comparisons within treatments for each treatment				
0 vs 5 min	Saline	0.012	1,22	0.912
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8--GnRH	120.517	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	73.095	1,22	0.000*
0 vs 10 min	Saline	4.164	1,22	0.053
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	113.108	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	54.338	1,22	0.000*
0 vs 20 min	Saline	3.618	1,22	0.070
	2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	28.018	1,22	0.000*
	5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	12.316	1,22	0.002*
Comparisons between treatments for each time				
0 min	Saline vs 2.5 μg	1.384	1,22	0.252
	Saline vs 5 μg	9.577	1,22	0.005*
	2.5 vs 5 μg	5.519	1,22	0.028*
5 min	Saline vs 2.5 μg	55.900	1,22	0.000*
	Saline vs 5 μg	59.679	1,22	0.000*
	2.5 vs 5 μg	0.093	1,22	0.764
10 min	Saline vs 2.5 μg	49.311	1,22	0.000*
	Saline vs 5 μg	43.325	1,22	0.000*
	2.5 vs 5 μg	0.290	1,22	0.595
20 min	Saline vs 2.5 μg	6.646	1,22	0.017*
	Saline vs 5 μg	7.588	1,22	0.012*
	2.5 vs 5 μg	0.047	1,22	0.831

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

5.3.5 LH responses to repeated injections of 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH

LH concentrations 5, 10 and 20 minutes after injection were higher after the first GnRH injection at 2.5 μg/kg than after subsequent injections (Figure 5.9A; see Table 5.6 for statistics). LH responses to the second, third and fourth injections did not differ.

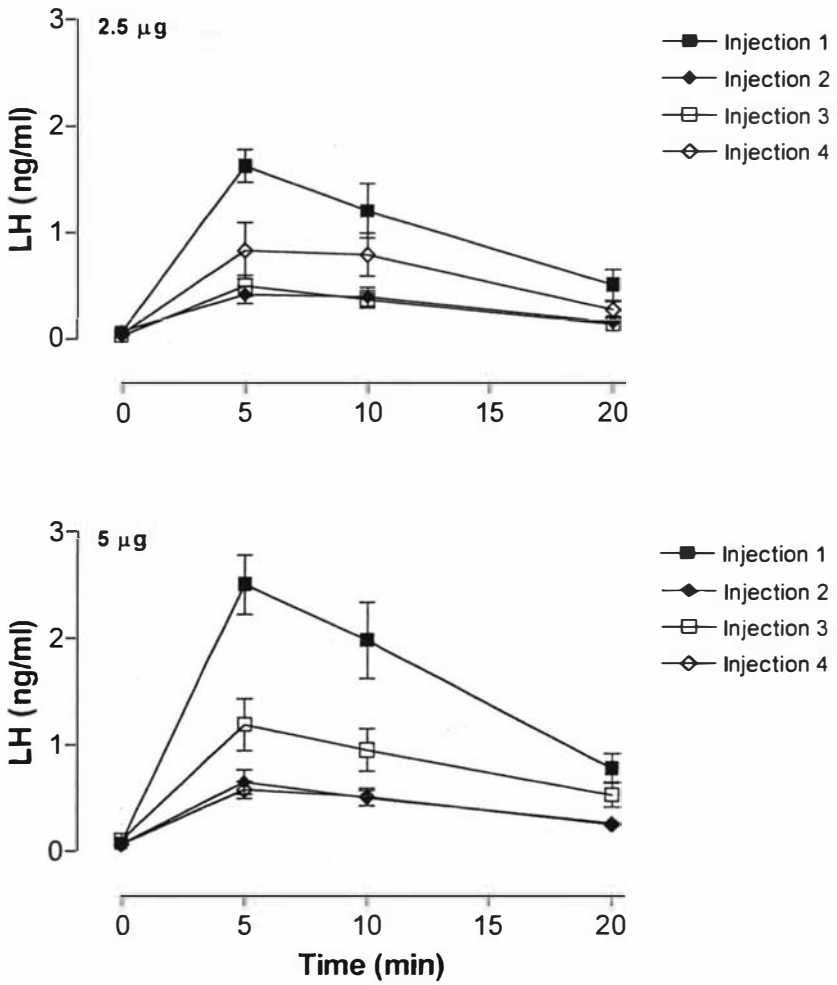

Figure 5.9. Changes in mean LH concentrations in birds after one, two, three or four injections of 2.5 (A) or 5 (B) µg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg given six hours apart. Results are shown as mean ±standard error.

Table 5.6. Two way single measures ANOVA for LH concentrations in female quail after one, two, three or four subcutaneous injections (one injection every six hours) of 2.5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight.

Effect		LH F	df	*p*
Injection		27.754	3,144	0.000*
Time		5.361	3,144	0.000*
Interaction of treatment and time		2.566	9,144	0.068
Comparisons between treatments for each time				
0 min	Injection 1 vs injection 2	66.029	1,144	0.000*
	Injection 1 vs injection 3	47.916	1,144	0.000*
	Injection 1 vs injection 4	18.037	1,144	0.000*
	Injection 2 vs injection 3	1.449	1,144	0.231
	Injection 2 vs injection 4	15.045	1,144	0.000*
	Injection 3 vs injection 4	7.156	1,144	0.008*
5 min	Injection 1 vs injection 2	13.307	1,144	0.000*
	Injection 1 vs injection 3	13.669	1,144	0.000*
	Injection 1 vs injection 4	8.399	1,144	0.004*
	Injection 2 vs injection 3	0.002	1,144	0.961
	Injection 2 vs injection 4	0.562	1,144	0.455
	Injection 3 vs injection 4	0.638	1,144	0.426
10 min	Injection 1 vs injection 2	21.657	1,144	0.000*
	Injection 1 vs injection 3	23.624	1,144	0.000*
	Injection 1 vs injection 4	10.941	1,144	0.001*
	Injection 2 vs injection 3	0.043	1,144	0.837
	Injection 2 vs injection 4	1.812	1,144	0.180
	Injection 3 vs injection 4	2.411	1,144	0.123
20 min	Injection 1 vs injection 2	49.478	1,144	0.000*
	Injection 1 vs injection 3	52.559	1,144	0.000*
	Injection 1 vs injection 4	22.215	1,144	0.000*
	Injection 2 vs injection 3	0.047	1,144	0.830
	Injection 2 vs injection 4	5.386	1,144	0.022*
	Injection 3 vs injection 4	6.434	1,144	0.012*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

5.3.6 LH responses to repeated injections of 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH

The LH response to the first injection of 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH was greater than responses to subsequent injections (Figure 5.9B, see Table 5.7 for statistics). The responses to the second and fourth injection were similar whereas there was a larger response to the third injection than to the second and fourth injections.

Table 5.7. Two way single measures ANOVA for LH concentrations in female quail after one, two, three or four subcutaneous injections (one injection every six hours) of 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight.

Effect		LH F	LH df	LH *p*
Injection		32.187	3,140	0.000*
Time		173.259	3,140	0.000*
Interaction of treatment and time		2.739	9,140	0.006*
Comparisons between treatments for each time				
0 min	Injection 1 vs injection 2	0.676	1,140	0.412
	Injection 1 vs injection 3	1.005	1,140	0.318
	Injection 1 vs injection 4	0.290	1,140	0.591
	Injection 2 vs injection 3	3.331	1,140	0.070
	Injection 2 vs injection 4	0.069	1,140	0.794
	Injection 3 vs injection 4	2.292	1,140	0.132
5 min	Injection 1 vs injection 2	37.186	1,140	0.000*
	Injection 1 vs injection 3	12.147	1,140	0.001*
	Injection 1 vs injection 4	34.808	1,140	0.000*
	Injection 2 vs injection 3	6.826	1,140	0.010*
	Injection 2 vs injection 4	0.001	1,140	0.972
	Injection 3 vs injection 4	6.288	1,140	0.013
10 min	Injection 1 vs injection 2	29.890	1,140	0.000*
	Injection 1 vs injection 3	10.415	1,140	0.002*
	Injection 1 vs injection 4	27.955	1,140	0.000*
	Injection 2 vs injection 3	5.017	1,140	0.027*
	Injection 2 vs injection 4	0.001	1,140	0.973
	Injection 3 vs injection 4	4.606	1,140	0.034*
20 min	Injection 1 vs injection 2	17.863	1,140	0.000*
	Injection 1 vs injection 3	3.452	1,140	0.065
	Injection 1 vs injection 4	22.806	1,140	0.000*
	Injection 2 vs injection 3	5.609	1,140	0.019*
	Injection 2 vs injection 4	0.438	1,140	0.509
	Injection 3 vs injection 4	8.803	1,140	0.004*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

5.3.7 LH concentrations at the end of the experiment

LH concentrations before the first injection did not differ between birds to be treated with saline or 2.5 or 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH, nor did they differ between groups 24 hours after the beginning of the experiment (Figure 5.10; see Table 5.8 for statistics).

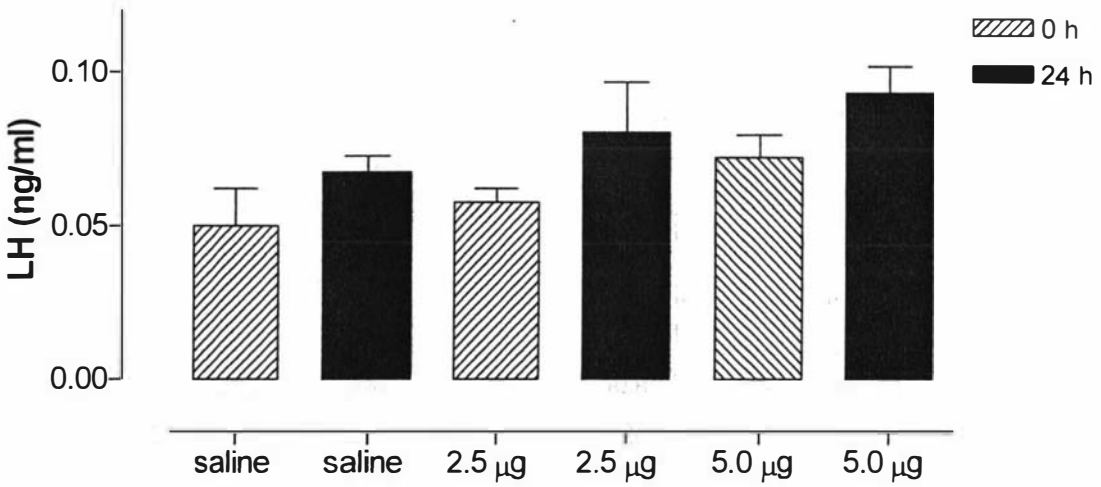

Figure 5.10. Mean LH concentrations in birds before treatment (0 h) and at the end of treatment (24 h). Results are shown as mean ± standard error.

Table 5.8. Two way single measures ANOVA for LH concentrations in female quail after four subcutaneous injections (one injection every six hours) of 5 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg body weight.

Effect		LH F	LH df	LH *p*
Group		2.632	2,43	0.083
Treatment		2.950	1,43	0.093
Interaction of group and treatment		0.332	2,43	0.719
Comparisons between treatments for each time				
Before experiment	Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	0.589	1,43	0.447
	Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	2.489	1,43	0.122
	2.5 vs 5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	0.985	1,43	0.326
End of experiment	Saline vs 2.5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	0.099	1,43	0.755
	Saline vs 5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	1.294	1,43	0.262
	2.5 vs 5 μg D-Lys^{6}Trp^{7}Tyr^8–GnRH	3.084	1,43	0.086
Comparisons within treatments				
	Saline 0 h vs saline 24 h	1.559	1,43	0.219
	2.5 μg at 0 h vs 2.5 μg at 24 h	0.194	1,43	0.662
	5 μg at 0 h vs 5 μg at 24 h	1.486	1,43	0.230

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

5.4 Discussion

The administration of subcutaneous injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH at six-hour intervals did not increase baseline concentrations of LH in sexually regressed female Japanese quail over a 24-hour period, although consecutive GnRH injection continued to stimulate LH secretion. Administration of the first D-Lys^{6}Trp^{7}Tyr^8-GnRH injections of 2.5 or 5 μg/kg caused a greater LH response in terms of peak concentrations and sustained elevation of LH, than subsequent injections. Birds injected with 5 μg did however have higher LH concentrations than birds treated with 2.5 μg or saline prior to the third and fourth injections. LH concentrations did not however differ between groups 24 hours after the start of treatment. LH did not return to initial concentrations 20 minutes after GnRH injection, so the full duration of the LH responses is not known. In the previous chapter LH concentrations in response to the same D-Lys^{6}Trp^{7}Tyr^8-GnRH doses were decreasing but remained elevated at the end of the sampling period (20 minutes). Delobelle *et al.* (1995) found a single injection of D-Lys^{6}Trp^{7}Tyr^8-GnRH maintained elevated LH concentrations for more than 60 minutes after injection in laying hens. It is possible that LH in the quail remained elevated for at least one hour after each GnRH injection.

The decreased LH responses with repeated D-Lys^{6}Trp^{7}Tyr^8-GnRH injections could indicate desensitisation of the pituitary gland. Desensitisation in birds involves both a gradual loss of pituitary responsiveness to GnRH because of reduced numbers of GnRH receptors, an alteration in post-receptor metabolic events and depletion of a previously formed pool of pituitary LH (King *et al.,* 1986). A study in immature female Japanese quail treated with two successive intravenous injections of synthetic mGnRH found LH responses to a second injection 30 and 60 minutes after the first were significantly reduced whereas 90 minutes after the first injection the LH response did not differ from the response to the first injection (Davies *et al.,* 1979). The results from the present study differ from those of Davies *et al.* in that responses to a second injection six hours after the first were reduced. The GnRH analogue used here is a more potent stimulation of LH release in birds than mGnRH and it may be that it induced down-regulation of GnRH receptors in the quail pituitary gland.

It is unclear from the present study whether six hourly injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH for a week or longer would have resulted in increased baseline LH concentrations and ovarian growth. Repeated subcutaneous injections of D-Arg^6-cGnRH-II or buserelin in incubating bantams at seven to nine hour intervals for nine days resulted in the loss of pituitary gland responsiveness and thus failed to maintain elevated plasma LH concentrations or induce ovarian growth (Sharp *et al.,* 1986). Similarly, twice daily intravenous injections of synthetic mammalian GnRH for six days did not induce ovarian development in turkey hens (Burke and Cogger, 1977). In contrast Minoia *et al.* (1984) reported moderate development of ovarian follicles and oviducts of pheasants and partridges after five, 10 and 15 days of treatment with 3.8 μg mGnRH every eight hours. LH concentrations were not measured in this study. The pheasants and partridges were held under conditions of natural light so ovarian growth might have been stimulated, at least in part, by long daylength.

5.5 Conclusion

Repeated injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH stimulated acute LH responses but did not induce an overall increase in baseline LH concentrations in sexually regressed female Japanese quail over a 24-hour period. The results of the current study do not indicate that repeated injections of a GnRH analogue over a period of weeks are not

warranted given the lack of evidence for a GnRH effect on ovarian stimulation and the possible adverse effects on the birds. Continuous infusions of low concentrations of GnRH are another method that has been used to successfully increase LH and FSH concentrations leading to ovarian growth and ovulation in seasonally anoestrous mammals and in reptiles. The effects of continuous delivery of GnRH in birds were therefore examined in the study described in the next chapter.

6 Effect of D-Lys^{6}Trp^{7}Tyr^8-GnRH delivered by osmotic pump on LH secretion and ovarian growth

6.1 Introduction

Follicular growth and ovulation in seasonally anoestrous domestic mammals can be induced by continuous infusion of mGnRH and GnRH analogues (Wright *et al.*, 1983; Jagger *et al.*, 1989; Dodson *et al.*, 1990; Ainsworth and Hyland, 1991), although this treatment is not always successful (Lincoln *et al.*, 1986; Jagger *et al.*, 1989). It was shown in the previous two chapters that subcutaneous injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH stimulated LH secretion but would not induce ovarian growth in sexually regressed Japanese quail when administered once per day or increase baseline LH concentrations when administered at six hour intervals during a 24 hour period. It was therefore considered important to investigate and compare an alternative method for delivering known concentrations of D-Lys^{6}Trp^{7}Tyr^8-GnRH to sexually regressed quail.

Osmotic mini-pumps have been used in many experiments to deliver peptides and proteins to animals including treatment of Japanese quail with pregnant mare serum gonadotropin (Wakabayashi *et al.*, 1996; Bennett, 2002). Although minor surgery is required, once implanted, osmotic pumps provide reliable constant hormone release and birds do not need to be captured and handled until the pump has expired and needs to be replaced or removed. This convenience would be particularly useful in situations where birds being treated were endangered species or where it is difficult or stressful for birds to be captured and handled regularly.

In vitro and *in vivo* studies in which GnRH solutions were continuously infused have had limited success in birds. *In vitro* studies have shown both sustained dose-dependent

GnRH-induced LH and FSH secretion in Japanese quail anterior pituitary cells (Hattori *et al.,* 1986a) and reduced LH secretion due to the effects of desensitisation in chicken pituitary cells (Millar and King, 1984). Infusion of mGnRH over seven days in turkey hens did not induce LH secretion for more than 16 hours and did not lead to ovarian growth (Burke and Cogger, 1977). Whilst both continuous and pulsatile infusion of GnRH via osmotic pumps did not significantly increase ovary weights there was some stimulation of ovarian growth in ducks treated with high doses (500 ng/hour)(Cockrem *et al.,* unpublished observations). Although endogenous GnRH secretion is pulsatile in nature the results of Cockrem *et al.* indicated that it was worthwhile to examine the use of pumps as a potential method for the stimulation of ovarian growth in quail.

The current experiment was designed to investigate the effects of D-Lys^{6}Trp^{7}Tyr^{8}-GnRH administered via osmotic mini-pump on plasma LH concentrations and ovarian growth over 14 days. Studies in which GnRH was administered to mammals by osmotic minipump have demonstrated the importance of administering the correct dosage of GnRH at a constant and controlled rate. There were no previous data for birds on the delivery of GnRH analogues in pumps. Doses were therefore chosen based on a range of doses used in a range of bird species to stimulate LH concentrations via subcutaneous, intravenous and intramuscular injection.

6.2Materials and Methods

6.2.1 Animals and housing

Six week old sexually mature female Japanese quail (*Coturnix coturnix japonica*) with wildtype plumage were purchased from a commercial source (Rangitikei Game Birds, Bulls, New Zealand). Before the start of the quail were identified and housed as described in section 3.2.1.

Birds were held under a long day photoperiod until they reached eight weeks of age. This treatment allowed the quail to be maintained with sexually active reproductive systems and daily egg laying to be recorded for each bird. At eight weeks of age the birds were transferred to a short day photoperiod at 10 ± 2 °C. This treatment caused regression of the reproductive system. Body weight and cloacal diameter measurements were measured weekly and egg laying continued to be recorded daily. Sexually

regressed birds were determined to be those that had not laid eggs for more than 10 days and had cloacal diameters less than 9.0 mm. The experiment began after five weeks of short days and only sexually regressed birds were included in the experiment.

6.2.2 Experimental Design

Fifteen week old female Japanese quail were divided into six groups of eight birds and one group of five birds (Table 6.1).

Table 6.1. Experimental groups

Group	Photoperiod	Dose of D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg/day (μg)
1	Euthanased at start of experiment	
2	8L:16D	5
3	8L:16D	10
4	8L:16D	15
5	8L:16D	20
6	8L:16D	-
7	16L:8D	-

Group one was euthanased at the start of the treatment period to ensure that ovaries and oviducts were regressed prior to the start of treatment. Birds in groups two to five received an osmotic mini-pump loaded with a GnRH solution to deliver either 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg/day. Birds in group six remained on short days, received no treatment and were euthanased at the end of the experiment. Birds in group seven were transferred to a long day photoperiod at 20 °C on the day pumps were implanted into birds in groups two-five. The rates of oviductal and ovarian growth stimulated by the long day photoperiod provided targets for the GnRH treatment to achieve. Sham treated groups of birds where pumps infused saline solution were not included in this experiment. Previous experiments in our laboratory have shown that the width of the cloacal opening, ovary and oviductal weights and plasma oestradiol concentrations were not different between non-treated birds maintained on 8L:16D at 10 °C and sham treated birds on 8L:16D at 10 °C with pumps infusing 0.9 % saline (Bennett, 2002).

Blood samples were collected from each bird on days 0 (immediately before pumps were inserted or before transfer to 16L:8D), 1, 4, 7, 10 and 14. Plasma concentrations of LH were measured in all blood samples.

All experimental procedures were approved by the Massey University Animal Ethics Committee.

6.2.3 Hormone preparation and administration

Solutions were made from a 20 μg/ml D-Lys^{6}Trp^{7}Tyr^8-GnRH stock solution by dilution with 0.9 % saline where necessary. The GnRH solutions were prepared the day before the experiment and kept at 4 °C overnight in glass flasks. The D-Lys^{6}Trp^{7}Tyr^8-GnRH solutions were administered using Alzet mini-osmotic pumps (Alzet, Model 2002; Alza Pharmaceutical, Palo Alta CA, USA). The model 2002 pumps were designed to continuously release their contents at 0.5 μl/hour over a 14 day period at a body temperature of 37 °C.

To prepare the pump the D-Lys^{6}Trp^{7}Tyr^8-GnRH solution was injected using a 1 ml insulin syringe and special filling tube to fill the osmotic pump. The flow moderator (a 21 gauge stainless steel tube with a plastic end-cap) was inserted through the exit port in the plug until the plastic end-cap was flush with the top of the pump. The flow moderator prevents diffusive and convective losses of drug solution from the pump during operation so that the delivery profile is osmotically controlled. As the osmotic pump comes in contact with an aqueous environment, water is transported across the semi-permeable membrane into the osmotic layer and causes the osmotic layer to swell thereby generating constant pressure to collapse the flexible drug reservoir uniformly.

Osmotic pumps were implanted subcutaneously after local anaesthesia using Lopaine (lignocaine hydrochloride USP) at 10 μg/g body weight. A small incision was made on the left ventral surface of the abdomen and pumps inserted beneath the skin. The incision was closed using a surgical wound clip (Autoclip, Becton Dickinson, USA) and a topical antibiotic cream applied (Bactroban, Smith Kline Beecham).

6.2.4 Data collection

The width of the cloacal opening was measured before each blood sample on days 0, 1, 4, 7, 10 and 14 using vernier callipers. Blood samples were collected by puncture of the

ulnar vein with a 27 gauge needle and approximately 150 μl of blood was collected into heparinized capillary tubes. Blood was expelled into heparinized 1 ml tubes and kept on ice until centrifugation. On day 14 the final blood sample was collected following euthanasia by stunning then decapitation. Trunk blood was collected into heparinized 10 ml polypropylene centrifuge tubes and kept on ice until centrifugation. Samples were centrifuged at 1 900 g for 15 minutes (Beckman TJ-6 centrifuge). Plasma was removed with a glass Hamilton syringe, stored in 1.5 ml polypropylene Eppendorf tubes and frozen at – 20 °C until assay. Ovaries and oviducts were excised and weighed. The number of yellow ovarian follicles was recorded.

6.2.5 LH radioimmunoassays

LH concentrations were determined by radioimmunoassay using the methodology outlined in section 4.2.5.1.

6.2.6 Statistics

The experiment was arranged as a randomised block design and the birds were placed at random, in cages on one rack consisting of four tiers of 10 cages per tier. Data were transformed to logarithms to reduce heteroscedascity where necessary. All variables were tested for homogeneity of variance using the Levenes test. Repeated measures two way ANOVA with time and treatment as the grouping factors was used to compare groups. Comparisons between times within each treatment and between treatments for each time were examined with post-hoc repeated measures contrasts. Ovary and oviduct weights were analysed using single measures ANOVA followed by Tukey's post hoc tests to determine differences between groups. A value of $p < 0.05$ was taken as statistically significant and indicated by an asterisk (*).

ANOVAs followed by post hoc tests were performed using Systat Version 8 (SPSS Inc., 1988). Data are presented as individual points (raw data) or as means ± standard error (group data) using GraphPad Prism Version 3.0 (GraphPad Software Inc., 1999).

6.3 Results

One bird implanted with an osmotic pump (10 μg/kg/day) was withdrawn from the experiment because the wound clip failed to maintain the pump in position.

6.3.1 Width of the cloacal opening

The width of the cloacal opening increased after four days in birds transferred to long days and continued to increase (Figure 6.1; see Table 6.2 for statistics), whereas there were no significant changes in width in birds held on short days or treated with GnRH.

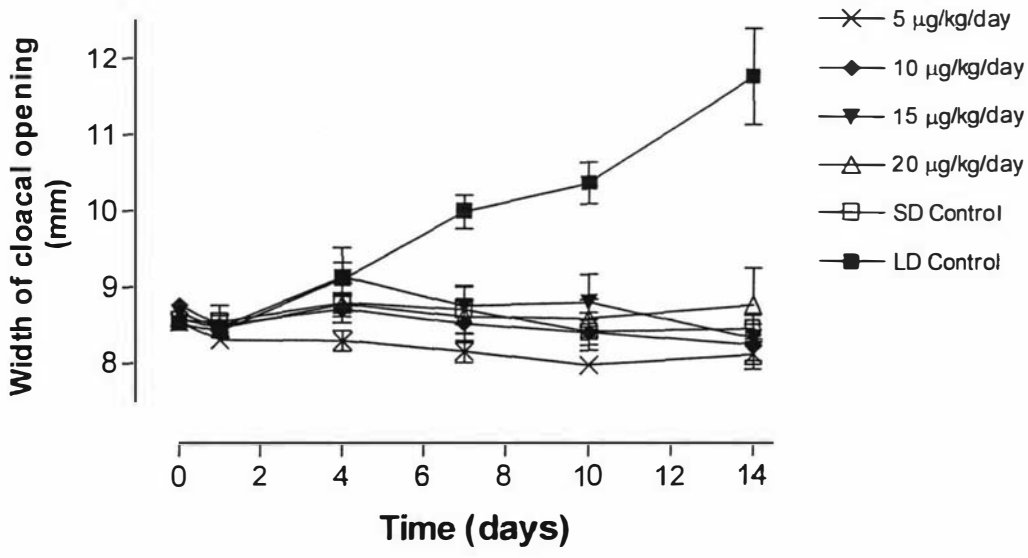

Figure 6.1. Mean width of cloacal opening in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 µg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg/day for 14 days by osmotic pump. Results are shown as mean ± standard error.

Table 6.2. Two way repeat measures ANOVA for the width of the cloacal opening in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH /kg/day for 14 days by osmotic pump.

		Width of cloacal opening		
Effect		**F**	**df**	***p***
Treatment		9.043	5,41	0.000*
Time		3.497	5,205	0.005*
Interaction of treatment and time		7.992	25,205	0.005*
Comparisons within treatments for each treatment				
Day 0 vs 1	5 μg/kg/day	8.846	1,41	0.005*
	10 μg/kg/day	9.797	1,41	0.003*
	15μg/kg/day	5.975	1,41	0.019*
	20 μg/kg/day	2.916	1,41	0.095
	Short day control	0.228	1,41	0.635
	Long day control	1.569	1,41	0.218
Day 0 vs 4	5 μg/kg/day	1.797	1,41	0.188
	10 μg/kg/day	0.095	1,41	0.759
	15μg/kg/day	4.051	1,41	0.050
	20 μg/kg/day	0.709	1,41	0.405
	Short day control	1.055	1,41	0.310
	Long day control	8.128	1,41	0.007*
Day 0 vs 7	5 μg/kg/day	4.029	1,41	0.051
	10 μg/kg/day	1.515	1,41	0.225
	15μg/kg/day	0.071	1,41	0.792
	20 μg/kg/day	0.001	1,41	0.977
	Short day control	0.170	1,41	0.683
	Long day control	41.393	1,41	0.000*
Day 0 vs 10	5 μg/kg/day	8.532	1,41	0.006*
	10 μg/kg/day	2.979	1,41	0.092
	15μg/kg/day	0.160	1,41	0.691
	20 μg/kg/day	0.030	1,41	0.864
	Short day control	0.821	1,41	0.370
	Long day control	65.096	1,41	0.000*
Day 0 vs 14	5 μg/kg/day	1.899	1,41	0.176
	10 μg/kg/day	2.358	1,41	0.132
	15μg/kg/day	1.288	1,41	0.263
	20 μg/kg/day	0.050	1,41	0.824
	Short day control	0.160	1,41	0.691
	Long day control	62.093	1,41	0.000*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

6.3.2 Luteinising hormone (LH)

LH concentrations did not differ between groups at the start of the experiment (Figures 6.2 and 6.3; see Table 6.3 for statistics). LH concentrations in quail maintained on short days did not change during the experiment. LH in quail transferred to long days increased significantly after 24 hours to a peak at 2.43 ± 0.30 ng/ml on day four then declined, although concentrations remained higher than those in all other groups. LH did not differ between days 0 and one in birds treated with 5, 10 or 15 μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH /kg/day then declined to concentrations significantly lower than pre-treatment values and lower than concentrations in birds on short days or long days. LH concentrations in birds treated with 20μg/kg/day decreased significantly after one day of treatment and were not different to 5, 10 or 15 μg treatment groups on days 10 and 14.

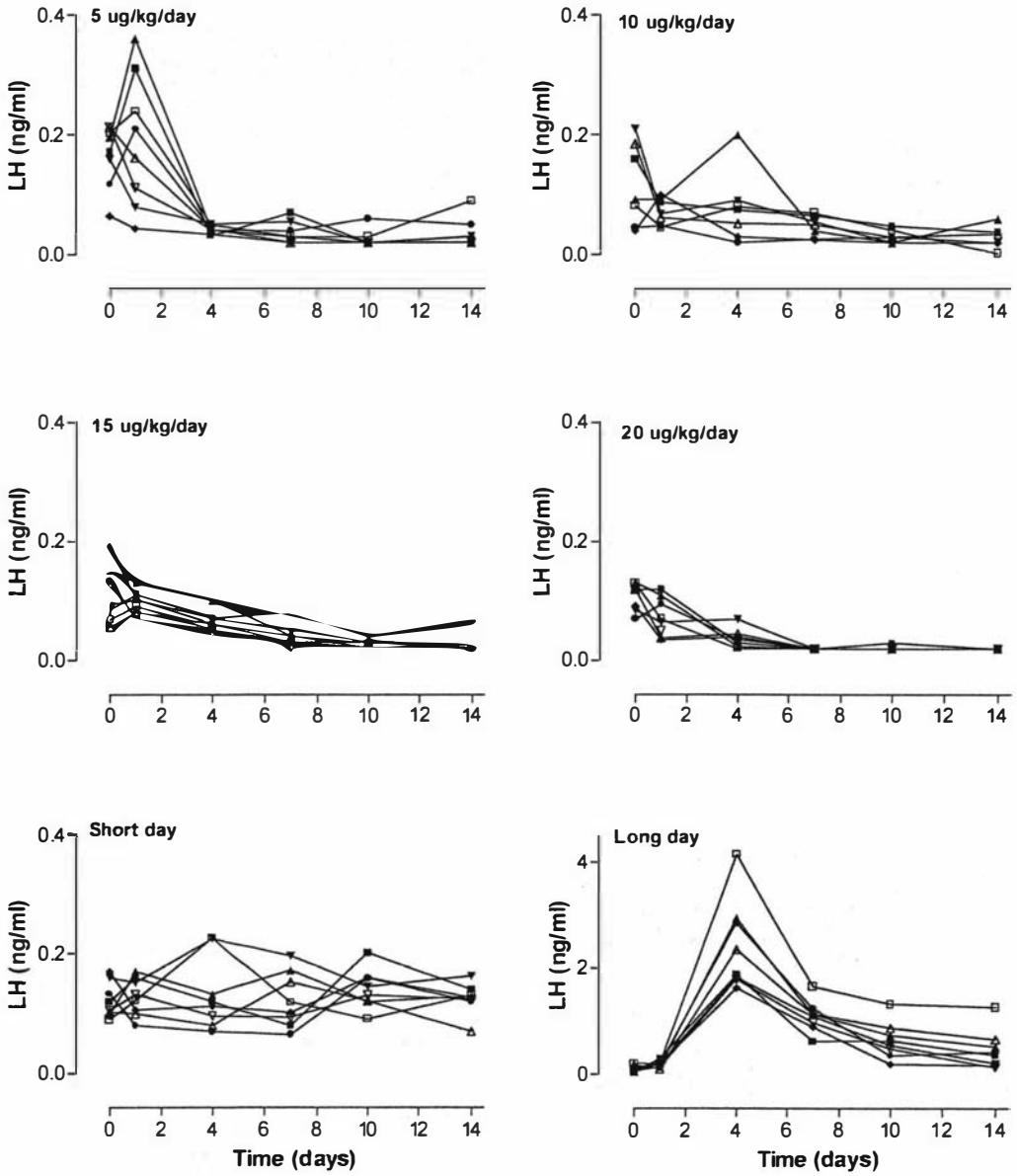

Figure 6.2. Individual LH concentrations in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg/day for 14 days by osmotic pump. Note the use of a different y axis scale for the birds transferred to long days.

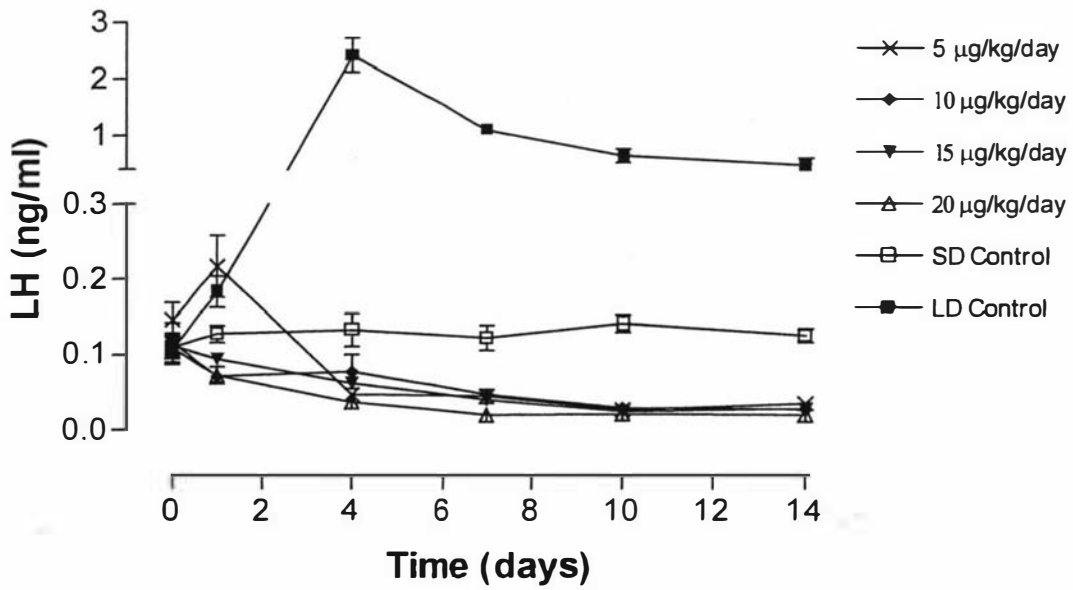

Figure 6.3. Mean LH concentrations in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 µg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg/day (A) and in treated and non-treated quail held on short days only (B) for 14 days by osmotic pump. Results are shown as mean ± standard error.

Table 6.3. Two way repeat measures ANOVA for LH concentrations in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH /kg/day for 14 days by osmotic pump.

Effect		LH F	LH df	LH *p*
Treatment		104.458	5,41	0.000*
Time		35.157	5,205	0.000*
Interaction of treatment and time		23.105	25,205	0.000*
Comparisons within treatments for each treatment				
Day 0 vs 1	5 μg/kg/day	2.298	1,41	0.137
	10 μg/kg/day	2.143	1,41	0.151
	15μg/kg/day	0.295	1,41	0.590
	20 μg/kg/day	3.848	1,41	0.055
	Short day control	0.913	1,41	0.345
	Long day control	7.522	1,41	0.009*
Day 0 vs 4	5 μg/kg/day	24.701	1,41	0.000*
	10 μg/kg/day	4.243	1,41	0.046*
	15μg/kg/day	6.695	1,41	0.013*
	20 μg/kg/day	28.859	1,41	0.000*
	Short day control	0.869	1,41	0.357
	Long day control	232.219	1,41	0.000*
Day 0 vs 7	5 μg/kg/day	35.183	1,41	0.000*
	10 μg/kg/day	13.683	1,41	0.001*
	15μg/kg/day	26.786	1,41	0.000*
	20 μg/kg/day	69.400	1,41	0.000*
	Short day control	0.443	1,41	0.509
	Long day control	143.221	1,41	0.000*
Day 0 vs 10	5 μg/kg/day	57.691	1,41	0.000*
	10 μg/kg/day	29.653	1,41	0.000*
	15μg/kg/day	47.218	1,41	0.000*
	20 μg/kg/day	57.909	1,41	0.000*
	Short day control	2.197	1,41	0.146
	Long day control	68.989	1,41	0.000*
Day 0 vs 14	5 μg/kg/day	35.735	1,41	0.000*
	10 μg/kg/day	37.670	1,41	0.000*
	15μg/kg/day	33.690	1,41	0.000*
	20 μg/kg/day	48.341	1,41	0.000*
	Short day control	0.675	1,41	0.416
	Long day control	31.043	1,41	0.000*

Table 6.3. cont. Two way repeat measures ANOVA for LH concentrations in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg/day for 14 days by osmotic pump.

Comparisons between treatments for each time		LH F	LH df	LH *p*
Day 0	5 vs 10 μg/kg/day	1.066	1,41	0.308
	5 vs 15 μg/kg/day	0.643	1,41	0.427
	5 vs 20 μg/kg/day	0.524	1,41	0.473
	5 μg/kg/day vs short day	0.497	1,41	0.485
	5 μg/kg/day vs long day	1.301	1,41	0.261
	10 vs 15 μg/kg/day	0.067	1,41	0.798
	10 vs 20 μg/kg/day	0.111	1,41	0.741
	10 μg/kg/day vs short day	0.123	1,41	0.727
	10 μg/kg/day vs long day	0.005	1,41	0.945
	15 vs 20 μg/kg/day	0.006	1,41	0.938
	15 μg/kg/day vs short day	0.009	1,41	0.923
	15 μg/kg/day vs long day	0.115	1,41	0.736
	20 μg/kg/day vs short day	0.000	1,41	0.985
	20 μg/kg/day vs long day	0.174	1,41	0.679
	Short day vs long day	0.190	1,41	0.665
Day 1	5 vs 10 μg/kg/day	18.596	1,41	0.000*
	5 vs 15 μg/kg/day	9.531	1,41	0.004*
	5 vs 20 μg/kg/day	21.360	1,41	0.000*
	5 μg/kg/day vs short day	0.942	1,41	0.337
	5 μg/kg/day vs long day	0.009	1,41	0.927
	10 vs 15 μg/kg/day	1.768	1,41	0.191
	10 vs 20 μg/kg/day	0.023	1,41	0.879
	10 μg/kg/day vs short day	11.387	1,41	0.002*
	10 μg/kg/day vs long day	17.832	1,41	0.000*
	15 vs 20 μg/kg/day	2.355	1,41	0.133
	15 μg/kg/day vs short day	4.480	1,41	0.040*
	15 μg/kg/day vs long day	8.968	1,41	0.005*
	20 μg/kg/day vs short day	13.330	1,41	0.001*
	20 μg/kg/day vs long day	20.513	1,41	0.000*
	Short day vs long day	0.771	1,41	0.385
Day 4	5 vs 10 μg/kg/day	1.758	1,41	0.192
	5 vs 15 μg/kg/day	1.734	1,41	0.195
	5 vs 20 μg/kg/day	1.457	1,41	0.234
	5 μg/kg/day vs short day	20.926	1,41	0.000*
	5 μg/kg/day vs long day	311.856	1,41	0.000*
	10 vs 15 μg/kg/day	0.003	1,41	0.958
	10 vs 20 μg/kg/day	6.210	1,41	0.017*
	10 μg/kg/day vs short day	9.571	1,41	0.004*
	10 μg/kg/day vs long day	247.586	1,41	0.000*
	15 vs 20 μg/kg/day	6.371	1,41	0.016*
	15 μg/kg/day vs short day	10.612	1,41	0.002*
	15 μg/kg/day vs long day	267.080	1,41	0.000*
	20 μg/kg/day vs short day	33.428	1,41	0.000*
	20 μg/kg/day vs long day	355.950	1,41	0.000*
	Short day vs long day	171.215	1,41	0.000*

Table 6.3. cont. Two way repeat measures ANOVA for LH concentrations in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg/day for 14 days by osmotic pump.

Comparisons between treatments for each time		LH F	df	*p*
Day 7	5 vs 10 μg/kg/day	0.307	1,41	0.583
	5 vs 15 μg/kg/day	0.061	1,41	0.806
	5 vs 20 μg/kg/day	9.552	1,41	0.004*
	5 μg/kg/day vs short day	18.861	1,41	0.000*
	5 μg/kg/day vs long day	234.241	1,41	0.000*
	10 vs 15 μg/kg/day	0.628	1,41	0.433
	10 vs 20 μg/kg/day	12.529	1,41	0.001*
	10 μg/kg/day vs short day	13.263	1,41	0.001*
	10 μg/kg/day vs long day	202.553	1,41	0.000*
	15 vs 20 μg/kg/day	8.088	1,41	0.007*
	15 μg/kg/day vs short day	21.065	1,41	0.000*
	15 μg/kg/day vs long day	241.853	1,41	0.000*
	20 μg/kg/day vs short day	55.257	1,41	0.000*
	20 μg/kg/day vs long day	338.394	1,41	0.000*
	Short day vs long day	120.165	1,41	0.000*
Day 10	5 vs 10 μg/kg/day	0.281	1,41	0.599
	5 vs 15 μg/kg/day	0.070	1,41	0.792
	5 vs 20 μg/kg/day	0.970	1,41	0.331
	5 μg/kg/day vs short day	70.614	1,41	0.000*
	5 μg/kg/day vs long day	262.827	1,41	0.000*
	10 vs 15 μg/kg/day	0.619	1,41	0.436
	10 vs 20 μg/kg/day	2.195	1,41	0.146
	10 μg/kg/day vs short day	57.580	1,41	0.000*
	10 μg/kg/day vs long day	228.981	1,41	0.000*
	15 vs 20 μg/kg/day	0.517	1,41	0.476
	15 μg/kg/day vs short day	75.146	1,41	0.000*
	15 μg/kg/day vs long day	271.505	1,41	0.000*
	20 μg/kg/day vs short day	88.132	1,41	0.000*
	20 μg/kg/day vs long day	295.724	1,41	0.000*
	Short day vs long day	60.977	1,41	0.000*
Day 14	5 vs 10 μg/kg/day	1.486	1,41	0.230
	5 vs 15 μg/kg/day	0.248	1,41	0.621
	5 vs 20 μg/kg/day	1.643	1,41	0.207
	5 μg/kg/day vs short day	14.940	1,41	0.000*
	5 μg/kg/day vs long day	57.499	1,41	0.000*
	10 vs 15 μg/kg/day	0.544	1,41	0.465
	10 vs 20 μg/kg/day	0.000	1,41	0.985
	10 μg/kg/day vs short day	24.533	1,41	0.000*
	10 μg/kg/day vs long day	73.011	1,41	0.000*
	15 vs 20 μg/kg/day	0.614	1,41	0.438
	15 μg/kg/day vs short day	19.039	1,41	0.000*
	15 μg/kg/day vs long day	65.301	1,41	0.000*
	20 μg/kg/day vs short day	26.493	1,41	0.000*
	20 μg/kg/day vs long day	78.583	1,41	0.000*
	Short day vs long day	13.820	1,41	0.001*

NOTE: The first three rows show the results of the ANOVA for the main effects and their interaction. The remaining rows show the results of *post hoc* comparisons.

6.3.3 Ovary weight

The ovaries of quail maintained on short days with no treatment or treated with D-Lys^{6}Trp^{7}Tyr^{8}-GnRH remained small and mean ovary weights were not significantly different between groups (Figure 6.4; see Table 6.4 for statistics). Mean ovary weight of birds transferred to long days was significantly greater than ovary weights of birds in all other groups. Yolk filled follicles were observed in four of these birds.

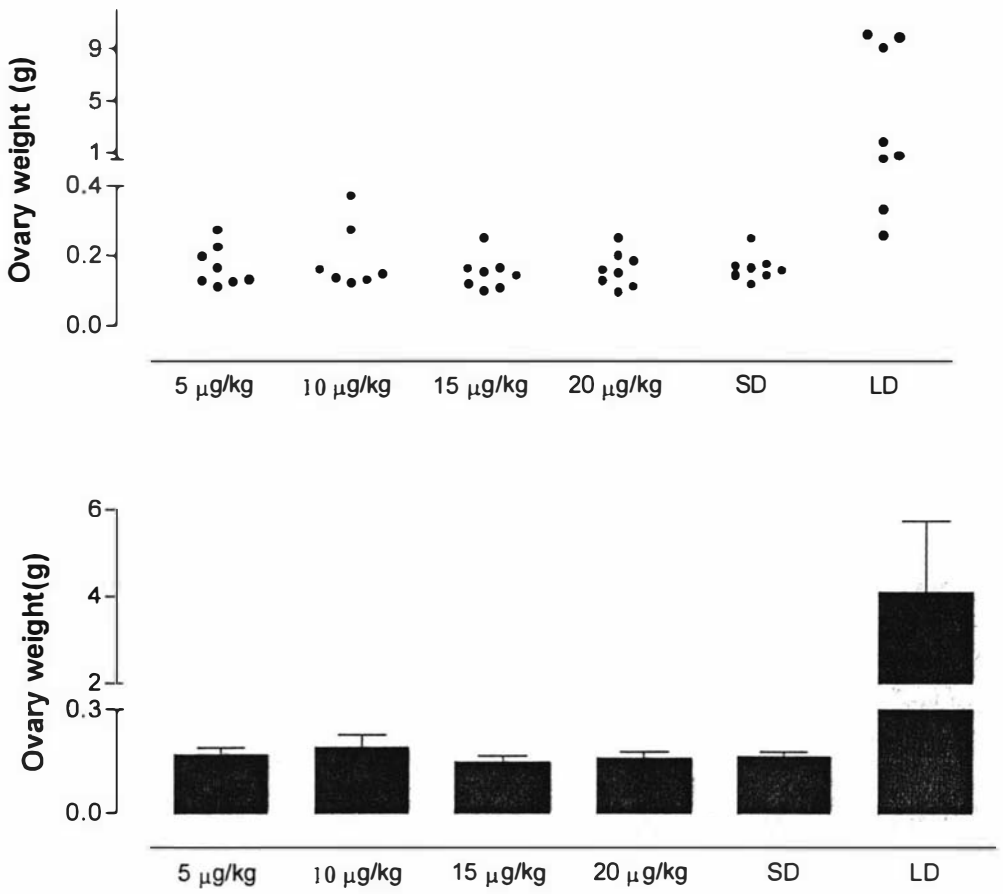

Figure 6.4. Ovary weights of female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^{8}-GnRH/kg/day for 14 days by osmotic pump. Results are shown as individual and mean ± standard error.

Table 6.4. Single measures ANOVA for ovary weights in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg/day for 14 days by osmotic pump.

	Ovary weight		
Effect	**F**	**df**	***p***
Treatment	14.781	5,41	0.000*
Comparisons between treatments			
5 μg vs 10 μg /kg/day			1.000
5 μg vs 15 μg/kg/day			0.999
5 μg vs 20 μg/kg/day			1.000
5 μg vs short day			1.000
5 μg vs long day			0.000*
10 μg vs 15 μg/kg/day			0.994
10 μg vs 20 μg/kg/day			0.999
10 μg vs short day			1.000
10 μg vs long day			0.000*
15 μg vs 20 μg/kg/day			1.000
15 μg vs short day			0.999
15 μg vs long day			0.000*
20 μg/kg/day vs short day			1.000
20 μg/kg/day vs long day			0.000
Short day vs long day			0.000*

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

6.3.4 Oviduct weight

Oviducts of quail maintained on short days with no treatment or treated with D-Lys^{6}Trp^{7}Tyr^8-GnRH remained small and mean oviduct weights were not significantly different between groups (Figure 6.4; see Table 6.4 for statistics). Mean oviduct weight of birds transferred to long days was significantly greater than oviduct weights of birds in all other groups.

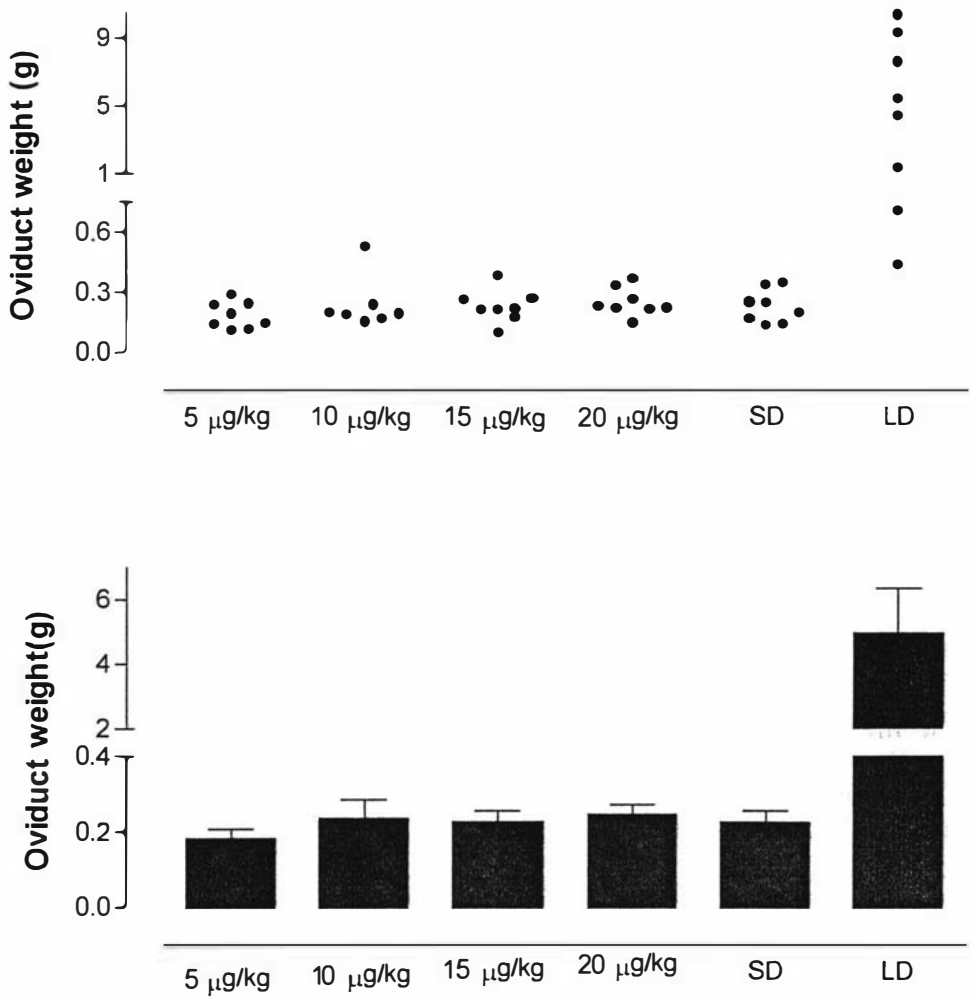

Figure 6.5. Oviduct weights of female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg/day for 14 days by osmotic pump. Results are shown as individual and mean ± standard error.

Table 6.5. Single measures ANOVA for oviduct weights in female quail held on short days (8L:16D), long days (16L:8D) and short days with treatment of 5, 10, 15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH /kg/day for 14 days by osmotic pump.

	Oviduct weight		
Effect	**F**	**df**	***p***
Treatment	25.624	5,41	0.000*
Comparisons between treatments			
5 μg vs 10 μg/kg/day			0.981
5 μg vs 15 μg/kg/day			0.981
5 μg vs 20 μg/kg/day			0.903
5 μg vs short day			0.982
5 μg vs long day			0.000*
10 μg vs 15 μg/kg/day			1.000
10 μg vs 20 μg/kg/day			1.000
10 μg vs short day			1.000
10 μg vs long day			0.000*
15 μg vs 20 μg/kg/day			0.999
15 μg vs short day			1.000
15 μg vs long day			0.000*
20 μg/kg/day vs short day			0.999
20 μg/kg/day vs long day			0.000*
Short day vs long day			0.000*

NOTE: The first row shows the results of the ANOVA for the main effect. The remaining rows show the results of *post hoc* comparisons.

6.4 Discussion

This is the first study in which a GnRH analogue was administered to Japanese quail using an osmotic minipump. Continuous infusion of 5, 10,15 or 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH/kg/day in sexually regressed female Japanese quail for 14 days caused a significant depression of pituitary function relative to short day control birds as reflected by decreased LH secretion and did not induce ovarian growth. LH secretion of birds transferred to long days was significantly increased 24 hours after transfer as previously described in other studies (Robinson and Follett, 1982; Follett and Pearce-Kelly, 1990).

It is unlikely the decline in LH concentrations in birds infused with D-Lys^{6}Trp^{7}Tyr^8-GnRH was due to the failure of the osmotic minipump. The precision and reliability of these devices in the delivery of their contents have been well established. The site of implantation and the positioning of the pump were chosen to minimise discomfort to the

bird and was satisfactory in previous experiments in which pregnant mare serum gonadotropin induced ovarian growth in Japanese quail (Wakabayashi *et al.,* 1996; Bennett, 2002).

The fate of D-Lys^{6}Trp^{7}Tyr^8-GnRH once it had left the pump was unknown but presumably it moved from interstitial fluid into the blood. At the time of dissection in the current study each pump was empty and there was no evidence of fibrous tissue encapsulation or fluid accumulation at the site of implantation. Several studies in mammals have however described the release of GnRH solutions from minipumps as haphazard, perhaps due to instability of GnRH in solution or to its pooling at the site of the pump (Hyland *et al.,* 1987; Boyle *et al.,* 1991; Turner and Irvine, 1991).

The LH concentrations of birds treated with 5, 10 or 15 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH were not different to pre-treatment values after 24 hours whilst 20 μg D-Lys^{6}Trp^{7}Tyr^8-GnRH caused significantly lower LH concentrations after 24 hours. LH concentrations in all treated birds were significantly lower than short day control birds by day four of the treatment period. These results suggest that in the current study GnRH was successfully delivered by the mini-pumps and as the birds were sexually regressed it was unlikely endogenous sex steroid hormones were influencing pituitary function.

Experiments in which redwinged blackbirds were injected daily with high doses of GnRH agonist (D-Trp^{6}Pro^{9}N-ethyl amide-GnRH) resulted in severely blunted LH secretion by day four (Lacombe *et al.,* 1992). However once treatment ceased LH concentrations returned to pre-treatment concentrations. Infusion of mGnRH in turkey hens by subcutaneous gel implants for seven days also resulted in decreased LH concentrations within eight hours of beginning the study and did not induce ovarian growth (Burke and Cogger, 1977). The decline in LH concentrations in the current study was somewhat slower in the birds treated with the lower doses of D-Lys^{6}Trp^{7}Tyr^8-GnRH and may reflect differences in GnRH purity, dose rate, species and implant type. Biodegradable implants in comparison to osmotic minipumps do not release hormones in a precise and controlled manner (Amkraut *et al.,* 1990).

Dose rates in the current study were calculated using a flow rate of 0.5 μl/h specified by the manufacturer. The actual flow rate may have been approximately 25 % higher due to the higher body temperature of birds compared with mammals (41 ° compared to 37 °

C; Girling *et al.*, 2002). However the range of doses used still covered the dose (approximately 12 μg/kg) of GnRH that stimulated some ovarian growth in ducks (Cockrem *et al.*, unpublished observations). The reductions in LH in treated birds in the current study probably resulted from down regulation of the number of pituitary receptors or alterations in post-receptor metabolic effects previously described as desensitisation (King *et al.*, 1986; chapters four and five). It is possible that a less potent form of GnRH might reduce or avoid the desensitisation seen in the current study.

It is unclear from the current study how long the pituitary remained responsive to D-Lys^{6}Trp^{7}Tyr^8-GnRH infusion and whether pituitary responsiveness is dose dependent, as LH concentrations were decreased in some birds when the first blood sample was collected. It would have been of interest to determine the short-term effects of continuous infusion of D-Lys^{6}Trp^{7}Tyr^8-GnRH to compare with LH concentrations after injection. Short-term intravenous infusion of GnRH in the female bullfrog was no more effective in elevating plasma gonadotropin concentrations than a single intravenous injection of GnRH (McCreery *et al.*, 1982; Licht *et al.*, 1983).

6.5 Conclusion

The use of osmotic minipumps for the delivery of D-Lys^{6}Trp^{7}Tyr^8-GnRH in birds was practical and convenient. D-Lys^{6}Trp^{7}Tyr^8-GnRH did not induce ovarian growth in sexually regressed birds, but D-Lys^{6}Trp^{7}Tyr^8-GnRH at a lower dose or other forms of GnRH might prove effective. The results do indicate that effects of exogenous GnRH-on LH secretion are markedly different in the bird compared to some mammalian species.

7 General Discussion

The goal of this thesis was to determine the efficacy of GnRH treatment for the stimulation of ovarian growth in endangered birds such as the kakapo. Japanese quail were used as study birds. The endocrinology of the annual cycle of quail outdoors was described and endocrine and gonadal changes during photoperiodically induced gonadal growth and regression determined. These studies were a prerequisite to the experimental treatment of quail with exogenous hormones.

The gonadal and endocrine changes and associated reproductive behaviours described in chapter two form the first study of annual patterns of hormones and behaviour in female Japanese quail living in a group outdoors with access to nesting materials and nest sites. Reproductive behaviours such as nest building and the incubation of eggs are said to have been bred out of this domestic species (Ball and Balthazart, 2002). However this is clearly not correct as the quail in this study exhibited a rich repertoire of reproductive behaviours including nesting activities. These results appear to contradict the opinion that domestication of birds causes the degeneration of 'normal reproductive behaviours'. The lack of reproductive behaviours in the domestic environment is likely due to the lack of appropriate environmental conditions.

Clear seasonal patterns of changes in the width of the cloacal opening (indicative of oviduct size and weight), and in plasma FSH concentrations were observed, whilst LH concentrations were low during winter and increased during spring and summer. Prolactin concentrations were higher in nesting quail and in quail caring for young compared with non-nesting quail. Sample sizes were too small to distinguish clear patterns in T4 and T3, which was unfortunate as experimental studies continue to

indicate that thyroid hormones are essential for the initiation of photoperiodic-stimulated gonadal growth in birds.

Studies in natural and semi-natural environments provide opportunities to establish relationships between behavioural events and associated changes in endocrinology, which may not be observed in the laboratory. In the semi-natural environment study the importance of the social contact with male quail for the initiation of nesting activity by females is unknown. A number of studies have established that a mature male provides environmental information for female quail which, in conjunction with environmental stimuli such as stimulatory photoperiod and food supply initiates an increase in reproductive activity (for review see Wingfield and Marler, 1988). The mechanism by which this occurs remains to be elucidated.

The study of gonadal and endocrine changes in quail moved from short to long or long to short photoperiods described in chapter three is the first description of changes in gonad size and plasma concentrations of gonadotropins, gonadal steroids, prolactin and thyroid hormones measured together. These results provide basic avian endocrine information and serve as a reference for subsequent studies in which non-breeding quail were treated with exogenous GnRH with the aim of stimulating gonadal growth. They also confirmed the Japanese quail is a good model species for photoperiodic studies. Variation between birds in gonadal growth and regression rates and associated endocrine changes in individual birds observed during the photoperiodic studies were of particular interest. Individual differences in gonadal responses to stimulatory photoperiodic conditions in untreated birds suggest the likelihood of variation in response to exogenous hormone treatment. Individual responses to changes in photoperiod might be useful for determining dose rates for treatment with exogenous hormones.

There is currently no reliable method for inducing ovarian growth and ovulation in birds. In the sexually immature Japanese quail administration of pregnant mare serum gonadotropin (PMSG) by subcutaneous injections and osmotic pumps has successfully stimulated ovarian development, ovulation and oviposition (Wakabayashi *et al.,* 1996; Bennett, 2002). However in these studies and others in which mammalian gonadotropins have been administered, results have ranged from no ovarian stimulation

to excessive follicular development and breakdown of the follicular hierarchy (turkey, Zadworny and Etches, 1988; domestic fowl, Palmer and Bahr, 1992; Hocking and McCormack, 1995; Japanese quail, Wakabayashi *et al.,* 1992). These studies do however illustrate the potential importance of increased LH concentrations as observed during photostimulation.

GnRH was chosen for the studies in this thesis for several reasons. Firstly, GnRH is the principal hormone responsible for the control of reproduction in birds. GnRH solutions should therefore stimulate the hypothalamic-pituitary-gonadal axis, with little risk of overstimulation of the ovary. The large number of GnRH analogues which bind to the GnRH receptors with high specificity, affinity and activity to compensate for the rapid and short-lived pituitary response to native GnRH peptides provide an array of GnRH hormones for use in reproductive studies. Additionally GnRH and GnRH analogues administered to seasonally anoestrous domestic mammals and non-breeding reptiles have been used successfully to induce follicular growth and ovulation. Although caution must be used when interpreting the results of mammalian studies with respect to the possibilities of adopting similar methods in birds, mammalian studies do provide useful information and insights when considering the development of clinical treatments in birds.

Administration by subcutaneous injection of the native GnRH peptides, cGnRH-I and cGnRH-II, buserelin (mammalian GnRH analogue) and D-Lys^{6}Trp^{7}Tyr^{8}-GnRH stimulated the secretion of LH in sexually regressed female Japanese quail. These results indicated that cGnRH-II was more potent than cGnRH-I *in vivo.* Both buserelin and D-Lys^{6}Trp^{7}Tyr^{8}-GnRH stimulated greater LH responses than the native peptides. The cGnRH-II analogue used in the studies in this thesis, D-Lys^{6}Trp^{7}Tyr^{8}-GnRH, has only been studied in laying chickens in which dose-response studies reported elevated LH concentrations for more than 60 minutes (Dellobelle *et al.,* 1995).

Increased FSH concentrations in response to cGnRH-I and cGnRH-II have been reported *in vitro* in chicken pituitary cells (Millar *et al.,* 1986) and *in vivo* in male Japanese quail (Hattori *et al.,* 1985; 1986a). The FSH responses were smaller than the LH responses. *In vitro* studies using quail pituitary cells reported FSH release in response to the GnRH analogue D-Ala^{6}-desGly^{10}-GnRH (Hattori *et al.,* 1985). FSH

concentrations in response to buserelin or D-Lys^{6}Trp^{7}Tyr^8-GnRH have not been reported in birds. FSH controls follicular maturation and differentiation and it would have been useful to measure FSH concentrations in the studies where exogenous GnRH was administered to sexually regressed Japanese quail. This was not possible however as the FSH assay in a Japanese laboratory was no longer available when the GnRH studies were conducted.

The use of exogenous GnRH has been investigated in a large number of different species with a view towards developing therapies for pathological conditions and methods to assist reproduction. In seasonally anoestrous mammals repeated injections and continuous infusions of GnRH can stimulate gonadotropin release without down-regulation and induced ovulation (McLeod *et al.,* 1983; Wright *et al.,* 1983; Jagger *et al,* 1987; Lamming and McLeod, 1988). Few studies however have been designed to stimulate ovarian growth and oviposition in non-breeding birds. Ovarian growth in response to mGnRH has been reported in a single study (pheasants and partridges; Minoia *et al.,* 1984). In the current series of experiments single subcutaneous injections of GnRH solutions induced increases in LH concentrations, but short term repeated injections of D-Lys^{6}Trp^{7}Tyr^8-GnRH did not maintain elevated LH concentrations. Daily injections of GnRH and continuous delivery of D-Lys^{6}Trp^{7}Tyr^8-GnRH by osmotic pumps for 14 days did not stimulate ovarian development in sexually regressed Japanese quail.

It is likely that the repeated injection of quail with GnRH or infusion of GnRH with osmotic pumps caused desensitisation of anterior pituitary GnRH receptors. Desensitisation could be via several mechanisms. Many studies refer to the down-regulation of GnRH receptor numbers (King *et al.,* 1986; Davidson *et al.,* 1994a; McArdle *et al.,* 2002) due to internalisation, increased degradation or decreased synthesis. Changes in post-receptor events have also been included as part of the desensitisation mechanism. Such events may include the (1) uncoupling of the receptors from their associated G-proteins, (2) modulation of signalling components such as G-proteins, phospholipases, calcium channels and pumps, (3) decreased responsiveness of exocytotic regulatory proteins to calcium, (4) depletion of releasable gonadotropin pools and (5) synthesis of gonadotropins with low biologic activity (Millar *et al.,* 1986; Davidson *et al.,* 1994a; McArdle *et al.,* 2002). Studies focussed on

the GnRH ligand and receptor binding continue to determine the roles of specific amino acids in this interaction and may in the future provide more insight into the mechanisms involved in the phenomenon of desensitisation. These structure-function studies will lead to the development of new GnRH analogues that might be more effective than the current analogues for the stimulation of reproductive function in birds.

It might be necessary to mimic the normal pattern of GnRH secretion during photostimulation to increase gonadotropin secretion in order to achieve ovarian growth. GnRH secretion is thought to be pulsatile in birds as LH secretion is pulsatile. Endogenous LH secretory patterns in blood collected via cannulae have been determined in turkey hens transferred from short days (6L:18D) to long days (14L:10D). High amplitude LH pulses superimposed on a low LH baseline changed to few low amplitude LH pulses superimposed on a relatively high LH baseline (Chapman *et al.,* 1994; Bacon and Long, 1995; Applegate *et al.,* 1997). The transition between the two secretory patterns is thought to occur between days two and five after photostimulation as no changes in LH pulses were observed during the first 48 hours after photostimulation (Bacon and Long, 1995). It may be possible to mimic this pattern of LH secretion by injecting a short-lived form of GnRH, such as cGnRH-I in a gradually increasing frequency. It would then be necessary to determine the effects of this treatment on both LH and FSH secretion, and if follicular growth was achieved, determine whether the administration of oestradiol/progesterone would be necessary to induce a preovulatory surge.

The quail treated with GnRH in the current studies were held on short days. There was no photoperiodic drive in the hypothalamo-pituitary-gonadal axis and the birds had regressed gonads. This was equivalent to treating free-living birds in winter outside their normal breeding season and was therefore a challenging situation in which to test GnRH treatment. The situation was chosen because the greatest need for stimulation of breeding in kakapo using exogenous hormone treatment was to promote breeding in females that had not bred for five – 10 years or even longer. It was assumed that these birds would have regressed ovaries. However, a recent study in our laboratory (Hawke, 2002) has raised the possibility that female kakapo may undergo some photoperiodically induced ovarian growth each year, even if breeding does not occur.

Furthermore in 2002 some females that had not bred for 20 years laid eggs. Exogenous hormone treatment for kakapo could therefore be tested in future in birds that had undergone some ovarian growth rather than regressed ovaries as in the current quail studies.

Further studies of GnRH treatment of quail on marginally stimulatory photoperiods are therefore warranted. GnRH treatment in quail exposed to increasing photoperiods would better mimic the environmental conditions that kakapo are exposed to each breeding season than the short days used in the current studies. GnRH treatment in birds who, when exposed to stimulatory photoperiods do not always respond with ovarian growth, may induce follicular development. Administration of single daily injections of D-Tle^{6}-GnRH has induced accelerated sexual development and egg laying in Japanese quail transferred to long days at the start of treatment (Zeman *et al.,* 1991). These results suggest treating birds exposed to increasing photoperiods may be potentially promising when developing a hormonal treatment programme to stimulate ovarian growth.

The gonadal axis is classically considered to be stimulated by a hypothalamic releasing factor (GnRH). However an inhibitory factor (gonadotropin inhibitory hormone, GnIH), has recently been discovered. GnIH is the first hypothalamic neuropeptide inhibiting gonadotropin release to be described in vertebrates and appears to be an important factor for the regulation of avian reproduction (Tsutsui *et al.,* 2000). Injections of GnIH have *in vivo* anti-gonadotropic activity in both the laboratory and field in song sparrows and Gambel's white-crowned sparrows (Osugi *et al.,* 2004). These results indicate that a thorough knowledge of the effects of GnIH during photoinduced growth and regression will be important for the future development of a GnRH-based treatment to stimulate ovarian growth.

Whilst Japanese quail are an excellent model species for photoperiodic studies and the GnRH dose response challenges, there were limitations for their use in studies in which repeated blood sampling was required. Limitations arose from the small size of the quail, risks of infections due to multiple injections and blood sampling, and possible adverse effects of repeated handling. Avian species that could be a useful alternative to the quail for development of a GnRH treatment programme is the turkey. In addition to

being easily maintained, gonadal growth and regression is readily achieved in turkeys through manipulations of photoperiod. A chronic cannulation system for serial bleeding has been developed in the domestic turkey (Chapman *et al.,* 1994) and successfully used to collect serial blood samples from non-restrained males and females (Bacon, 2001). This method has been used to measure secretory profiles of LH, testosterone, estradiol and progesterone over several days, and could potentially provide the opportunity to profile LH secretory patterns in response to both repeated injections and continuous infusions of GnRH. The large size of turkeys would, however be a disadvantage in terms of costs of obtaining and maintaining birds and the larger quantities of hormones needed for treatments.

Kakapo are a long-lived bird and many females are more than 30 years old. It is therefore important to consider changes in the dynamics of the hypothalamic-pituitary-gonadal axis as birds age, as responses to GnRH treatment may depend on the age of the treated birds. GnRH-induced gonadotropin secretion has been reported to diminish as birds mature (Bonney *et al.,* 1974; Knight *et al.,* 1985; Wilson *et al.,* 1989). This may reflect the effects of higher concentrations of gonadal steroids in older birds, possibly via effects on the binding characteristics of pituitary receptors for GnRH (Kawashima *et al.,* 1992). Reproductive aging and senescence of the reproductive system has been studied in detail in Japanese quail (Ottinger *et al.,* 1983; Ottinger and Balthazart, 1986; Ottinger, 1992; 1996; Ottinger *et al.,* 1997; 2002). Young Japanese quail have a higher cGnRH-I content in the POA regardless of reproductive state, than older birds. However, sexually active old Japanese quail had higher cGnRH-I content than inactive old Japanese quail (Ottinger, 1992). These results suggest that there is a decline in GnRH system as birds age, but that it may not necessarily be of significance until all reproductive activity has ceased.

The practicalities of treating an endangered bird such as the kakapo would require careful consideration. Although kakapo are intensively managed they are free living and a hormone treatment programme would require regular handling. It would be of particular interest to know how endogenous LH and FSH are affected by capture and handling in these birds prior to hormonal treatment. The response of the birds to changes in environment alone may not be conducive for stimulating reproduction.

A crucial requirement for hormone stimulation programmes is to ensure the ovaries and oviducts of treated birds are normal. Histological studies of ovaries of treated and untreated birds would determine this. Studies would need to ensure that treated birds would undergo normal gonadal regression at the end of treatment and that the birds would be responsive to future treatments or to increases in photoperiods. For a hormonal treatment programme to be deemed successful it would need to be shown that successive treatments would stimulate ovarian growth and did not compromise future reproduction (Bennett, 2002). Further studies on the potential development of a hormone treatment programme will continue to offer a promising future for endangered avian species including the New Zealand kakapo.

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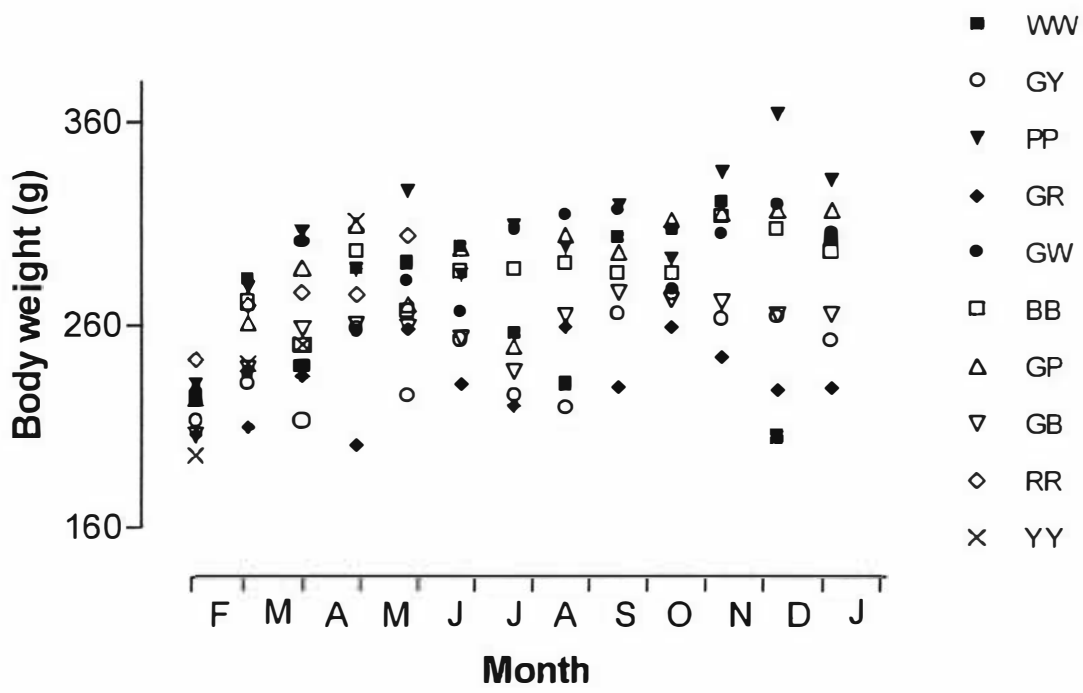
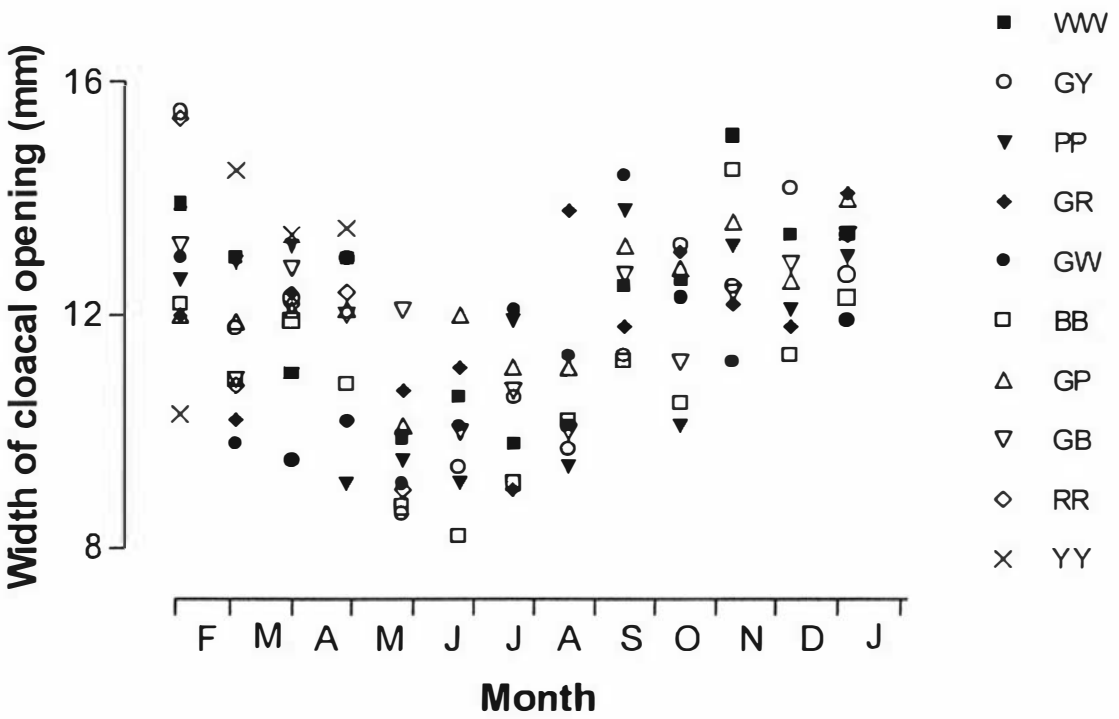
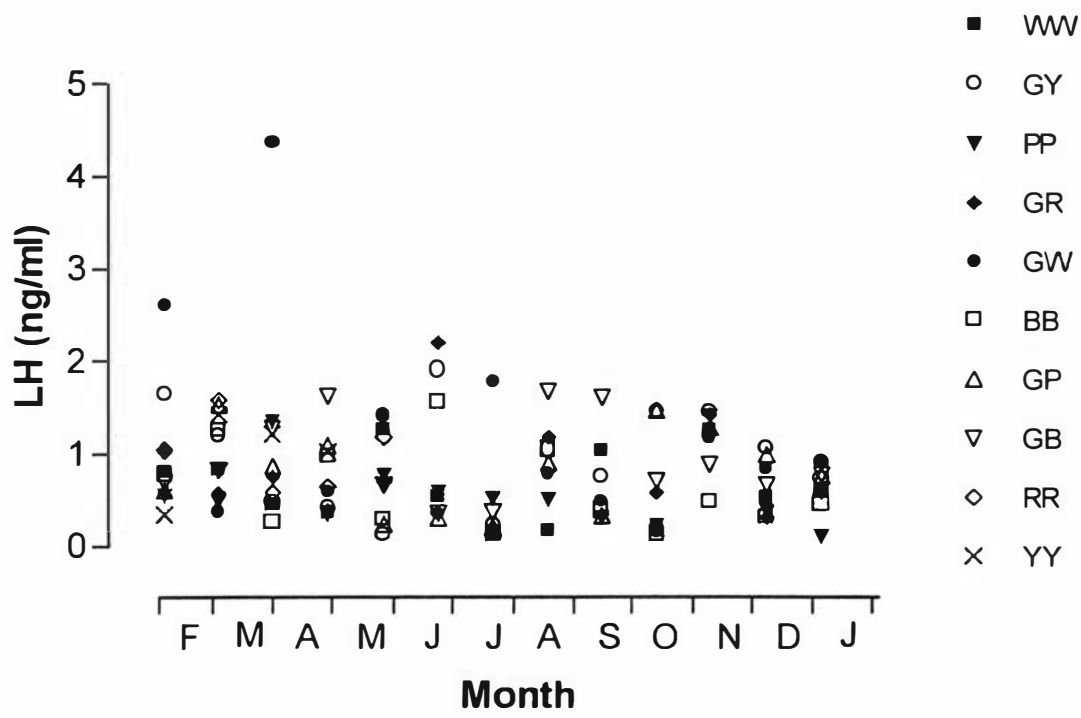
Appendix 1: Annual reproductive cycle of female Japanese quail held in a semi-natural environment



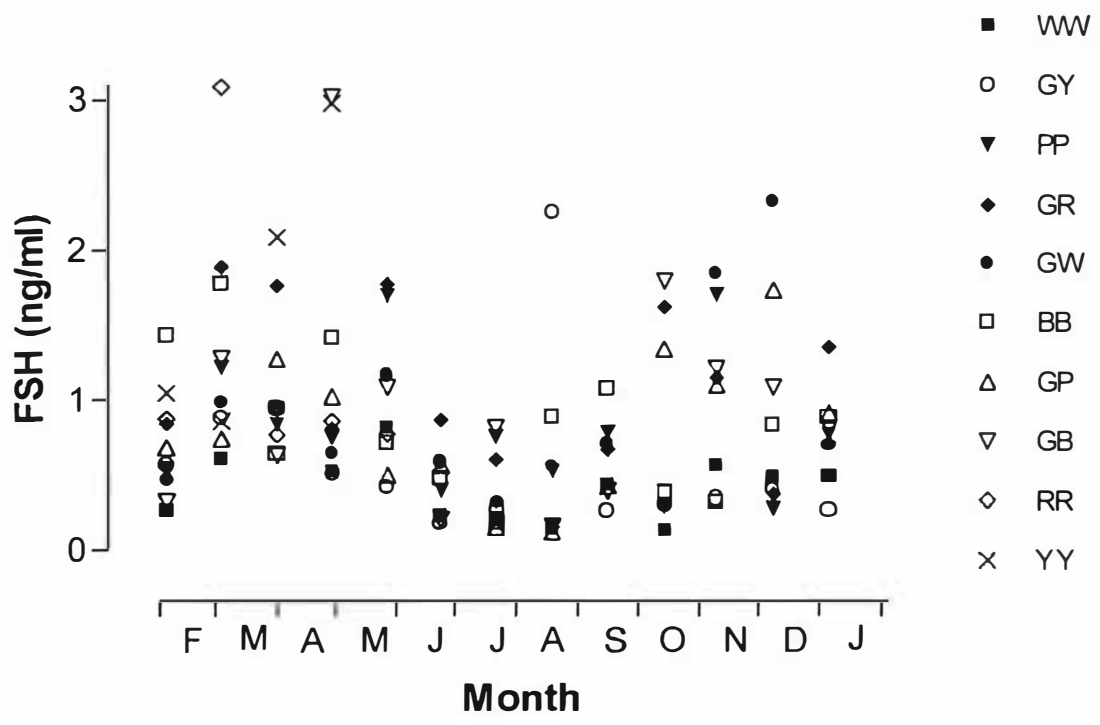
Appendix 1.1. Individual body weights of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.



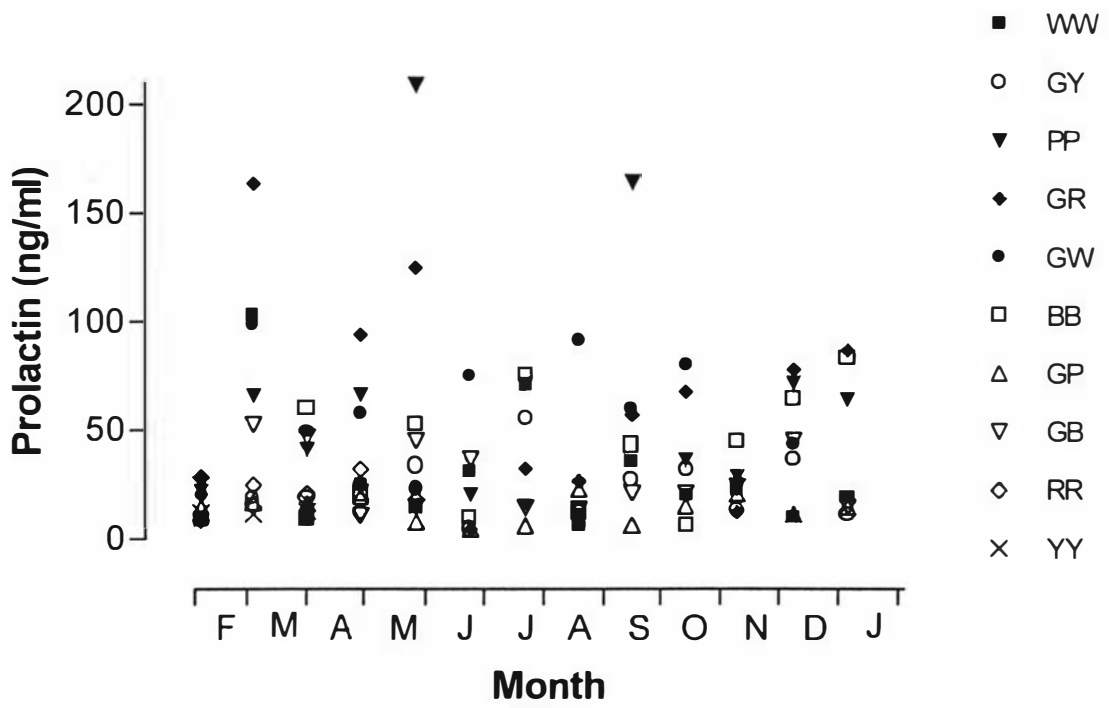
Appendix 1.2. Individual width of cloacal openings of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.



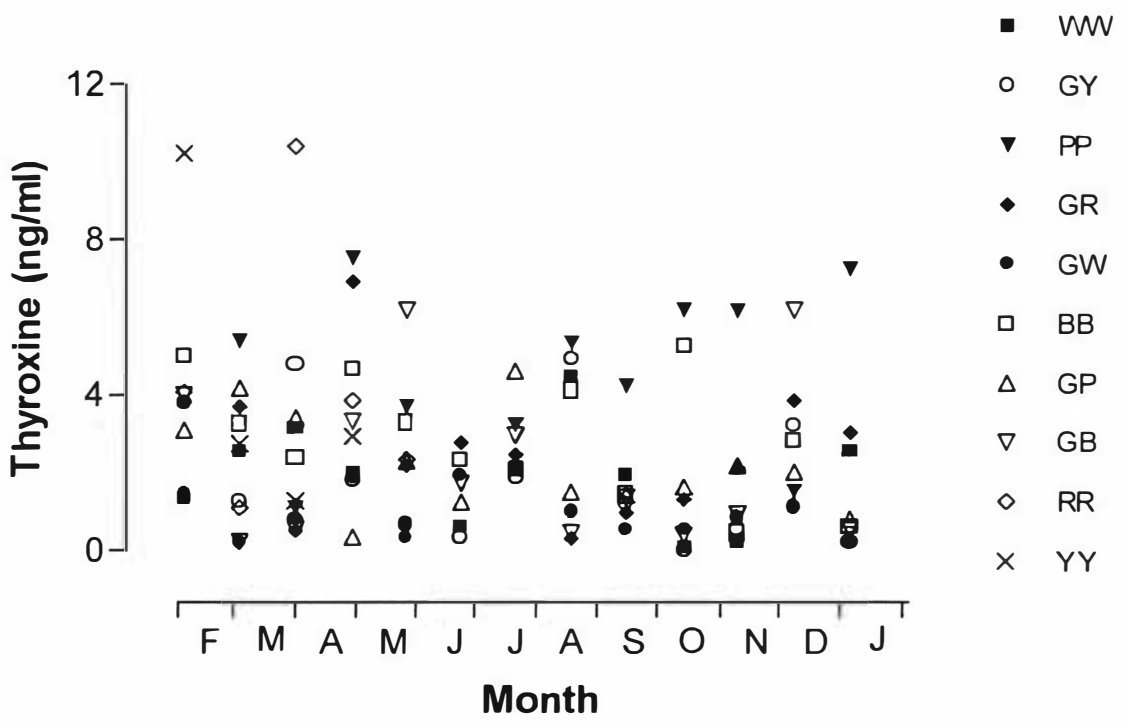
Appendix 1.3. Individual LH concentrations of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.



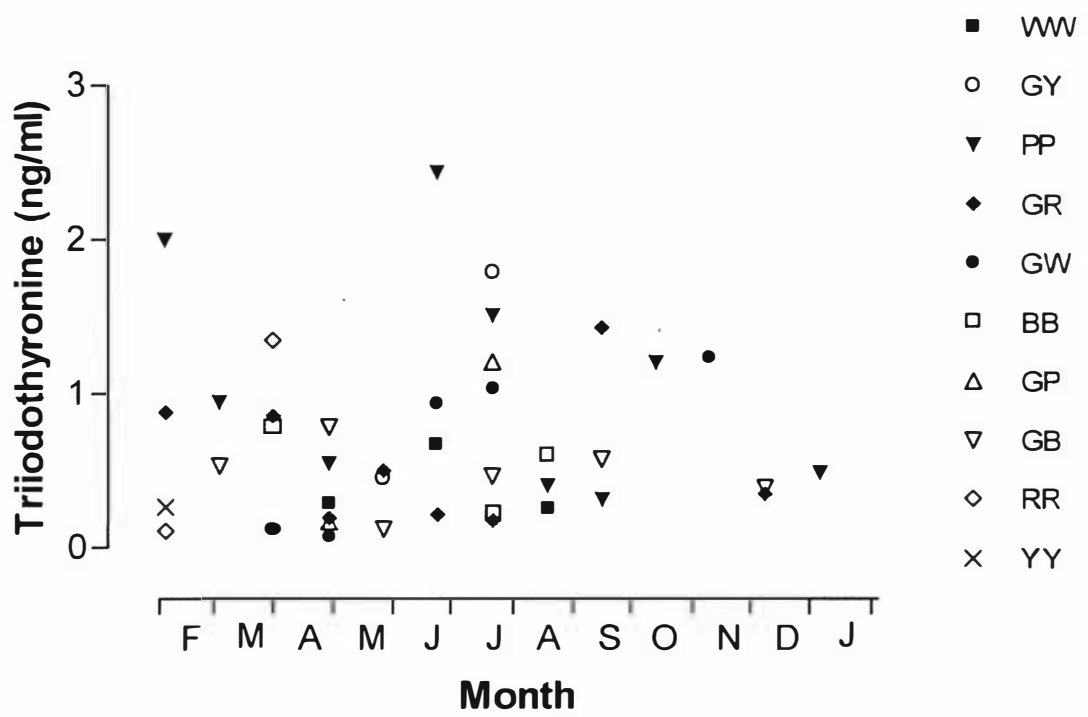
Appendix 1.4: Individual FSH concentrations of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.



Appendix 1.5: Individual prolactin concentrations of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.



Appendix 1.6: Individual T4 concentrations of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.



Appendix 1.7: Individual T3 concentrations of female quail maintained in a semi-natural environment under natural conditions from 11 January 1999 to 6 January 2000.

Appendix 2: Papers in preparation for publication

Henare, S.J., Cockrem, J.F., Bennett, E.J., Kikuchi, M. and Talbot, R. Annual changes in plasma gonadotropins, prolactin, body weight and the width of the cloacal opening in Japanese quail (*Coturnix coturnix japonica*) housed outdoors in a semi-natural environment.

Henare, S.J., Bennett, E.J. and Cockrem, J.F. Annual variation in reproductive behaviour in Japanese quail (*Coturnix coturnix japonica*) housed outdoors in a semi-natural environment.

Henare, S.J., Cockrem, J.F., Kikuchi, M., Talbot, R. Physiology and endocrinology of gonadal growth and regression of male Japanese quail (*Coturnix coturnix japonica*).

Henare, S.J., Cockrem, J.F., Kikuchi, M., Talbot, R. Physiology and endocrinology of gonadal growth and regression of female Japanese quail (*Coturnix coturnix japonica*).

Henare, S.J. and Cockrem, J.F. Effects of cGnRH-I, cGnRH-II and D-Lys^{6}Trp^{7}Tyr^8-GnRH on plasma luteinising hormone concentrations in non-breeding female Japanese quail (*Coturnix coturnix japonica*).

Henare, S.J. and Cockrem, J.F. Effects of D-Lys^{6}Trp^{7}Tyr^8-GnRH delivered by mini osmotic pump on plasma luteinising hormone concentrations in non-breeding female Japanese quail (*Coturnix coturnix japonica*).