Aspects of Puberty and Growth in pasture-raised New Zealand Thoroughbreds born in spring and autumn

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Abstract

This thesis reports a series of studies conducted to examine the growth and onset of puberty of pasture-raised Thoroughbred horses born in spring and autumn. Current knowledge of the growth characteristics of pasture-raised Thoroughbreds is limited, and there is little information on puberty in horses. An understanding of aspects of growth and the onset of puberty in young, pasture-raised Thoroughbreds born in spring and autumn may be of importance to the New Zealand Thoroughbred industry, with the aim of producing horses for sale in the Northern Hemisphere.

Aspects of growth including the effect of sex, age, birth weight, month of birth, weaning and castration were examined in spring-born Thoroughbreds between birth and 16 months of age, and compared with those born in autumn. All foals had similar growth rates between birth and 5 months of age, after which growth rates were related to seasonal factors. There was little difference in mean body weight between spring and autumn-born horses at the end of the study, despite the 4-month age difference. Body weights were similar to horses in other studies, including supplementary grain-fed Northern Hemisphere horses. Autumn-born horses weaned in the spring had increased growth rate after weaning, whereas spring-born horses had decreased growth rate after weaning in the autumn, indicating that post-weaning growth rate was a factor of seasonal changes.

Puberty was determined by measurement of plasma testosterone and progesterone concentrations. Spring-born fillies and colts were older and heavier than autumn-born fillies and colts at puberty. However, in both spring and autumn-born horses, puberty occurred in October (New Zealand spring) indicating that seasonal changes affect the timing of puberty onset. GnRH challenges were performed every 8 weeks in spring and autumn-born colts from four months of age. Luteinising Hormone concentration followed a seasonal pattern from 4 months of age and was increased in the spring and summer and decreased in the winter months. Testosterone was first detectable in the spring (at 8 and 12 months of age in autumn and spring-born horses, respectively), possibly after a period of Leydig cell maturation.

It appears that, as in some other seasonal-breeding species, horses must reach a threshold body weight at the same time as increasing photoperiod in the spring for puberty to occur, but proof of this requires further study.

The possible implications of this work to the New Zealand Thoroughbred industry, and the suggestions for further research are discussed.

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Chapter 1

Introduction, literature review and objectives of the study

1.1 Introduction

The New Zealand Thoroughbred breeding industry is internationally renowned for producing top quality bloodstock to international standards.

The sale of yearling horses is an important event for breeders of Thoroughbred horses, with the national yearling sales held at Karaka in February attracting international attention/buyers. The 2002 premiere sale grossed $NZ37.4 million from the sale of 364 yearlings. The Select and Festival yearling sales grossed $NZ17.3 million from the sale of 807 yearlings. From the 2002 yearling sales, 352 horses were sold to overseas buyers (Australia (212), Hong Kong (41), Singapore (31), Malaysia (24), Philippines (20), Macau (11), England (9), New Caledonia (3), and USA (1)).

The bloodstock industries of Europe and America are worth billions. The leading sire of yearlings at the Keeneland September 2001 yearling sale in Kentucky was Storm Cat. The sale of 13 yearlings sired by Storm Cat grossed over $US22 million and averaged $US1.7 million. In comparison, the leading sire at New Zealand's premiere yearling sale, held at Karaka in February 2002, was Zabeel. The sale of 42 yearlings sired by Zabeel grossed $NZ9.1 million and averaged $NZ222,524. The sale of 6 yearlings by Danehill grossed $NZ2.5 million and averaged $NZ410,000. New Zealand has never been fully competitive in the Northern Hemisphere with regard to the sale of yearling horses and in 2-3 year old Thoroughbred racing, due to the differences in the breeding season of Northern and Southern Hemispheres.

The Northern Hemisphere is seasonally 6 months different from the Southern Hemisphere. Horses in the Southern Hemisphere have their birthdays on 1 August, whereas in the Northern Hemisphere, the universal birthday is on 1 January. New Zealand horses are actually 6 months younger and smaller than the equivalent horse born in the Northern Hemisphere. Although the official birthday is 1 August in the Southern Hemisphere, up until the year 2000, foals born on 31 July turned 1 year old the following day. The Australian and New Zealand Stud books changed regulations in 2000 to allow horses to be born from 1 July, as long as they were conceived after 1 September in the previous breeding season.

An important objective of any breeding farm in New Zealand is to produce a horse that will ultimately gain an acceptable price when it is sold. Sale price is greatly influenced by general appraisal, with size being a positive factor during assessment. Consequently the majority of Thoroughbred breeders aim for their foals to be born in August or September, as earlier-born foals will be older and as a result, considerably larger and well-grown at the time of sale. These foals are then likely to be more popular and will have enhanced trading potential. A late foal will be disadvantaged, as it will have had less time to mature and grow than its earlier born peers (Jackson, 1998).

Thoroughbred breeders must manage their production systems to compensate for industry regulations of the “official age”. The official birthday of 1 August in New Zealand takes no cognisance of the true age or maturity of a horse, and produces a situation whereby at the time of yearling sale or 2 and 3-year-old racing the more mature animals have a competitive advantage (MacCarthy and Mitchell, 1974). Mares that do not conceive in the first heat following foaling (approximately 9 days after birth) have to wait one oestrous cycle (21 days) until service. If they do not conceive to 30-day service, foaling in the following year will be later by nearly one month. This may not become a problem if the mare originally foaled close to 1 August, but if the mare foaled late, in December for example, it would be necessary to become pregnant to first service at foal heat to ensure the next foal is not later still.

In New Zealand the foaling season runs from 1 July to 30 June and horses are branded as being born in the year of the first month of the foaling season. A horse born in March will still be branded as being born in the foaling season beginning the year before. For example a horse born in March 2001 will be branded as being born in the 2000-birth season.

Horses exported to a different hemisphere automatically conform to that Hemisphere’s age rules. This leaves them with a distinct disadvantage because they are now 6 months younger than their counterparts. An example of this would be if a horse born in the USA in January 2002 were imported into New Zealand as a weanling with the intention of racing. It will officially turn 1 year old on 1 August

2002, when it is actually only 7 months old. Similarly, when horses from the Southern Hemisphere are exported to the Northern Hemisphere they automatically have another year added to their age each 1 January.

Late or autumn-born horses foaling after 1 January in the Southern Hemisphere, when exported to the Northern Hemisphere, turn 1 year old the next 1 January. Therefore, Southern Hemisphere autumn-foals are recognised as being born to Northern Hemisphere time and do not have the 6 month age disadvantage that spring-born Southern Hemisphere horses have when exported to the North.

1.1.1 Why breed New Zealand horses to Northern Hemisphere time?

Investigation into the breeding of horses so that they foal out of season and studies into the subsequent growth of these horses could allow the New Zealand bloodstock industry to sell horses competitively in the Northern Hemisphere. Breeding "out of season" and exporting to the opposite hemisphere could be a strategy to avoid losing a season of breeding from a mare that does not conceive soon after 1 September (Southern Hemisphere countries), and allows more choice to breeders as to when to stop a season. It could potentially allow breeders to manage two foaling herds – specifically bred for Southern or Northern Hemisphere sales and racing. This could improve access for New Zealand-bred horses into the Northern Hemisphere's multi-billion dollar bloodstock industry, or at least reduce prejudice against New Zealand foals, which may not be significantly smaller or less mature than their Northern Hemisphere counterparts of similar "age".

1.2 Time related growth patterns

One objective of animal study is to determine the effects of one or more variables on the growth of an animal. Growth of the animal is measured as change in weight over time, and there are several mathematical expressions used to describe this. The most usual way is to make a series of measurements as the body accumulates new tissue and to plot these against time. If growth is normal, the curve is a sigmoid curve with the slope of the curve being the accumulative growth rate (Davies, 1989). The age-growth curve can be divided into two stages, the first of increasing slope and the second of stabilised or decreasing slope. The inflection point between these two phases represents the time of maximum growth velocity and occurs between 30-50% of the animals' mature size (Brody, 1945).

Animal growth can also be studied by deriving mathematical equations to fit growth curves. A growth model is a function describing how an animal grows and is derived from the mathematics of the physical process producing the data. Growth models include factors that affect growth, such as birth weight and sex. Growth models are usually three or four parameter non-linear exponential equations, with an inflection point coinciding with the time or age of maximum growth rate (Bridges et al., 2000).

Growth curves have many practical applications including:

- Measurements of the performance of an animal or group of animals with respect to time;
- Comparison of the growth of individuals to an expected or "normal" curve of performance for different genotypes;
- Use in research to determine animal responses to different nutritional trials and environmental conditions; and
- A predictor of future performance where no animal measurements or data may exist (Bridges et al., 2000).

1.3 Growth of the Thoroughbred horse

The growth of the Thoroughbred horse has not been well defined, and there has been no equine growth model published.

Observed measurements of Thoroughbred growth include body weight, wither and hip height and measurement of defined body parts such as cannon circumference and chest width. Weight has been expressed as absolute weight in kilograms (kg) (MacCarthy and Mitchell, 1974; Hintz, 1978; Hintz et al., 1979; Thompson, 1995; Jelan et al., 1996; Pagan et al., 1996) and growth rate expressed as average daily gain (ADG) (Jelan et al., 1996; Pagan et al., 1996) or percentage of increase (Δ%) (Nogueira et al., 1997). Height and cannon circumference have also been documented (Green, 1969; Green, 1976; Hintz, 1978; Hintz et al., 1979; Thompson, 1995; Pagan et al., 1996), as have linear measurements to assess conformation (Mawdsley et al., 1996; Hunt et al., 1999).

Thoroughbred growth data have been collected primarily from Northern Hemisphere horses raised under stud farm conditions and fed supplementary grain (Green, 1969; MacCarthy and Mitchell, 1974; Hintz et al., 1979; Thompson, 1995; Jelan et al., 1996; Pagan et al., 1996). There are much less data available on growth of Thoroughbred horses raised at pasture (Grace et al., 1999; Nash et al., 2001; Grace et al., 2002).

Studies of growth in the horse have not been extensive because the horse is not primarily a meat-producing animal. In meat-producing systems, high growth rates are considered desirable (Purchas et al., 1989). This is not necessarily true in the horse, since rapid growth in the immature horse has been associated with high weight gain and compromised skeletal growth, predisposing the animal to developmental orthopaedic disease (Lewis, 1980; Thompson et al., 1988; Savage et al., 1993; Pagan et al., 1998). The regular weighing of growing horses is recommended (Pagan,1996) but is not routinely performed on most stud farms in New Zealand. The monitoring of an animal's weight to follow the normal growth curve allows growth rates to be controlled. This may reduce the incidence of developmental orthopaedic disease (DOD) (Jelan et al., 1996).

Colts are generally heavier than fillies at equivalent ages (Thompson, 1995; Pagan et al., 1996), but Green (1969) found no sex difference in foal size at birth and no significant difference in the average height and girth between fillies and colts. Body weights of commercially raised Thoroughbred fillies and colts are summarised in Table 1.1. Furthermore, there is little robust evidence of the mature body weight of the Thoroughbred. Typically studies have assumed Thoroughbred mature weights to be 500kg (Hintz et al., 1979; Frape, 1998). However, Pagan et al. (1996) measured body weights of 472 brood-mares and 25 breeding stallions and reported a mature body weight of 570 kg and 580 kg for mares and stallions respectively, and concluded that the mature body weight of a Thoroughbred in Kentucky as being 575 kg.

Table 1.1 Summary of body weights (kg) collected from Thoroughbred horses raised on Northern Hemisphere stud farms.

Study (kg)	2 months		6 months		10 months		12 months	
	fillies	colts	fillies	colts	fillies	colts	fillies	colts
(MacCarthy and Mitchell, 1974)	-	-	-	-	307	326	-	-
(Hintz et al., 1979)	135	137	236	245	311	325	329	345
(Thompson, 1995)	134.2	132.9	256.4	260.5	325.7	337.2	363.3	364.5
(Pagan et al., 1996)	-	-	250.7	255.9	311	322	335.2	349.2

Weight and height data were collected from 798 Irish Thoroughbreds between 1988-1992, from birth to 20 months of age (Jelan et al., 1996). The body weight of these horses was represented by a smooth, almost linear curve, the gradient of which was steepest up to 10 months of age, followed by a reduction in growth rate to 13-14 months and then an increase in growth rate to 15-16 months. ADG was described in four phases; birth to 1, 1-12, 12-15 and 15-20 months of age. The most rapid growth occurred in the first month after birth where ADG was 1.6 kg/day (kg/d). ADG at 12 months was 0.5 kg/d, which increased to 0.7 kg/d at 15 months. Height data showed rapid growth in the first three months of age followed by a regular decrease until 14-15 months, with a plateau between 14 and 20 months. The average height at birth (104.4 cm) was approximately 67% of the height attained at 20 months. The height at 6 and 12 months (132 cm and 146 cm respectively) was approximately 85% and 95% of height at 20 months of age.

Weight, height, chest and cannon circumference were measured in 10 Thoroughbred fillies from birth to two years of age (Nogueira et al., 1997). At birth the fillies were 66% of their height attained at 21 months, 63% of their cannon circumference, 44% of their chest circumference and 14% of weight. Average height at birth was 100 cm, which increased by 23% during the first 3 months, by 7% during the second three months and by 5% during the third three-month period. Average birth weight was 64 kg, and at 3 months, fillies weighed 151 kg. Monthly growth rate decreased continuously between 2 and 14 months, with the lowest weight gain occurring during the 14th month, at which age onset of reproductive activity was confirmed by plasma progesterone measurement. Assessment of this study (Nogueira et al., 1997) is difficult as the nutritional management of the fillies was not discussed.

There are limited data available on pasture-raised horses. Australian colts raised on pasture had a greater average daily gain than fillies between 7 and 14 months of age (0.77 kg/d vs. 0.73 kg/d) (Nash et al., 2001). New Zealand horses raised on pasture had a weight gain of 1.86 kg/d between 1-7 days of age and 1.31 kg/d between 8-28 days, and an ADG of 1.14 kg/d from birth to weaning (5 months) (Grace et al., 1999). ADG of 14 pasture-raised New Zealand yearlings averaged 0.6 kg/d when horses were between 10-17 months of age (Grace et al., 2002).

The time of year of foaling influences birth weight and the subsequent growth curve of the horse. The effect on month of foaling on body weight is shown in Table 1.2.

The data from the Kentucky foals (Pagan et al., 1996) were in contrast to the studies of MacCarthy and Mitchell (1974) and Hintz et al. (1979). Birth weights of the 4 groups of Kentucky foals were not supplied. The growth rates of foals born January/February, March, April of May/June were all reduced during winter regardless of birth month, and increased during April and May the following year. Growth rate in these horses was more related to season than to age, with growth patterns following changes in temperature and pasture growth.

Table 1.2 The effect of month of birth (Northern Hemisphere foaling season) on body weight of young Thoroughbred horses.

Study	Country	Observation
(MacCarthy and Mitchell, 1974)	Ireland	January/February-born foals were heavier than March to May-born foals.
(Hintz et al., 1979)	Canada	January/February/March-born foals were heavier than April/May/June-born foals from 1 to 2.5 months of age.
(Pagan et al., 1996)	Kentucky, USA	January/February-born foals were lighter than March-born foals at 2 weeks, and remained lighter until 9 months, at which time mean body weight was the same as that of the March-born foals.

1.4 Puberty

The onset of puberty has many definitions encompassing a variety of morphological, physiological and behavioural observations.

Published definitions of puberty include:

- The period in the male when the accessory organs and secondary sexual characteristics develop under the influence of the testis and when the animal first becomes fertile (Lincoln, 1971).
- The stage at which a person's reproductive organs are in the process of becoming mature and he or she becomes capable of producing offspring (Hawkins, 1987).
- The period of transition from a state of reproductive immaturity to a state of full reproductive competence (Cameron, 1990).

- The result of a gradual adjustment between increasing gonadotrophic activity and the ability of the gonads to simultaneously assume steroidogenesis and gametogenesis (Hafez, 1993).
- The time of human life during which the secondary sex characteristics begin to appear and the capability of sexual reproduction is possible (Tortora and Grabowski, 1996).
- The period of life at which the ability to reproduce begins. It is a stage of development when genitalia reach maturity and secondary sexual characteristics appear (Anderson et al., 1998).

1.4.1 The physical signs and identification of puberty

Puberty in humans is characterised by the development of secondary sexual characteristics and acceleration of linear growth (Tanner, 1981; Cutler et al., 1986; Attie et al., 1990; Rogol, 1996; Zhang et al., 2000). Pubertal development begins earlier and does not last as long in females as in males (Kacsoh, 2000). The pubertal growth spurt, although occurring in all children, varies in intensity and duration from one child to another (Tanner, 1981). In girls, growth velocity increases at approximately 10 years of age, reaching a peak of approximately 10.5 cm/year at 12 years, and decelerates toward zero at 15 years. In males, the growth spurt occurs approximately two years later reaching a peak of 12 cm/year at 14 years of age, decelerating towards zero at 17 years (Rogol, 1996). The pubertal growth spurt contributes to approximately 15% - 18% of adult height (Cutler et al., 1986).

Development of the secondary sexual characteristics (breast and pubic hair development in females, genital [penis, testis, scrotum] and pubic hair development in males) has been divided into the 5 Tanner stages, which are used to assess the stage of pubertal development (Tanner, 1981). The first sign of puberty is defined as an increase in testicular volume from 1mL to 3mL or breast development (Kacsoh, 2000). The appearance of spermatozoa in urine samples of males (spermarche) and onset of menses (menarche) in the female, are developmental milestones but do not necessarily mark the final stage of puberty in all humans. Average age at menarche is 12.8 years and generally occurs within 5 years of beginning of breast development (Kacsoh, 2000).

In domestic animal species, secondary sexual characteristics such as growth of pubic hair and breast development do not occur or are difficult to quantify. The animal is defined to have reached puberty when it is able to release gametes and to manifest sexual behaviour (Hafez, 1993).

In the female animal, puberty can be defined as the first ovulation and/or first period of oestrus, which can be determined by observations of oestrous behaviour and/or measurement of plasma progesterone. Initiation of corpus luteum function following an ovulation, hence onset of puberty, was defined as having occurred when concentrations of progesterone first exceeded 1ng/ml in ewes (Suttie et al., 1991b), heifers (Roberson et al., 1991; Bergfeld et al., 1994) and mares (Nachreiner and Hyland, 1993). Puberty in female ponies has been reported to occur when progesterone concentrations are greater than 2ng/ml and at least 3 times greater than the previous progesterone level (Wesson and Ginther, 1981).

Puberty in male animals cannot be determined with the same degree of accuracy as that of females, because it is generally regarded as a phase of development rather than a sudden event and, hence, many descriptions are used. Puberty is the time when the testes become androgenically active, with spermatozoa appearing some time after the onset of puberty (Skinner and Rowson, 1968). More recent descriptions of puberty in males include measurements of fertility not necessarily including hormonal observations. For example, Naden et al. (1990a) defined age at puberty in the male horse as when the ejaculate contains 50×10^6 spermatozoa, with >10% motile. Sex steroid hormones increase around 15 months of age in the colt, but very few spermatozoa are produced at that age. Reproductive capacity increases once testosterone secretion is established, with stallions reaching sexual maturity or maximal reproductive capacity between 2-4 years (Amann, 1993).

Because of the difficulty in determining when reproductive structure and function begins in animals, it is perhaps more appropriate to use an endocrinological parameter to define puberty in the horse. Thus, in this thesis, the definition of Wesson and Ginther (1981) has been used to determine puberty in the female, namely that the onset of puberty in the filly is the first significant increase in progesterone concentration (>2ng/ml) associated with luteal activity (immediately following the first ovulation), provided that it is at least three times greater than the previous concentration. Onset of puberty in the colt is the first significant increase in testosterone concentration (associated with a decrease in the response to the inhibitory feedback action of gonadal steroids). The criterion that defines the first significant increase in testosterone is derived from that of Goodman and Karsch (1980), namely that the hormone concentration exceeds the 95% confidence limits (2 standard deviations) of the baseline concentration (in this study the baseline is 0.05ng/ml).

1.5 Control of reproduction

1.5.1 The hypothalamic-pituitary-gonadal axis

Reproduction is regulated by the hypothalamus, pituitary gland and the gonads (hypothalamic-pituitary-gonadal axis), and the onset of puberty is regulated by the maturity of the link between these structures. See Figure 1.1.

The hypothalamus is a small portion of the diencephalon of the brain and is a major regulator of homeostasis. The main functions of the hypothalamus are to: (i) Control the activity of the autonomic nervous system, which regulates contraction of smooth and cardiac muscle and acts to regulate heart rate, movement of food through the gastrointestinal tract and contraction of the urinary bladder; (ii) Regulate emotional and behavioural patterns; (iii) Regulate hunger and satiety; (iv) Control body temperature; and (v) Regulate diurnal rhythms and states of consciousness (Tortora and Grabowski, 1996).

The hypothalamus is also an important endocrine gland that synthesises and secretes specific releasing hormones, evoking physiological responses. Hormones released by the hypothalamus include gonadotrophin releasing hormone (GnRH), growth hormone releasing hormone (GHRH), thyrotrophin releasing hormone (TRH), somatostatin or growth hormone inhibiting hormone, and corticotrophin releasing hormone (CRH). The hypothalamus also synthesises oxytocin and vasopressin, which are stored in the posterior lobe of the pituitary gland. The role of the hypothalamus in reproduction involves the control of the release of pituitary gonadotrophins and the stimulatory effect of steroid hormones on sexual behaviour.

The pituitary gland is located in the sella turcica of the sphenoid bone at the base of the brain and is attached to the hypothalamus via a stalk known as the infundibulum. The gland has two separate portions, known as the anterior and posterior lobes of the pituitary. The anterior lobe of the pituitary gland (anterior pituitary gland) accounts for about 45% of the total weight and secretes a range of hormones that regulate many bodily functions. The release of hormones from the pituitary gland is stimulated by releasing hormones and suppressed by inhibiting hormones from the hypothalamus. Hypothalamic hormones reach the pituitary gland through portal blood vessels that directly connect the two structures. The pituitary gland consists of 5 types of cells: (i) Somatotrophs – that produce growth hormone (GH); (ii) Thyrotrophs – that synthesise thyroid stimulating hormone (TSH); (iii)Gonadotrophs – that produce follicle-stimulating hormone (FSH) and luteinising hormone (LH); (iv) Lactotrophs – that synthesise prolactin; and (v) Corticotrophs – that synthesise adrenocorticotrophic hormone (ACTH) (Hafez, 1993). Secretion of hormones from the pituitary gland is regulated in two ways: by hypothalamic hormones and by negative feedback from target gland hormones.

Hormones that influence another endocrine gland are called trophic hormones. LH and FSH are trophic hormones and act to regulate the function of the gonads (ovaries and testes) hence they are termed gonadotrophins. Negative feedback systems decrease the secretory activity of gonadotrophs when levels of their target gland hormones rise.

GnRH and ovarian hormones control the reproductive axis of the female. GnRH stimulates FSH and LH secretion from the anterior pituitary gland. FSH stimulates the initial secretion of oestrogen from the developing follicles. LH stimulates further development of the follicles and their full secretion of oestrogen. LH also brings about ovulation, promotes formation of a corpus luteum and stimulates the production of oestrogens, progesterone, relaxin and inhibin by the corpus luteum (Tortora and Grabowski, 1996).

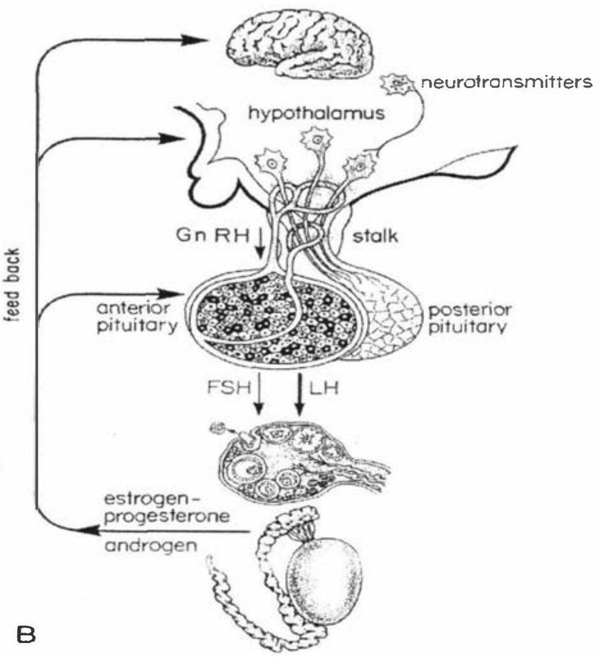

Figure 1.1 The hypothalamic-pituitary-gonadal axis. Source: Hafez (1993).

There are three major oestrogens in the female – beta-estradiol, estrone and estriol (Kacsoh, 2000). The oestrogens are secreted by the follicular cells and act to:
(i) Promote growth and development of the reproductive structures; (ii) Control fluid and electrolyte balance; (iii) Increase protein anabolism; and (iv) Lower blood cholesterol level. Progesterone is secreted by the cells of the corpus luteum and acts with oestrogen to prepare the uterus for the implantation of a fertilised ovum. High levels of progesterone and moderate levels of oestrogen act to inhibit GnRH and LH secretion.

LH stimulates the Leydig cells of the testes to secrete testosterone in the male. Testosterone is synthesised from cholesterol and is the main male sex hormone (androgen). FSH stimulates spermatogenesis by acting with testosterone on the Sertoli cells of the testes. High concentrations of testosterone act on the hypothalamus and pituitary to inhibit the secretion of GnRH and LH, in a negative feedback mechanism (Tortora and Grabowski, 1996).

1.6 The onset of puberty

There are two theories concerning factors that control the onset of puberty. The "missing link" concept assumes that one or more components of the hypothalamic-pituitary-gonadal axis are incapable of functioning in an adult fashion until at or near the time of puberty. The endocrine system of the pubertal animal may be mature but one or more specific components are inhibited from functioning prior to puberty (Squires, 1993). There is evidence that exogenous GnRH causes LH release prior to the time of puberty in ewe lambs (Foster et al., 1986), and male and female horses (Naden et al., 1990b and 1990c). This indicates that the pituitary can synthesise and secrete gonadotrophins during the prepubertal period and some other component of the hypothalamic-pituitary-gonadal axis inhibits the onset of puberty.

The "gonadostat" theory states that before puberty, the hypothalamic-pituitary-gonadal axis is extremely sensitive to the negative feedback effect of gonadal steroids (testosterone in males, and oestrogen in females), resulting in low tonic LH and FSH secretion. During puberty, there is a decrease in the responsiveness to the inhibitory actions of gonadal steroids, resulting in increased tonic gonadotrophin secretion sufficient to initiate reproductive activity (Olster and Foster, 1986). The onset of puberty is regulated by the maturity of the hypothalamic-pituitary-gonadal axis interconnections rather than by the pituitary not producing gonadotrophins or by gonadal insensitivity to their effects (Squires, 1993).

However, these theories appear to be not all that different to each other. The evidence supports the missing link theory early in an animal's life, when removal of a negative feedback will not stimulate onset of puberty because other factors are not permissive i.e. body weight (Foster, 1981). However, the gonadostat theory appears to be true closer to puberty onset, where a negative feedback mechanism is removed or sensitivity is decreased so that other permissive conditions allow puberty to start (Adam, 1992).

The frequency of LH pulses increase as the onset of puberty approaches (Squires, 1993; Hafez, 1993). In the pre-pubertal animal, pulses of LH are maintained at low frequencies and amplitudes. The GnRH pulse generator is a theorised mechanism governing GnRH secretion (Foster et al., 1986). It is thought that this GnRH pulse generator is highly sensitive to the negative feedback effects of estradiol and testosterone in the pre-pubertal animal. In females, ovarian oestrogen is inferred to act on the hypothalamus to suppress the generation of GnRH pulses that maintain the secretion of low-frequency LH pulses, preventing growth of follicles. The activity of the generator increases during puberty and requires higher levels of gonadal steroids to decrease the pulsatile secretion of GnRH. Increased GnRH pulse generator activity results in high frequency LH pulses (Foster et al., 1986). The trigger for the increased activity has not been defined, but is thought to involve nutrition, growth factors and photoperiod (in seasonal breeders), and the interaction between them (see Figure 1.2).

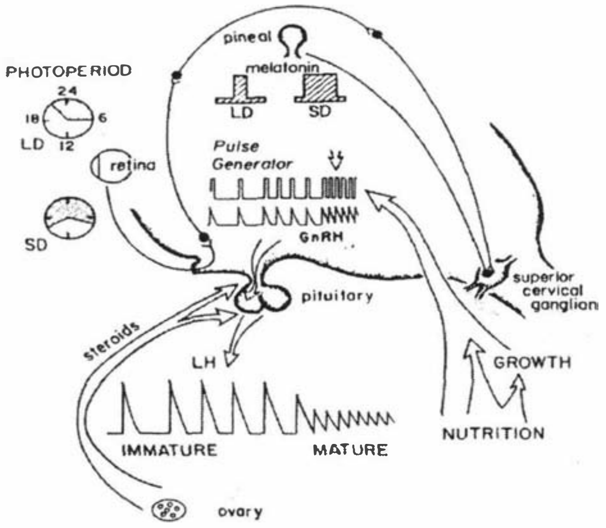

Figure 1.2 Proposed mechanism for the onset of puberty in the sheep by control of the GnRH pulse generator. Source: Foster et al. (1985).

At the onset of puberty, the frequency of LH pulse-generation increases. Follicles then develop to a more advanced stage, secreting progressively higher concentrations of oestrogen, stimulating uterine growth. At a certain point where LH pulse frequencies reach a threshold for both frequency and concentration to drive ovarian growth to a pre-ovulatory stage, oestrogen induces a pre-ovulatory surge of gonadotrophins and ovulation occurs (Squires, 1993). See Figure 1.3.

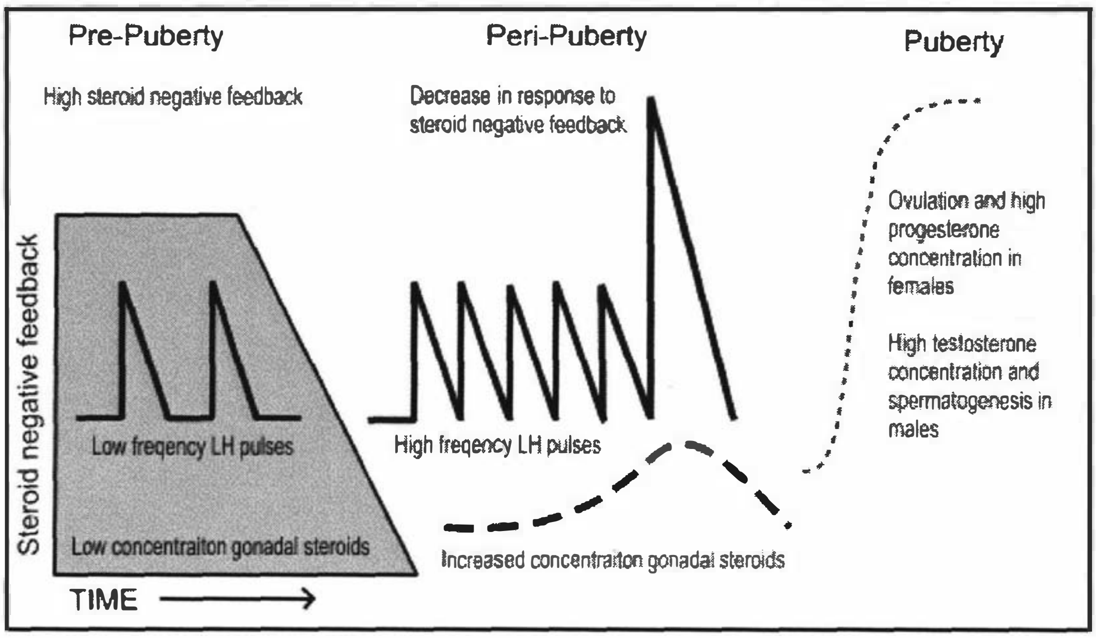

Figure 1.3 Diagram of the gonadostat theory for the onset of puberty. Decrease in response to negative feedback of gonadal steroids results in increased LH pulse frequency, culminating in ovulation in females and high testosterone concentrations and spermatogenesis in males.

This process is very similar in the male, where testosterone concentration progressively rises in response to increasing gonadotrophin secretion. In the male, the Leydig cells require high levels of LH for testosterone secretion, which increases as puberty advances. Testosterone concentration remains elevated once puberty has been reached (Hafez, 1993).

Castrated animals show a greater pre-pubertal rise in LH than intact animals due to the absence of the negative feedback from testicular androgens (Hafez, 1993).

The mechanism for reduced negative feedback effect of oestrogen and testosterone on LH as puberty approaches has not been determined. It could be due to reduced number or activity of steroid receptors on the hypothalamus as puberty approaches. Therefore less oestrogen/testosterone can bind, exerting less of a negative feedback on LH release. This effect on the receptors could be induced by many factors including growth rate, age, body-composition such as fat/lean tissue ratio, photoperiod (number of hours of light in a day), seasonal changes or pheromones.

However, these factors generally fall into two categories – nutritional effects and seasonal effects together with the interactions between them.

1.6.1 Nutritional effects and the onset of puberty

A threshold minimum age and minimum weight may be required to trigger onset of puberty. Other factors such as the level of nutrition, growth rate, body composition and metabolic factors may also have a stimulatory or inhibitory effect on puberty in animals of sufficient age and weight (Kirkwood and Aherne, 1985).

In well-nourished heifers, a high rate of weight gain results in younger, heavier animals at puberty and under-nutrition can delay onset of puberty (Foster and Yellon, 1985; Foster et al., 1985; Schillo et al., 1992; Barash et al., 1994; Bergfeld et al., 1994; Adam and Robinson, 1994; Bwire and Wiktorsson, 1996).

Ovarian follicular development differed in heifers fed diets with two different energy levels (Bergfeld et al., 1994). Heifers fed a high energy diet had larger dominant follicles. Heifers fed a low energy diet had smaller follicles and reached puberty approximately 63 days later than the high-energy group. In another study, heifers fed a low energy diet followed by a high energy diet to produce compensatory growth, had puberty delayed by one month compared with a control group maintained on one energy level (Barash et al., 1994). Despite this, puberty occurred at the same body weight (270 kg) in both groups.

Age at the onset of puberty did not differ in female pigs fed two different energy levels, but the percentage of pubertal animals at 28 days was greatest in those fed a high energy diet. Delayed puberty in the low energy group was associated with a lower body weight and a lower fatness (Klindt et al., 1999). Age at puberty in Holstein bulls was 41 weeks if fed above maintenance (Killian and Amann, 1972), and 37 weeks if fed *ad libitum* (Almquist, 1982). It was observed that body weight was more important than age on the onset of puberty. Rapidly growing ram lambs had an earlier onset of pulsatile LH secretion than slowly growing lambs (Foster et al., 1978).

A critical weight, also called threshold body weight or critical minimum weight for puberty, has been proposed (Keane, 1974; Suttie et al., 1991b). Threshold bodyweight for puberty in Irish bred lambs declined from 40kg in November to 33kg in December, contradicting this theory (Keane, 1974). In pubertal female lambs, body weight *per se* was not the primary cue for puberty, as animals at an intermediate level of nutrition adapted to under-nutrition and achieved puberty (Suttie et al., 1991b). A relative lack of influence of nutritional status on the timing of puberty in the highly photoperiodic Soay ewe lamb was demonstrated compared with the Suffolk x Greyface ewe (Adam et al., 1998). Mean pubertal live weight of feed-restricted Soay ewe lambs averaged 76% of if their *ad libitum* fed counterparts and puberty occurred on the same date for both groups. However, puberty in food restricted Suffolk cross lambs was delayed by two weeks compared with *ad libitum* fed ewes of the same breed and pubertal live weight of feed-restricted ewes was 73% of *ad libitum* fed ewes (Adam et al., 1998). Thus, growth restriction delayed the date of pubertal onset in Suffock cross ewes, but did not influence the timing of puberty in the Soay ewe lamb, raising the question of breed differences in the relative importance of photoperiod versus nutrition in the timing of puberty in female sheep.

The time of puberty onset has been attributed to actual body weight or the total amount of growth achieved, rather than to the rate of growth or when it occurred. There was no difference between age at puberty in heifers fed to maintain a constant growth rate during the 90 days after weaning, compared with heifers fed for rapid growth during the first or second half of the post-weaning period (Clanton et al., 1983).

Body composition may also control puberty onset. In humans, both body weight and body composition have an effect on the onset of puberty. Obesity in young girls results in younger age at puberty (Hopwood et al., 1990). Conversely, intensive physical training, chronic stress and low body weight and/or low body fat, cause a delay in the onset of puberty. Late menarche is common in athletes and is related to low body fat, delayed skeletal maturation and intensive physical training (Georgopoulos et al., 1999).

1.6.2 Metabolic factors and the onset of puberty

The reproductive system of the mammal is sensitive to available energy. Changes in an animal's energy status can perturb the hypothalamic-pituitary-gonadal axis.

Circulating concentrations of hormones/growth factors such as growth hormone (GH), insulin-like growth factor (IGF-1) and leptin, are often associated with the nutritional status and body composition of the animal. These metabolites may exert an influence on the hypothalamic control of LH secretion, or provide a signal linking metabolic status of the body to the hypothalamus and affect GnRH secretion (Anderson et al., 1998).

1.6.2.1 Insulin-like growth factor-1 and Growth hormone

In humans, plasma IGF-1 levels increase from birth and reach peak concentrations in preadolescent years, after which they slowly decrease (Kacsoh, 2000). GH and IGF-1 mediate the rapid pubertal growth spurt in humans and GH insufficient children have failure of growth at puberty (Rogol, 1996). The preadolescent peak of IGF-1 is accompanied by increased GH secretion. GH is under the negative feedback control of IGF-1; thus the associated rise in both IGF-1 and GH during puberty implies that the set point of negative feedback becomes elevated around puberty (Kacsoh, 2000). Mean circulating concentrations of GH increase prior to the pubertal growth spurt and return toward pre-pubertal levels shortly after the cessation of linear growth (Rogol, 1996).

In the rat, puberty is characterised by an increase in IGF-1 concentration (Handelsman et al., 1987). Castration at birth enhanced the IGF-1 surge that occurs around puberty showing that neither testicular secretion of testosterone, nor the acute effects of testicular steroids on non-testicular tissues are responsible for the peri-pubertal surge of IGF-1 in the maturing rat. The increased concentration of IGF-1 seen at puberty must therefore be mediated by a neuroendocrine mechanism that occurs in parallel with, but independent of testicular hormonal status.

Onset of puberty in female sheep was associated with significantly increased IGF-1 levels (Roberts et al., 1990). IGF-1 acts directly on the median eminence (containing the highest density of type 1 IGF-1 receptors in the brain) to elicit a dose-related release of GnRH (Hiney et al., 1991). IGF-1 induced LH secretion in juvenile and peri-pubertal rats by stimulating GnRH release, significantly advancing onset of puberty (Hiney et al., 1996), showing that IGF-1 may play a role in facilitating peri-pubertal changes in GnRH release.

Decreased IGF-1 concentrations are positively associated with delayed puberty in heifers (Schoppee et al., 1996). Onset of puberty was earlier and IGF-1 concentrations were higher in heifers fed to achieve high growth rates than in those fed to maintenance levels (Yelich et al., 1996). IGF-1 concentration and ovarian follicular fluid volume were positively related indicating that IGF-1 may stimulate oestrogen production. Low levels of nutrition, resulting in low IGF-1 concentration, may cause reduced ovarian estradiol synthesis, thereby delaying the pre-pubertal LH surge (Yelich et al., 1996).

There was also a close relationship between GH and IGF-1 concentrations, live weight gain and increasing daylength in peri-pubertal red deer stags (Suttie et al., 1989).

1.6.2.2 Leptin

Leptin is an adipocyte-derived signalling molecule seemingly secreted in proportion to adiposity, which limits food intake and increases energy expenditure. Fasting decreases leptin levels leading to reduced energy expenditure thereby conserving energy stores. Conversely, increased food intake can result in increased adiposity and increased leptin levels. Obese humans have elevated concentrations of leptin with a greater percentage of body fat, suggesting a resistance to the actions of leptin or a decreased sensitivity to leptin, which maintains increased adipose-tissue mass (Roemmich and Rogol, 1999; Kacsoh, 2000).

The hypothalamus is the target for the satiety effects of leptin and has receptors located on the capillary endothelial cells. Leptin inhibits the synthesis of Neuropeptide Y (NPY), which occurs in the hypothalamus. NPY is a potent stimulator of food intake. Leptin causes a decrease in NPY production and a reduced appetite. Leptin receptors are also found in peripheral tissues. Leptin acts on cultured hepatocytes, adipocytes, haemopoietic cells and pancreatic islet cells (Auwerx and Stales, 1998). Leptin inhibits intracellular lipid accumulation in these cells by reducing fatty-acid synthesis and increasing lipid oxidation, possibly through inhibition of acetyl-CoA carboxylase activity (the rate-limiting enzyme in fatty acid synthesis).

Leptin concentrations are high in the new-born, which decline to relatively low levels in childhood, are similar in both sexes until puberty, and increase at puberty onset. In males, the pre-pubertal rise in leptin concentration is followed by a decline because the pubertal increase in androgens results in increased lean body mass and decreased fat. In females, leptin concentration remains elevated or increases due to oestrogen-stimulated increase in body fat deposition. Leptin concentrations are higher in women than men of similar age (Kacsoh, 2000).

Leptin may be a permissive signal for the onset of puberty (Figure 1.4), by acting with other factors such as glucose availability, photoperiod and growth-related cues as co-determinants of the initiation of reproductive cycles through timing the expression of the high-frequency GnRH pulses (Foster and Nagatani, 1999).

Initiation of puberty requires a minimal level of adiposity and an associated increase in plasma leptin levels. After puberty, if lipid stores are severely depleted (e.g. in certain athletes), the decrease in leptin shuts down reproductive function via GnRH (Kacsoh, 2000).

The pubertal rise in leptin concentration does not occur in the rat or Rhesus monkey. Serum leptin concentrations in rats remained constant between 10 and 50 days of age (puberty) (Cheung et al., 2001). In male Rhesus monkeys, serum leptin concentrations remained unchanged during onset of puberty while testosterone concentration increased (Plant and Durrant, 1997). These reports suggest that leptin is not a trigger for the onset of puberty in the primate and rat, although leptin is thought to act as a permissive factor whose presence is important but not sufficient (Cheung et al., 2001).

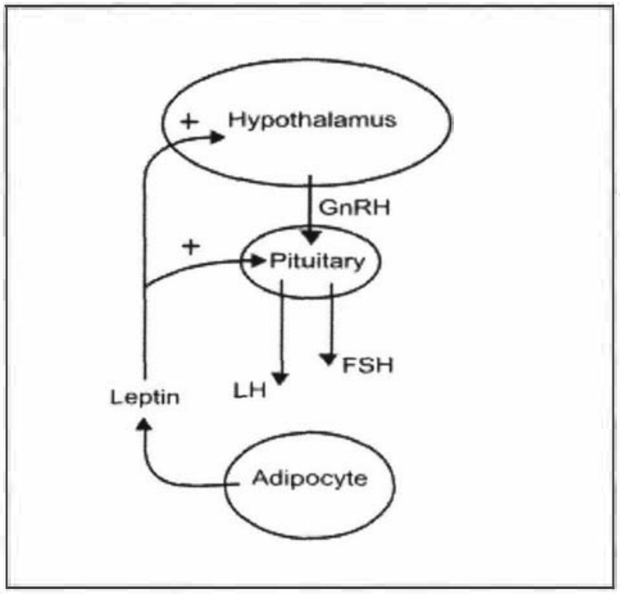

Figure 1.4 Diagram of the probable action of leptin to stimulate GnRH and LH/FSH release. Adapted from: Yu et al. (1997).

Leptin is a product of the obese *(ob)* gene. Mice carrying the *ob/ob* gene are obese due to a mutation in the gene, resulting in failure of the secretion of leptin from the adipocytes (Yu et al., 1997). Mice carrying the *ob/ob* gene fail to undergo normal sexual maturation and remain infertile throughout life (Barash et al., 1996). Chronic leptin treatment of the *ob/ob* mouse reduces food intake and weight and restores reproductive function (Barash et al., 1996; Cheung et al., 2001). These results indicate that leptin may be a critical signal informing the reproductive axis about the body's nutritional state, allowing reproduction to occur if nutritional reserves are sufficient and blocking it if reserves are low or the metabolic system is stressed.

Leptin administered to pre-pubertal mice accelerated pubertal onset (Ahima et al., 1997). Female mice injected with leptin had a lower growth rate, reproduced 9 days earlier and had earlier maturation of the reproductive tract than controls (Chehab et al., 1997). Leptin may be permissive to pubertal maturation (in that it allows puberty to commence if and when metabolic resources are adequate) although its presence alone in the well-fed pre-pubertal animal is not sufficient to initiate puberty before the time it would normally occur (Cheung et al., 1997; Gruaz et al., 1998).

Given the limited and inconsistent literature on the effect of leptin on the timing of pubertal onset in rodents and the lack of increased plasma leptin concentration prior to puberty, conclusions about the possible role of leptin in controlling the timing of puberty onset should be treated with some caution (Cheung et al., 2001). Although species differences could account for some of the inconsistencies, experimental method and the different sources of recombinant leptin are also likely to contribute to the difference in results.

1.6.3 Pheromones and the onset of puberty

The term pheromone refers to air-borne chemical substances secreted externally in urine, faeces, or cutaneous gland secretions, which cause a specific reaction in a receptive individual of the same species. The reaction involves either the expression of a specific behaviour or physiological change in the recipient's endocrine or reproductive system (Izard, 1983).

In domestic animals, priming pheromones from the male can influence the induction of puberty, the termination of seasonal anoestrus, or shortening of postpartum anoestrus. An androgen-dependent priming pheromone present in the urine of adult male mice can accelerate puberty in the female (Rekwot et al., 2001). The male pheremonal stimulation hastens the final maturation of the positive feedback system controlling ovarian function by inducing LH release and stimulating oestrogen secretion (Bronson and Desjardins, 1974). The time of onset of puberty in gilts is advanced in the presence of a boar (Brooks and Cole, 1970) (Thomson and Savage, 1978). Priming pheromones of the ram and buck hasten puberty onset in female sheep and goats (Rekwot et al., 2001) and social interaction between bulls and heifers resulted in earlier puberty onset in the heifers (Roberson et al., 1991).

1.7 Onset of puberty in seasonal breeders

Most domestic species and many wild species are seasonally, not continuously, fertile. This is an evolutionary adaptation that ensures birth is at a time when climatic and nutritional factors maximise survival chances of the new-born (Hafez, 1993).

The environmental cue that has the greatest influence on seasonal breeding is photoperiod, which varies markedly with season in temperate zones. Seasonal breeding animals with shorter gestational periods, such as the sheep and red deer, breed in autumn so that the young are born in the spring and are termed "short day breeders". Animals with longer gestations, such as the horse, breed and give birth in the spring and are termed "long-day breeders".

Photoperiod influences the onset of puberty in seasonal breeding animals and puberty occurs in the normal breeding season. In well-nourished spring-born lambs, the decrease in daylength in autumn provides the appropriate photoperiod cues for the first ovulation around 30 weeks of age (Foster and Yellon, 1985) but there are major variations between breeds. Red deer are normally born in the long days of summer, exhibit slow growth during the autumn and winter, grow rapidly in the following summer and achieve puberty in their second autumn around 16 months of age (Adam et al., 1996). In the horse, puberty occurs in the second spring of life at approximately 12-15 months of age (Wesson and Ginther, 1981).

1.7.1 Sheep

1.7.1.1 Puberty in the ewe

Onset of puberty in the ewe is influenced by seasonal changes in daylength, and therefore age at puberty will differ depending on when the lamb is born. Spring-born and autumn/winter-born lambs will attain puberty at the same time of the year, namely in the breeding season (autumn) (Foster, 1981; Foster et al., 1986). Lambs born in late summer may not achieve sexual maturity on time because their small physiologic size delays puberty until the following breeding season.

The conclusion that daylength is the seasonal factor controlling puberty onset arose from studies establishing that various artificial photoperiod regimens altered the age at pubertal onset. The age at puberty in natural photoperiod was determined in spring-born and autumn-born lambs and in autumn-born lambs raised in a seasonally reversed photoperiod (Foster and Ryan, 1981). Spring-born lambs reached puberty in their first autumn at approximately 30 weeks of age, autumn-born lambs at about 50 weeks and autumn-born lambs raised under a seasonally reversed photoperiod at 30 weeks (Foster et al., 1986).

Studies were conducted to determine whether long daylength prevented puberty, or short daylength stimulated puberty, or a combination of both short and long daylength was required for puberty (Yellon and Foster, 1985; Foster et al., 1985). Spring-born lambs under continuous long days (15 hours of light: 9 hours of dark) from birth had delayed onset of puberty, isolated reproductive cycles and short luteal phases (Yellon and Foster, 1985). This indicates that long photoperiod prevented puberty onset, supporting the view that the long days of summer prevent onset of puberty. However, the short daylength in autumn is not the stimulatory factor for the onset of puberty, as lambs raised under artificial short-days (9 hours of light: 15 hours of dark) also showed delayed onset of puberty (Foster et al., 1988).

Under natural conditions, spring-born lambs experience increasing daylength in spring and summer prior to decreasing daylength in autumn before the onset of puberty. Lambs were raised to experience various combinations of long and short daylength (Yellon and Foster, 1985). Spring-born lambs were raised in artificial short days with either no long days, or with 10 weeks, 5 weeks or 1 week of long days (15 hours of light: 9 hours of dark) at 12-22, 18-22, and 21 weeks respectively. In all regimes exposing the lambs to some long days (for 1, 5 or 10 weeks), lambs established reproductive cycles. This indicates that lambs may maintain a “photoperiod history” and that the long days of summer are a reference point and must be experienced in order for puberty to occur in the shorter days of autumn.

Lambs raised under artificial photoperiod (either short or long days) from birth did not achieve puberty at the same age as lambs raised under natural photoperiod (Foster et al., 1988). Ewes raised under natural photoperiod and then housed under artificial short or long daylength followed the same seasonal pattern of reproduction as ewes under natural daylength (Robinson and Karsch, 1988).

Ewes may have an in-built tendency to oscillate between periods of breeding and non-breeding. Animals maintained for extended periods in constant photoperiods either from being blinded, pinealectomised, or held in a constant daylength continued to have cycles of breeding (Robinson and Karsch, 1988). Thus, the seasonal reproductive rhythm of the ewe, rather than being generated by the 24 hour daylength, may be entrained to the annual photoperiodic cycle over 365 days (Robinson and Karsch, 1988).

1.7.1.2 Puberty in the ram

In spring-born ram lambs, spermatogenesis begins in early summer, at approximately 10 weeks of age. The effect of photoperiod on the attainment of puberty in the ram has been considered relatively minor in comparison with the female (Adam and Robinson, 1994), because puberty occurs at the same age regardless of daylength (Wood et al., 1991). The age at puberty was similar in rams raised in natural photoperiod (Foster et al., 1988), simulated natural photoperiods, or seasonally reversed changes in photoperiod (Herbosa et al., 1995; Herbosa and Foster, 1996).

Male lambs were raised from 2 weeks of age in either an increasing daylength followed by a decreasing daylength or an opposite regimen (Wood et al., 1991). Ram lambs raised in the decreasing photoperiod followed by increasing photoperiod showed a delay in puberty onset of only 3 weeks. This suggests a sex difference in the photoperiodic regulation of puberty, as male lambs begin sexual maturation in the presence of a photoperiod which is inhibitory to puberty in the female. Neuroendocrine sexual maturity in the male begins at approximately 5-10 weeks, regardless of season, indicating that although photoperiod has an influence on the timing of puberty in male lambs, the effect is minimal (Wood et al., 1991).

There is increasing evidence that male and female sheep have different photoperiod requirements for onset of puberty (Kosut et al., 1997) and that male sheep are far less dependent on changes in daylength compared with female sheep where puberty is highly influenced by photoperiod. Reproductive maturity in male lambs begins during long days of summer, whereas puberty in the female lamb requires the change to decreasing photoperiod in the autumn. Moreover, unlike the female lamb that requires the change in daylength to stimulate pubertal onset, puberty is not delayed in ram lambs maintained in constant long or short days (Wood et al., 1991).

Onset of puberty in both sexes is driven by increased secretion of LH as a result of reduced sensitivity to negative feedback of gonadal steroids, but there is evidence that the events leading up to onset of puberty in sheep are different between males and females. Male sheep undergo a reduction in sensitivity to steroid feedback at around 10 weeks of age, whereas ewe lambs remain responsive to steroids until 30 weeks of age (Wood and Foster, 1992). This difference is attributed to exposure to androgens *in utero*. Testosterone administered to gonadectomised, estradiol-treated female sheep *in utero* advances neuroendocrine puberty and reduces responsiveness to photoperiod (Kosut et al., 1997) .

LH and testosterone response to exogenous GnRH was examined in ram lambs between 6 and 32 weeks of age (Wilson and Lapwood, 1979). Peak LH concentrations were highest at six weeks and decreased progressively until 32 weeks. A delay in peak LH response to GnRH occurred from 15 min at 6-18 weeks, to 45 min at 22 weeks and 60 min at 30-32 weeks. Testosterone response to GnRH was lowest (3.6 ng/ml) at 6 weeks and progressively increased to maximal (9.9 ng/ml) at 32 weeks. The decreased and delayed LH secretory response to GnRH as the animals aged was due to a change in pituitary responsiveness to the negative feedback effects of testosterone. Season had a greater effect than genotype on individual differences in LH response to GnRH in lambs (Tyrrell et al., 1980). In December, when lambs were 9 weeks old, 85% attained peak LH concentrations within 60 minutes of GnRH administration, whereas only 28% did so when 13 weeks or older (the peak being reached within 90-120 min). The greatest LH response in ram lambs, measured as area under the curve (AUC) occurred in late summer (February) just prior to puberty onset, when they were 17-20 weeks old. LH response decreased during April and May when the lambs were 26-33 weeks old and pubertal. These results are similar to those of Wilson and Lapwood (1979), who found the time taken to reach peak LH response increased as the animals aged, reflecting increasing maturity of the lambs.

1.7.2 Cattle

Cattle are not seasonal breeders although there have been several studies which indicate some seasonal variation in bovine reproductive activity. Season affects the secretion of LH in both the beef and dairy cow (Stumpf et al., 1993). Patterns of LH and testosterone secretion vary with season in the bull, as concentrations of LH are higher in spring than in winter and testosterone concentrations are higher in summer (Stumpf et al., 1993).

The endocrine events associated with the onset of puberty were studied using GnRH-stimulated LH and testosterone release in 1-2 year old *Bos indicus* bulls (Wildeus et al., 1984). Basal testosterone concentrations were low before the onset of puberty (as detected by ejaculatory behaviour) and gradually increased until 24 months of age. Peak GnRH-stimulated testosterone response provided an estimate of testosterone secretory activity and was different from basal patterns, increasing throughout the experimental period. Peak testosterone concentration was a direct indicator of increased Leydig cell sensitivity to GnRH-stimulated LH, as LH concentration decreased with advancing age. The decrease in LH response to GnRH as the animals' aged indicated an inhibitory effect of increased testosterone secretion on pituitary function.

A similar decrease in LH response to GnRH accompanied by increasing testosterone secretion was found in peri-pubertal Brahman bulls (Rutter et al., 1991). LH response to GnRH (200 μg) in bulls was similar when challenged (a) before puberty, (b) at the time when sperm cells were first observed in the ejaculate, and (c) post puberty, but there was no testosterone response until sperm were first detected in the ejaculate. As puberty approached there was a lower LH response to GnRH and a significant negative correlation between the area under the curves of LH and testosterone. This indicates an increasing responsiveness of the testes to LH, probably through increased Leydig cell responsiveness with advancing age.

In spring and autumn-born calves, mean serum LH concentrations increased from 4 to 18 weeks after birth and then decreased by week 24 in spring-born calves, but remained elevated until week 44 in autumn-born calves. Season of birth had no effect on mean testosterone concentrations, which remained low until 22 weeks after birth and then increased steadily in both groups of calves (Aravindakshan et al., 2000). The mean age and body weight at puberty for the two groups was not significantly different (46.1 ± 1 weeks and 342 ± 4 kg for spring-born and 45.7 ± 4 weeks and 343

± 11kg for autumn-born), but variance was greater in the autumn born calves (Aravindakshan et al., 2000).

Age and weight at puberty did not differ between spring and autumn-born heifers, but there was greater variance in the autumn-born heifers (Honaramooz et al., 1999). In contrast, some studies in dairy and beef heifers have shown that spring-born heifers were younger at first oestrus than heifers born at other times of the year (Menge et al., 1960; Arije and Wiltbank, 1971; Roy et al., 1980). Autumn-born Angus x Holstein heifers attained puberty at a younger age than heifers born in spring (Schillo et al., 1982). Many factors could contribute to the inconsistent information on the effect of birth season on pubertal onset in the heifer, including differences in breed, the latitude/location of the work and management practices.

1.7.3 Deer

Red deer calves are born in the summer following the autumn breeding season. Puberty is not reached until autumn of the second year when the deer are approximately 16 months old (Adam et al., 1996). The red deer stag undergoes three phases of sexual maturation (Suttie et al., 1991a). The first is in the autumn following birth, characterised by an increase in LH pulse frequency and increased testosterone production. During late winter and spring there is a decline in testosterone concentration, which increases during the stag's second autumn, when puberty is achieved. Male and female deer born out of season in the late winter (4 months before normal calving) were heavier at weaning and showed precocious puberty in their first autumn at approximately 8-9 months old (Adam et al., 1992). Winter-born deer achieve sufficient live weight to support puberty onset and respond to the decrease in photoperiod in the first autumn. Deer born in the normal birth season (summer) do not achieve sufficient live weight for puberty in the first autumn and must wait for the decreasing daylength in the second autumn to complete sexual maturity.

Stags and hinds raised in constant artificial daylength (12 hours light: 12 hours dark) from the time of birth exhibited different photoperiodic responsiveness and sexual maturity (Adam et al., 1995). Stags raised at constant daylength showed greater sensitivity at an earlier age to gonadotrophin stimulation, indicative of their uninterrupted progressive reproductive development. However, in female deer, rearing photoperiod had no effect on LH pulse frequency and ovarian activity (Adam et al., 1996). These data differ from ewes which require specific photoperiodic cues to time puberty onset in the early autumn and ram lambs which attain puberty at the same age under a variety of photoperiodic regimens (Wood et al., 1991).

1.7.4 Horses

1.7.4.1 Puberty in the filly

Puberty in the filly has been reported in the spring, between the ages of 10 and 25 months (Wesson and Ginther, 1981; Palmer and Draincourt, 1983; Adams and Bosu, 1988; Adams and Bosu, 1988; Naden et al., 1990b; Souza et al., 1997; Nogueira et al., 1997; Camillo et al., 2002). Spring and summer-born (April and June) pony fillies had their first ovulation during the following late spring or early summer, whereas late-born fillies (August and September) did not (Wesson and Ginther, 1981). The mean date for the first ovulation (onset of puberty) did not differ between spring and summer-born ponies so age at puberty onset was therefore different between the two groups (410.8 ± 5.5 days and 341.0 ± 16.5 days). The failure of late-born fillies to ovulate in the first spring was attributed to exposure to decreasing daylength in the first 6 months of life. The authors proposed that the sensitivity of the pre-pubertal pony to increasing photoperiod accounted for the attainment of puberty during the next breeding season (Wesson and Ginther, 1981).

The effect of season of birth and the age at puberty in fillies was studied in Welsh Ponies, which are known to have a high incidence of ovarian inactivity in winter (Palmer and Draincourt, 1983). Ovarian activity was compared between spring-born foals and June-born foals. All spring-born fillies had their first ovulation in the following spring/summer when they were approximately 14.5 months old. Only 4 of 10 June-born fillies showed luteal activity in the first spring after birth, with only one filly showing a complete breeding season with regular cycles. Palmer and Draincourt (1983) postulated that the effect of birth season on ovarian activity in the pre-pubertal filly is regulated by synchrony of a potential puberty age (approximately 14 months) with the increase in daylength. The June-born fillies would receive the stimulus of increasing daylength before their sensitivity was established.

Body weight at puberty did not differ between 9 spring and 3 summer-born fillies (Wesson and Ginther, 1981). Body weight of 2 late-born (August-September) fillies that failed to achieve puberty was not noted. It is possible that autumn-born fillies that grow well or are born early in the autumn reach a threshold weight and ovulate in the spring at the same time as the spring-born foals from the year before. There has been no threshold age or weight proposed for onset of puberty in the horse, but ponies maintained on a low plane of nutrition with decreased growth rates between 6 and 12 months had a delayed onset of puberty (May to July) compared with ponies that grew faster (April to May). Unfortunately body weight at puberty in these ponies was not recorded (Ellis and Lawrence, 1978).

The sexually mature mare has, on average, a 21 day long ovulatory cycle, consisting of a 14 day luteal phase (diestrus) and 7 days of oestrus when the mare is sexually receptive (Hammond, 1940; Ginther, 1992). GnRH is the major regulator of LH and FSH secretion in the mare, with pulses of the gonadotrophins accompanied by a major episode of GnRH release (Alexander and Irvine, 1996). Reproductive activity in the female horse is highly seasonal with low numbers of ovulations in winter, when daylength is short, and increased ovulatory activity in the spring. At the onset of the breeding season, the frequency of FSH and LH pulses increase prior to the first ovulation (Alexander and Irvine, 1991). At the end of the breeding season, decreasing daylength precedes the decline in ovulatory activity resulting in a lag of several months, at which stage the ovulatory LH surge is absent resulting in diminished ovarian function and no ovulation (Irvine et al., 2000).

1.7.4.2 Puberty in the colt

Puberty in colts, as determined by semen characteristics, occured at 12 to 15 months in Welsh Pony colts (Skinner and Bowen, 1968) and at 14 to 24 months in Quarter Horse colts (Naden et al., 1990c). It is possible that stimulation for sexual maturity of the male and female horse differ in a similar way to the sheep. Male puberty is a gradual process of increasing tonic secretion of LH and testosterone. An increase in pulse frequency and concentration of LH and FSH occurs between 8 and 10 months with no significant testosterone secretion. Concentrations of LH average 4-8 ng/ml (Naden et al., 1990a), much lower than those in the adult stallion in the breeding season (30-50ng/ml) (Harris et al., 1983) and indicate one or more of (i) insufficient LH to stimulate testosterone secretion; (ii) Leydig cell immaturity, or (iii) seasonal inhibition of testosterone secretion. In Quarter Horse colts, gonadotrophin levels decreased after 10 months due to the negative feedback of testosterone on the hypothalamus (Naden et al., 1990a). FSH concentrations increased from approximately 16 months of age, whereas LH concentrations remained low until about 20 months, possibly indicating independent control mechanisms. After 18 months, testosterone concentration increased, accompanied by rapid testicular growth. Naden et al. (1990a) stated that increased LH pulse frequency is the driving factor in attainment of puberty in colts, but they failed to discuss a trigger for the increase in LH pulse frequency, which could be increasing daylength, nutrition or breed effects.

In the stallion, semen characteristics and hormonal concentrations show seasonal patterns. Gel-free semen volume, spermatozoa per ejaculate and libido all increased during the spring and summer months, accompanied by elevated FSH, LH and testosterone concentrations (Harris et al., 1983). Clay et al. (1988) demonstrated a stimulatory effect of increased daylength (16 hours light: 8 hours dark) after 20 weeks of short days (8 hours light: 16 hours dark) on FSH, LH and testosterone concentration. Increase in LH concentration at the onset of the breeding season is due to an increased amount of LH released and not increase in LH pulse frequency (Clay et al., 1989). The subsequent increase in testosterone concentration is due to the enhanced stimulation of Leydig cells by the increased amount of LH. Increasing daylength in spring is thought to a positive stimulus for, or removes an inhibition of, LH release (Clay et al., 1989). However, seasonal control of LH secretion in the stallions has been debated and it was suggested that it was mares that influenced the seasonal nature of stallions' sexual activity (Irvine and Alexander, 1988). LH secretion was not found to follow a seasonal pattern of release in geldings (castrated males) except when testosterone was administered (Irvine and Alexander, 1982), and stallions, when exposed to oestrous mares, exhibited rapid release of GnRH and LH pulses (Irvine and Alexander, 1991).

1.8 Photoperiod and reproduction

Photoperiod is a stimulus by which an organism measures time and synchronises biological rhythms. The earth's axis is not perpendicular to the plane of orbit around the sun and thus season and latitude dictate the number of daylight hours within the 24 hour day. In the Southern Hemisphere, the summer solstice (longest day) and winter solstice (shortest day) occur on the 21 December and 21 June, respectively, opposite to the Northern Hemisphere. The equinotes (when hours of daylight and darkness are equal) occur on the 21 March and 21 September in both Northern and Southern Hemispheres.

Most organisms possessing an internal pacemaker or clock which responds to the daily cycle of light and dark by generating and regulating a circadian rhythm of metabolic activities, including wakefulness/sleep, excretion, hormone production and behaviour (Follett and Follett, 1981). Photoperiod is the most important factor regulating circadian and annual cycles (circannual) (Goldman and Nelson, 1993). Non-light cues, including food intake, onset of sleep, or temperature, can also act to entrain (reset daily) the pacemaker's circadian rhythm of activity (Kacsoh, 2000). Without entrainment, the pacemaker possesses its own intrinsic rhythmic pattern of activity. In vertebrates, the internal pacemaker consists of the retina, the hypothalamic suprachiasmatic nucleus (SCN) and the pineal gland (Kacsoh, 2003).

1.8.1 The pineal gland and melatonin

The pineal gland lies within the epithalamus and protrudes from the dorsal midline of the third ventricle and consists of masses of neuroglia (cells of the nervous system) and pinealocytes (secretory cells) (Tortora and Grabowski, 1996). Light signals from the retina stimulate the gland to synthesise and secrete melatonin (Kacsoh, 2000).

The Greek philosopher Galen, c. 170 AD, undertook the first in-depth study of the pineal gland, but it was not until 1958 that scientists first identified melatonin, a biogenic amine (simple structured hormone) produced by the pineal gland (Kennaway and Rowe, 1995) .

Photic cues detected by the retina cause electrical signals to pass to the SCN via the retinohypothalamic projection (specialised nerve fibres). The electrical impulses are then passed from the SCN via the paraventricular nuclei (in the hypothalamus) and spinal cord to the superior cervical ganglia of the sympathetic nervous system, terminating at the pinealocytes (Yu et al., 1993). Within the pineal gland, the amino acid tryptophan is converted to serotonin with 5-hydroxytryptophan as an intermediate. These two steps are catalysed by the enzymes tryptophan hydroxylase and 5 hydroxytryptophan carboxylase respectively. Serotonin is then converted to N-acetylserotonin by the enzyme N-acetyltransferase, which is subsequently converted to melatonin (Reiter, 1980). The pathway of melatonin biosynthesis is summarised in Figure 1.5.

The pineal gland secretes but does not store melatonin, which has a half life of approximately 25 minutes (Bartsch et al., 1992; Kacsoh, 2000).

Besides the pineal gland, melatonin is reported to be produced in a number of extrapineal sites including the gastrointestinal tract (Bubenik, 2002) and reproductive organs (Tijmes et al., 1996), where it is thought to act as an intracellular mediator or paracrine signal in addition to its endocrine effects (Stefulj et al., 2001). There is 400-times more melatonin in the gastrointestinal tract than in the pineal, and it is thought to exert a local effect regulating the tone of the gastrointestinal muscles, regeneration of the epithelium and enhancing the immune system of the gut (Bubenik, 2001). The secretion of gastrointestinal melatonin is not related to photoperiod, but the periodicity of food intake. Food intake as well as long-term food deprivation resulted in an increase in tissue and plasma melatonin (Bubenik, 2002). Less is known about the gonadal production of melatonin, but the interstital cells of the rat testes have been shown to have to capacity to synthesise melatonin, which may act to regulate testosterone production (Tijmes et al., 1996).

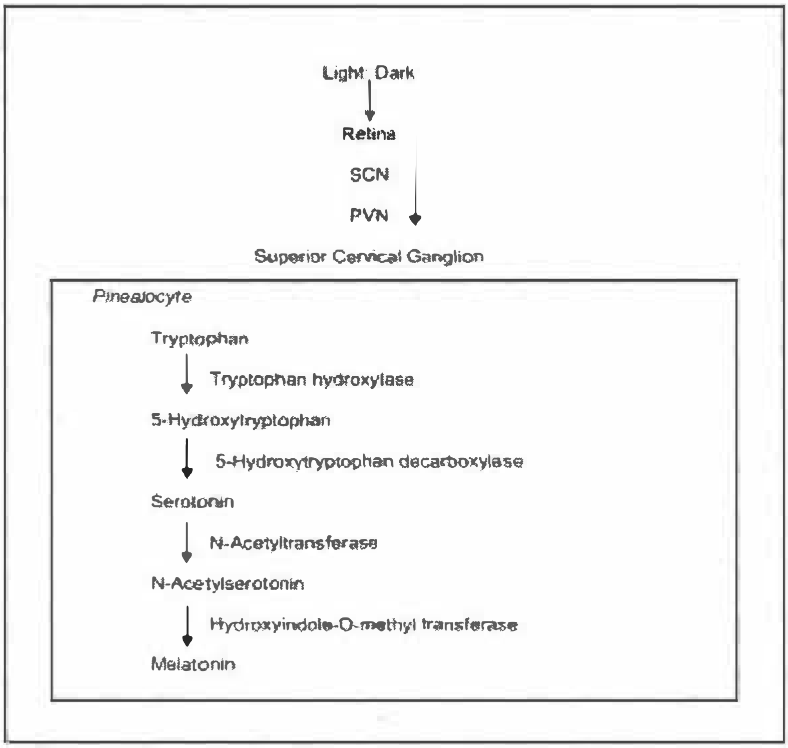

Figure 1.5 The neuropathway between the eye and the pineal gland leading to melatonin secretion. Adapted from: Yu et al. (1993).

The effect of melatonin on seasonal reproduction has been extensively studied, but melatonin's site of action has not been established. Studies on melatonin binding sites have provided evidence that melatonin may act on the tuberal region of the hypothalamus and neurological tissue within the SCN (Kennaway and Rowe, 1995).

1.8.2 Regulation of melatonin secretion

In the absence of light (e.g. blind animals and those kept in total darkness), melatonin concentration maintains a circadian rhythmic pattern. This is referred to as a "free-running" circadian rhythm because without entrainment of the pacemaker by a 24-hour light-dark cycle, it will maintain its intrinsic cycle closer to 25 hours.

In the normal mammal, melatonin production and secretion is precisely synchronised with the alternating periods of light and dark (Reiter and Lerchl, 1993). Melatonin concentration in the plasma is lowest during light, with an increased production and secretion during darkness (Kacsoh, 2000). Exposing mares to approximately 2 hours of artificial light (100 watt bulb in a 12x12 ft stable) beginning at the time of sunset, inhibited the appearance of melatonin in plasma until the light was removed (Sharp et al., 1993). However, artificial light of similar duration and intensity applied prior to sunrise had no effect on melatonin concentrations (Palmer and Guillaume, 1992).

Similarly, melatonin concentrations drop rapidly if animals are exposed to a light pulse during the night (Reiter and Lerchl, 1993). For instance, a 1 second light pulse with an intensity of 32,000 microwatt/cm^2 (in comparison, sunlight has an intensity of 50,000 microwatt/cm^2) during the night can cause melatonin concentrations in the rat to drop to daytime levels within 30 min, after which melatonin concentration increases (Reiter et al., 1986).

In sheep, plasma melatonin concentrations are usually undetectable but at night can reach 300 pg/ml (Rollag and Niswender, 1976). In contrast, pony mares exhibit a daytime melatonin concentration of 20-40 pg/ml, rising to 100-150 pg/ml during the night (Berglund et al., 1981). Night peak melatonin concentrations reach 150 pg/ml in 3-month-old children, and are at a maximum (800 pg/ml) between 1 and 5 years; adult levels are 60-100 pg/ml.

1.8.3 Manipulation of photoperiod and melatonin in the horse

Artificial light is the most common method used by horse breeders to advance the onset of the breeding season. The process takes 6-8 weeks and to be effective in the Southern Hemisphere, breeders begin the artificial lighting program in July to breed in September. An average light intensity of 107 lux (generated by one 100W bulb in the centre of a 12x12 ft stable) is sufficient to cause photo-stimulation (Sharp et al., 1993). In Florida, breeding season was induced by exposing anoestrous mares to two hours of artificial light beginning at the time of sunset for eight weeks (Sharp et al., 1993). A one hour light pulse can also induce breeding season onset but only if the light pulse is timed effectively, at approximately 10 hours after the onset of darkness (Palmer and Guillaume, 1992).

Lighting was manipulated to mimic the transfer of stallions from one hemisphere to the other at the summer solstice of the former hemisphere (Cox et al., 1988). The effect of immediately shortening the daylength caused a dramatic decrease in testosterone concentration to levels comparable to those found in stallions in winter. A second regime was studied to ascertain if maintaining stallions on long days when transported to the Southern Hemisphere in mid-June (Northern Hemisphere summer) would result in maintained testosterone secretion. The normal decline in testosterone concentration in autumn occurred in the horses maintained on long days, indicating that the natural decrease in testosterone concentration at the end of the breeding season is maintained in the absence of reducing daylength.

Circulating melatonin concentration in the mare is inversely related to hypothalamic GnRH concentration (Cleaver et al., 1991) . Melatonin implants in pony mares during summer caused decreased hypothalamic GnRH concentration (Strauss et al., 1979). Mares maintained in constant light for 30 days (in comparison to control mares that were kept in 12 hours light and 12 hours dark) showed higher plasma LH and hypothalamic GnRH concentration and lower melatonin concentration (Cleaver et al., 1991). A circadian secretion of melatonin in the horse has been suggested (Kilmer et al., 1982) but a consistent nocturnal rise in melatonin concentrations was not evident in Quarter Horse mares (Diekman et al., 2002). Serum melatonin concentrations were higher in spring than autumn (Sharp et al., 1980; Diekman et al., 2002). In autumn, increased secretion of melatonin is related to the onset of the breeding season in sheep, whereas in horses decreased secretion of melatonin in spring is associated with the onset of the breeding season. Therefore, there could be a practical need for a melatonin-secretion and/or receptor-binding antagonist to advance the onset of the breeding season in horses (Sharp and Cleaver, 1993).

Administering oral melatonin to horses in the evening prevents stimulation of reproductive function (Guillaume and Palmer, 1991). Stallions (kept in 16 hours light and 8 hours dark) given melatonin daily for 9 days, showed a 50% decrease in testosterone concentration suggesting that melatonin may play a role in the termination of seasonal reproductive activity in the horse (Argo et al., 1991).

Removal of the pineal gland in the horse, or severing the pathway by which light cues reach the pineal (superior cervical ganglionectomy) did not interfere with breeding season onset during the first year post-surgery (Sharp et al., 1993). However, the onset of the breeding season was delayed the following year suggesting that when the gland is removed, entrainment to the environmental cues does not occur and the circannual rhythm becomes free running.

1.8.4 Difficulties associated with photoperiod and equine reproduction

Much of equine reproduction research to date has been undertaken in temperate areas with definite seasonal changes in daylength. There is little information on equine seasonal breeding in countries of extreme latitude, which have poorly defined seasons.

Photoperiod at the equator is always 12 hours long, whereas at higher latitudes there are more extreme variations in daylength. At the poles, photoperiod fluctuates between 24 hours in summer and 0 hours in winter. Previous research on birds including the Song thrush, Prothonotary warbler and California white-crowned sparrow, found that latitude dictates the start of the breeding season (Whitfield et al., 1981). Seasonal breeding species in temperate zones (60-70°N/S) have clearly defined breeding seasons whereas closer to the equator (where daylength is relatively constant), species show less synchrony in their breeding (Hafez, 1952; Whitfield et al., 1981). See Figure 1.6.

The duration of light exposure required to induce cycling in the mare is thought to be at least 14.5 hours of light per day (Sharp et al., 1993). However, mares in the tropics (30°S-30°N) establish reproductive activity without ever experiencing more than 14 hours of daylight. Although the natural breeding season of mares in Mexico (15°-22°N) (Saltiel et al., 1982), California (38°N) (Hughes et al., 1972) Wisconsin (43°N) (Ginther., 1974), and England (51°N) (Arthur, 1958) began at a similar time (April to May), the daylength at the start of the breeding season was 12, 13, 14, and 16 hours respectively. Therefore, other environmental factors such as light intensity, nutrition, local climatic conditions and temperature may have an influence on breeding season onset. The change of daylength at the winter solstice may also provide a signal for beginning the reproductive season.

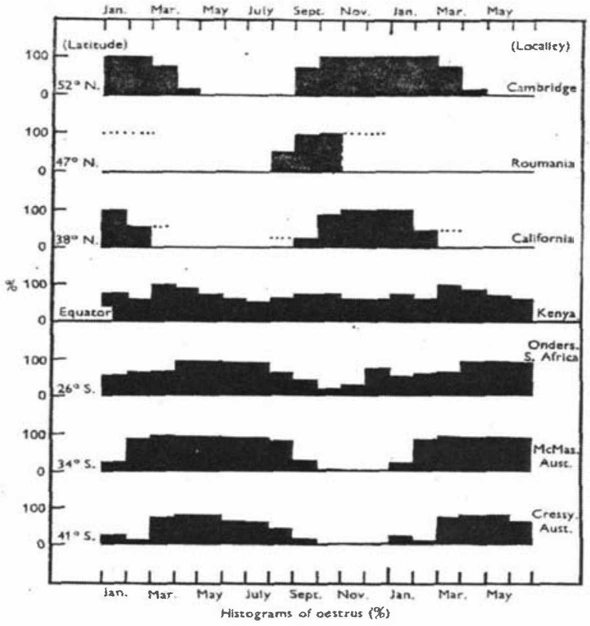

Figure 1.6 Timing and duration of breeding seasons in female sheep at different latitudes. Source: Hafez (1952).

Typically sunrise and sunset are used as reference points in defining daylength, irrespective of the partial illumination beginning in the morning before sunrise (dawn twilight) and in the evening after sunset (dusk twilight). Astronomical twilight ends when the sun is 18° below the horizon, which marks the onset of night (Cotton, 1947). At the equator, the sun descends vertically and twilight duration is 1 hour and 12 minutes. Other than at the equator, the sun sets below the horizon at an oblique angle resulting in longer twilight, with twilight in high latitudes lasting all night in summer. The effect of twilight on equine reproductive activity has not been examined, however as it is an illumination >100 lux for at least part of the time, it may have an effect on seasonal breeding.

1.9 Conclusions from literature review

Reproductive maturation has been intensively studied in production animals, and mechanisms controlling seasonal breeding and onset of puberty are well understood. Study on puberty in the horse has been limited. Horses generally do not foal until at least 2 years after they have reached puberty, because they must first prove performance ability and breeding worth. Horse breeders have therefore not needed to understand the process of pubertal development and the topic has remained relatively uninvestigated.

As horses are seasonal breeders (Ginther, 1992), information on puberty in other seasonal breeding animals (such as sheep and deer) is relevant to forming an understanding of the possible mechanisms for its onset in horses (Squires, 1993). The horse produces low frequency LH pulses from an early age, which are probably maintained before puberty by the GnRH pulse generator, as suggested by Foster et al. (1986) in the sheep. Increased activity of the GnRH pulse generator results in high frequency LH pulses, but the trigger for increased the activity has not been defined.

Growth and nutrition-related cues affect the activity of the GnRH pulse generator in seasonal breeding animals (Foster et al., 1986). Growth-retarded female lambs do not express the high-frequency LH pulses required for the development of pre-ovulatory follicles. Therefore, growth-related cues such as body weight and metabolic index of growth (e.g. leptin or IGF-1) apparently affect puberty onset. However, a critical weight for initiation of puberty does not exist in the lamb (Foster, 1981) and has not been determined in other seasonal breeding species.

For seasonal breeders to reach puberty, both body weight and photoperiod cues need to be permissive and co-ordinated (Foster et al., 1985; Foster et al., 1986; Suttie et al., 1992; Adam and Robinson, 1994). Without this, activity of the GnRH pulse generator does not increase, the slow LH pulse frequency (characteristic of reproductive immaturity) is maintained, and production of gonadal steroids remains low, at least in sheep (Foster et al., 1985) and red deer (Adam et al., 1996). Sheep reach puberty in the autumn regardless of season of birth, and winter-born lambs are thus older at puberty than spring-born lambs (Foster et al., 1985). Puberty in red deer occurs in the autumn, and is more dependent on photoperiod and body weight than age. When photoperiod cues are first permissive for reproductive activity (first autumn of life), the animal has not yet reached a weight that will support puberty and remains in a sexually immature state. By the time deer reach a threshold weight for puberty (spring), the daylength is inhibitory for pubertal onset therefore the animal must wait until the following autumn (Adam et al., 1996). These findings suggest that in the sheep and deer at least, long daylength maintains gonadotrophin secretion at a low level preventing puberty from occurring.

The extent to which age, body weight and photoperiod influence the onset of puberty in horses has not been documented in the literature. Age at puberty in fillies was 10 to 24 months (Wesson and Ginther, 1981), 12 months (Adams and Bosu, 1988; Camillo et al., 2002), 14 months (Nogueira et al., 1997), 15 months (Palmer and Draincourt, 1983), 15 to 25 months (Souza et al., 1997) and 21 to 24 months (Naden et al., 1990b). Puberty in Welsh Pony colts was 12 to 15 months (Skinner and Bowen, 1968) and 14 to 24 months in Quarter Horse colts (Naden et al., 1990c). This difference in the age at puberty in fillies and colts may be due to differences in breed, management systems and nutritional factors, but could be due to how puberty was defined.

There is limited literature on body weight and nutritional factors relating to puberty in horses. Spring-born ponies reached puberty in spring at 56% of mature body weight (Wesson and Ginther, 1981), whereas puberty in Thoroughbred fillies occurred in the spring at 61% of mature body weight (Nogueira et al., 1997) and at 59% of mature body weight in 8 of 20 Brazilian Thoroughbred fillies (Souza et al., 1997). However, puberty in the 12 remaining Brazilian Thoroughbreds fillies occurred at 71% of mature weight, in the next season, approximately 10 months after pubertal onset in the initial 8 fillies. These data show clear differences between the studies, which were all carried out in grain-supplemented horses. There is very little knowledge on the growth and pubertal development of pasture-raised horses.

There is also inconsistent and little research on the effect of season of birth on the onset of puberty in horses. Palmer and Draincourt (1983) reported that summer-born fillies did not attain puberty in the following spring whereas, Wesson and Ginther (1981) claimed that season of birth did not affect the date of puberty onset (spring and summer-born female ponies reached puberty at the same date and body weight but at different ages). There has been no research on puberty onset in Thoroughbreds born out of season in the autumn, which may be of importance to the New Zealand Thoroughbred industry.

The only consistency in the literature pertaining to puberty onset in horses is that it occurs in spring (Wesson and Ginther, 1981; Palmer and Draincourt, 1983; Naden et al., 1990b; Naden et al., 1990c; Souza et al., 1997; Nogueira et al., 1997). This aspect of reproductive function may be influenced by the interaction between photoperiod and maturity (of which body weight and age are factors), but it is unknown if these signals are equal or hierarchical in their influence. It is also unclear if there is a particular "trigger" for the onset of puberty or if it is a gradual transition.

1.9.1 Objectives of the current study

The review of relevant literature in the area of growth and reproductive development in the horse has pointed to an absence of a substantial, unified, consistent body of knowledge on the subject.

The objectives of the project described in this thesis, comparing the growth and reproductive development of pasture-raised Thoroughbreds born in spring and autumn, were; (i) to test the hypotheses by conducting suitable designed experiments; (ii) to present the practical relevance of the findings to the New Zealand Thoroughbred industry; and (iii) to make recommendations on further work which could potentiate or maximise the benefits of the research line to the equine industry.

The hypotheses to be tested are:

1. That there are statistically significant differences between growth rates of healthy horses in the same environment.

2. That foal birth weight has a significantly positive effect on postnatal growth.

3. That there are significant differences in growth rates between colts and castrates from 6-18 months of age.

4. That there is no significant difference in body weight at the same age between spring and autumn-born foals.

5. That puberty occurs at the same time of year in autumn-born foals and spring-born foals.

6. That autumn-born and spring-born colts show a similar pattern of LH secretion (baseline and in response to GnRH challenge) at equivalent times of the year.

The following experiments were undertaken to test the hypotheses:

1. Body weight was measured at birth and fortnightly intervals in 47 growing Thoroughbred horses born in spring 1999 and 2000 up to 17 months of age. The effects of sex, season, weaning and castration (in 10 of the colts) on body weight and average daily gain were examined. (Chapter 2). 3

2. The effect of season of birth on aspects of growth was examined in the same experimental horses as in Chapter 2, as well as from a further 16 horses born in either spring 2001 or autumn 2002. (Chapter 3). 4

3. Puberty onset was studied in 23 colts and 41 fillies born in spring 1999, 2000, 2001 and autumn 2002. Horses were weighed and blood sampled fortnightly between birth and 17 months of age, and testosterone and progesterone assays conducted (Chapter 4). 5

4. The LH and testosterone response to 5 doses of a GnRH analogue (buserelin) was determined in 8 colts born in 1999 and 5 colts born in 2000 (Chapter 5), as pilot

work to determine an appropriate dose of buserelin and sampling regimen for use in the experiment described in Chapter 6.

5. GnRH challenges were conducted at 8-week intervals between December 2001 and December 2002 to determine LH and testosterone response in 6 colts born in spring 2001 and 5 colts born in autumn 2002. Season and age effects on both hormones were analysed to assess the change in function of the pituitary-gonadal axis of colts born in autumn and spring (Chapter 6).

Chapter 2

Aspects of Growth in pasture-raised Thoroughbred horses between birth and 16 months of age[1]

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[1] Submitted as C. G. Brown Douglas, E.C. Firth, T.J. Parkinson, and P.FF. Fennessy (2003) *Equine Veterinary Journal.*

2.1 Abstract

Growth data were obtained from 47 pasture-raised Thoroughbred horses between birth and approximately 16 months of age. Growth rate in the first 5 months of age was similar in all horses, whereas post-weaning growth rate was a function of seasonal factors. All horses, regardless of age showed equivalent decreases in average daily gain (kg/d) between May and August and increases in the spring (September-October). The effect of castration pre and post-puberty was also observed in 10 colts. Pre-pubertal castration did not have a significant effect on growth. Post-pubertal castration resulted in an immediate reduction in body weight after surgery, but a greater average daily gain, and cumulative weight gain in the two to three months after surgery. Although body weights were slightly higher than those reported for grain-fed horses in Northern Hemisphere studies, the horses in this study exhibited no clinical symptoms of developmental orthopaedic disease suggesting that horses raised in a pasture system can achieve and tolerate high growth rates without compromising the development of the musculo-skeletal system.

2.2 Introduction

Information on the growth of young Thoroughbred horses has primarily been collected from animals raised under commercial stud farm conditions and fed supplementary grain (Green, 1969; MacCarthy and Mitchell, 1974; Hintz et al., 1979; Thompson, 1995; Jelan et al., 1996; Pagan et al., 1996) in the Northern Hemisphere. Much less information is available on growth of Thoroughbred horses raised exclusively on pasture (Grace et al., 1999; Grace et al., 2002).

Since the horse is not primarily a meat-producing animal the study of its growth has not been extensive. The higher growth rates required in meat-producing systems (Purchas et al., 1989) are not necessarily desirable in horses, as rapid growth in the immature horse has been associated with high weight gain and compromised skeletal growth, predisposing the animal to developmental orthopaedic disease (DOD) (Lewis, 1980; Thompson et al., 1988; Savage et al., 1993; Pagan et al., 1998).

In general, the growth pattern of the young horse can be described as exhibiting two phases of postnatal growth. Firstly, there is the early phase in the first 5-6 months of life when the foal is with its mother. Average daily gain (ADG) during this stage starts at around 1.5 kg/d in the first few weeks after birth and falls to about 0.7 kg/d. Thereafter over the next 12 months, growth rates of about 0.5 kg/d are generally observed. This pattern of initial rapid growth followed by a decrease in growth rate is consistent across studies (Hintz et al., 1979; Thompson, 1995; Pagan et al., 1996; Jelan et al., 1996; Grace et al., 1999; Grace et al., 2002). Growth rate is also influenced by factors such as sex, birth month, and management factors such as weaning, level of nutrition and castration.

Colts are generally heavier than fillies at equivalent ages. Mean body weight of Kentucky colts and fillies at 2 weeks of age was 83.5 kg and 81.5 kg respectively, and between 38 and 46 weeks of age fillies weighed 9 - 19 kg less than colts (Thompson, 1995). These figures were slightly higher than those reported for 700 Kentucky Thoroughbreds born between 1993 and 1995 (Pagan et al., 1996), in which the mean body weights of 2 week old colts and fillies were 77.7 kg and 76.1 kg respectively. The 1.6 kg difference between colts and fillies increased to 8.5 kg by 38 weeks of age, when weights and heights were similar to those of the Thoroughbreds studied by Thompson (1995).

There has been little direct study of the growth of horses at pasture. Australian colts raised on pasture had a greater ADG than fillies between 7 and 14 months of age (0.77 kg/d vs. 0.73 kg/d) (Nash et al., 2001). New Zealand horses raised on pasture had a weight gain of 1.86 kg/d between 1 and 7 days of age and 1.31 kg/d between 8 and 28 days, and an ADG of 1.14 kg/d from birth to weaning (5 months) (Grace et al., 1999). ADG between 10 and 17 months of age of 14 pasture raised New Zealand yearlings averaged 0.6 kg/d (Grace et al., 2002).

The time of year at which foals are born influences birth weight and the subsequent growth curve of the horse. Earlier born horses are generally, but not always, lighter than those born later in the foaling season. At 3 weeks of age, Canadian Thoroughbred foals born in April, May or June were 2.4-5.6 kg heavier than foals born in January, February, or March and remained heavier until the study ended when the horses were 73 weeks old (Hintz et al., 1979). At 2 weeks of age, Kentucky foals born in January/February were on average 6.8kg lighter than March-born foals, and remained lighter until 9 months, at which time mean body weight was the same as that of the March-born foals (Pagan et al., 1996). However, Irish foals born in January/February were significantly heavier than those born in March to May (MacCarthy and Mitchell, 1974).

Nonetheless, month of birth did not affect the rate of growth in Kentucky horses during the first months of life (Pagan et al., 1996). Average daily gain for these Kentucky foals was 1.5-1.7 kg/d during the first month and declined to 0.7-0.8 kg/d at 7 months. Regardless of birth month, growth rates of all groups were less during winter, and increased during April and May the following year. Growth rate in the yearlings was more related to season of year than to age, with growth patterns following changes in temperature and pasture growth.

Castration is a management practice which affects growth and body composition of males because it involves the complete removal of the testes and, hence, the major source of the testicular androgens, testosterone, dihydrotestosterone and androstenedione. Little work has been done on the effect of pre- or post-pubertal castration in the horse, but Heusner et al. (1997) reported that pre-pubertal castration of colts between two days and two months of age resulted in no significant differences in body weight between early-castrated colts and entire colts up to 12 months of age. However, castrated male cattle, sheep, deer and pigs showed decreased rate of growth, poorer feed conversion efficiency, and a greater deposition of carcass fat compared with entire males (Price and Yeates, 1969; Drew et al., 1978; Knudson et al., 1985; Arnold and Meyer, 1988; Knight et al., 1999). Entire males experience an androgenic effect resulting in a higher growth rate than castrates (Gazzola et al., 2002).

cattle

The purpose of this study was to obtain growth data from a group of pasture-raised thoroughbred horses, for reference purposes since the New Zealand production system is pasture-based. This paper reports a retrospective examination of growth records from birth to approximately 16 months of a group of pasture-raised Thoroughbred horses grown under typical New Zealand conditions.

2.3 Materials and methods

2.3.1 Animals

The study consisted of 47 Thoroughbred horses (17 colts and 30 fillies) from two consecutive foal crops (1999 - 2000). The average date of birth for the two seasons foals were 7 November 1999 (7 colts, 9 fillies) and 28 October 2000 (10 colts, 21 fillies). The 10 colts born in 2000 were castrated in either August of or December 2001 (see below). The horses were bred and reared at Flock House Thoroughbred Stud, Bulls, New Zealand, close to the 40th parallel. Mares and foals were raised on tall fescue dominant pasture, and approximately 1 kg meadow-hay per horse was offered daily during the winter months of May to July. Weaning of the 1999-born horses was by date and occurred between 7 and 14 May 2000, whereas weaning of the 2000-born horses was by age and occurred at 133 ± 9.5 days of age. Horses were grazed on two-hectare paddocks in mobs of about 8-9 horses, paddocks were changed weekly or more frequently when required as assessed by the stud manager.

2.3.2 Weighing and blood sampling

Horses were observed daily for general health and more closely during monthly clinical examination. All foals were weighed at birth and thereafter at fortnightly intervals, at a similar time of day, using electronic scales (Tru-test 703, Tru-test, Auckland, New Zealand). The scales were checked with a known weight at each fortnight before weighing. Horses were also blood-sampled fortnightly from birth until approximately 16 months for endocrinology study elsewhere (See Chapter 3). Blood samples were collected by jugular venepuncture using evacuated collection tubes (10ml; lithium-heparin anticoagulant). After collection, samples were placed on ice, and transported to the laboratory where plasma was separated by centrifugation at 1000g/10 min within 2 hours of collection and stored at -20°C for later assay.

2.3.4 Castration

The ten colts born during spring (September-November) 2000 were allocated into two groups that were castrated either before (early-castration; n=5) or after puberty (late-castration; n=5). The 5 early-castrated horses (mean birth date 2 October 2000) were surgically castrated on 30 August 2001, whilst the 5 late-castrated horses (mean birth date 14 November 2000) were castrated on 6 December 2001. Castration was performed using a conventional field, surgical, semi-closed castration method. The early-castrated group was recumbent under general anaesthesia, and the late-castrated group was castrated standing using local analgesia and a combination of chemical and physical restraint (n=4) or physical restraint only (n=1).

2.3.5 Hormone assay and puberty status

Testosterone data from the colts born in 1999 were used to define the time of onset of puberty. It was expected that the 2000-born colts would exhibit similar testosterone concentration profiles as the 1999-born colts. Date of castration was therefore decided upon based on testosterone data and time of puberty in the 1999-born colts. Colts whose testosterone concentrations were ≤0.1 ng/ml were considered pre-pubertal, whereas those animals with ≥0.25 ng/ml were considered pubertal. Testosterone was measured using a coated-tube RIA kit assay (Testo-ctk, P3093, DiaSorin, Italy), carried out in accordance to the manufacturer's instructions. The minimum detectable level of testosterone was 0.05 ng/ml. The inter- and intra-assay coefficients were 13.0% and 13.2% respectively. Assays were performed in December 2000 and 2001 when the two crops of colts were yearlings. Pubertal behaviour was noted at the time of blood sampling but this was not carried out systematically due to labour and time constraints.

2.3.6 Statistical analysis

ADG was calculated by the formula (body weight – previous recorded body weight)/(date – previous date that the body weight was recorded). Body weight and ADG data for the two seasons were initially analysed separately, but as there were no statistically significant differences for any parameter between the two years ($p > 0.05$), data were thereafter combined. Data for weight and ADG of all horses were subjected to generalised linear model analysis, with respect to sex and linear and quadratic effects of age. Birth weight was treated as a covariate. Differences of regression lines between groups were quantified using analysis of variance.

Weight data from the 10 castrated colts were also analysed by generalised linear modelling, with respect to group (early or late-castration), age and calendar month, in a repeated measures analysis. Data for growth were also subjected to analysis of variance with respect to the same factors, but using age as a covariant. Differences in regression lines between the two groups were quantified using analysis of variance. Differences in the growth rate for three time periods (98 days before the early group was castrated, 98 days between the early and late castrations, and 98 days after the late group was castrated), were tested using an analysis of variance.

Results are expressed as mean ± SEM and differences were considered significant at $p<0.05$.

2.4 Results

2.4.1 Animals

All horses remained healthy throughout the study. No abnormalities in growth were noted, and no foals exhibited clinical signs of lameness or ill health. None of the castrated colts showed signs of ill health after castration. Post-operative care was provided by the stud manager who ensured that the surgical site was kept clean and that the horses were walked daily in the weeks after surgery.

2.4.2 Hormone data/puberty

Prior to castration on 30 August, testosterone concentrations in the early-castrated group averaged 0.06 ng/ml. Testosterone concentrations in the late-castrated group increased from 0.05 ng/ml in the winter months (April-August) to 0.2 ± 0.05 ng/ml in late September (an increase of 0.15 ng/ml above baseline) and thereafter remained elevated until castration on 6 December. In both groups, concentrations underwent a precipitous decline after castration, as expected (Figure 2.1). The late-castrated colts were also observed to be exhibiting pubertal behaviour in the weeks before castration, including vocalisation to mares, penile erection and attempts to mount.

After castration the colts did not display any signs of male sexual behaviour that were observed before surgery.

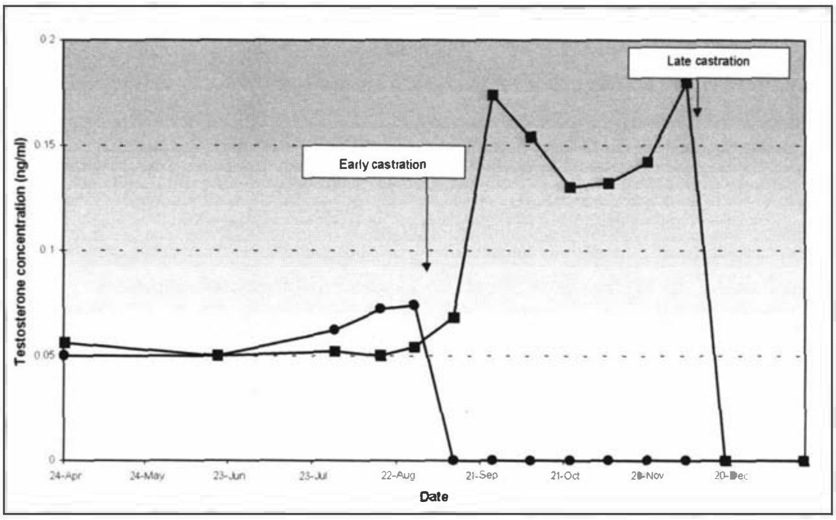

Figure 2.1 Mean concentration of plasma testosterone and time of castration in of Thoroughbred colts castrated before puberty (●) or after puberty (■).

2.4.3 Statistical analysis of body weight and ADG

Birth weight had a significant ongoing effect on body weight ($P<0.01$) but did not affect ADG ($p=0.80$). For body weight, but not for ADG, there was a significant ($p<0.001$) main effect of sex, whilst both body weight and ADG exhibited significant (both $p<0.001$) quadratic relationships with age. Furthermore, the changes of both ADG and body weight that occurred throughout the (calendar) year were reflected by a significant effect of the random variable, calendar month ($p<0.001$). Summary data from the analysis of variance of body weight and ADG of all horses is shown in Table 2.1. The goodness of fit of the predicted body weight and ADG derived from the regression coefficients generated during the analysis (Table 2.1) were examined by correlating actual and predicted data. Thus, using the example of October-born colts, predicted and actual body weights were highly correlated ($r^2=0.999$; $p<0.001$), as was the predicted and actual ADG ($r^2=0.953$; $p<0.001$).

Statistical analysis of the castrated colts' body weight data revealed significant age and date and time of castration effects ($p<0.001$). Body weight increase over time was significantly ($p<0.001$) related to the time of castration when allowance had been made for the effect of age as a covariate ($p<0.05$). Summary data from the analysis of variance in weight between the two groups is shown in Table 2.2.

Table 2.1 Summary of ANOVA for body weight (kg) and average daily gain (kg/d) of pasture-raised fillies and colts. The model for body weight and ADG of fillies and colts were based on the coefficients of the ANOVA. All regression equations, whether linear or quadratic were significantly different from zero ($p<0.05$). * = random effect, varying month by month.

Factor Body weight	F value (complete model)	p	coefficient
birth weight	402.16	0.001	1.1847
sex	45.93	0.001	colts –12.97 fillies –21.50
age (linear)	30900.74	0.001	1.0478
age (quadratic)	366.76	0.001	-6.88×10^{-4}
month	8.97	0.001	*

Factor Average daily gain	F value (complete model)	p	coefficient
birth weight	0.33	n/s	1.53×10^{-3}
sex	0.67	n/s	0.012
age (linear)	581.93	0.001	-5.75×10^{-3}
age (quadratic)	175.16	0.001	7.07×10^{-6}
month	11.36	0.001	*

Table 2.2 Summary of ANOVA for body weight of two groups of Thoroughbred colts castrated either before the onset of puberty (early castration) or after puberty onset (late castration). All linear regression equations were significantly different from zero ($p<0.001$). * = Random effect, varying for each date.

Factor	F Value (complete model)	p	coefficient
Castration group	31.3	0.001	Early –87.3 Late –45
+ date of weighing	44.68	0.001	*
+ age	144.9	0.001	1.603

2.4.4 Body weight and ADG

There were no significant effects of sex upon foals' birth weights ($p>0.05$), although there were trends for fillies to be lighter than colts (57.21 ± 1.10 vs 57.44 ± 1.40 kg). Overall mean body weight data for colts and fillies are illustrated in Figure 2.2.

Between birth and 4 months of age, overall mean ADG in all horses declined from 1.5 kg/day to 1.0 kg/d. Thereafter, from 5 months of age until the completion of the study, ADG varied between 0.5 and 0.7 kg/day (Figure 2.3). The effect of age and month can be seen in Figures 2.4 and 2.5, in which the ADG of animals born in September, October, November and December are illustrated separately. Figure 2.4 shows that ADG was similar in the four groups during the first three months of life, but thereafter effects of calendar month complicated the effect of age. Figure 2.5 shows that ADG was low between the months of May and August reaching a nadir in late July. Thereafter, ADG increased somewhat during September and October, before again decreasing. Therefore, the decrease in ADG that occurred in September-born foals at 11 months of age, and the decrease in ADG that occurred in December-born foals at seven months of age both correspond to the July-August period when average daily gains were low (Figure 2.5).

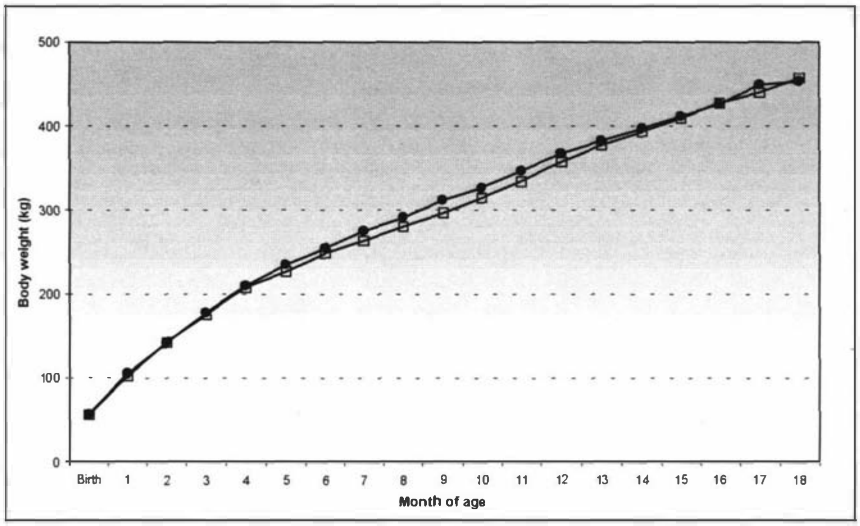

Figure 2.2 Mean body weight (kg) of pasture-raised Thoroughbred colts (●) and fillies (□).

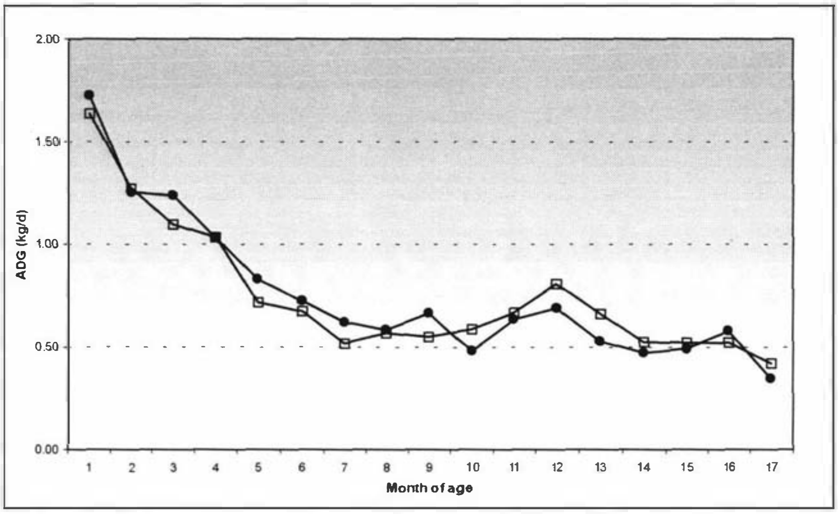

Figure 2.3 Mean ADG (kg/d) of pasture-raised Thoroughbred colts (●) and fillies (□).

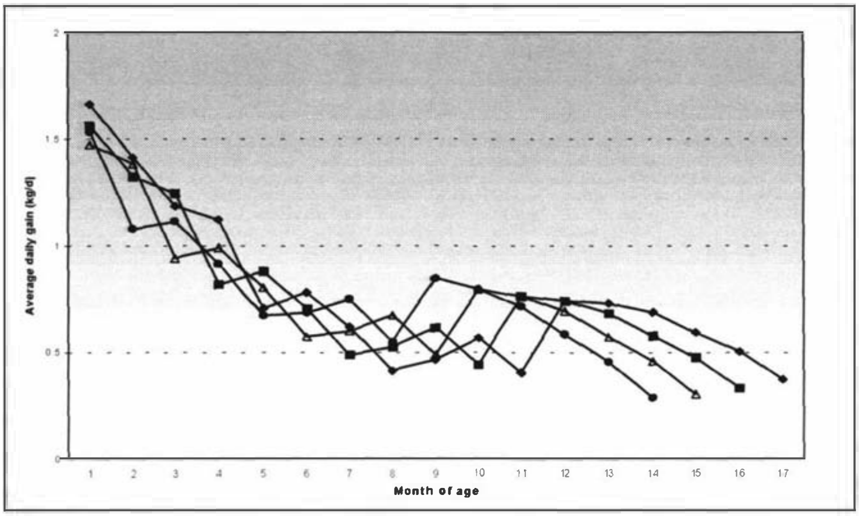

Figure 2.4 The effect of age on ADG (kg/d) in four groups of pasture-raised foals born in September (♦), October (■), November (Δ), and December (●).

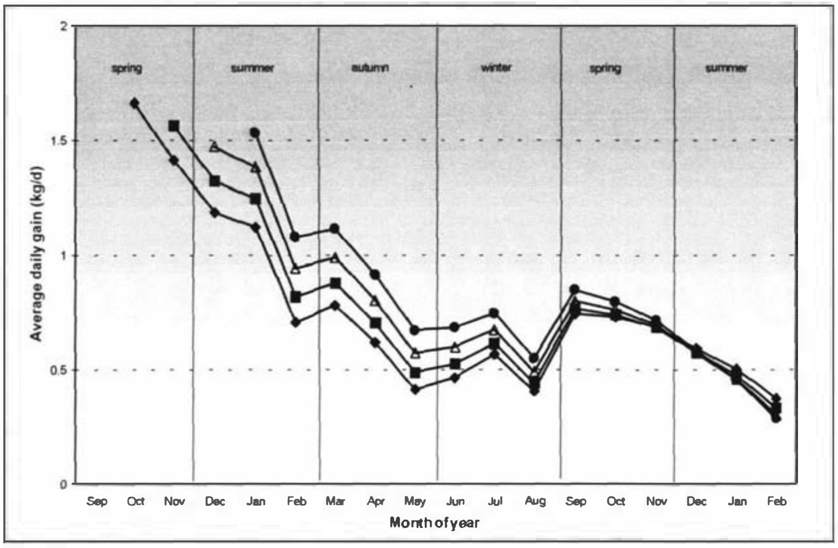

Figure 2.5 The effect of season on ADG (kg/d) in four groups of foals born in September (♦), October (■), November (Δ), and December (●).

2.4.5 Castration

Colts castrated at 330 days (pre-puberty) weighed on average 334 kg at castration and two weeks after castration weighed 339 kg. Colts castrated at 385 days (after puberty onset) weighed on average 384 kg at castration and lost weight in the two weeks post-surgery to weigh 380 kg. By 428 days of age there was only 2 kg difference in mean body weight between the two groups. Body weight curves in relation to date and age of the two groups are shown in Figures 2.6 and 2.7.

There was no significant difference in ADG between the two groups of colts until 98 days after the late group was castrated, when the ADG of the late-castrated group was significantly higher than the early-castrated group (0.47 ± 0.05 kg/d vs. 0.62 ± 0.02 kg/d) (Table 2.3).

Cumulative weight from May (3 months before castration of the early group) did not differ significantly between the early and late castrated colts until 2 weeks before, and 8 weeks after the late group was castrated, when late-castrated horses had gained significantly more weight than early castrated horses (116.5 kg vs. 98.7kg and 155.1 kg vs. 140.3 kg: $p<0.001$). Cumulative weight from May prior to castration for the two groups of colts is shown in Figure 2.8.

Table 2.3 Average daily gain ± SEM (kg/d) of two groups of male Thoroughbred horses over three 98 day time periods. The early-castrated group was castrated on 30/31 August 2001 and the late-castrated group was castrated on 6 December 2001. (* Indicates a significant difference ($p<0.05$) in ADG between the two groups of colts at that particular time point).

	24 May 2001 – 30 August 2001	**30 August 2001 – 6 December 2001**	**6 December 2001 – 14 March 2002**
Early castrated (n=5)	0.50 ± 0.02	0.64 ± 0.04	0.47 ± 0.05*
Late castrated (n=5)	0.57 ± 0.04	0.71 ± 0.03	0.62 ± 0.02*
SED		0.052	

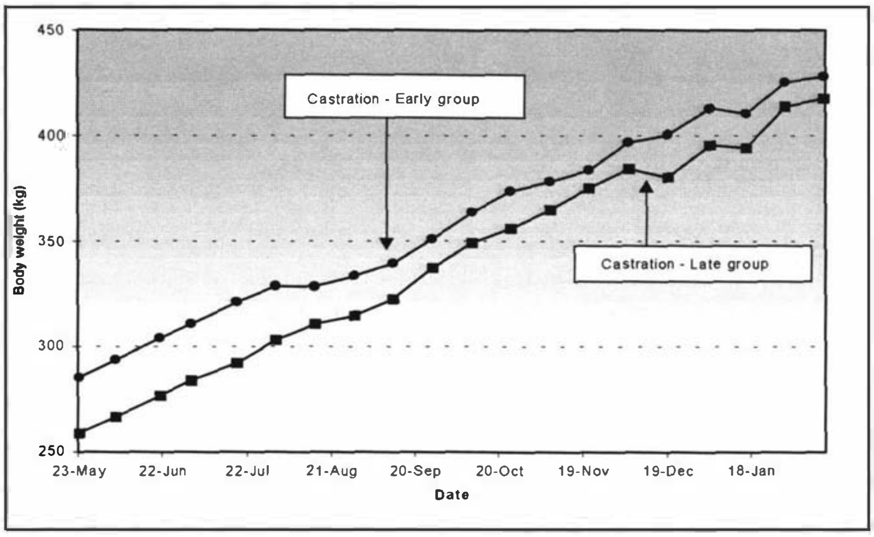

Figure 2.6 Mean body weight (kg) of two groups of Thoroughbred colts castrated either before the onset of puberty (early castration ●) or after puberty onset (late castration ■). On 30 August, early and late-castrated colts weighed 333.5 ± 14.8 kg and 314.7 ± 11.8 kg respectively, and on 6 December, they weighed 396.6 ± 15.0 kg and 384 ± 14.3 kg. At the time of castration early-castrated colts averaged 54 days younger and 50kg lighter than late-castrated colts.

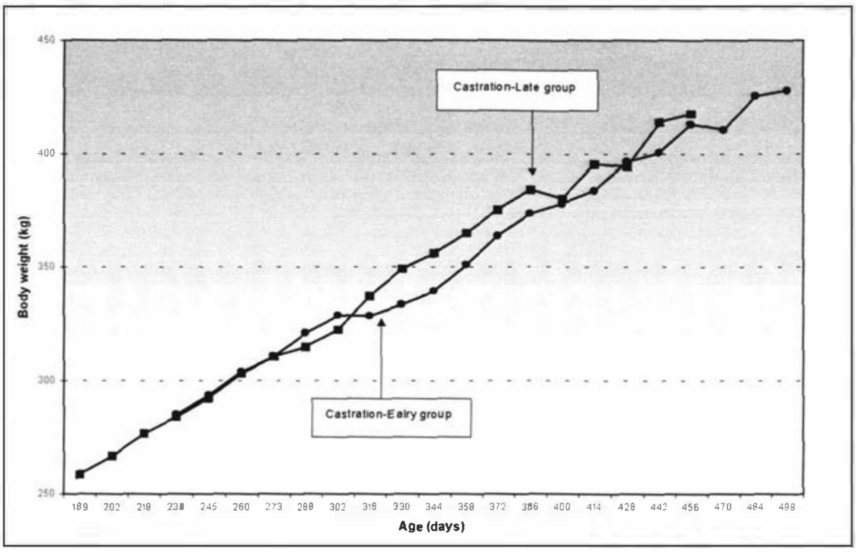

Figure 2.7 Mean body weight (kg) of two groups of Thoroughbred colts castrated either before the onset of puberty (early castration ●) or after puberty onset (late castration ■) in relation to age.

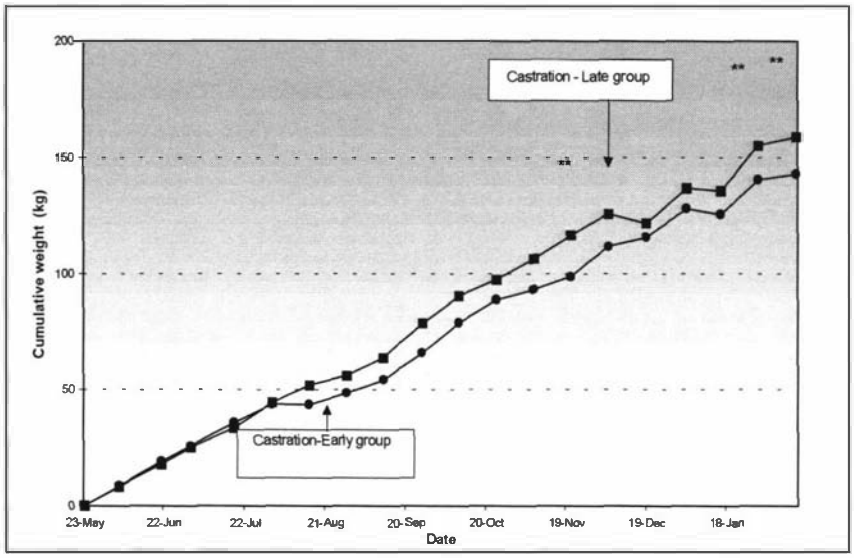

Figure 2.8 Cumulative weight change (kg) from 23 May of two groups of colts castrated either before the onset of puberty (early castration ●) or after puberty onset (late castration ■). ** Indicates a significant ($p<0.001$) difference between the two groups.

2.5 Discussion

Body weight and ADG of horses in this study were similar to those previously recorded from the same stud (Grace et al., 1999), but slightly less than reported for animals reared at pasture in Australia (Nash et al., 2001). The Australian experiment used a lower stocking rate (1.25 hectares/horse) than the present experiment (average of 4.5 horses/hectare) which may have allowed for greater intake in the Australian horses and, hence, greater ADG. Differences in pasture type and pasture quality may also have been a factor. Surprisingly, body weights in the present study were slightly higher than have been reported for animals that received supplementary grain and/or concentrates during rearing in Canada (Hintz et al., 1979), Ireland (Thompson, 1995), and Kentucky (Pagan et al., 1996; Jelan et al., 1996) . Body weight of foals in the present study compared with data from other growth studies is shown in Table 2.4.

Table 2.4 Comparison of body weight (kg) data from four Northern Hemisphere growth studies and the present study. Note: Hintz data collected on days 32, 62, 187, and 352, Thompson data on days 28, 56, 182 and 364, and Pagan data on days 183 and 350.

Month of age	**1**		**2**		**6**		**12**	
	Colts	**Fillies**	**Colts**	**Fillies**	**Colts**	**Fillies**	**Colts**	**Fillies**
Present study	105.8	102.4	142.2	142.4	254.4	248.6	361.1	357.3
(Hintz et al., 1979)	98	97	137	135	245	236	345	329
(Thompson, 1995)	98.2	100.5	132.9	134.2	260.5	256.4	364.5	363.3
(Jelan et al., 1996)	-	-	131.5	-	240.5	-	340.0	-
(Pagan et al., 1996)	-	-	-	-	255.9	250.7	349.2	335.2

Colts and fillies born between September and December had similar ADG over the first six months of their life. In this six-month period, ADG declined at a constant rate, from maximum values immediately after birth, in all animals. Since body weight, and the rate of decline of ADG, over the first six months of life were similar in all animals, it is probably a reflection of the degree to which their diet is composed of dams' milk. Growth rate of young horses is largely determined by the amount of milk produced by their dams (Frape, 1998), with the peak of lactation occurring at two months postpartum and the greatest intakes of milk in the second and third months after birth (Oftedal et al., 1983).

Thereafter the relationship between age and body weight, although still highly statistically significant, was also affected by other factors that were independent of age. Weaning was likely to have had an influence upon growth rate, since, at least for foals in Kentucky, weaning is associated with a significant check to ADG (reducing from 0.8 to 0.6 kg/day (Warren et al., 1997). In the present study, growth rate in the month prior to weaning at 5 months of age was 0.8 kg/d, after which it decreased to 0.6 kg/d, supporting the view that weaning is associated with a check in growth rates.

Of greater significance to the post-weaning growth rates were effects that were related to calendar month. Pasture growth rates in the district where Flock House Thoroughbred stud is located are typically lowest between May and August (10-30 kg DM/ha/day), and highest in the spring and early summer (50-70 kg DM/ha/day) (Holmes et al., 2002). The decline in ADG observed in December and January may be attributed to the dry summer climate in this area, and decreased pasture quality associated with flowering. Summer rainfall averages 200mm in the area where the stud is located (NIWA, 2003), but severe drought in the summer of 2000/2001 resulted in 45% of normal rainfall, significant soil moisture deficits and decreased pasture growth. However, there were no significant differences in body weight or ADG between the two years, and animals received no supplementary feed.

Never the less, it may not be reasonable to attribute the decline in growth rates through the winter entirely to pasture quality and availability, since many domestic herbivores exhibit a period of relative inappetance and consequent low growth rates during the mid-winter period (Brown et al., 1979; Simpson et al., 1984; Suttie and Kay, 1985; Webster et al., 1997; Webster et al., 1998). It is easy to see the biological advantage to animals of having a physiologically-entrained reduction of demand for feed during periods when herbage is in relatively short supply, yet, since (as least in meat-producing animals) winter inappetance is determined at least partly endogenously, it is also clear that maintenance of growth rates simply by adding more feed into the system is unlikely to be very successful (Webster et al., 2000).

Moreover, a period of slower growth rate may confer advantages upon growing horses. Rapid growth rates have been associated with DOD; DOD is a collective term that includes several diseases in growing horses related to abnormalities in the development and maturation of cartilage and bone. The diseases include physitis, osteochondrosis, osteochondrosis dissecans, and acquired flexural deformities (Hurtig and Pool, 1996). The there are many suggested causal factors of DOD, which may include improper nutrition (deficiencies or imbalances of major and trace minerals, and several vitamins), faulty conformation, muscle imbalance, trauma and excessive body weight (Knight et al., 1985). Contributory factors to DOD include fast growth rate, intensive feeding practices, endocrine disorders, faulty conformation and lack of exercise (Hurtig and Pool, 1996).

DOD is of great significance when one considered the prevalence of such problems in intensively managed foals. For example, Pagan and Jackson (1996) found a 10% prevalence of DOD in Kentucky-born foals, as diagnosed radiographically after a foal displayed lameness or joint effusion. The affected foals tended to be large at birth, grew rapidly from 3-8 months and were heavier than the average population at weaning. Irish foals with DOD were also found to be heavier than non-affected foals between 6-12 months, but this difference was not significant (Jelan et al., 1996). Even so, body weight itself is not the sole determinant of whether such problems will develop, since the foals in the present study were slightly heavier than both the Kentucky and Irish animals and displayed no clinical symptoms of DOD as defined by Pagan and Jackson (1996). The foals in the present study, at least during spring when pasture quality is high, may have been on a diet of equivalent digestible energy to the animals fed hay plus concentrates in Kentucky (11.3 MJ DE/kg DM [Grace et al., 2002] vs. 11.68 – 11.95 MJ DE/kg DM; [Pagan and Jackson, 1996]). On the other hand, the animals in the present study were able to exercise freely for 24 hours a day, whereas the animals in Kentucky were box stalled for 17 hours a day as young foals, and 6-7 hours daily when older than 4 weeks of age (Pagan and Jackson, 1996) whilst Irish yearlings were box stalled for up to 18 hours a day (Jelan et al., 1996).

Exercise was, however not shown to affect prevalence or severity of osteochondrosis in Warmblood horses (VanWeeren and Barneveld, 1999). It is suggested that whilst body weight is a contributory factor to DOD, a period of growth check in winter, plus unrestricted opportunity for exercise, means that higher overall growth rates (i.e. over the entire rearing period) can be tolerated in pasture-raised foals than in animals that are grain fed without prejudice to their final musculo-skeletal development.

Pre-pubertal castration at approximately 10 months of age did not result in any short-term weight-loss immediately post surgery. Post-pubertal castration, in contrast, resulted in short-term weight loss. This immediate decrease in growth rate following castration could be indicative of the stress of surgery, because these findings support those of Cosgrove et al. (1996), who found that post- but not pre-pubertal castration of bulls resulted in short-term weight loss and reduced weight gain. General anaesthesia is known to produce a stress response (Taylor, 1989; Luna et al., 1996) and it would be expected that the stress of general anaesthesia would be greater than in local anaesthesia used in the later castrations. All surgical procedures produce a metabolic/stress response however, in this case the surgery was identical in both groups, but there was a difference in the method of anaesthesia. However, analgesia was administered prior to general anaesthesia in the early group, which is known to reduce the stress associated with surgery under general anaesthesia (Taylor, 1998). The early-castrated colts were castrated under general anaesthesia and the late-castrated colts were castrated under local anaesthesia, therefore the age at surgery seems to have a more important effect on the stress response than the general anaesthetic.

Colts castrated before puberty showed less ADG than colts castrated after puberty. Pre-pubertal castration of cattle and sheep resulted in a lower growth rate compared with entire males (Cosgrove et al., 1996; Knight et al., 1999; Gazzola et al., 2002).

The early-castrated group showed a decrease in cumulative weight gain before castration, between 1 and 15 August, but this was not significantly different to the cumulative weight gain achieved by the late-castrated group at this time. However, it resulted in the cumulative weight gain curve of the early group diverging from, and remaining below, the curve of the late-castrated group until the end of the study (Figure 2.8). The reason for this drop in cumulative weight gain is due to three of the five colts losing between 0.5 and 8 kg of weight between the two weighing periods. This resulted in a mean decrease in weight of 0.2 kg compared with the increase of 7.5 kg in the late castrated group at the same time. This observed decrease in weight was unrelated to castration and the reason for the drop in weight of the three horses is unknown (they were all housed in the same paddock and received the same treatment at this particular time).

Results indicated that pre-pubertal castration had no significant effect on body weight compared with animals castrated after puberty at the same time. However, colts castrated after puberty lost body weight immediately after castration, resulting in convergence of the body weight curves at this time. After castration, late-castrated colts showed significantly greater ADG, resulting in greater cumulative weight gain two months after castration compared with early-castrated colts. Differences in cumulative weight gain between the two groups of colts reached significance only immediately prior to castration of the late group. It is therefore possible that the second group of colts were castrated too soon after the pubertal increase in testosterone concentration to observe any androgenic effect on growth. It is also possible that the horses were not studied for long enough after castration to observe any long term differences in growth between the groups.

In conclusion, horses raised solely on pasture achieve growth rates that are commensurate to those attained elsewhere using diets of forage plus concentrates. Although the data pertain to only one stud, they do provide an important evaluation of, and reference to, the normal growth pattern of pasture-raised Thoroughbreds.

Chapter 3

Aspects of Growth in pasture-raised Thoroughbred horses born in spring and autumn

3.1 Abstract

Growth data from 63 pasture-raised Thoroughbred horses born in either spring or autumn, were collected from birth to approximately 13 and 17 months of age respectively. There was no difference in birth weight or subsequent growth at equivalent ages between spring and autumn-born horses. Spring-born horses were weaned in autumn and had decreased post-weaning ADG compared with autumn-born horses that were weaned in early spring and had increased post-weaning ADG. At the completion of the study, the mean body weight of the two groups was within 52 kg of each other, despite the 4-month age difference. This indicated that the autumn-born horses had ”caught up” with the spring-born horses to achieve similar body weights at time of yearling sale in New Zealand. Autumn-born foals, on the basis of body weight alone, could be marketed for late Southern Hemisphere sales (March-April). Furthermore, autumn-born horses could also be competitive in Northern Hemisphere industry (sales or racing), as they would be competing against horses of the same official age.

3.2 Introduction

The Thoroughbred industry has set regulations so that horses share the same official birth date regardless of actual age of the animal. This official birth date is 1 January and 1 August of the year of birth in Northern and Southern Hemispheres respectively. Thoroughbred breeders therefore aim to have horses born as soon as possible after this date to maximise growth time and body size at the time of sale as yearlings, which is said to influence racing prospects as two or three-year-olds.

This generic birth date requires New Zealand mares to be bred in early September. Mares that foal late in the year are usually not bred and consequently miss the following breeding season to prevent progressively later foaling dates. This results in financial loss to breeders due to the cost of maintaining dry mares until mating the following spring, and loss of income from progeny.

The opportunity for out of season breeding has been considered in many farmed species including sheep (Stritzke and Whiteman, 1982; Adam et al., 1998), deer (Adam et al., 1992), cattle (Montgomery and Davis, 1987; Morrison et al., 1992) and goats (Deveson et al., 1992; Papachristoforou et al., 2000), and is an accepted management practice in many production animals including sheep and cattle, where autumn rather than spring births are often required to meet production targets.

There have been inconsistent reports on the productivity and growth of animals born out of season. Spring-born lambs were heavier at birth and weaning than autumn-born lambs, but autumn-born lambs gained weight more quickly after weaning and reached market weight at a younger age (Blackwell and Henderson, 1955; Gould and Whiteman, 1971). In addition, winter-born lambs were heavier at birth than summer and autumn-born lambs, and had greater average daily gain than summer and autumn-born lambs. Consequently, winter-born lambs reached market weight 30 and 16 days earlier than summer or autumn-born lambs respectively (Stritzke and Whiteman, 1982). Likewise, calves born in autumn were heavier at birth than spring-born calves, but grew 20% slower and had a lower average weaning weight (Montgomery and Davis, 1987).

Information in the literature on the growth of Thoroughbred horses is restricted mainly to those born in spring and summer, and has primarily been collected from animals raised under commercial stud farm conditions and fed supplementary grain (Green, 1969; MacCarthy and Mitchell, 1974; Hintz et al., 1979; Thompson, 1995; Jelan et al., 1996; Pagan et al., 1996). Much less information is available on growth of Thoroughbred horses raised exclusively on pasture (Grace et al., 1999; Nash et al., 2001; Grace et al., 2002) and there are limited data about the growth of foals born in autumn.

Horses born early in the breeding season (close to the official birth date of 1 January and 1 August in Northern and Southern Hemispheres respectively) are generally, but not always, lighter than those born later in the foaling season. Foals born in Canada in late winter (January-March) were 2.4-5.6 kg lighter at 3 weeks of age than foals born in April, May or June (Hintz et al., 1979). Early-born Kentucky foals were on average 6.8 kg lighter at 2 weeks of age than March-born foals, and remained lighter until 9 months, at which time mean body weight had equalised (Pagan et al., 1996). In contrast, Irish foals born in January/February were significantly heavier than those born in March to May (MacCarthy and Mitchell, 1974). The late-born Irish horses remained lighter than early-born horses, but then grew faster (compensatory growth), reducing the weight differences between the early and late-born foals: May-born fillies gained approximately 4.1 kg/week until September (16 months of age) whereas January-born fillies gained 3.3 kg/week. In November of the year of birth the May-born foals were 73-96 kg lighter than the January-born foals (because of being 4 months younger) but by yearling sale in September the following year the difference in body weight had reduced to 41-53 kg (MacCarthy and Mitchell, 1974).

Horses exhibit a seasonal growth pattern. Regardless of birth month, growth rates of Kentucky horses were low during winter and increased during April and May the following year. Monthly growth rate in yearlings was related more to season of year than to age, with growth patterns following changes in temperature and pasture growth (Pagan et al., 1996).

Anecdotally, New Zealand Thoroughbred stud masters believe that autumn-born horses do not grow as well as spring-born horses, would be an inferior product, and therefore can not be marketed because their body weight would be sub-optimal by the time of sale. However, no data are available to verify this opinion. If the facts were shown to be otherwise, then considerable opportunity might exist for breeders to produce offspring from late foaling mares or mares that fail to conceive early in the breeding season, and to manage two groups of foaling mares each suited to differing markets. This could result in production increase and reduction of losses from empty mares, both of which would contribute to improved economic value.

Given the limited information available about growth of New Zealand Thoroughbred horses born out-of-season and raised at pasture, the purpose of this study was to obtain growth data from groups of pasture-raised thoroughbred horses born in spring and autumn.

3.3 Materials and methods

The study consisted of 63 Thoroughbred horses (28 colts and 35 fillies) born in the spring seasons of 1999 (n=16), 2000 (n=31), 2001 (n=9) and autumn-2002 (n=7). Average birth dates for the three spring-born and one autumn-born foal crops were 7 November, 28 October, 16 October and 25 February respectively. The horses were bred and reared at AgResearch Flock House Thoroughbred Stud, Bulls, New Zealand, close to the 40th parallel. Mares and foals were raised on tall fescue dominant pasture, and foals were weaned at 4.5 to 6 months of age. Weaning of the 1999, 2001 and autumn-2002-born horses was by date and occurred between 7 and 14 May, 23 April, and 31 August respectively, whereas weaning of the 2000-born horses was by age and occurred at 133 ± 9.5 days of age. Horses were grazed on two-hectare paddocks in mobs of 8-9 horses, and were shifted to different paddocks weekly or more frequently when required as assessed by the stud manager. Approximately 1 kg meadow-hay per horse was offered daily during the months of May to July. Horses were observed daily for general health and more closely at monthly clinical examination. Ten colts (born-2000) were castrated in either August or December 2001 (see Chapter 2).

Foals were weighed at birth and thereafter at fortnightly intervals until March, when the spring and autumn-born horses averaged 17 and 13 months of age respectively. Weighing occurred at a similar time of day, using electronic scales (Tru-test 703, Tru-test, Auckland, New Zealand). The scales were checked with a known weight before each fortnightly weighing.

Data for the three spring foal crops were initially analysed separately, but, as there were no statistically significant differences for weight or growth rate between the three years ($p>0.05$), data were thereafter combined. Fortnightly data for weight and growth rate (average daily gain, ADG) were subjected to generalised linear model analysis, with respect to sex, and season of birth, as well as the linear and quadratic effects of age. Calendar month was added to the model as a random effect. Differences of regression lines between groups were quantified using analysis of variance. Differences were considered significant at $p<0.05$, unless otherwise stated.

3.4 Results

There were no significant effects of season or sex on foals' birth weights ($p>0.05$), although there were trends for fillies to be lighter than colts (53.5 ± 2.28 kg vs. 56. 7 ± 1.65 kg) and autumn-born foals to be lighter than those born in spring (52.55 ± 2.97 kg vs. 57.99 ± 0.92 kg; $p= 0.08$). Mean body weight was similar in both spring and autumn-born horses at equivalent ages (Figure 3.1).

Statistical analysis of body weight and ADG are shown in Table 3.1. Body weight was significantly related to age ($p<0.001$), age^2 ($p<0.001$), season of birth ($p<0.05$) and calendar month ($p<0.001$) and there was also a significant season by age interaction ($p<0.001$). For ADG, only the effects of age, age^2 and calendar month were significant ($p<0.001$), although there was also a significant season by age interaction ($p<0.001$). Thus although birth season of birth and sex affected absolute body weight, they did not affect the foals' rate of growth.

Between birth and 5 months of age, overall mean ADG declined from 1.5 kg/day to approximately 0.8 kg/d in both spring and autumn-born foals. Thereafter, from 6 months of age, ADG in the autumn-born horses increased to 0.9 kg/day at 8 months and then decreased gradually to 0.3 kg/day at 11 months. In the spring-born horses, ADG from 6 to 10 months of age decreased steadily to 0.47 kg/d (Figure 3.2). The effects of age and calendar month are shown in Figures 3.2 and 3.3, in which the mean ADG of spring and autumn-born horses are illustrated with respect to age and month of the year respectively. ADG rates were similar in spring and autumn-born foals during the first six months of life (Figure 3.2) but, thereafter, effects of season were associated with lower ADG rates between May and August, reaching a nadir in late July (Figure 3.3). ADG of autumn-born foals were 0.2 - 0.4 kg/d greater than the ADG of spring-born foals during these winter months. Thereafter, ADG increased during September and October, and then declined in summer. ADG of spring and autumn-born foals were never more than 0.15 kg/d different to each other from October to March. Therefore, the low ADG that occurred in spring-born foals at 10 months of age, and in autumn-born foals at six months of age both correspond to the period in July and August when ADG was at its minimum since birth. The lowest ADG in both spring and autumn-born foals occurred in December/January.

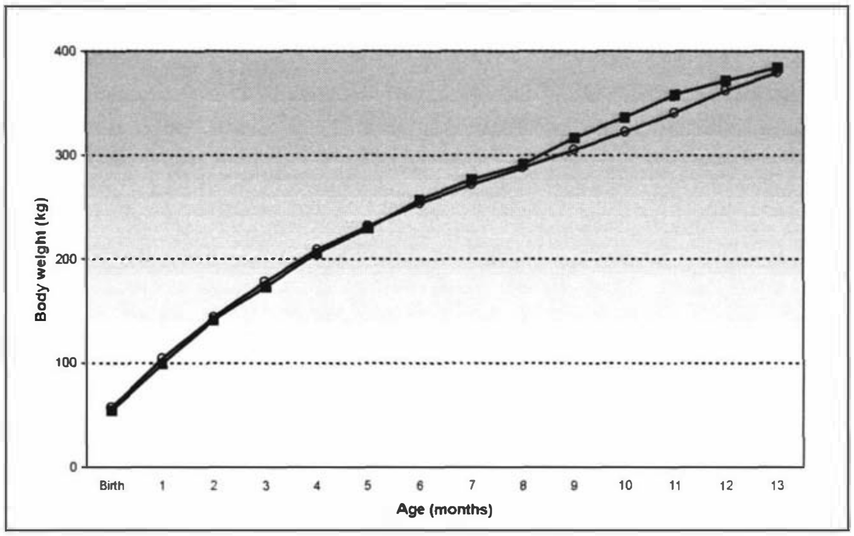

Figure 3.1 Body weight (kg) of pasture-raised Thoroughbreds born in spring (○) and autumn (■).

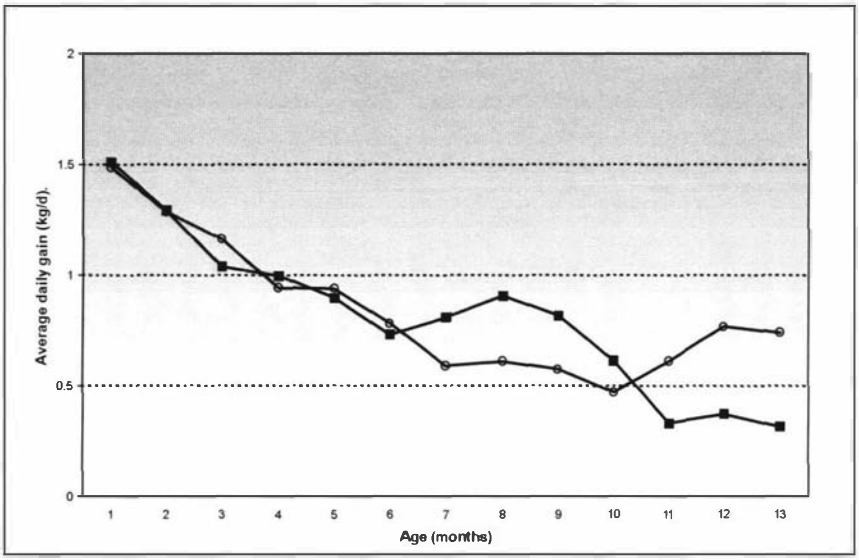

Figure 3.2 Mean ADG (kg/d) of pasture-raised Thoroughbreds born in spring (○) and autumn (■).

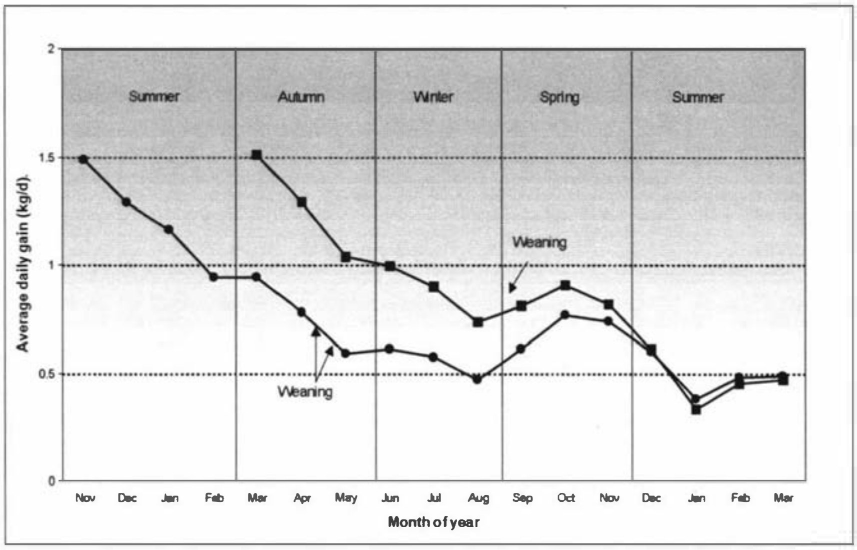

Figure 3.3 Mean ADG (kg/d) of pasture-raised Thoroughbreds born in spring (○) and autumn (■) with respect to month of year. Spring-born horses were weaned between April and May, and autumn-born horses were weaned at the end of August, as indicated by arrows.

Table 3.1 Summary of ANOVA for body weight (kg) and average daily gain (kg/d) of New Zealand pasture raised fillies and colts born in spring or autumn. The model for body weight and ADG of spring and autumn-born horse were based on the coefficients of the ANOVA. * = Random effect, varying month by month.

Factor	F Value	p	Coefficient
Body weight	**(Complete model)**		
birth weight	439.92	0.001	1.913
sex	37.70	0.001	fillies –43.50
			colts –36.49
age (linear)	25507.08	0.001	1.104
age (quadratic)	323.28	0.001	-6.32×10^{-4}
birth season	15.78	0.001	12.86
age by birthseason	3.75	0.05	0.0191
calendar month	6.09	0.001	*

Factor	F Value	p	Coefficient
Average Daily Gain	**(Complete model)**		
birth weight	3.68	n/s	2.61×10^{-3}
sex	4.3×10^{-4}	n/s	-3.1×10^{-3}
age (linear)	418.52	0.001	-4.56×10^{-3}
age (quadratic)	108.22	0.001	6.16×10^{-6}
birth season	4.2×10^{-4}	n/s	0.189
age by birthseason	4.38	0.05	-7.05×10^{-4}
calendar month	14.78	0.001	*

3.5 Discussion

Body weights and growth rates of horses in this study were similar to those previously recorded from the same stud in the 1994 season (Grace et al., 1999). Although spring-born foals were slightly heavier at birth than autumn-born foals, differences were not significant. More importantly, for the first six months of life, spring and autumn-born foals had similar growth rates. The increase in body weight and the rate of decline of growth rate over the first six months of life was the same in the two groups of animals, probably reflecting the degree to which their diet was composed of milk and increasing amounts of pasture. Foals begin to nibble on grass at 1 week of age, and by 5 months 50% of their time is spend grazing (Frape, 1998). Mare milk yields start to wane at 2-3 months of lactation and by month 4-5, milk yields are 1/3 that of the first month (Gibbs et al., 1982). Therefore after 2-3 months of age, pasture becomes increasingly important as a nutrient supply to the growing foal.

It is clear from the ADG data shown in Figures 3.2 and 3.3 that ADG in the first six months of life is more a function of age than season, since both spring and autumn-born foals exhibit identical growth-rate curves. However, from six months of age ADG was more a function of seasonal factors than of age, the increase in autumn-born horses coinciding with the onset of spring pasture growth. In spring-born foals ADG remained low between 6 and 10 months of age with the increase in ADG after this time concurring with the spring pasture flush. Another contributing to the increase in ADG observed in September and October in both spring and autumn-born horses may be a peri-pubertal growth spurt, such as known to occur in children (Tanner, 1981; Cutler et al., 1986; Rogol, 1996). Onset of puberty in these horses occurred in October (see Chapter 4), when spring and autumn-born horses were 12 and 8 months of age respectively. Average daily gain between August and October increased by 0.2 and 0.3 kg/d in spring and autumn-born horses respectively, which could be indicative of a peri-pubertal growth spurt.

Weaning can significantly affect ADG. For example, in Kentucky foals weaning reduced ADG from 0.8 to 0.6 kg/day (Warren et al., 1997). This can be seen in the spring-born foals in this study, which as with common stud practice, were weaned in the autumn onto pasture of declining quality and quantity. By contrast, the autumn-born foals were weaned in spring when pasture quality and growth is at its highest. Thus, ADG in the month prior to weaning in spring-born foals averaged 0.8 kg/d, after which it decreased to 0.6 kg/d. However, in the autumn-born foals, ADG in the month before weaning was 0.75 kg/d after which it increased to 0.8 and 0.9 kg/d one and two months after weaning respectively. It could therefore be suggested that the weaning-associated check in ADG might be alleviated or abolished by nutritional management, as suggested by Bagley et al. (1987) and Boulton et al. (1987). Similar results have been reported from beef production systems in which autumn calving has been associated with superior growth characteristics, including higher weaning weights (Bagley et al., 1987) and higher post-weaning growth rates (Boulton et al., 1987) than occurs in spring-born calves.

Some pasture characteristics at the stud emphasises this observation. Pasture growth rates in the area where the present study took place are typically at a low in late winter (10-30 kg DM/ha/day) (July and August) with pasture growth reaching maximum in the spring and early summer (50-70 kg DM/ha/day) (Holmes et al., 2002). Pasture nutritive quality (digestibility, energy content and protein content) is highest in the spring and lowest in the late summer due to stem elongation and flowering. The AgResearch Flock House Stud is located on coastal sandy soil, which can get very dry in summer resulting in a decreased pasture growth. Summer rainfall in this area averages 200mm, but during the summers of 2000/2001 and 2002/2003 severe lack of rainfall (45% of normal) resulted in significant soil moisture deficits (NIWA, 2003). The dry conditions resulted in reduced pasture growth rates. The lower ADG in December and January is consistent with reduced rainfall and pasture growth at this time of year on coastal sand country.

It is also clear that the spring and autumn-born horses had similar mean body weights at the same age up to eight months (differences were never greater than 5 kg). Between 9 and 12 months the mean body weights of autumn-born horses were 10-18 kg heavier than that of the spring-born horses at the same age. At the completion of this study (20 March), when the spring and autumn-born horses were 17 and 13 months old respectively, the mean body weight of the spring-born horses was only 52 kg greater than that of the autumn-born horses mean. In other words the autumn-born horses 'caught up' to achieve 88% of spring-born horses body weight, while being four months younger in age. This has been observed in May-born Irish yearlings, which grew faster than those born early in the breeding season (January/February) thereby reducing the mean body weight difference between the early and May-born foals to only 41 kg for fillies and 53 kg for colts in September of their second year (MacCarthy and Mitchell, 1974).

When the individual body weights were considered, rather than the group means, the three heaviest autumn-born horses, born respectively on 1 March 2002, 31 January 2002 and 5 February 2002, weighed 431, 391 and 383 kg on 20 March 2003, i.e. no less than 51 kg different to the spring-born horses (who weighed on average 434 ± 7.9 kg on 20 March). Thus, selected autumn-born horses would be eligible (in terms of weight) for late Southern Hemisphere sales, and certainly for Northern Hemisphere sales. Furthermore, some individuals were of similar weight to the mean of the spring-born horses in March, and perhaps the others could have been if supplemented in December-January. These animals and their dams were grown on pasture with some hay offered in the winter months, and no supplementary grain was provided. Individual management was not undertaken because the aim of the study was to assess the growth of horses solely raised on pasture.

Clearly, weight targets could be met by appropriate individual nutritional management over a period of several months. Also, there seems little evidence so far, which shows whether there is such as thing as a "minimum" age, which precludes attainment of a suitable stage of musculo-skeletal development for successful sale. Appropriate conditioning regimens to advance such musculo-skeletal development are currently being investigated, and may, with suitable breeding, suitable timing, and individual management, result in prestige stock being produced from autumn-foaling.

The results from this study clearly show that a) horses born in autumn can achieve growth rates comparable with horses born in the spring and b) that there is little difference in body weight between spring and autumn-born horses at the time of yearling sales in New Zealand (February). This means that late-born horses, on the basis of weight and body condition alone, could be marketed for late Southern Hemisphere sales (March-April). Furthermore, pasture-raised autumn-born horses achieve body weights comparable to those attained in the Northern Hemisphere (Table 3.2). Although these autumn-born foals were not studied beyond 13 months of age, they could perhaps be marketed at Northern Hemisphere yearling sales (September of the year following birth, at approximately 17-18 months old). Horses in the Southern Hemisphere turn a year older every 1 August due to the official birth-date imposed on Thoroughbreds, therefore autumn-born horses would be more competitive in Northern Hemisphere industry as they would be competing against horses of the same official age.

Table 3.2 Comparison of body weight (kg) data from four Northern Hemisphere growth studies on spring-born foals with the present study (spring and autumn-born foals). Note: All data are the average of fillies and colts. Hintz data collected on days 32, 62, 187, and 352, Thompson data on days 28, 56, 182 and 364, and Pagan data on days 183 and 350.

Month of age	n	1(30d)	2(60d)	6(180d)	12(360d)
Present study (spring-born)	56	104	144	253	362
Present study (autumn-born)	7	99	141	257	372
(Hintz et al., 1979)	1992	97.5	136	240.5	337
(Thompson, 1995)	106	99.4	133.6	258.5	363.9
(Jelan et al., 1996)	798	-	131.5	240.5	340.0
(Pagan et al., 1996)	350	-	-	253.3	342.2

The results indicate that late-born horses need not have the prejudice attached to them as in the past and, therefore, progressively later foaling mares need not necessarily loose a breeding season. Economic loss from the retention of dry mares would be reduced, and production would be increased from mares that, in the past, would have not produced a foal until the breeding season two years later. This is particularly important with regard to valuable mares as well as optimising production from mares known to be of superior breeding-age: Mares between the ages of 7 and

11 years produce significantly heavier foals, a difference that persists until the foal is over 2 years of age (Hintz et al., 1979). Foals of low birth weight are known to have inferior growth and performance characteristics than foals of heavier birth weight (Platt, 1978).

Chapter 4

Onset of puberty in pasture-raised Thoroughbred horses[1]

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[1] Submitted as: C.G. Brown Douglas, P.F. Fennessy, E.C. Firth and T.J. Parkinson (2003) *Equine Veterinary Journal*.

4.1 Abstract

The age, body weight and date of onset of puberty were studied in 51 pasture raised Thoroughbred horses born in spring and autumn. Spring-born fillies and colts were older and heavier than autumn-born fillies and colts at puberty. The age at onset of puberty in spring and autumn-born foals was 291 - 408 days and 212 - 270 days respectively. The weight at puberty in the spring -born foals was 302 - 409 kg, and in autumn-born foals was and 277 - 344 kg. However, the mean date at onset of puberty was not significantly different between spring and autumn-born horses, with puberty occurring in October (New Zealand spring). Results indicated that the seasonal changes in photoperiod affect the timing of onset of puberty. It is proposed that the minimum threshold body weight for the onset of puberty in Thoroughbreds is around 280 kg and no less than 49% of mature weight. Spring-born horses reached this threshold weight during the winter months and remained reproductively inactive until after the stimulus of increasing daylength occurred. The autumn-born horses reached the threshold weight to support puberty simultaneously with stimulatory photoperiod, and therefore reached puberty significantly younger and lighter than the spring-born horses. Therefore, the synchrony of a threshold weight with increasing photoperiod could provide the cue for onset of puberty in horses.

4.2 Introduction

Horses are long-day seasonal breeding animals with reproductive activity generally occurring in the spring and summer (Daels and Hughes, 1993). A period of anoestrus generally occurs in mares in the winter months (Sharp and Davis, 1993). Likewise, seminal characteristics and sexual behaviour of stallions roughly coincide with the natural breeding season of mares, but many stallions are known to be sexually competent all year round and therefore less seasonal than mares (Pickett, 1993).

Puberty is the period of transition from a state of reproductive immaturity to a state of full reproductive competence (Cameron, 1990). An animal is defined to have reached puberty when it is able to release gametes and to manifest sexual behaviour (Hafez, 1993). Onset of puberty in the female may be defined as the time of the first ovulation, which can be determined by measurement of plasma progesterone. In many species, including horses, a progesterone concentration greater than 2ng/ml is indicative of a luteal phase following an ovulation (Roberson et al., 1991; Suttie et al., 1991; Nachreiner and Hyland, 1993; Bergfeld et al., 1994). Puberty in male animals is difficult to determine with the same degree of accuracy, because it represents a process of development rather than a definable single event. However, puberty has been defined as the time when the testes become androgenically active producing testosterone concentrations greater than 0.5 ng/ml (Naden et al., 1990a), with spermatozoa appearing some time after the onset of puberty (Skinner and Rowson, 1968).

Puberty in animals is difficult to determine. In humans, secondary sexual characteristics such as growth of pubic hair and breast development are indicative of puberty but in domestic animal species, these sexual characteristics do not occur or are difficult to quantify. Endocrine patterns associated with puberty have been studied in many domestic species, including sheep, cattle, pigs, and horses (Pelletier et al., 1981; Schams et al., 1981; Wesson and Ginther, 1981; Naden et al., 1990). Therefore, it is perhaps more appropriate to use an endocrinological parameter to define puberty in the horse.

There are few data on the age, weight and time of year of puberty in Thoroughbred horses. A major difficulty with interpretation of such data as do exist is the wide variety of detection methods and descriptions of the onset of puberty, and puberty itself, resulting in inconsistencies between studies. For example, published ages at puberty in pony fillies are 10 to 24 months (Wesson and Ginther, 1981), or 15 months (Palmer and Draincourt, 1983); in Thoroughbred fillies it occurs between 12 months (Adams and Bosu, 1988; Camillo et al., 2002) and 14 months (Nogueira et al., 1997); in Quarter Horse fillies it occurs between 21 and 24 months (Naden et al., 1990b). Puberty in colts, as determined by semen characteristics, occurred at 12 to 15 months in Welsh Pony colts (Skinner and Bowen, 1968) and at 14 to 24 months in Quarter Horse colts (Naden et al., 1990a).

In seasonal breeders, there is evidence for the involvement of both photoperiodic cues and degree of maturity (defined as weight as a proportion of expected mature weight) in the onset of puberty. Regardless of season of birth, sheep reach puberty in the autumn and winter-born lambs are thus older at puberty than spring-born lambs (Foster et al., 1985). In lambs born in late summer and autumn, puberty onset is delayed until the following autumn, when the lambs are older than 1 year of age (Foster, 1981). In horses, Wesson and Ginther (1981) claimed that the date at onset of puberty in female ponies was unaffected by the season of birth: the mean date and body weight at the first ovulation were not significantly different in spring and summer-born ponies but mean age at puberty was significantly different (411 ± 6 vs. 341 ± 17 (SEM) days respectively).

Genetic influences may also affect the timing of the onset of puberty, at least in sheep. Sheep are short-day breeders, and onset of puberty in males occurs in the first autumn of life if body weight is 35 - 40 kg or more (approximately 40-70% of adult live weight depending on breed (Brown, 1994). In contrast, there are major breed differences in the onset of puberty in female sheep. Selection for high growth rates has led to pubertal onset at younger ages and occasionally lighter live weights, and therefore lower percentage of mature weight than ewes in the unselected populations from which such animals have been derived (Lawrence and Fowler, 1997). Therefore breeds selected for high growth rates may reach puberty at a relatively immature live weight. For example, Finnish Landrace and East Friesian ewes reach puberty in the first autumn of life at 50% of their mature live weight, but the proportion of New Zealand Romney ewe lambs attaining puberty at 50% of their mature body weight is much lower (McMillan et al., 1998) . It appears that the minimum weight for onset of puberty, expressed as a percentage of the mature weight, is higher in Romney ewes than in Finn or East Friesian ewes.

There is a dearth of data around puberty in the horse, so it is not surprising that there is no evidence of breed differences in terms of relative maturity (expressed as a percentage of mature weight). Spring-born ponies and Thoroughbred fillies reached puberty in spring at 56% and 61% of mature body weight for their breeds respectively (Wesson and Ginther, 1981; Nogueira et al., 1997). Furthermore, (assuming a 575 kg mature body weight (Pagan, 1996)) puberty in 8 of 20 Brazilian Thoroughbred fillies occurred at 59% of mature body weight in spring (mean age of 15 months) and in the 12 remaining fillies the following spring at 71% of mature weight (mean age of 25 months) (Souza et al., 1997). It appeared that the 12 fillies did not reach a weight permissive for pubertal onset (threshold weight) in the first spring, and remained reproductively inactive until the photoperiod was stimulatory the following spring. Thus there is evidence for both photoperiodic cues and 'maturity' factors in the timing of pubertal onset in the horse.

The objectives of this study were to ascertain the effect of season of birth (spring and autumn) on the age, weight and date at puberty onset in Thoroughbred horses bred and reared on pasture in New Zealand and to provide reference data on threshold age and weight for the onset of puberty in this environment.

4.3 Materials and methods

4.3.1 Animals

The study comprised 51 Thoroughbred horses (18 colts and 33 fillies) born in the spring of 1999 (n=16), 2000 (n=26), 2001 (n=9) and 8 (5 colts and 3 fillies) born in autumn 2002 (n=8). Average birth-dates for three spring-born crops and the one autumn-born foal crops were 7 November, 2 November, 16 October, and 25 February respectively. The horses were bred and reared at the AgResearch Flock House Thoroughbred Stud, Bulls, New Zealand, close to the 40th parallel. Horses were raised on tall fescue dominant pasture and were weaned at 4.5 - 6 months of age.

4.3.2 Data collection

Horses were blood-sampled and weighed fortnightly from birth until the second March in all foal crops at a similar time of day using electronic scales (Tru-test 703, Tru-test, Auckland, New Zealand). Blood samples, collected by jugular venepuncture (10ml; lithium-heparin anticoagulant), were placed on ice, and within 2 h plasma was separated by centrifugation at 1000g/10 min and stored at -20°C for later assay. Horses were observed daily for general health and more closely during monthly clinical examination. All animals remained healthy throughout this study which ended in March 2003.

4.3.3 Hormone assays

Fortnightly samples collected were assayed for either progesterone (fillies) or testosterone (colts) concentrations. The frequency of blood sampling (fortnightly) was considered appropriate to detect an ovulation as the luteal phase of the mare is 14 to 15 days (Daels and Hughes, 1993).

Progesterone was measured using a coated-tube RIA kit assay (Progesterone-RIA Kit, CA-1724, DiaSorin, Stillwater, MN, USA), carried out in accordance to the manufacturer's instructions. The minimum detectable level of progesterone was 0.11ng/ml at 95% confidence limit. The mean inter-assay coefficient of variation was 15.3%, while the mean intra-assay coefficient of variation was 16.8%.

Testosterone was measured using a coated-tube RIA kit assay (Testo-ctk, P3093, DiaSorin, Italy), carried out in accordance to the manufacturer's instructions. The minimum detectable level of testosterone was 0.05 ng/ml. The inter- and intra-assay coefficients were 13.0% and 13.2% respectively.

All assays were performed in duplicate and all samples from a given horse were analysed in a single assay.

4.3.4 Data analysis

Baseline hormone concentrations were defined as the minimum detectable concentration of the hormone (i.e. sensitivity of the assay: 0.05 ng/ml for testosterone and 0.11 ng/ml for progesterone). Fillies were considered pubertal when they (a) exhibited a progesterone concentration greater than 2 ng/ml, (b) which was at least 3 times greater than the previous progesterone concentration, which is indicative of a luteal phase of an ovulatory cycle in fillies. Colts were considered pubertal when they exhibited a change in testosterone concentration greater than twice the standard deviation (of each individual colt's assay) above the baseline concentration. Testosterone pulses were also observed in colts and were defined as any concentration greater than 0.1 ng/ml. Testosterone pulses were not indicative of the onset of puberty, unless the concentration of the pulse was within the criteria for onset of puberty as described above.

4.3.5 Statistical analysis

The three sets of spring data were analysed separately. There were no significant differences between the three spring seasons so the data were pooled thereafter.

Filly and colt growth puberty data were analysed separately using a Univariate General Linear Model, with respect to body weight, age and date at puberty. Year and season of birth were treated as fixed effects. All statistical analyses were performed with SPSS V10.10 (Chicago, Il). Data are expressed as mean ± SEM and differences were considered significant at $p<0.05$ unless otherwise stated.

Body weight data were subjected to a generalised linear model analysis with respect to sex, season of birth, as well as linear and quadratic effects of age.

4.4 Results

Progesterone concentrations were not detectable until the spring after birth when all fillies (except one autumn-born filly) exhibited progesterone concentrations > 2 ng/ml (data from 12 fillies are shown in Figure 4.1). The first spring-born fillies (4 of 33) to exhibit a pubertal increase in progesterone concentration did so in late September and the majority (28 of 33) had done so by late October. The two autumn-born fillies that reached puberty did so in late October. The number of fillies that had reached puberty, based on plasma progesterone concentrations greater than 2 ng/ml at any one sampling, is shown in Table 4.1.

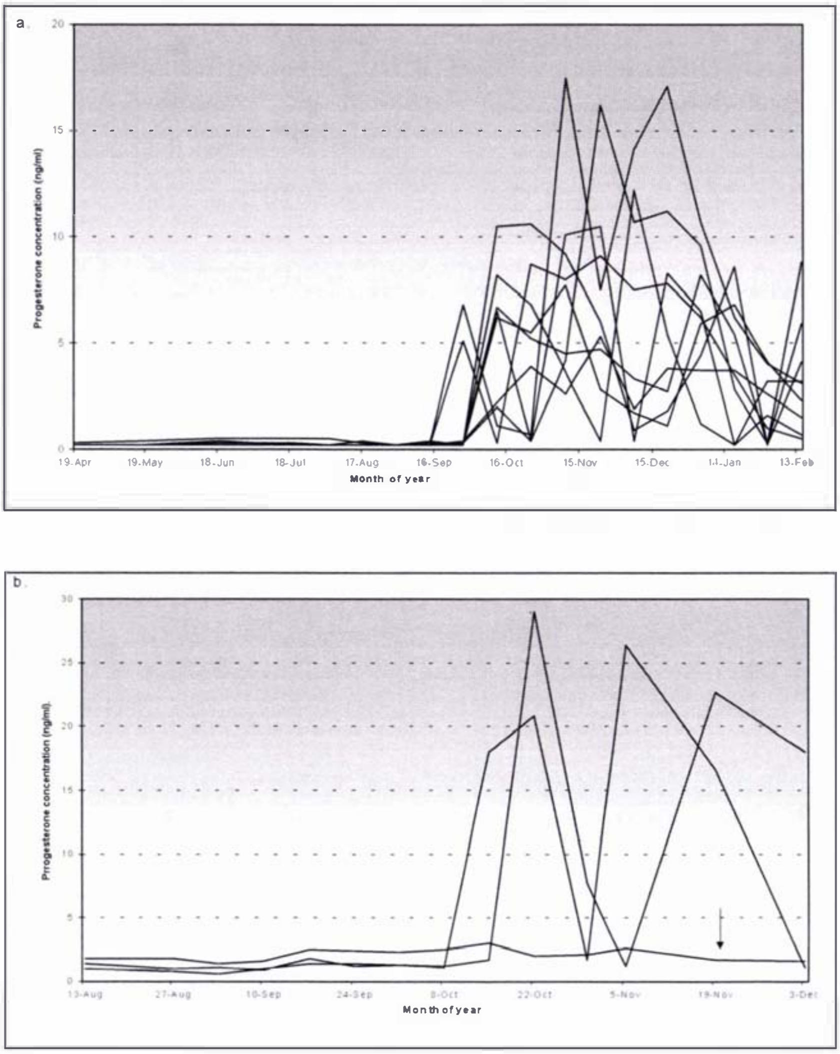

Figure 4.1 Fortnightly plasma progesterone concentrations of (a) 9 spring-born fillies and (b) 3 autumn-born fillies.

For clarity, in figure 4.1 (a), curves of only 9 randomly selected spring-born fillies are shown to illustrate patterns of progesterone concentrations, which up until September, were less than 1 ng/ml. The arrow indicates the one autumn-born filly that failed to reach puberty.

Table 4.1 Numbers of spring and autumn-born Thoroughbred colts and fillies with hormonal activity during spring and mid summer in a single blood sample taken from each horse at each time (For inclusion criterion, see text).

Date	Number and Proportion (%) of colts showing evidence of a T pulse in a single blood sample (>0.1ng/ml),		Number and Proportion (%) of colts showing evidence of the onset of puberty.		Number and Proportion (%) of fillies showing progesterone concentration > 2 ng/ml	
	Spring-born (n=18)	Autumn-born (n=5)	Spring-born (n=18)	Autumn-born (n=5)	Spring-born (n=33)	Autumn-born (n=3)
Mid August	3 (17)	0	0	0	0	0
Late August	6 (33)	0	0	0	0	0
Mid September	7 (39)	0	0	0	0	0
Late September	16 (89)	4 (80)	7 (39)	0	4 (12)	0
Mid October	15 (83)	4 (80)	11 (61)	0	18 (55)	0
Late October	16 (89)	4 (80)	16 (89)	1 (20)	28 (85)	2 (67)
Mid November	16 (89)	4 (80)	17 (94)	5 (100)	33 (100)	2 (67)
Late November	16 (89)	4 (80)	17 (94)	5 (100)	33 (100)	2 (67)
Early December	16 (89)	5 (100)	18 (100)	5 (100)	33 (100)	2 (67)

The proportion of colts showing evidence of testosterone pulses at the particular sampling is shown in Table 4.1. The first spring-born colts (3 of 13) showing testosterone pulses did so in mid-August, and by late-September 15 of the 18 colts showed evidence of testosterone pulses. The first testosterone pulses in the 5 autumn-born colts were detected in late-October, and all colts showed testosterone pulses by mid-November. Mean testosterone concentrations in spring and autumn-born colts are shown in Figure 4.2.

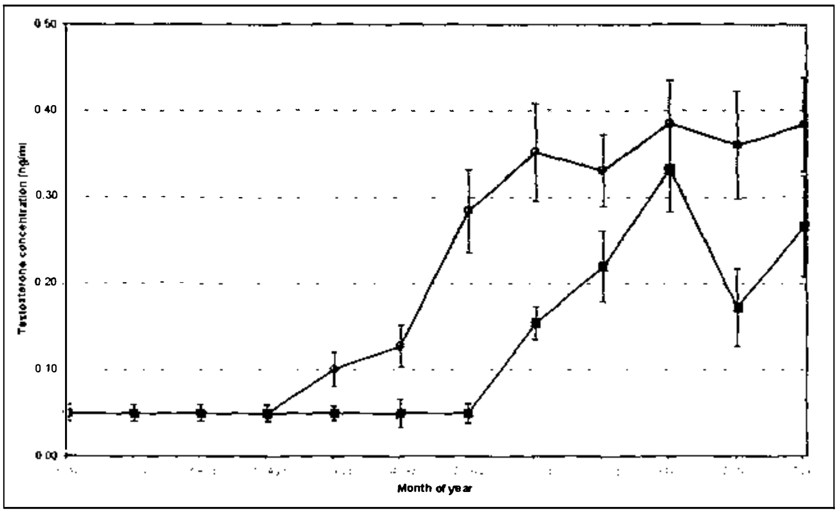

Figure 4.2 Mean ± SEM fortnightly plasma testosterone concentration of 18 spring-born (○) and 5 autumn-born (■) Thoroughbred colts.

Mean fortnightly testosterone concentration increased steadily in the spring-born colts from mid August and rapidly from mid September (11 months of age) whereas testosterone concentration in all the autumn-born colts increased rapidly in early October (7 - 8 months of age). Testosterone concentration decreased in November in autumn-born colts, while concentrations remained elevated in spring-born colts into December. Mean testosterone concentration in September and October was lower ($p<0.05$) in autumn-born than in spring-born colts.

All spring-born fillies (n=33) and colts (n=18), and all autumn-born colts (n=5) achieved puberty, whereas two of three autumn-born fillies reached puberty by the end of the study. Spring-born fillies and colts were significantly ($p<0.001$) older at puberty than autumn-born fillies and colts. At puberty, spring-born fillies (nearing significance; $p=0.06$) and colts ($p<0.05$) were heavier than autumn-born fillies and colts. Mean date at onset of puberty was not significantly different between spring and autumn-born horses. Age, weight and date at puberty are shown in Table 4.2 and Figures 4.3 and 4.4.

Table 4.2 Mean ± SEM and range of age, weight and date at puberty in Thoroughbred fillies and colts born in spring or autumn.

Fillies	Age at puberty (d)		Weight at puberty (kg)		Date at puberty	
	Spring	Autumn	Spring	Autumn	Spring (n=33)	Autumn (n=2)[2]
Mean	352±5.2	235±22.5	350 ± 5.4	284 ± 7.3	16 Oct ± 2.2	18 Oct ± 3.5
Range	298–408	212–257	302–419	280–292	24 Sept – 9 Nov	15 Oct – 22 Oct
SED	27.9 (p<0.001)		28.8		10.8	

Colts	Age at puberty (d)		Weight at puberty (kg)		Date at puberty	
	Spring	Autumn	Spring	Autumn	Spring (n=18)	Autumn (n=5)
Mean	341 ± 6.7	256 ± 6.0	355 ± 5.8	305 ± 12.4	11 Oct ± 5.1	30 Oct ± 2.6
Range	291– 405	231– 270	323 – 415	283 – 344	21 Sept – 4 Dec	22 Oct – 5 Nov
SED	17.7 (p<0.001)		14.6 (p<0.05)		9.2	

The youngest autumn-born foal, a filly, failed to reach puberty during the study, therefore data from this filly was removed from the analysis. Plasma progesterone concentration of this filly did not increase greater than 4 ng/ml, averaged 2.2 ng/ml over the study period until March 2003, and no sample was ever greater than three times the previous. On 18 October (average date of puberty in the autumn-born fillies), this filly was 191 days of age and weighed 262 kg; approximately 43 days younger and 29 kg lighter than the other autumn-born fillies.

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[2] One filly did not attain puberty in the period to 31 March 2003 (at an age of 13 months).

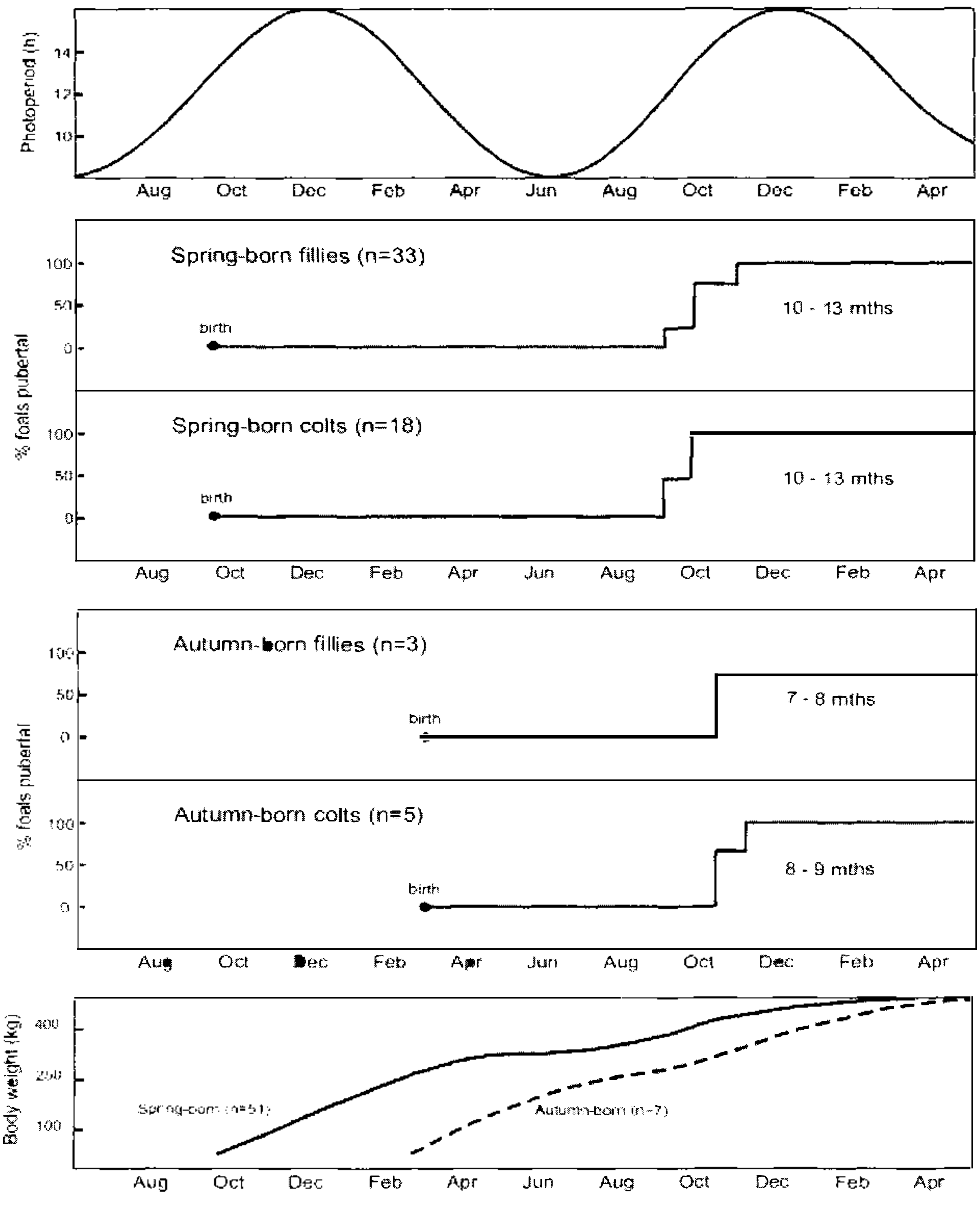

Figure 4.3 Onset of puberty (expressed as the percentage of animals that have reached puberty) in spring and autumn-born Thoroughbred foals in relation to Southern Hemisphere photoperiod, date, age, and body weight.

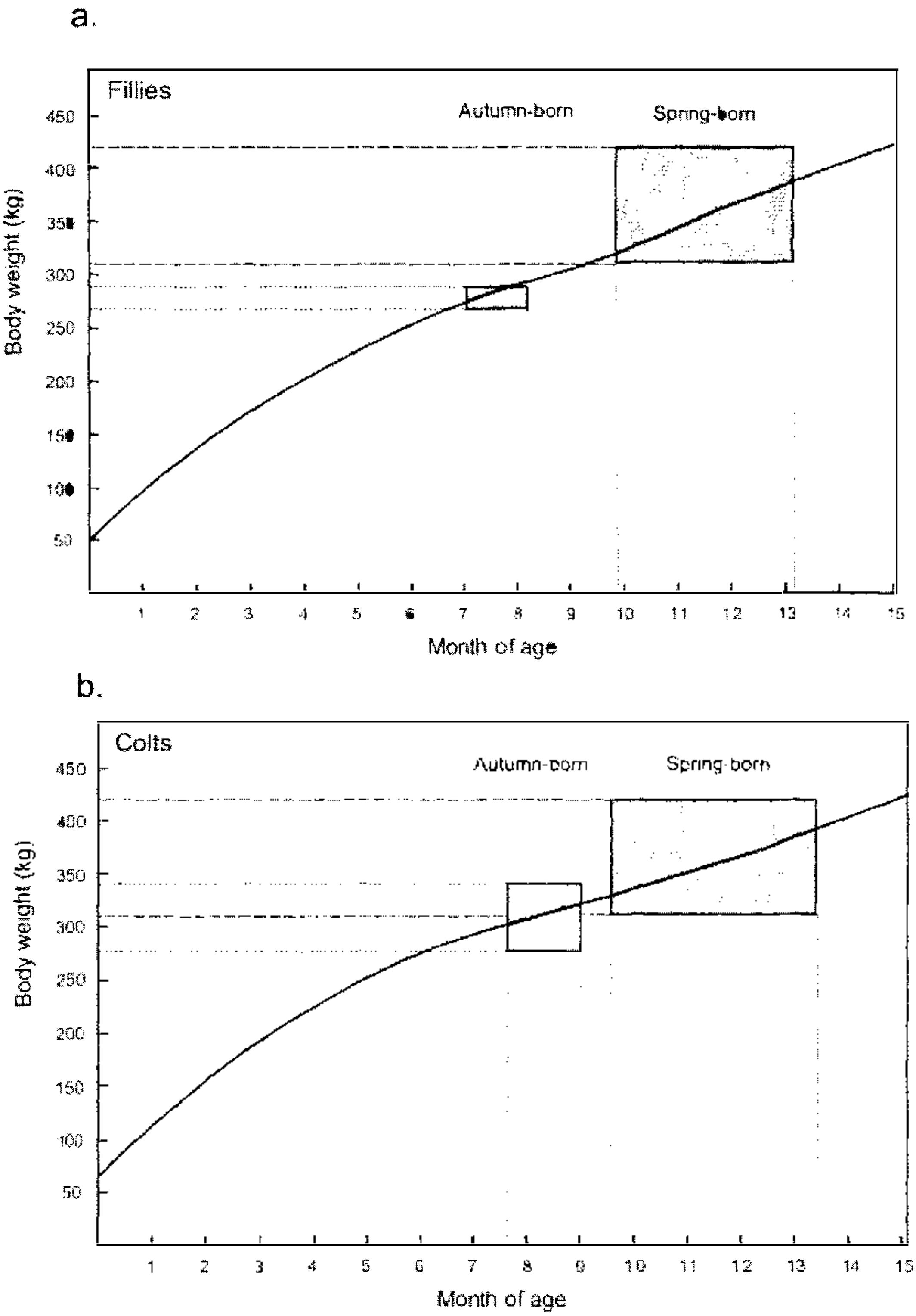

Figure 4.4 Range of age and body weight at puberty (▨) in spring and autumn-born Thoroughbred fillies (above) and colts. For mean ± SEM, see Table 4.1.

4.5 Discussion

Onset of puberty in spring-born fillies and colts and autumn-born fillies and colts occurred in the spring following birth. At the onset of puberty, the mean age of spring and autumn-born horses was 11 and 8 months of age respectively. These results clearly show that season of birth affects age at puberty.

The age and weight at onset of puberty in spring-born foals was 291 - 408 days and 302 - 409 kg, and in autumn-born foals was 212 - 270 days and 277 - 344 kg respectively (Figure 4.4). From these data it is proposed that the minimum threshold weight for the onset of puberty is around 280 kg (lightest fillies and colts were 277 kg and 283 kg respectively). Assuming a 575 kg mature weight (Pagan, 1996) then the minimum threshold body weight for puberty onset is no less than 49% of mature weight. However, onset of puberty only occurs if photoperiodic cues are permissive. Spring-born foals reached 49% of mature weight between 6 to 9 months of age, but this occurred in May to June where daylength was decreasing and not stimulatory for reproductive activity, and therefore foals remained reproductively inactive.

The youngest autumn-born filly did not reach puberty. At the mean date of pubertal onset in the autumn-born fillies, this filly was approximately 43 days younger and 29 kg lighter than the other autumn-born fillies. It is likely that this filly either failed to grow sufficiently to support gonadotrophin activity or was reproductively abnormal. There is evidence that retarded growth in lambs results in delayed puberty (Foster et al., 1985) and it was postulated that growth-retarded female lambs can not express the high-frequency LH pulses required for the development of a preovulatory follicle due to hypersensivity to steroid negative feedback. In late November, December and late February the filly weighed 284, 303 and 355 kg respectively, which are easily within the values of all the other animals at pubertal onset, and thus presumably a weight that was permissive for pubertal onset. Thus, some other factor must have prevented pubertal onset. Either the daylength, or daylength increase, in late November/December (summer), by which time body weight was permissive, was not stimulatory for reproductive development, or some other factor such as a genetic abnormality may have contributed (XO karyotype – Turner's syndrome).

Puberty in the filly is characterised by increasing pulsatile LH secretion culminating in a preovulatory LH surge and subsequent ovulation. In pre-pubertal fillies, LH secretion was under a seasonal influence: concentrations increased during the spring immediately after birth and were low during the winter months before increasing again the following spring when puberty occurred (Naden et al., 1990b). In the pre-pubertal state, pulses of LH are maintained at low levels due to high sensitivity to ovarian oestrogen feedback. As maturation advances, this sensitivity is decreased allowing increased LH secretion, which in turn stimulates follicular growth culminating in ovulation (Naden et al., 1990b; Squires, 1993). This is different to mares during the transition into the breeding season, when a pre-ovulatory follicle is present and when there is no measurable increase in mean daily LH concentration until several days before ovulation (Fitzgerald et al., 1987; Alexander and Irvine, 1991).

In colts, a similar pre-pubertal and seasonal change in LH pulse frequency and amplitude to mares is known (Naden et al., 1990b; Naden et al., 1990c). Serum LH was low from birth until 8 months of age, when an elevation was observed in spring; concentrations then decreased during the winter months until 20 months of age, when it rapidly increased. Testosterone concentrations did not rise concurrently with the initial spring increase of LH, but increased slightly from 15 months of age, and significantly from 18 to 20 months in the following spring (Naden et al., 1990a), indicating that photoperiod and, possibly, low body weight have an inhibitory action on testosterone secretion. LH secretion in mature stallions is highly seasonal, with peak concentrations occurring in late spring (Johnson and Thompson, 1983; Clay et al., 1988). Stallions also exhibit seasonal patterns in the secretion of testosterone, which mirrors patterns of LH secretion (Clay et al., 1988).

Increased daylength in spring is thought to provide a stimulus for, or remove an inhibition of, LH release from the pituitary gland in the mature stallion which in turn is responsible for increased testosterone concentrations observed in the spring (Clay et al., 1989). However, photoperiodic control over seasonal reproductive activity may be questionable as LH concentration in geldings (castrated males) is constant throughout the year, which reverts to a seasonal pattern of secretion after testosterone administration (Irvine et al., 1986). Moreover, in the presence of an oestrous mare, stallions became sexually aroused, inducing pulses of GnRH, and LH (Irvine and Alexander, 1991). It was therefore suggested that reproductive activity in stallions is less influenced by photoperiod than that of mares and it is the seasonal pattern of mare receptivity that regulates the observed breeding season in stallions (Irvine and Alexander, 1988; Irvine and Alexander, 1991).

For seasonal breeders to successfully become reproductively active, both body weight and photoperiod cues need to be permissive. This has been shown in sheep (Foster and Yellon, 1985) and red deer (Adam et al., 1996). In the lamb, if body size is inadequate to begin reproduction, stimulatory photoperiodic cues are overridden and puberty is delayed (Foster and Yellon, 1985). Puberty in red deer occurs in the autumn, and is more dependent on photoperiod and body weight factors than age. When the photoperiodic regimen is first permissive for reproductive activity (first autumn of life), the animal has not yet reached a weight that will support onset of puberty and remains in a sexually immature state. By the time the stag reaches a threshold weight for the onset of puberty (spring), the daylength is inhibitory for puberty therefore the animal must wait until the following autumn (Adam et al., 1996).

Testosterone pulses were observed in July in several of the spring-born colts in the present study, but it was not until September that testosterone concentrations reached pubertal levels. It is possible that these spring-born colts reached adequate body weight to support reproductive activity in the winter, but photoperiodic cues were not permissive. The autumn-born colts reached puberty in the spring (mean age of 8 months) and there was little evidence of testosterone pulses before this time in these colts. It could be suggested that the autumn-born colts reached the threshold weight to support puberty simultaneously with stimulatory photoperiodic cues. Similar work in deer has shown that male and female deer born out of season, in late winter (4 months before normal calving), showed precocious puberty in their first autumn at approximately 8-9 months old (Adam et al., 1992). Winter-born deer achieved sufficient body weight and responded to the decrease in photoperiod in the first autumn to complete puberty. Deer born in the normal birth season (summer) did not achieve sufficient body weight to complete the process of puberty in the first autumn and remained sexually immature until the second autumn of life.

Daylength is an important environmental cue timing puberty, but it is unclear how photic cues transduce into hormonal signals to time puberty at the same time of year. Photoperiod affects onset of puberty in seasonal breeding animals such as the sheep and deer (Yellon and Foster, 1985; Foster et al., 1985; Foster et al., 1986; Foster et al., 1988; Wood et al., 1991; Adam et al., 1992; Suttie et al., 1992; Adam and Robinson, 1994). Hours of daylight (photoperiod) are the same for any year and times of sunrise and sunset at one location will not vary by more than a minute or two on the same date from year to year. At the summer solstice on 21 December at the latitude 40°S, the ratio of day light hours to darkness is 14:10, and at the winter solstice on 21 June, the ratio of day light hours to darkness is 10:14. At the equinoxes, on 21 March and 21 September, the hours of daylight and darkness are equal at 12 hours. It appears that these horses reached puberty in the month following the spring equinox where day light hours equal darkness hours and therefore 4 months after the winter solstice.

The exact photoperiodic regimen to stimulate onset of puberty in horses is not known, but extensive work in the sheep has shown that exposure of lambs to short days early in postnatal life is not necessary for them to recognise subsequent long days (Foster et al., 1988). Once some long days have been experienced by lambs, a sequence of neuroendocrine events occur which lead to puberty. In addition, the normal initiation of reproductive cycles after long days can occur in the absence of any further photoperiodic information. Therefore, in lambs raised under natural conditions, short days are merely permissive to puberty, and the long days of summer followed by their disappearance, time the onset of puberty. Despite the horse and sheep being different (long-day breeders vs. short-day), it maybe possible the mechanisms are "inversely different" and that, in horses, exposure to only some short days are required to stimulate pubertal onset; once the horse has detected the disappearance of the short days the process of pubertal onset begins.

In this study, autumn and spring-born foals attained puberty at the same time of year at significantly different ages, indicating that seasonal changes in photoperiod affect the onset of puberty. Palmer and Draincourt (1983) hypothesised that the effect of birth-season on ovarian activity in the pre-pubertal horse is regulated by synchrony of a potential puberty age (approximately 14 months) with the increase in daylength. The idea of a potential puberty age is disregarded, because the mean age of autumn-born foals at onset of puberty was 8 months, and no less than 3 months younger than spring-born horses.

Therefore, it could be suggested that is the synchrony of a threshold weight with increasing photoperiod (after the spring equinox i.e. long-days) that provides the cue for onset of puberty in horses. Spring-born horses normally achieve the threshold weight during the winter months, therefore did not reach puberty until after the stimulus of long-days occurred in the spring. The autumn-born horses reached the threshold weight to support puberty simultaneously with stimulatory photoperiod, therefore reaching puberty at a significantly younger age and lighter body weight than spring-born horses. It is likely that it is the change from short-days to long-days after the spring-equinox is the stimulus for puberty onset, because the mean date of pubertal onset did not differ between spring and autumn-born foals.

Chapter 5

LH and testosterone responses to 5 doses of a GnRH analogue (buserelin acetate) in 12 month old Thoroughbred colts[1]

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[1] Accepted for publication as: C.G. Brown Douglas. T.J. Parkinson. P.F. Fennessy. and E.C. Firth (2003) *Theriogenology*.

5.1 Abstract

Thirteen yearling Thoroughbred colts were challenged with 5 doses (0.5, 1, 5, 10 and 40 μg) of the GnRH analogue, buserelin. Jugular venous blood samples were taken at −10, 0, 10, 20, 30, 40, 60, 120 and 180 min after treatment and measured for luteinising hormone (LH) and testosterone concentrations. Significant increases ($P<0.05$) in LH concentrations occurred in colts that received 5, 10 and 40 μg buserelin but not in those receiving 0.5 or 1 μg. Peak LH concentrations and mean area under the curve were significantly higher ($P<0.05$) in animals receiving 40 μg buserelin than in those receiving 0.5 or 1 μg. Significant increases ($P<0.05$) in testosterone concentrations occurred in colts that received 1, 5, 10 or 40 μg buserelin. Neither peak concentrations of testosterone nor mean area under the curve were significantly different between doses of buserelin. The percentage of horses that responded to the buserelin increased with increasing dose, with only the highest dose (40 μg), eliciting LH and testosterone responses in all colts. In conclusion, pubertal colts exhibit a dose response of LH to buserelin, but animals appear to respond in an "all or nothing" manner, such that each horse either showed an LH response or it did not. Some colts that exhibited a significant LH response did not have a parallel testosterone response, possibly indicating that the pituitary LH response may not have been great enough to stimulate the testes in these individuals.

5.2 Introduction

Luteinising hormone (LH) response to exogenous gonadotrophin-releasing hormone (GnRH) has been used to determine hypothalamic-pituitary function in sheep (Wilson and Lapwood, 1979; Haley et al., 1989; Xu et al., 1993; Thomas and Brooks, 1997), bulls (Mongkonpunya et al., 1975; Tannen and Convey, 1977; Wildeus et al., 1984; Abdel Malak and Thibier, 1985; Miller and Amann, 1986; Byerley et al., 1990), deer (Fennessy et al., 1988; Adam et al., 1996), boars (Andersson et al., 1998) and both the male and the female horse (Alexander and Irvine, 1986; Clay et al., 1989; Naden et al., 1990b and 1990c; Pinaud et al., 1991; Seamans et al., 1991; Parlevliet et al., 2001). This has most commonly been performed on mature animals, in conjunction with other methods, to assess the activity of the hypothalamic-pituitary-gonadal axis (Parlevliet et al., 2001). The activity of the axis is on interest in investigations of sexual development (puberty) and seasonality. Some examples of such studies around puberty include Wilson and Lapwood (1979) in sheep, Wildeus et al. (1984) in bulls, Adam et al. (1994) in deer, and Andersson et al. (1998) in boars.

In some of these investigations, large doses of GnRH have been administered. However, it has been suggested that, in studies of the physiology of the hypothalamic-pituitary gonadal system, smaller doses of GnRH, which induce physiological LH responses, may be preferable to large doses, since the latter may result in a level of stimulation to which the pituitary is never naturally exposed and to which it may respond in a non-physiological manner (Alexander and Irvine, 1986).

A commercially available alternative to native GnRH is the synthetic analogue buserelin. Buserelin has been used in the adult stallion to assess pituitary-hypothalamic activity (Parlevliet et al., 2001) and in the mare to assess follicular responses (McCue et al., 1991). Buserelin is reported to have similar effects on release as native GnRH in terms of maximal response and the shape of the LH response curve, although buserelin shows a higher resistance to degradation (de Koning et al., 1984). It is also reported that buserelin is 10 times more potent as native GnRH (Koiter et al., 1988). There has been no published work investigating GnRH or buserelin responses in young male horses.

The aim of this study was to examine the LH and testosterone responses of yearling colts to a range of doses of buserelin, and to determine a dose that is suitable for use in investigation of physiological changes of pituitary responsiveness to buserelin during the peri-pubertal period.

5.3 Materials and methods

5.3.1 Animals

Thirteen yearling Thoroughbred colts were bred and reared at Flock House Thoroughbred Stud, Bulls, New Zealand. The colts were considered pubertal as their mean basal plasma testosterone concentrations had increased from 0.10 ± 0.01 ng/ml (mean ± SEM) in the winter months (April-August) to 0.37 ± 0.09 ng/ml in late September and had thereafter remained elevated throughout the duration of the experiment (late November/early December) (See Chapter 4). The colts were also observed to be exhibiting pubertal behaviour around the time of the experiment, including vocalization to mares, penile erection and attempts to mount.

The colts had been weaned at approximately 6 months of age and raised on tall fescue pasture. Horses were observed daily for general health and more closely during monthly clinical examination. Horses were weighed, condition scored and blood-sampled fortnightly. All remained healthy throughout the trial and exhibited no adverse response to the treatment during this study.

5.3.2 Buserelin challenge

The colts were challenged with buserelin, a synthetic GnRH analogue (Receptal®, Hoechst Roussel, Intervet, Auckland, NZ) in the last week of November, at a mean age of 383 ± 4.8 days and a mean weight of 387 ± 11.5 kg.

Prior to the start of blood sampling, an indwelling canula was placed in the left jugular vein of each colt using local infiltration of local anesthetic at the site of the venapuncture.

Five doses of Buserelin were used: 0.5 µg (n=6), 1 µg (n=5), 5 µg (n=8), (d) 10 µg (n=4) and 40 µg (n=3). Each animal received two of the doses of Buserelin. Eight colts received the second dose after a 1.25h rest period following the last sample, the remainder on a separate occasion at least 24 hours after the first challenge. The eight colts that received two doses on one day received the lower dose first and the LH concentration had returned to baseline before administration of the second dose.

Two blood samples (7-10ml) were collected, 10 min apart, prior to buserelin administration. Subsequent samples were collected at 10, 20, 30, 40, 60, 120 and 180 min after administration. Samples were immediately transferred to a 10 ml lithium-heparin vacutainer tube (Becton Dickinson, NJ, USA) and placed on ice. Samples were transferred to the laboratory where plasma was separated by centrifugation at 1000g for 10 min and stored at -20°C in three aliquots for later assay.

5.3.3 Hormone assays

Luteinising hormone (LH) was measured by radioimmunoassay (RIA) as described previously (Irvine et al., 1998; Irvine et al., 2000) using equine LH (Papkoff e263B) for standards and labelled hormone, monoclonal antiserum against the β-subunit of bovine LH (AFP #518137), and separation of free and bound hormone with goat anti-mouse gamma globulin (Calbiochem-Novabiochem Pty, Croydon, Victoria, Australia). Inter-assay and intra-assay coefficients of variation for LH assays were 7% and 8% respectively. The detection limit for LH was 0.36 ng/ml.

Testosterone was measured using a coated-tube RIA kit assay (Testo-ctk, P3093, DiaSorin, Italy), carried out in accordance to the manufacturer's instructions. The minimum detectable level of testosterone was 0.05 ng/ml at 95% confidence limit. The mean within-assay coefficient of variation was 13.2%, while mean between-assay coefficient of variation was 13.0%.

All assays were performed in duplicate and all samples from a given challenge were assayed together.

5.3.4 Data Analysis

Baseline hormone concentrations were defined as the average of the two pre-treatment samples. Peak LH and testosterone response was defined as the sample with the highest concentration of hormone achieved at any time after buserelin administration. Responses to the buserelin challenge were measured by calculating the area under the curve to give the magnitudes of the LH and testosterone responses.

The intervals between buserelin administration and LH and testosterone peak concentrations were also calculated. A significant response to each dose of buserelin was identified using criteria derived from those of Goodman and Karsch (1980). Briefly, the hormone concentration at the peak response had to exceed the 95% confidence limits (+ 2SD) of the average baseline concentration.

5.3.5 Statistical analysis

Data were analyzed using a repeated measures analysis of variance with respect to dose and time, with animal nested within treatment. Baseline concentrations from each horse that received two doses on one day were analyzed using a one way repeated measures analysis of variance. Peak concentration data were normalised by logarithmic ($\log_e$) transformation prior to analysis of variance. Results are expressed as the mean ± SEM.

5.4 Results

Pre-treatment plasma concentrations of LH and testosterone did not differ significantly ($P>0.05$) between animals receiving different doses of buserelin. Pre-treatment LH concentrations were not statistically different ($P=0.77$) between doses, for horses receiving two doses on one day. Significant increases in LH concentrations occurred in colts that received 5, 10 and 40 μg buserelin, but not in those receiving 0.5 or 1 μg (Figure 5.1). Thus, 30 min after buserelin administration, concentrations in colts that received 40 μg buserelin were significantly ($P<0.05$) higher (4.07 ± 0.83 ng/ml) than pre-treatment values (0.90 ± 0.17 ng/ml) and higher than in animals which had received other doses of buserelin (10 μg: 2.30 ± 0.24 ng/nl, 5 μg: 2.64 ± 0.94 ng/ml). Peak concentrations were also higher in animals receiving 40μg buserelin than in those receiving 0.5 or 1 μg. The mean areas under the curve of LH responses were likewise significantly ($P<0.05$) greater in colts that received 40 μg buserelin (6.15 ± 1.69 ng/ml.h) than in those receiving 0.5 μg (0.33 ± 0.23 ng/ml.h) or 1 μg (1.51 ± 0.86 ng/ml.h). Values in animals receiving 5 μg (3.27 ± 1.3 ng/ml.h) or 10 μg (3.35 ± 0.45 ng/ml.h) were intermediate between other groups and significantly different from neither (Figure 5.2).

Significant increases in testosterone concentrations occurred in colts that received 1, 5, 10 and 40 μg buserelin, but not in those receiving 0.5 μg. Thus, 180 min after buserelin administration, testosterone concentrations in colts that received 10 and 40 μg buserelin were significantly ($P<0.05$) higher (0.53 ± 0.09 and 0.65 ± 0.06 ng/ml respectively) than pre-treatment values (0.22 ± 0.04 and 0.28 ± 0.08 ng/ml). Also, 120 min after buserelin administration, testosterone concentration in colts that received 1 buserelin were significantly ($P<0.05$) higher (0.60 ± 0.09 ng/ml) than base concentrations (0.36 ±0.06 ng/ml). Peak concentrations of testosterone were not significantly different between the doses. Dose response, defined in terms of the area under the curve of testosterone, did not reach statistical significance ($P=0.067$) (Figure 5.2).

The percentage of horses that responded to buserelin was 33.3, and 87.5 % for 0.5 and 5 μg respectively, while 100% of horses challenged responded to 1, 10 and 40 μg based on LH concentration. Based on testosterone response, 33.3, 60, 62.5, 75 and 100% of horses challenged responded to 0.5, 1, 5, 10 and 40 μg challenges respectively (Table 5.1).

All horses that exhibited a significant LH and testosterone response to the lower of the two allocated doses also exhibited a significant response to the higher dose, except for one horse that failed to show a significant testosterone response to the second dose (5 μg), while having a significant LH response for both doses and a significant testosterone response to the first dose (1 μg).

Table 5.1 The number of horses showing significant LH and testosterone responses to 5 doses of buserelin. A significant response was defined as the peak concentration exceeding the 95% confidence limits (+2SD) of the average baseline concentration.

Dose (μg)	n	Response	
		LH	Testosterone
0.5	6	3	2
1	5	5	4
5	8	6	4
10	4	4	3
40	3	3	3

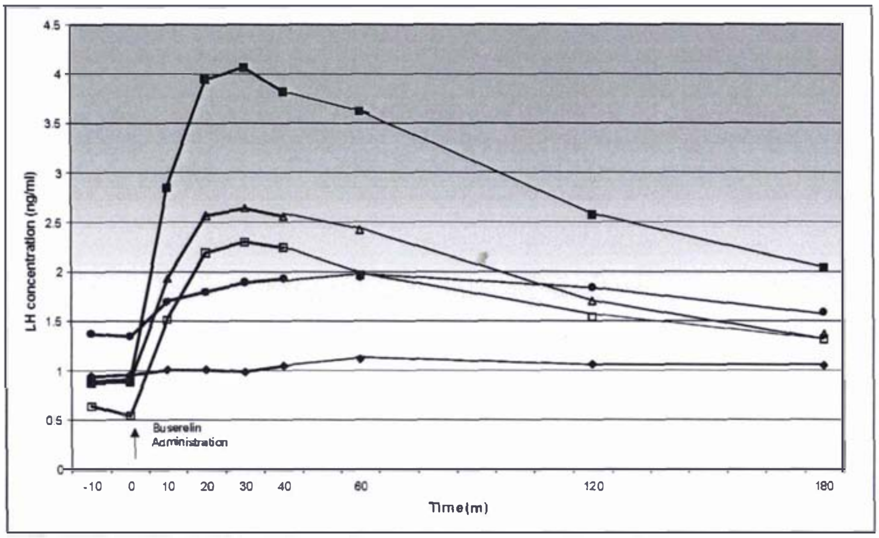

Figure 5.1 Mean LH concentrations (ng/ml) in yearling colts before and after stimulation with 5 doses of buserelin. (◆ = 0.5 µg, ● = 1 µg, △ = 5 µg, □= 10 µg, and ■ = 40 µg). SED = 0.97

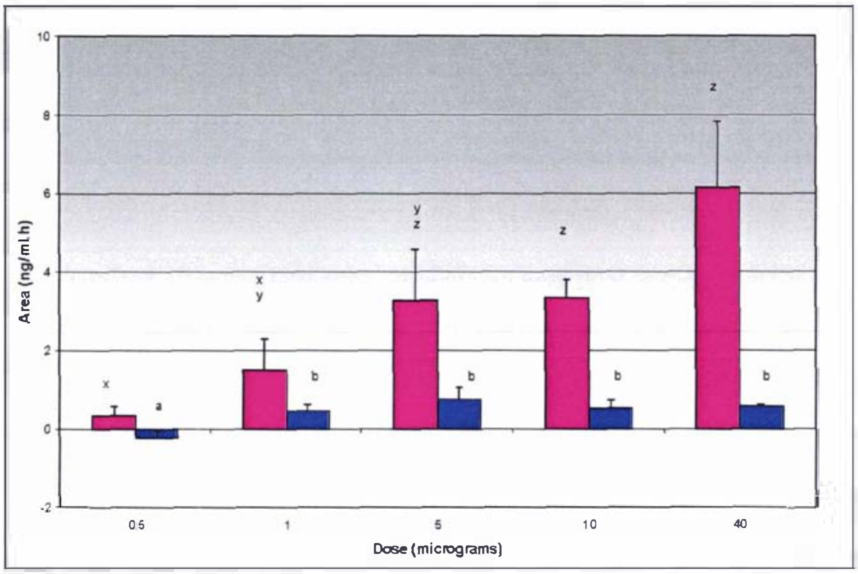

Figure 5.2 Mean ± SEM AUC (ng/ml.h) of LH (■) and testosterone (■) to five doses of buserelin.

5.5 Discussion

Previous studies have demonstrated that high doses (400 µg-1 mg) of GnRH and analogues stimulate a significant and supra-physiological LH response in the adult male horse (Burns and Douglas, 1984; Evans and Finley, 1990). The present study has shown that buserelin causes a dose-related release of LH in pubertal colts as three relatively low doses (5, 10 and 40 µg) induced significant LH and testosterone responses.

As no direct comparisons have been made between the effects of buserelin and GnRH upon LH and testosterone in the male horse, it is difficult to compare results from different trials. In the work of Clay et al. (1989) and Naden et al. (1990c) maximal LH responses were claimed with doses of 2 µg/kg body weight and 5 µg/kg body weight in mares and pre-pubertal colts respectively. The doses used in the present experiment are much lower as the highest dose of 40 µg is equivalent to approximately 0.1 µg/kg body weight. Lower doses of 25 µg GnRH and 40 µg buserelin have also been shown to demonstrate a significant LH response in the stallion (Seamans et al., 1991; Parlevliet et al., 2001).

Peak concentrations of LH at the highest dose (40 µg) were considerably lower (4.07 ± 0.83 ng/ml) than previously reported (15 ng/ml) for male horses of similar age (Naden et al., 1990c). However, that study administered a very large dose of GnRH (5 µg /kg body weight), claiming to stimulate a maximal release of LH in pre-pubertal stallions. In the present study, peak concentrations of LH increased with dose, albeit not significantly, indicating the presence of large individual animal variation in the response to buserelin administration.

In terms of dose-response, the highest dose (40 µg) induced a significantly greater LH response than both 0.5 and 1 µg buserelin ($P<0.05$). The dose-response effects on testosterone were nearing significance ($P=0.067$). This indicates that the testes may only have a limited capacity to respond to increasing concentrations of LH with increasing testosterone release and that a higher dose of buserelin may be needed to stimulate the pituitary to produce LH which in turn stimulates the testes effectively.

The percentage of horses that responded to the buserelin increased with increasing dose, with only the highest dose (40 μg), eliciting LH and testosterone responses in all colts. Fifteen of the 17 colts challenged with 1, 5, or 10 μg buserelin showed a significant LH response but only 11 of the 17 colts exhibited a testosterone response to those doses. In contrast, only 3 of the 6 colts challenged with 0.5 μg produced a significant LH response. These results suggest that a threshold must be reached for each horse to elicit a LH response to buserelin and that the LH response to buserelin is either all or nothing. Increasing dose response of LH to GnRH was observed in male deer (Fennessy et al., 1988) and lambs (Tyrrell et al., 1980), but no comment has been made on a threshold level of GnRH to elicit a response in the horse.

All horses that exhibited a significant LH and testosterone response to the first dose also exhibited a significant response to the second dose, except for one, indicating that the effect was the dose and not the horse.

Of the 21 colts to respond to the buserelin challenge with an increase in LH, 16 also exhibited a testosterone response, which may indicate that the pituitary LH response to the buserelin may not have been great enough to stimulate a response in the testes. Such a pattern of a low testosterone response coupled with a high LH response to GnRH, has been observed in adult male deer during antler growth in early spring (Fennessy et al., 1988), a known period of testicular regression accompanied by low plasma testosterone concentrations. Seasonal fluctuations in testosterone response in the stallion to exogenous GnRH have been reported, with maximum LH and testosterone responses occurring in mid summer, in conjunction with maximum basal plasma concentrations of these hormones (Clay et al., 1989).

Lack of a significant testosterone response to GnRH administration has been reported in pre-pubertal bulls (Mongkonpunya et al., 1975) and boys (Roth et al., 1973). This observation was thought to be associated with an inability of the Leydig cells to respond to gonadotrophins, either through a limited number of, or availability of LH receptor sites as opposed to a low level of circulating LH (Roth et al., 1973; Sharpe et al., 1973).

In summary, the results have shown that peri-pubertal colts elicit a dose response of LH to buserelin, but these responses were of an "all or nothing" manner where each horse either showed a maximal LH response to the dose or failed to exhibit any increase in concentration. Some colts that elicited a significant LH response did not elicit a significant testosterone response. It is therefore concluded that a dose of 10 μg buserelin is appropriate for testing the responsiveness of the pituitary to GnRH in young colts.

Chapter 6

Influence of season of birth on the pituitary and testicular responses to GnRH challenge in Thoroughbred colts between 4 and 14 months of age

6.1 Abstract

GnRH challenges were carried out every 8 weeks from 4 months of age on 11 Thoroughbred colts born in spring (n=6) or autumn (n=5). In the prepubertal colts, LH secretion followed a seasonal pattern, with high baseline and peak concentrations in the spring and summer and low concentrations in the winter. Testosterone concentrations were undetectable until the spring in both groups of colts. Hence the autumn-born colts were younger than spring-born colts when a testosterone response was first observed. Autumn-born colts weighed on average 300 kg at the first detectable testosterone response in October. Spring-born colts had reached this weight in the winter months before day length had increased, and did not exhibit a significant testosterone response until the spring. It is proposed that the horse must achieve a threshold body weight simultaneously with stimulatory photoperiod for onset of puberty to occur.

6.2 Introduction

Stallions exhibit seasonal patterns of reproductive hormonal activity mediated by changes in the secretion of LH and testosterone (Thompson et al., 1977; Harris et al., 1983; Irvine et al., 1985; Thompson et al., 1985; Irvine et al., 1986; Clay et al., 1988).

In the normal responsive male, secretion of GnRH causes immediate release of LH from the anterior pituitary gland, which in turn causes testosterone release from the testes. Evaluation of the endocrine function of the hypothalamic-pituitary-gonadal system can be achieved using a GnRH challenge test and measuring the response of LH and testosterone. GnRH challenges can be used to assess changes in the hypothalamic-pituitary-gonadal axis during pubertal development and have been performed in rams (Wilson and Lapwood, 1979; Tyrrell et al., 1980), bulls (Wildeus et al., 1984; Rutter et al., 1991), stags (Suttie et al., 1991; Adam et al., 1996), boars (Andersson et al., 1998), and stallions (Naden et al., 1990c). GnRH challenges have also been used to study the seasonal and photoperiodic effects on the hypothalamic-pituitary-gonadal axis in the stag (Fennessy et al., 1988), mare (Alexander and Irvine, 1986; Silvia et al., 1987; Nequin et al., 1990) and stallion (Clay et al., 1989).

Prepubertal gonadotrophin and testosterone patterns have been examined in colts (Wesson and Ginther, 1980; Johnson and Thompson, 1983; Naden et al., 1990a and 1990c), but the influence of season and age on the maturity of the equine hypothalamic-pituitary-gonadal axis at puberty is less well understood. The effect of season of birth on pubertal changes in LH and testosterone secretion, and pubertal maturation of the hypothalamic-pituitary-gonadal axis has not been described in horses. GnRH challenges were therefore used to estimate indirectly the changes in the hypothalamic-pituitary-gonadal axis in young Thoroughbred colts during the first year of life.

6.3 Materials and methods

6.3.1 Animals

Thoroughbred colts born in either spring (mean birth-date 21 October 2001; n=6) or autumn (mean birth-date 17 February 2002; n=5) were bred and reared at the AgResearch Flock House Thoroughbred Stud, Bulls, New Zealand, close to the 40th parallel. Mares and foals were raised on tall fescue dominant pasture and foals were weaned at 6 - 6.5 months of age. Spring-born colts were weaned on 23 April 2002, and autumn-born colts were weaned on 31 August 2002. Horses were weighed, condition scored and blood-sampled fortnightly, and were also subjected to GnRH challenges (see below). Horses were observed daily for general health and more closely during monthly clinical examination.

6.3.2 GnRH challenge

The colts were challenged with 10 μg the GnRH analogue buserelin (Receptal®, Hoechst Roussel, Intervet, Auckland, New Zealand) at 8-weekly intervals from 4 months of age. The dates of GnRH challenge were 21 February 2002, 20 April 2002 (spring-born colts) and 20-21 June 2002, 22-23 August 2002, 24-25 October 2002, 19-20 December 2002 (all colts) (Figure 6.1).

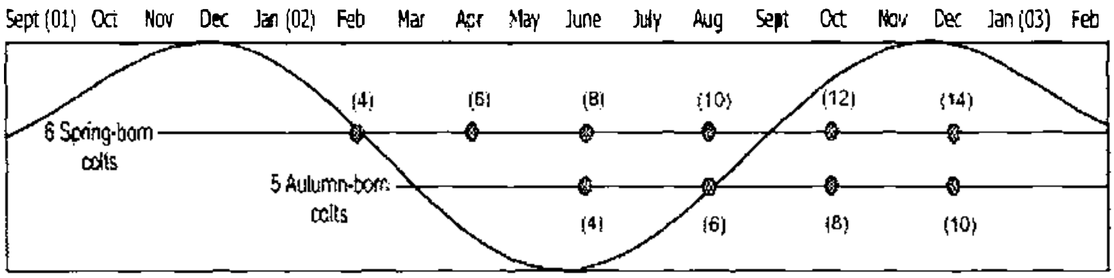

Figure 6.1 Timeline to show the date and mean age (months) at GnRH challenge (●) of 6 spring-born and 5 autumn-born colts.

Two blood samples (10ml lithium-heparin vacutainer tube) were collected by jugular venepuncture, 10 min apart, the second only seconds prior to GnRH administration. At time zero, 10 μg of GnRH analogue was administered intravenously to each animal. Subsequently 10 ml blood samples were collected at 30, 60, 120, 150 and 180 min. Samples were immediately placed on ice and transferred to the laboratory, where plasma was separated by centrifugation (within 2 h of the completion of each challenge) at 1000g for 10 min and stored at -20°C in three aliquots for later assay.

6.3.3 Hormone assay

Luteinising hormone (LH) was measured by radioimmunoassay (RIA) as described previously (Irvine et al., 1998; Irvine et al., 2000) using equine LH (Papkoff e263B) for standards and iodination, monoclonal antiserum against the β-subunit of bovine LH (AFP #518137), and separation of free and bound hormone with goat anti-mouse gamma globulin (Calbiochem-Novabiochem Pty, Croydon, Victoria, Australia). Inter-assay and intra-assay coefficients of variation for LH assays were 7.0% and 8.0% respectively. The detection limit for LH was 0.36 ng/ml.

Testosterone was measured using a coated-tube RIA kit assay (Testo-ctk, P3093, DiaSorin, Italy), carried out in accordance to the manufacturer's instructions. The minimum detectable level of testosterone was 0.05 ng/ml at 95% confidence limit. Inter-assay and intra-assay coefficients of variation for testosterone assays were 13.0% and 13.2% respectively.

All assays were performed in duplicate and all samples from a given challenge were assayed together.

6.3.4 Data analysis

Baseline hormone concentrations were defined as the average of the two pre-treatment samples. Peak LH and peak testosterone concentrations were the highest concentration of hormone achieved at any time after GnRH administration. The magnitude of the response to GnRH challenge was measured by calculating the area under the curve (AUC) from 0 to 180 min. A significant response to each dose of GnRH was identified using criteria derived from those of Goodman and Karsch (1980). Briefly, the peak hormone concentration had to exceed the 95% confidence limits (± 2 standard deviations) of the average baseline concentration, and be greater than 0.1 ng/ml. Results are expressed as the mean ± SEM.

6.4 Results

6.4.1 LH concentration

Regardless of season of birth, there was seasonal fluctuation in the plasma LH baseline and peak concentrations in all colts ($P<0.05$). Peak LH concentration in each colt occurred at 30 minutes after administration of GnRH. Both spring and autumn-born colts exhibited lowest mean baseline LH and lowest mean peak LH concentrations in response to GnRH in June, at 8 and 4 months of age respectively. After June, mean peak LH concentration was greatest in October when spring-born colts were 12 months of age and autumn-born colts were 8 months of age (Figure 6.2).

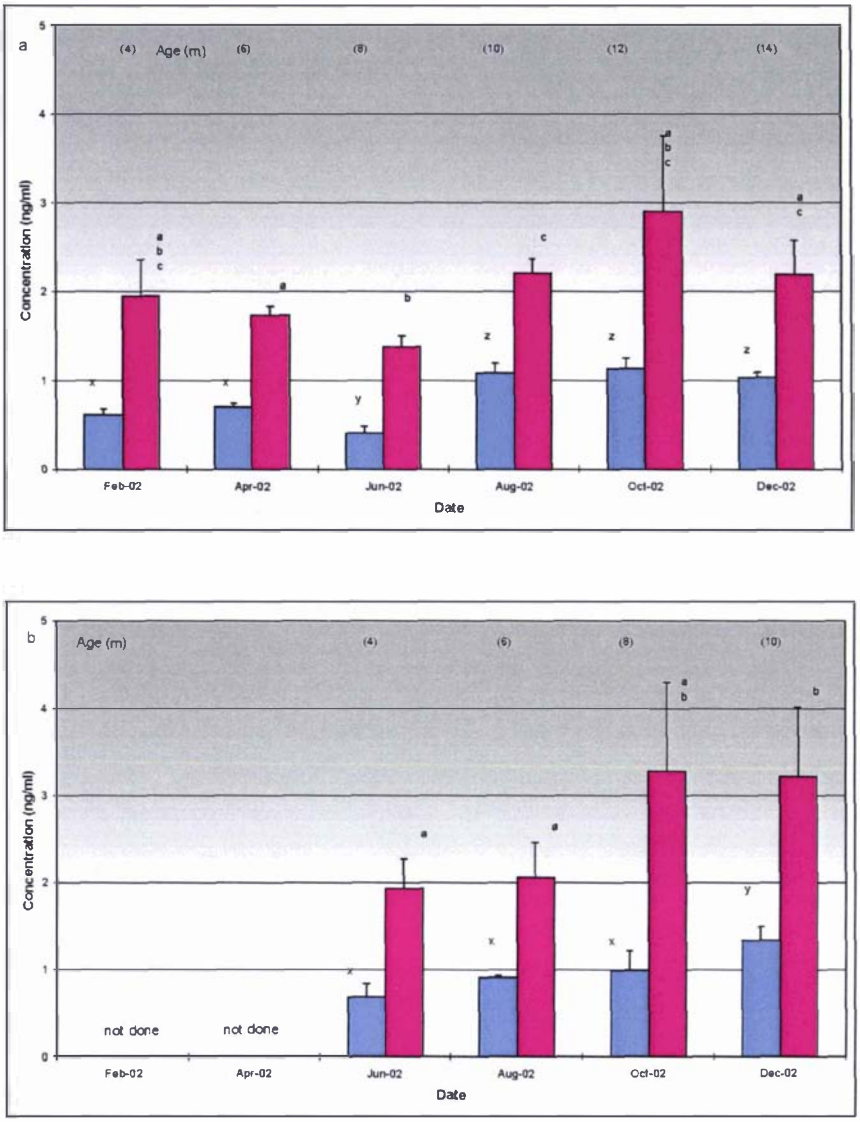

Figure 6.2 Mean ± SEM baseline (■) and peak (■) concentrations of Luteinising Hormone (LH) in response to GnRH in (a) 6 spring and (b) 5 autumn-born colts in relation to date and mean age (in brackets).

6.4.2 Testosterone concentration

In both spring and autumn-born colts, mean peak testosterone concentration increased and decreased in synchrony with the seasonal fluctuation of mean baseline testosterone concentrations. Testosterone baseline concentrations were low (<0.05 ng/ml) in the spring-born colts from 4 to 8 months of age (February to June) and at 10 months of age (August) were > 0.2 ng/ml. At 14 months of age (December), baseline testosterone concentrations in spring-born colts were 0.8 ng/ml.

In the autumn-born colts, mean baseline concentrations were low at 4 and 6 months of age (June and August), and they increased to 0.3 ng/ml at 8 months of age (Figure 6.3).

The first significant increase in mean baseline and mean peak testosterone response to GnRH occurred in August in the spring-born colts and October in the autumn-born colts. Autumn-born colts were approximately 4 months younger and in August weighed 270 kg compared to 350 kg in the spring-born colts. In October, autumn and spring-born colts weighed 298 and 380 kg respectively.

Time of peak testosterone differed with each individual colt and ranged from 120-180 minutes after GnRH administration. Peak values used in the analysis were those of each individual colt. Mean peak testosterone response followed the same trend as baseline concentrations. Spring-born colts had a significantly greater ($p<0.05$) mean peak testosterone response to GnRH challenge than autumn-born colts at the same date (1.06 ± 0.11 ng/ml vs. 0.51 ± 0.05 ng/ml in October, and 1.29 ± 0.16 ng/ml vs. 0.54 ± 0.08 ng/ml in December).

6.4.3 LH and testosterone response as area under the curve (AUC)

Mean area under the LH curve was not significantly different ($p>0.05$) between the spring and autumn-born colts at the same date. Greatest mean AUC for LH and testosterone, in both the spring and autumn-born colts, occurred in October, at 12 and 8 months of age respectively (Figure 6.4). However, the mean AUC for testosterone was significantly ($p<0.05$) greater in the spring-born colts than in the autumn-born colts in October (0.98 ± 0.21 ng/ml.hour vs. 0.36 ± 0.10 ng/ml.hour).

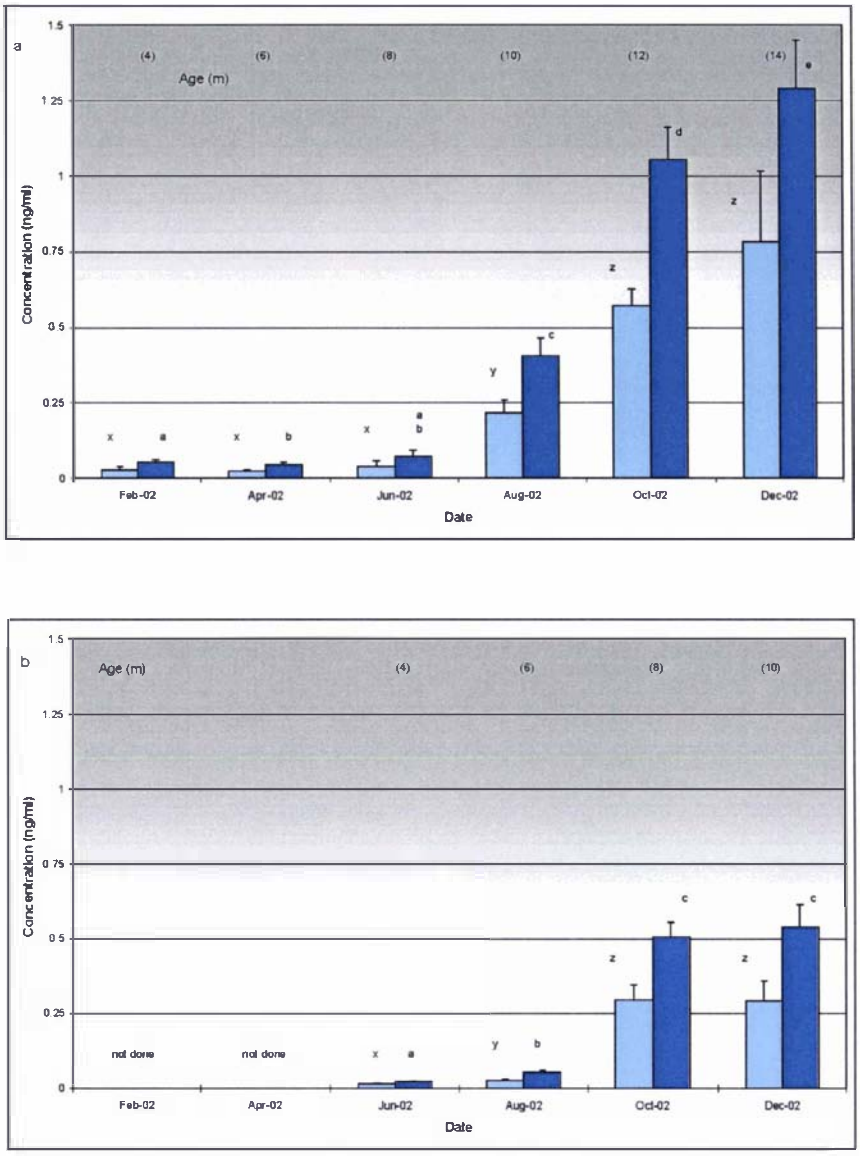

Figure 6.3 Mean ± SEM baseline (🟦) and peak (🟦) concentrations of testosterone in response to GnRH in (a) 6 spring and (b) 5 autumn-born colts in relation to date and mean age (in brackets).

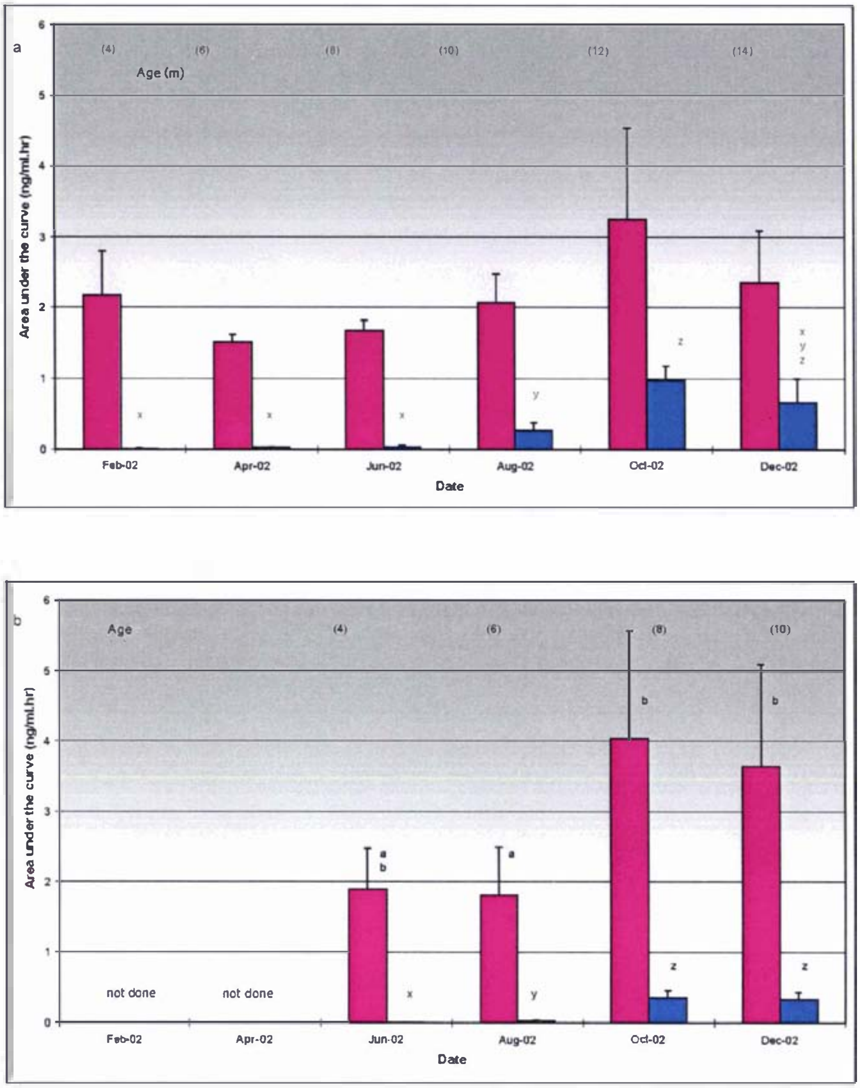

Figure 6.4 Mean ± SEM area under the curve for LH (■) and testosterone (■) in response to GnRH in (a) 6 spring and (b) 5 autumn-born colts.

6.4.4 LH/testosterone ratio

With increasing age in both spring and autumn-born colts, the ratio of LH and testosterone (both baseline and peak) decreased; that is, progressively lower concentrations of LH were associated with successively higher concentrations of testosterone (Table 6.1). In spring-born colts, mean peak testosterone was significantly greater in December than October, but mean peak LH was greatest in October and had decreased in December.

Table 6.1 Mean ± SEM baseline and peak LH : T ratio (see text) in response to a 10μg GnRH challenge in Thoroughbred colts born in spring and autumn.

	Spring-born colts (6)		**Autumn-born colts (5)**	
	Baseline	Peak	Baseline	Peak
February	39.1 ± 12.4	45.1 ±11.7	-	-
April	37.4 ± 8.0	61.5 ± 24.8	-	-
June	18.7 ± 4.5	29.4 ± 9.8	49.5 ± 10.1	91 ± 18.9
August	6.2 ± 1.5	5.9 ± 0.8	44.5 ± 11.4	42.5 ± 12.4
October	2.1 ± 0.4	2.9 ± 1.0	3.5 ± 0.7	6.7 ± 2.2
December	2.1 ± 0.7	1.8 ± 0.3	5.6 ± 1.3	6.1 ± 1.0

6.4.5 Responders/non-responders

Testosterone responses were detected in two of six spring-born colts in June, three of six in August and all six colts in October and December. The two exhibiting significant testosterone responses in the June challenge were 263 and 252 days old and weighed 371 and 318 kg, older and heavier than the four colts that did not (which were on average 235 ± 5.6 days of age and weighed 303 ± 4.1 kg). No autumn-born colts responded in April and June, but all exhibited significant testosterone responses in October and December. The proportion of colts exhibiting significant testosterone responses to GnRH challenge is shown in Table 6.2. Body weight at the GnRH challenge prior to, and at, the first significant T response is shown in Table 6.3.

Table 6.2 Number and proportion of spring and autumn-born colts exhibiting significant testosterone responses to challenge with 10 μg GnRH. Criteria of inclusion were a testosterone response greater than 2 standard deviations above the baseline concentration, provided the concentration was greater than 1ng/ml.

Challenge date	Number and proportion (%) of colts exhibiting T response	
	Spring-born colts (6)	Autumn-born colts (5)
21 February	0	-
20 April	0	-
21 June	2 (33)	0
22 August	3 (50)	0
24 October	6 (100)	5 (100)
19 December	6 (100)	5 (100)

Table 6.3 Month and body weights (kg) of spring (S) and autumn-born (A) colts at the GnRH challenge immediately prior to, and at the challenge which produced the first significant testosterone response

Horse ID	Challenge prior to the observation of the first testosterone response		Challenge resulting in the first testosterone response	
	Month	Body weight	Month	Body weight
S2	April	328	June	371
S4	April	384	June	318
S6	June	292	August	327
S7	August	338	October	369
S9	August	334	October	356
S10	August	342	October	374
A12	August	272	October	290
A13	August	281	October	308
A14	August	258	October	284
A15	August	252	October	282
A16	August	287	October	327

6.5 Discussion

The pituitary and testicular endocrine responses associated with puberty in Thoroughbred colts were assessed and it was determined whether these responses differed with time, and with season of birth.

In the spring-born colts, LH basal concentration, peak LH, and the LH AUC were related to photoperiod, with the lowest values in June, around the winter solstice. Significantly higher concentrations of LH (baseline, peak and AUC) were observed in spring and summer, i.e. in and after August. This pattern of LH secretion is not inconsistent with changes in baseline LH concentration observed from monthly blood samples from mature and peri-pubertal stallions (Harris et al., 1983; Thompson et al., 1985; Clay et al., 1988; Naden et al., 1990a) . Clay et al. (1989) found that both peak LH and peak testosterone exhibit a seasonal cycle in synchrony with that seen for baseline concentrations of each hormone.

Baseline LH concentrations increased in August and October in parallel with the peak LH response to GnRH, indicating increased pituitary sensitivity to GnRH with increasing day length in both spring and autumn-born colts. Increased LH concentrations (both baseline and peak response) as day length increases may be associated with a reduction and replenishment of pituitary LH in the spring (Clay et al., 1989), with the primary effect of increasing day-length being stimulation, and/or a removal of an inhibition, of LH release.

Mean peak LH and AUC were greatest in October and decreased in December. However, baseline and peak testosterone concentration increased in October in both spring and autumn-born colts and remained elevated in December. If the relationship between LH and testosterone were that of a negative feedback, in which testosterone exerts a negative feedback on LH release that changes with time of year and/or age (Irvine et al., 1986; Rutter et al., 1991), then one would expect this to be evident in the LH/T ratio. Mean LH/T ratio did exactly this and decreased with increasing photoperiod and age in both spring and autumn-born colts demonstrating that similar LH concentrations produced successively higher testosterone concentrations (Table

6.1). On an individual basis, ratios were as high as 81 and 78 in two colts at 4 months of age (In February in 1 spring-born colt and in June in 1 autumn-born colt). Ratios were as low as 0.65 and 2.4 in two colts in December (1 spring-born colt at 14 months of age, and 1 Autumn-born colt at 10 months).

Decreased mean baseline and peak LH accompanied by increased mean baseline and peak testosterone seen in these colts supports the theory of increasing Leydig cell responsiveness to similar or decreasing concentrations of LH with onset of puberty in bulls and rams (Mongkonpunya et al., 1975; Olster and Foster, 1986). However, this observation could also be indicative of decreasing pituitary sensitivity to GnRH and increased sensitivity to the negative steroidal feedback of testosterone reported in peri-pubertal animals such as the deer (Fennessy et al., 1988), ram (Lincoln, 1977), and bull (D'Occhio and Setchell, 1984). Moreover, seasonal changes in sensitivity to negative feedback of gonadal steroids have been reported in sheep (Pelletier and Ortavant, 1975; Legan and Karsch, 1980). In the stallion, Irvine et al. (1986) and Clay et al. (1989) suggested that testosterone blocks LH secretion in the non-breeding season, an effect which is removed when day length increases or as Irvine et al. (1986) suggested, switched to testosterone providing a positive stimulus for LH release. However, the mechanisms proposed by Irvine et al. (1986) and Clay et al. (1989) could not be regulating the seasonal LH secretion in the pre-pubertal colts in the present study because LH followed seasonal changes in secretion before testosterone was detectable above 0.05 ng/ml.

Mean baseline and peak testosterone concentrations were lowest in both spring and autumn-born colts until day length increased after mid-winter. Low baseline and peak testosterone concentrations were observed in young (4 to 6 month-old) colts, but baseline and peak testosterone was significantly greater in autumn-born colts than in spring-born colts at 8 months of age. Baseline and peak testosterone concentrations remained low in spring-born colts until 10-12 months of age (early spring). Failure of GnRH induced LH to stimulate testosterone in pre-pubertal bulls (Mongkonpunya et al., 1975) and boys (Roth et al., 1973) was attributed to an inability of the Leydig cells to respond to gonadotrophins. However in the colts in the present study, Leydig cell responsiveness to gonadotrophins must have been established at an early age because the baseline and peak testosterone concentration in the autumn-born colts' increased at 8 months of age. Testosterone secretion must have therefore been inhibited in spring-born colts until 10-12 months of age when either a stimulus and/or removal of an inhibition, occurs.

Naden et al. (1990a and 1990c) reported a rise in LH concentration in pre-pubertal colts between 9-10 months of age, which was not accompanied by an increase in testosterone. A second increase in LH occurred approximately one year later, after a period of low LH levels and coinciding with increased testosterone concentrations. Both periods of increased LH secretion occurred in April-May (Northern Hemisphere spring). It was postulated that the Leydig cells were unable to respond to the initial LH increase, and it was only when the colts were older (18-20 months) that the spring-increase in LH was able to stimulate a testosterone response and puberty was achieved (Naden et al., 1990a). In the autumn-born colts in the present study a pubertal increase in testosterone occurred at 8 months of age in the spring. It appears from the present study that seasonal factors, presumably day-length and other environmental factors such as temperature and management exert control over the secretion of gonadotrophins and testosterone. LH concentrations were high in the spring and summer and low in the winter, regardless of age and weight. Testosterone concentrations were low until seasonal cues and probably a threshold weight were permissive for secretion in the spring, presumably after a period of Leydig cell maturation in the first few months of life.

In June, the two heaviest and oldest Spring-born colts exhibited a significant testosterone response to GnRH challenge, but it was not until October that all spring-born colts exhibited significant testosterone responses. Testosterone responses were not detected in autumn-born colts in June or August, but all colts exhibited significant testosterone responses in October and December (Table 6.2). The spring-born colts that had testosterone responses in June and August may have reached a threshold weight earlier than the colts that did not. This threshold weight may be permissive for Leydig cell function (Olster and Foster, 1986). If horses are similar to red deer they must reach a threshold weight at a particular time of year for puberty to occur (Adam et al., 1992). Body weight at the GnRH challenge prior to, and at, the first significant testosterone response in each colt is shown in Table 6.3. GnRH challenges were 8 weekly therefore one must assume that colts were capable of exhibiting a testosterone response at any time between the first challenge that a response was observed and the challenge prior to it. Given this, the weight that spring and autumn-born colts exhibited a testosterone response was no less than 284 kg and 252 kg respectively – a difference of 32 kg. All spring-born colts had reached 252 kg by April, which may indicate that spring-born colts achieved the threshold weight in the autumn but photoperiod was not permissive for a testosterone response to occur. This would further support the idea of the integration between a threshold weight and stimulatory photoperiod for the onset of puberty to occur.

Secretion of testosterone has been shown to be photo-inducible (Cox et al., 1988; Clay et al., 1989). Basal testosterone concentration and the response to GnRH were induced by changing the photoperiodic regimen. Stallions housed in short day-length (8 hours light: 16 hours dark) for 20 weeks from the summer solstice and then exposed to long days (8 hours dark: 16 hours light) from near the winter solstice showed maximal baseline and testosterone response to GnRH four months earlier than control stallions housed in natural day-length (Clay et al., 1989).

The relationships between LH, testosterone response to GnRH and the seasonal change in day length in this study was very similar to that observed in mature stallions. However, in mature stallions, seasonal change in testosterone secretion was synchronous with that of LH (Johnson and Thompson, 1983; Harris et al., 1983; Clay et al., 1988; Clay et al., 1989). A testosterone response was not observed in the pre-pubertal colts in this study until spring, when spring-born horses were 10-12 months of age and autumn-born horses were 8 months. This is consistent with the hypothesis that photoperiod controls the seasonal nature of testosterone secretion (Clay et al., 1989), and probably controls the onset of puberty.

There are two current theories on the mechanisms controlling the onset of puberty. The "missing link" concept assumes that the endocrine system of the pubertal animal may be mature but one or more specific components are inhibited from functioning prior to puberty (Squires, 1993). The present study supports this theory, at least early in life. In the horses from the present study, LH was synthesised and secreted during the pre-pubertal period, without a testicular response, indicating that some other component of the hypothalamic-pituitary-gonadal axis is inhibitory to puberty onset. It is most likely that the initial factor inhibiting onset of puberty was relative immaturity (of which body weight is an index) and Leydig cell immaturity. In the spring-born colts, secretion of LH was not accompanied by testosterone release until horses were 10 months old. However, the results from the autumn-born colts in the present study indicate that the Leydig cells are capable of producing testosterone at 8 months of age provided the photoperiod is stimulatory. Autumn-born horses were approximately 50 kg lighter and 2-4 months younger than spring-born horses at the first significant testosterone response. This indicates that the threshold weight that allows pubertal onset in horses must be less than what is normally observed in spring-born horses at puberty.

The "gonadostat" theory states that before puberty, the hypothalamic-pituitary-gonadal axis is extremely sensitive to the negative feedback effect of gonadal steroids resulting in low tonic LH secretion. During puberty, there is a decrease in the responsiveness to the inhibitory actions of gonadal steroids, resulting in increased tonic LH secretion sufficient to initiate reproductive activity (Olster and Foster, 1986). The results from this study are not in agreement with the gonadostat theory, at least up until 8 months of age, since LH secretion was observed without a testicular response until mid spring. However, as the time of pubertal onset approaches, stimulatory conditions including increasing day length and greater body weight, affect the removal of, or decrease in, sensitivity to steroid negative feedback, culminating in puberty. Therefore the gonadostat theory may be true closer to the time of pubertal onset, where there is removal of a negative feedback mechanism, or decrease in sensitivity to it, so that other permissive conditions allow puberty to be initiated.

In conclusion, GnRH challenge of pre-pubertal colts has provided insights into the changes in the hypothalamic-pituitary axis during the pubertal phase of development in young Thoroughbred colts. LH secretion in pre-pubertal colts follows a seasonal pattern, with high baseline concentrations and peak response to GnRH in the spring and summer and low concentrations in the winter. The onset of pubertal testosterone secretion occurred in the spring, regardless of season of birth. Therefore autumn-born colts were significantly younger and lighter than spring-born colts at pubertal onset. Autumn-born colts weighed approximately 300 kg in October when they first exhibited significant testosterone increases. The spring-born colts reached this weight in May to June at a time when photoperiodic cues are inhibitory for onset of puberty, and did not show increases in testosterone concentration until the spring when they weighed on average 350 kg. Autumn-born colts reached a threshold weight for puberty at the same time as increasing day length in the spring (after the spring equinox i.e. long-days) and therefore reached puberty at a younger age and at a lighter body weight than spring-born colts.

Chapter 7

General Discussion

7.1 Introduction

For the New Zealand bloodstock industries to be competitive in Northern Hemisphere markets, and to reduce economic loss through progressively later foaling dates, horses need to be bred out of season. There is little information on the growth of pasture-raised horses, because extensive farm systems do not lend themselves to interventions such as regular weighing. Also, intake of pasture and nutrients are difficult to measure, and any measurement disturbs the system and the animals. Most study has therefore been performed on grain-fed horses raised in the Northern Hemisphere (Green, 1969; MacCarthy and Mitchell, 1974; Hintz et al., 1979; Thompson, 1995; Pagan et al., 1996; Jelan et al., 1996). In addition, study of reproductive development in horses has been limited, and factors associated with the onset of puberty in the young horse are unclear. Lastly, very little is known about the growth and development of horses born out of season.

The aim of this thesis was to examine the relationship between body weight, daily live weight gains, and the onset of puberty in pasture-raised yearling Thoroughbred horses grown under typical New Zealand stud farm conditions. Data from these studies were then compared to those from foals born in the Southern Hemisphere autumn, i.e. at the time of the Northern Hemisphere birth season.

Growth data were collected from birth until March (approximately 17 and 13 months of age respectively) from pasture-raised Thoroughbreds born in either spring or autumn so that aspects of growth of the Thoroughbred raised in New Zealand pasture conditions could be examined (Chapters 2 and 3). Body weights of spring and autumn-born horses were similar at equivalent ages and, at the completion of the study in March, there was little difference in body weight between the two groups of horses, despite the 4-month age difference. Horses reached puberty in the spring, and therefore autumn-born horses were younger and lighter than spring-born horses at puberty onset (Chapter 4). After pilot work was carried out to establish a dose rate and sampling regimen (Chapter 5), GnRH challenges were conducted every 8 weeks from 4 months old to 14 and 10 months old in spring and autumn-born colts respectively (Chapter 6). In spring-born colts, LH concentrations were elevated in

February (summer) at 4 months of age. In both spring and autumn-born colts, LH concentrations were low in June (winter) and increased in October (spring). Testosterone response to GnRH was not detectable until August in spring-born colts (10 months of age) and October in autumn-born colts (8 months of age).

The experiments described in this thesis allow comparison of growth characteristics and endocrine data from Thoroughbred horses born in three consecutive spring foalings and one autumn foaling season, and managed similarly on the same property between birth and a mean age of 13 – 17 months. The series of studies relied on accurate body weight and endocrine assay data. Body weight data were collected fortnightly, and prior to each weighing session the electronic scales were checked with a known weight to ensure accuracy. Horses were weighed at a similar time at each weighing so that variances in gut fill would be minimised.

Differences between spring and autumn-born horses' data sets are presented in ANOVA summary tables in the relevant chapters, and significance reported at the level of 5% or below. The 95% confidence intervals were calculated for all the spring and autumn-born body-weight data so that the level of variance within a sampling group could be observed (see appendix 2). The 95% confidence intervals for the autumn-born horses were approximately half those of the spring-born horses; on average 14% and 7% for spring and autumn-born fillies respectively, and 10% and 6% for spring and autumn-born colts respectively. The means ± 95% confidence intervals for the spring and autumn-born growth data clearly indicate that the margin of error was low, despite having only 7 autumn-born horses. It is therefore assumed that the same results would have been attained had numbers been greater.

The low numbers of horses born in autumn was due to misadventure not design. Three of twelve mares allocated to the autumn foaling trial aborted during gestation and one foal died within 24 hours of birth, leaving only three autumn-born fillies available for study. Furthermore, the youngest of these had severe congenital limb deformities and was therefore not solely pasture-raised. This filly did not reach puberty during the study, and her growth data were therefore not included in the analysis. Time and financial constraints prohibited subsequent seasons of autumn-born foals being bred and studied.

This general discussion addresses aspects of growth observed in horses born in spring and autumn. The date, body weight and age at puberty are then discussed, and relationships between growth and puberty are suggested. Possible mechanisms behind puberty onset are discussed, and a link between growth and reproductive development supported. It is concluded that for the onset of puberty, a threshold body weight must be attained at some stage during the time that day length is increasing. Finally, the implications of the research to the Thoroughbred industry are discussed, and areas for further research into the mechanisms of growth and pubertal development in horses born out of season are suggested.

7.2 Aspects of growth in young Thoroughbreds

These studies showed that New Zealand pasture-raised Thoroughbreds born in spring and autumn achieved body weights and weight gains comparable to horses in other published studies, including those raised in the Northern Hemisphere and fed supplementary grain (Hintz et al., 1979; Thompson, 1995; Pagan et al., 1996; Jelan et al., 1996). This may indicate that grain supplementation is not necessary to achieve an acceptable body weight for yearling sale.

Birth weight had a significant ongoing influence on body weight. Foals with higher birth weight were heavier throughout the study and, presumably, grow into heavier horses. This is important, since foals of low birth weight have inferior racing performance characteristics compared to foals that are heavier at birth (Platt, 1978). Therefore, breeders would be well advised to aim to have heavy foals at birth. Possible ways to achieve this might be supplementary feeding of the mare during gestation, ensuring the mare is healthy prior to and throughout gestation, selecting for large mares (the birth weight of a foal, independent of breed, is on average 10% of mare body weight, therefore larger mares deliver larger foals [Walton and Hammond, 1938; MacCarthy and Mitchell, 1974; Platt, 1978]), and selecting mares of optimum breeding age (mares between the ages of 7 and 11 years have foals of heavier birth weight [Hintz et al., 1979]).

It was notable that there were no differences in body weight between spring and autumn-born foals at equivalent ages. Regardless of season of birth or month of birth within a season, the monthly average daily gains (ADGs) between birth and 5 months of age were not significantly different between individual foals. The most likely explanation for this is that dam's milk provides a buffering effect from seasonal differences in pasture growth and quality. Mean daily milk yield decreases from 11-15 kg/day in the first few weeks after birth to approximately 8-10 kg/day in the 5th month of lactation (Gibbs, 1982; Frape, 1998). Milk yields of this order probably insulate the foal against the fall in amount and nutritive quality of pasture, which changes from high quality, abundant spring pasture to poorer quality pasture in the summer, and from high quality autumn pasture to winter pasture of low quality and volume.

After 5 months of age, ADG was related to season, as in the spring the monthly ADG of all horses increased by an average of 0.3 kg/d from August to October, independently of age. Conversely, in the summer (November/January), the ADG of all horses decreased by between 0.2 and 0.4 kg/d, also independent of age (Figures 2.5 and 3.3)

The increase in ADGs observed in all horses during the spring following birth (i.e. when spring-born horses were approximately 12 months of age and autumn-born horses were approximately 8 months of age) may not be desirable, since rapid growth rates may be associated with skeletal disorders (DODs) in young growing horses (Hurtig and Pool, 1996). There are many suggested causal factors of DOD, such as rapid growth rate, high body weight, intensive feeding practices and lack of exercise (Knight et al., 1985; Jeffcott, 1991). A 10% prevalence of DOD, defined as osteochondrotic (OCD) lesions in intensively managed Kentucky foals (Pagan and Jackson, 1996) indicates that these diseases are of great significance to the Thoroughbred industry. Affected Kentucky foals tended to be large at birth, grew rapidly from 3 to 8 months of age, weighed heavier than unaffected foals at weaning and were 14 kg heavier than the Kentucky average at 8 months of age. Monthly pooled ADGs from 1 to 11 months of age in Kentucky and Warmblood horses with OCD lesions were approximately 1.00 kg/d and 1.04 kg/d respectively (Pagan and Jackson, 1996; VanWeeren et al., 1999). This was greater than in the horses in the present study (0.87 kg/d). However, changes in monthly ADGs observed in the horses in the present study were of similar magnitude to those observed in normal Thoroughbreds (not showing evidence of OCD) at the same age (Pagan et al., 1996; Jelan et al., 1996). As a result body weights of the horses in the present study were similar to those of the Northern Hemisphere Thoroughbreds. Despite showing similar spring increases in ADGs and achieving similar body weights to grain-fed Thoroughbreds in Northern Hemisphere studies, no clinical signs of OCD were observed in the horses in the present study. This was probably because the serial monthly increase in ADG, beginning in September and continuing to increase to November inclusive, began respectively at 11 and 7 months of age in spring and autumn-born Thoroughbreds in the present study. This is at an age when, if it is to occur, OCD is already present, and if present is likely to be regressing (Dik et al, 1999; VanWeeren et al., 1999). OCD is reported to occur within a "window of vulnerability" in young foals, commonly between the ages of 3 and 6 months (Hurtig and Pool, 1996). Therefore, despite achieving body weights comparable to other studies, the seasonal increase in ADG observed may not affect the incidence of OCD because it occurred at an older age than suggested by Dik et al. (1999).

In addition, distal radial physitis or "blown knees", although one of the DODs, is a disease of the growth plate rather than of the joint cartilage. Unlike OCD, it appears to be almost certainly acquired in the spring/summer, most commonly in grain-fed yearling horses being prepared for sale. Distal radial physitis is most marked at 12 to 18 months of age (Lewis, 1995). The seasonal increase in ADG observed in spring in the present study might have been expected to contribute to distal radial physitis, but this disease was not seen in this or previous studies of pasture-raised Thoroughbreds on the same farm. Therefore, it appears that concentrate feeding, possibly combined with poor pasture management, might increase ADG to a greater extent than observed in the present study, and contribute to the occurrence of physitis of the distal radius. However, the nutritional effects associated with grain feeding, not just the increased ADG it may induce, could be of importance as well.

A further possible reason for the lack of clinical signs of DOD observed in the horses in the present study is perhaps because they were able to exercise freely for 24 hours a day, whereas the Kentucky horses were box stalled for up to 6-7 hours a day (Pagan and Jackson, 1996). However, exercise was shown not to affect the prevalence or severity of OCD in Warmblood horses (VanWeeren and Barneveld, 1999). It is therefore suggested that, while high body weight may be a contributing factor to DOD, unrestricted exercise and lack of grain supplementation apparently permits body weights as high or higher than recorded in other studies in pasture-raised Thoroughbred foals without severely or even obviously affecting their musculo-skeletal development (Pearce, 1998; Gee, 2003).

The spring and autumn-born horses were weaned at six months of age. In the autumn-born horses weaned in early spring, mean monthly ADG increased after weaning from 0.73 kg/d in August to 0.81 and 0.91 kg/d in September and October respectively (Figure 3.3). However, mean monthly ADG decreased in spring-born horses after weaning in the autumn from 0.78 kg/d in April to 0.59 and 0.61 kg/d in May and June respectively (Figure 3.3). This difference in post-weaning monthly ADGs (from weaning until 9 months of age) between the spring and autumn-born foals was related to seasonal factors, including availability of high quality feed, pasture growth rates, climatic conditions and, possibly, daylength. It is perhaps not surprising that ADG of spring-born foals was less after weaning, as they not only experienced the loss of dam's milk and assumed a solely pasture diet, but also the pasture was of decreasing quality and quantity. The cooler climatic conditions of the winter months may also have contributed. These points are in accordance with pasture characteristics observed at the stud, namely pasture growth rates that are typically low in the winter months (July and August) and highest in the spring and early summer (Holmes et al., 2002).

However, it is possible that the decrease in growth rate observed in the winter months was not entirely due to pasture quality and availability, since many domestic herbivores exhibit a period of seasonal inappetance and consequent low growth rates during winter (Brown et al., 1979; Simpson et al., 1984; Suttie and Kay, 1985; Webster et al., 1997; Webster et al., 1998). Winter inappetance has not been documented in horses.

By the completion of the study in March, the mean body weight of the autumn-born horses was 88% of that of the spring-born horses. However, several individual autumn-born horses had attained weights that were between 95% and 100% of the mean weight of spring-born horses. Perhaps other autumn-born horses could have achieved similar body weights to spring-born horses by March had they been fed concentrates during the summer. Individual management was not undertaken in this study because the aim of the study was to assess the growth of horses raised solely on pasture. Specific management of individual animals would increase the possibility of attaining the same body weight as spring-born horses by March, thus permitting at least some selected autumn-born horses to be marketed for late Southern Hemisphere yearling sales in March and April. Autumn-born horses could also be marketed in the Northern Hemisphere, where they would be competing against horses of the same official age. Anecdotally, New Zealand stud masters believe that late-born foals do not grow as well as spring-born horses, and therefore can not be marketed because their body weight would be sub-optimal by the time of sale. However, the present results indicate that autumn-born horses need not have the prejudice that has been attached to them in the past.

The results have a further implication in equine production. That is, that progressively later foaling mares need not necessarily lose a breeding season, because as shown above their progeny produced from breeding them in the autumn are not necessarily undesirable at sale. This would result in a production increase from mares that would not normally produce a foal until the breeding season two years later. The production and economic gain comes from the extra foals produced, as well as reduced economic loss from the retention of dry mares.

7.3 Onset of puberty in young Thoroughbreds

Spring-born fillies and colts were older and heavier than autumn-born fillies and colts at puberty. However, in all of the spring-born and in 7 of the 8 autumn-born horses, puberty occurred in October, indicating that the seasonal increase in photoperiod in the spring affects the timing of onset of puberty, providing that a threshold body weight has been attained. The lightest autumn-born horses weighed 280 kg at puberty. Assuming that Thoroughbreds have a mature body weight of 575 kg (Pagan et al., 1996), the minimum weight at the time of pubertal onset therefore appears to be approximately 50% of mature weight. On the other hand, all spring-born horses had reached 50% of their mature body weight before the winter solstice, and these horses remained reproductively immature until after the spring equinox (21 September). The onset of puberty in spring-born horses occurred between 21 September and 4 December at weights of 302-419 kg. Each autumn-born horse reached 280 kg (50% mature body weight) after the spring equinox and before the onset of puberty. The onset of puberty in autumn-born horses occurred between 15 October and 5 November at weights of 280-344 kg. Thus it is suggested that photoperiod parameters [one or more of daylength i.e. daylight>darkness after the spring equinox at 21 September, or increase in daylength before, at or around the spring equinox, or rate of photoperiod increase] all are the trigger for the onset of puberty after a threshold weight that is approximately 50% of mature body weight and is a minimum of 280 kg, has been achieved. In this respect, the onset of puberty in horses may be similar to that of sheep and deer, although horses are long-day breeders and sheep and deer are short-day breeders. Sheep and deer must reach a threshold weight of approximately 50% of mature body weight (Foster, 1981; Adam et al., 1992) in the autumn (decreasing photoperiod) for puberty to occur. It may be possible that the mechanisms which trigger the onset of puberty in horses are "inversely different" to sheep and deer and that, in horses, exposure to increasing photoperiod after the spring equinox is required to stimulate pubertal onset in animals of greater than the proposed threshold weight.

In pilot work that tested five doses of GnRH analogue (buserelin), spring-born yearling colts exhibited a dose-related LH response, and it was concluded that the

10μg dose was appropriate for use in serial stimulation challenges. Eight-weekly GnRH challenges from 4 months of age in 6 spring-born and 5 autumn-born colts (grouped for age) demonstrated that both baseline and peak LH concentrations follow a similar pattern to photoperiod. That is, in spring-born colts, LH concentrations were high in February (summer) at 4 months of age, and in both spring and autumn-born colts, LH concentrations were low in June (winter), and peaked in October (spring). This pattern of LH secretion is consistent with changes in baseline LH concentration observed from monthly blood samples from mature and peri-pubertal stallions (Harris et al., 1983; Thompson et al., 1985; Clay et al., 1988; Naden et al., 1990a).

Similar to the seasonal patterns of testosterone secretion observed in single blood samples from mature stallions i.e. high concentrations in the breeding season (spring) and low concentrations in the winter (Clay et al., 1988), baseline testosterone concentrations were low in June in both spring and autumn-born colts. Baseline testosterone concentrations indicative of puberty onset, as defined in Chapter 4, were not present in any of the colts until spring (October). Autumn-born colts exhibited increased baseline testosterone concentrations in October at 8 months of age and when they weighed between 284 and 327 kg, indicating that they responded to stimulatory photoperiods after the spring equinox. Spring-born colts in June were approximately 8 months old and of similar body weight to 8 months of autumn-born colts. But in June, photoperiod appears not to have been stimulatory for puberty to occur, and spring-born colts did not exhibit increased baseline testosterone concentrations until 12 months of age in the spring (when they weighed between 356 and 436 kg). These observations indicate that not only must a threshold body weight be attained but also that photoperiod must be permissive (one or more of absolute daylength, cumulative increase in daylength, or rate of change in daylength around the spring equinox) for testosterone secretion to be initiated.

Testosterone responses to GnRH administration in only the two heaviest spring-born colts (body weights of 318-371 kg), were first observed in June, and in all colts by August, when daylength was increasing, indicating that horse testes may not respond to LH until late winter/spring, and after the Leydig cell have matured (Naden et al., 1990a). It is likely that the two heaviest colts had achieved the threshold weight to permit Leydig cell maturity by winter (June), but the photoperiod was not permissive for spontaneous gonadotrophin secretion to result in testosterone concentrations increasing above baseline.

Furthermore, a decreasing ratio between GnRH induced LH and testosterone was observed as photoperiod and age increased in all colts (Table 6.1). This finding supports the previously suggested theory of increasing Leydig cell sensitivity to decreasing concentrations of LH as puberty begins (Olster and Foster, 1986). It may also indicate a decreased pituitary sensitivity to GnRH and increased pituitary sensitivity to the negative feedback of testosterone (Fennessy et al., 1988), or may just be indicative of decreased pituitary LH content (Muyan et al., 1993).

The occurrence of a peri-pubertal growth spurt, such as is known to occur in children (Tanner, 1981; Cutler et al., 1986; Rogol, 1996), has not been examined in domestic animals. However, the onset of puberty may influence weight gain in Brazilian Thoroughbred fillies, as growth rate significantly increased immediately after the onset of puberty as detected by increased progesterone concentration in fortnightly blood samples (Nogueira et al., 1997). In the present study, the onset of puberty, as defined by plasma gonadal steroid concentration increase, occurred in October in all horses (see Chapter 4). From August to October, mean monthly ADG increased by 0.2 and 0.3 kg/d in spring and autumn-born horses respectively, but this increase was not significant. Individual growth curves of most of the horses show that ADGs increased with time over August, September and October, preceding the onset of puberty. Hence, the relationship between growth rate and puberty is not clear, but it appears likely that the increase in growth rate observed in the spring was related to level of nutrition, and that the consequent ADG contributed to the attainment of the threshold body weight permissive for the onset of puberty.

7.4 Implications to industry and opportunities from this research

One of the main objectives of Thoroughbred breeders is to produce yearlings that will ultimately gain an acceptable price at yearling sales, which are held between late January and April in New Zealand and Australia. Sale price is greatly influenced by general appraisal, with size being a positive factor during assessment. Yearlings prepared for sale are intensively fed on concentrates and have limited access to pasture (MacCarthy and Mitchell, 1974). Remarkably, growth rates of the present pasture-raised horses were similar to those attained under systems using diets of forage plus concentrates (Hintz et al., 1979; Thompson, 1995; Pagan et al., 1996; Jelan et al., 1996), indicating that if nutritional management were to be individualised and optimised, there is little reason to not raise selected foals on pasture alone without concentrates (independent of season of birth), and still achieve suitable body weight for particular yearling sales.

General industry opinion is that autumn-born foals do not grow as well as spring-born foals and have less time to achieve an acceptable size for sale, and as a result can not be marketed. However, there is no data to verify this. Results from this study clearly show that autumn-born horses achieved growth rates comparable to horses born in the spring. Therefore, there is little reason to exclude autumn-born foals from late, or even the earlier, Southern Hemisphere sales. Furthermore, horses born in the New Zealand autumn to Northern Hemisphere time are also marketable to Northern Hemisphere industries, where they are competing against horses of the same age.

The results from these studies indicate that autumn breeding and autumn foaling need not have such a prejudice attached to it as in the past. The implications are that:

a) Autumn-born foals can be sold to the Northern Hemisphere, if commercial factors allow.
b) Autumn-born foals reach body weight suitable for the (late) Southern Hemisphere sales.
c) Progressively later foaling mares need not necessarily lose a breeding season.

With its temperate climate and pasture-based system, New Zealand is marketed as a nursery for young Thoroughbred horses. The New Zealand Standardbred industry uses the advantage the New Zealand horse-raising environment has to produce horses for the Northern Hemisphere market. Some Standardbred mares are bred to Northern Hemisphere time, foal in the New Zealand autumn and are exported to the Northern Hemisphere where they are raced as three-year olds. This study has shown that there is little reason why the Thoroughbred industry could not do the same and produce horses for yearling or ready-to-run sale.

In addition, the official birthday of Northern Hemisphere Thoroughbreds (1 January) falls in mid-winter, at a time when mares are not naturally reproductively active. Thus, most Northern Hemisphere foals are born later in the breeding season in March/April (MacCarthy and Mitchell, 1974; Hintz et al., 1979; Pagan et al., 1996). However, mares in New Zealand bred to Northern Hemisphere time are mated in the natural breeding season, when ovulation rates are high. As a result, Thoroughbred studs that breed horses in New Zealand to Northern Hemisphere time are more likely to achieve foaling dates closer to 1 January than in Northern Hemisphere countries. As well as foaling closer to the Northern Hemisphere birthday, New Zealand autumn-born horses also have the advantage of spring weaning, and rapid growth in the summer, compared with spring-born horses that are weaned in autumn and have a period of reduced growth in the winter. The result is thus very high potential for the New Zealand industry to grow heavier and more mature horses of the same official age as those in the Northern Hemisphere, which may be a competitive advantage.

The biological implications indicated here require further discussion and refinement by relevant industry sectors to determine if the appropriate increased production potential is consistent with economic aspects of on-farm management, sale and marketing.

7.5 Suggestions for further research

These studies raised a number of questions regarding the growth and reproductive development of young Thoroughbreds.
Future research might consider:

1. Growth in the grain-fed New Zealand Thoroughbred. Since many Thoroughbred foals are intensively managed in New Zealand, the growth of commercially raised Thoroughbreds needs to be investigated, with the possibility of individual management of autumn-born horses to optimise growth.

2. Growth in the autumn-born foal compared with spring-born foals. Further study should include (i) the effect of the increase in ADG in autumn-born horses at spring weaning on bone development, and (ii) the growth of New Zealand autumn-born horses compared with Northern Hemisphere spring-born horses up to the time of sale in September at 15-18 months of age (Northern Hemisphere sales).

3. The effect of photoperiod regimen on the onset of puberty in the horse. This should be examined by housing prepubertal horses indoors in opposite photoperiods to which they would normally be exposed between birth and the onset of puberty, in a similar concept to that described by Foster et al. (1985).

4. Measurement of other hormones associated with growth and onset of puberty in young horses including, GH, leptin and IGF-1.

Appendices

Appendix 1

95% Confidence intervals of spring and autumn-born growth data

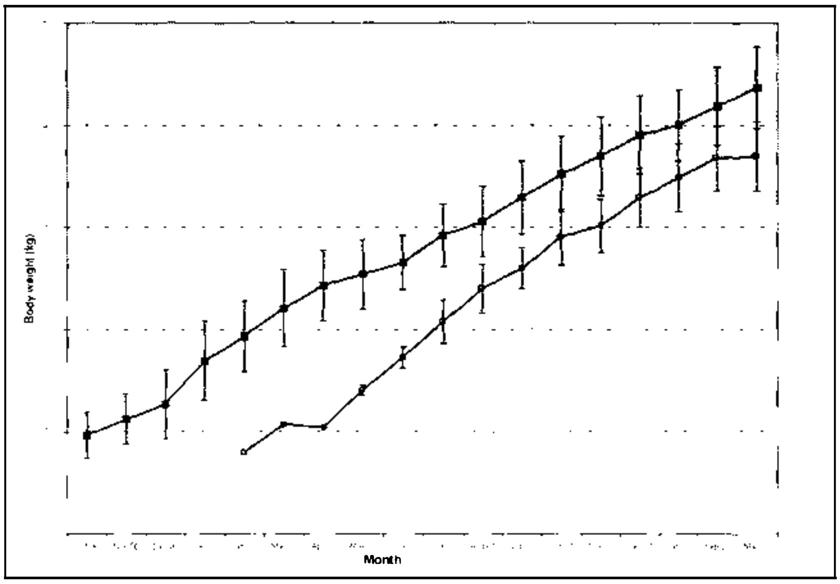

Figure 1 Mean ± 95% confidence interval for body weight (kg) of 35 fillies born in spring (■) and 2 fillies born in autumn (○).

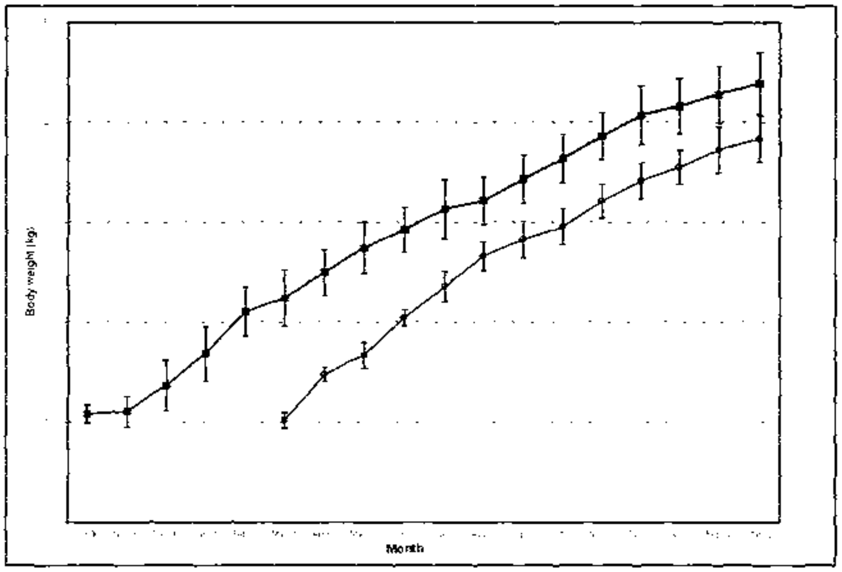

Figure 2 Mean ± 95% confidence interval for body weight (kg) of 23 colts born in spring (■) and 5 colts born in autumn (○).

Appendix 2

Glossary of terms

Androgen – Any steroid hormone that increases male characteristics.

ADG – Average daily gain (kg/day)

AUC – Area under the curve.

Castration – The excision of one or both testicles either surgically or chemically.

Circadian – A rhythmic pattern based on the 24-hour cycle, involving the repetition of certain physiologic phenomena such as sleeping, and eating.

Circannual – Any rhythmic pattern based on the 365 day yearly cycle.

Colt – Entire male horse under 3 years of age.

DOD – Developmental orthopaedic disease.

Endogenous – Originating or growing within the body; produced by internal causes.

Exogenous – Outside the body; originating outside the body; produced by external causes.

Filly – Entire female horse under 3 years of age.

FSH – Follicle Stimulating Hormone.

Gelding – Castrated male horse.

GH – Growth Hormone.

Gilt – A young sow (female pig).

GnRH – Gonadotrophin Releasing Hormone.

Homeostasis – The condition in which the body's internal environment remains relatively constant, within physiological limits.

IGF-1 – Insulin-like Growth Factor type 1.

LH – Luteinising Hormone.

Long day breeders – Seasonal breeding animals that mate when day light hours are longer than dark i.e. spring.

Luteal phase – The phase of the menstrual cycle after ovulation. The corpus luteum, stimulated by LH, develops from the ruptured follicle and secretes progesterone.

Menarche – the onset of menstruation/menses in the female.

Oestrous – The cyclic period of sexual activity in mammals other than primates.

Oestrus – The period of time in the oestrous cycle that the female is sexually receptive. In species with short oestrus, ovulation occurs after it ends and in species with long periods of oestrus, ovulation occurs during oestrus.

Ovulation – The release of an ovum from the ovary.

Peri-pubertal – Near or at the time of puberty.

Permissive signal – A signal that allows an event to occur.

Photoperiod – The number of hours of light in a day.

Photophase – Hours of light in the 24 hour cycle.

Post-partum – After the time of birth, usually defined by a number of days.

Pre-puberty – The period immediately before puberty. In people it is characterised by preliminary physical changes such as accelerated growth and appearance of secondary sexual characteristics that lead to sexual maturity.

Pubarche – Onset of puberty, marked by the beginning of the development of secondary sexual characteristics in the human.

Pubertal – Pertaining to puberty.

Pubescent – Pertaining to the beginning of puberty.

RHP – Retinohypothalamic projection.

RIA – Radioimmunoassay.

SCN – Suprachiasmatic nucleus.

Scotophase – Hours of dark in the 24 hour cycle.

SD – Standard deviation.

SED – Standard error of the difference.

SEM – Standard error of the mean.

SGX – Superior cervical ganglionectomy or removal of the superior cervical ganglia.

Short day breeders – Seasonal breeding animals that mate when day light hours are shorter than dark i.e. autumn.

Spermache – The appearance of spermatozoa in urine samples of the male.

Stallion – Entire male horse over 3 years of age.

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CERTIFICATE OF REGULATORY COMPLIANCE

This is to certify that the research carried out in the Doctoral Thesis entitled: "Aspects of Puberty and Growth in pasture-raised New Zealand Thoroughbreds born in spring and autumn" in the Institute of Veterinary, Animal and Biomedical Sciences at Massey University, New Zealand:

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SUPERVISOR'S DECLARATION

This is to certify that the research carried out for the Doctoral thesis entitled: "Aspects of Puberty and Growth in pasture-raised New Zealand Thoroughbreds born in spring and autumn" was done by Clarissa Brown Douglas in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Turitea Campus, New Zealand. The thesis material has not been used in part or in whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

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This is to certify that the research carried out for my Doctoral thesis entitled: "Aspects of Puberty and Growth in pasture-raised New Zealand Thoroughbreds born in spring and autumn" in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Turitea Campus, 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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