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EFFECTS OF MATERNAL BROMOCRIPTINE AND MELATONIN TREATMENTS ON FETAL DEVELOPMENT

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LIST OF ABBREVIATION

ACTH	adenocorticotrophic hormone
BN	binucleate
°C	degree Celsius
CIDR	controlled internal drug releaser
CRL	crown rump length
cm	centimetre
d	day
DNA	deoxyribonucleic acid
EGF	epidermal growth factor
g	gram
GH	growth hormone
bGH	bovine growth hormone
hGH	human growth hormone
GH-V	placental growth hormone
GnRH	gonadotrophin releasing hormone
h	hour
IU	international units
IGF	insulin-like growth factor
IGF-I	insulin-like growth factor-1
IGF-II	insulin-like growth factor-2
IGFs	insulin-like growth factors
IGFBPs	insulin-like growth factor-binding proteins
kg	kilogram
LWT	live weight
MFD	mean fibre diameter
mg	milligram
ml	millilitre
mm	millimetre
µm	micrometre
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
ng	nanogram
NZ	New Zealand
O ₂	Oxygen
PGE	prostaglandin E
PIF	prolactin inhibitory factor
PL	placental lactogen
hPL	human placental lactogen
oPL	ovine placental lactogen
PMSG	pregnant mare serum gonadotropin
PRF	prolactin releasing factor
PRL	prolactin
oPRL	ovine prolactin
PRP	prolactin-related protein

S/E	starvation/exposure
SBCRU	sheep and beef cattle research unit
T ₃	triiodothyronine
T ₄	thyroxine
TSH	thyroid stimulating hormone
vs	versus

Statistical:

GLM	general linear model
LSM	leastsquare mean
SAS	statistical analysis system
s.e.m.	standard error of the mean
n	number of experimental units
*	P<0.05
**	P<0.01
***	P<0.001

CHAPTER ONE

INTRODUCTION

1. PREAMBLE

A previous study (Jenkinson *et al.*, 1994) has proposed that lowered birth weights of autumn (May)-born lambs compared with those of spring (August)-born lambs are due to a direct seasonal effect on placental size, which in turn mediates a slower fetal growth. The effect of season on placental and fetal development is established early in pregnancy (by day 84 of gestation), and is suggested to be mediated by seasonal differences in circulating prolactin concentrations in the dams and/or fetuses (Jenkinson *et al.*, 1994; McCoard *et al.*, 1996).

Plasma prolactin concentrations are likely to be very high in autumn-lambing ewes (December-mated ewes) during the early- to mid-gestation period December-February due to a seasonal (primarily daylength) effect (Pearson *et al.*, 1993; Pearson *et al.*, 1996). The study described herein was carried out in an attempt to increase birth weights of autumn-born lambs by improving placental development and hence fetal growth via manipulation of maternal prolactin concentrations. Treatments with bromocriptine (a dopaminergic agonist) and melatonin early in gestation were tested for their ability to reduce maternal and/or their fetal circulating prolactin concentrations, improve placental development and hence increase fetal growth and birth weights.

2. AUTUMN-LAMBING SYSTEMS

2.1. Background

Relying only on a traditional lambing system (March - May mating) has created a highly seasonal killing pattern and marketing difficulties for the New Zealand sheep

industry. Extending the normal lambing pattern may overcome the problems. The use of hormones to induce oestrus in anoestrous ewes (“out-of- season breeding”) has enabled farmers to extend the normal breeding season and permitted mating of ewes earlier than normal (Robinson, 1954; Welch and Tervit, 1970; Welch, 1985). This has been practised for many years in New Zealand, and been driven by the development of new technology for storing chilled lamb cuts and by the market opportunities that have developed for chilled lamb (Morris, 1990). Thus, the application of both normal season and out-of-season lamb production has given opportunities to provide a year-round supply of chilled lamb, especially for overseas supermarkets. However, the system of out-of-season lamb production still has a problem relating to birth weights. Most experimental results have shown that lambs born to autumn-lambing ewes are up to 25-30% lighter at birth than those born to spring-lambing ewes (Reid *et al.*, 1988; Peterson *et al.*, 1990; Morris, 1993).

2.2. Autumn-born lambs

Birth weight in all species is a function of fetal growth rate (Robinson *et al.*, 1979; Mellor, 1983), which in turn is, in part, determined by the plane of maternal nutrition and placental size (Mellor, 1983). In practice they act simultaneously, and their effects may be confounded because a high plane of maternal nutrition can partly offset the growth-retarding effects of a small placenta, while a large placenta can partly offset the growth-retarding effects of maternal under-nutrition (Mellor, 1983). However, a variety of different fetal growth patterns could lead to similar weights in late gestation. It seems that there is a period of accelerated ‘catch-up’ growth that is involved in the attainment of the same final size, implying that birth weight, regardless of its ease of measurement, is at best a poor surrogate measure of fetal growth (Harding and Johnston, 1995). In the fetal sheep, it is possible to measure growth in terms of fetal crown-rump length (CRL) or fetal thoracic circumference (girth) on a day-to-day basis using a simple chronically implanted measuring device (Mellor and Matheson, 1979). The CRL or girth measurements of individual fetal sheep produce a more accurate measurement of fetal growth than does birth weight (Mellor, 1983).

Even though there is compensatory fetal growth, which may be, in part, due to the ability of the dams to buffer fetuses against changes in nutrition, it appears that there is a variation of birth weights between season of lambing and between years (Table 1). Autumn-born lambs have tended to have lower birth weights than spring-born lambs, although this effect is not always observed (Rumball, 1980; McQueen, 1986).

Table 1. Mean birth weight (\pm SE) data for lambs born in autumn or spring

Birth weight (kg)		Reference
Autumn-born lambs	Spring-born lambs	
3.9†	3.7†	Rumball (1980)
3.6 \pm 0.9	4.3 \pm 1.1	McQueen (1986)
4.6 \pm 0.8	4.4 \pm 1.1	McQueen (1986)
4.0 \pm 0.9	4.4 \pm 0.9	Reid <i>et al.</i> (1988)
4.3 \pm 0.2	4.8 \pm 0.1	Cruickshank & Smith (1989)
3.8 \pm 0.3	4.5 \pm 0.2	Peterson <i>et al.</i> (1990)
3.9 \pm 0.1	4.9 \pm 0.1	Morris (1992)
4.2 \pm 0.2 [‡]	5.1 \pm 0.2 [‡]	Jenkinson <i>et al.</i> (1994)
4.8 \pm 0.3 [‡]	5.6 \pm 0.3 [‡]	McCoard <i>et al.</i> (1996)

† No standard errors given

[‡] Fetal wet weights near term (day 140 of gestation)

Source: Table 1, p. 4, Jenkinson (1994).

A previous study (Jenkinson *et al.*, 1994) suggested that the lower birth weight of autumn-born lambs was due to a direct seasonal effect, especially the photoperiodic effect, on placental formation rather than to differences in maternal nutrition. It was found that there was a marked decrease in placentome number and total placental weight of autumn-lambing ewes compared with those of spring-lambing ewes (89.4 \pm 4.2 vs 106.9 \pm 4.3 and 564.7 \pm 34.0 g vs 679.0 \pm 34.9 g, respectively). The reduced placental size was apparently due to a reduction in maternal caruncle occupancy by fetal cotyledon (79 vs 88%). A subsequent study found that the seasonal differences in placental formation and fetal growth of autumn-lambing ewes/fetuses can be recognised as early as day 84 of gestation (McCoard *et al.*, 1996), implying that season is likely to exert its effects during early pregnancy.

Birth weight in sheep is a critical factor that will be subsequently correlated with lamb viability (survival) and post-natal growth (Robinson *et al.*, 1979; Mellor, 1983). High birth weights are associated with dystocia, especially in single male lambs, and low birth weights with starvation/exposure (S/E) (Dalton *et al.*, 1980). Dystocia accounted for 50.4% of all single deaths and S/E for 45.0% of all multiple deaths (Scales *et al.*, 1986). The highly susceptible birth weight groups for most breeds are those below 3.0 and those above 5.0 kg. These groups usually include only a small proportion of lambs born, but account for a much higher proportion of the total mortality (Scales *et al.*, 1986).

2.3. Perinatal and neonatal mortalities

Both perinatal and neonatal mortalities in sheep are recognised as the major form of reproductive wastage (Mellor, 1988). The mortality rate is highest at birth weight extremes, particularly at the lower extreme (Robinson *et al.*, 1979). Numerous factors which jeopardise the newborn have been identified (Alexander, 1984), and it is evident that lamb survival depends on the integration and success of physiological activities in four crucial areas, i.e., initiation and completion of the birth process, prenatal preparation of the lamb to face the challenges of a free-living existence, preparation of the ewe for her postnatal nutritive role, and establishment of a good ewe-lamb bond (Mellor, 1988).

The incidence of fetal death during late pregnancy is greater when fetal growth retardation is caused by a small placenta than when it is due mainly to maternal underfeeding (Mellor *et al.*, 1977; Mellor and Matheson, 1979; Mellor and Murray, 1981). Even though both types of fetus become hypoglycaemic, those with small placentae also become hypoxaemic, suggesting that fetal survival is jeopardised more by the combined effects of oxygen and nutrient deficiencies than by a nutrient deficiency alone (Mellor and Murray, 1982). Fetal hypoxaemia can occur chronically, or acutely during parturition. The effects of both types of hypoxaemia persist after birth (Mellor, 1983).

Either hypoxic lambs, or small lambs, regardless of which season of birth they belong to, are at a greater thermal disadvantage because of their high surface area to weight ratio and (sometimes) retarded coat development. The increased heat production required to maintain body temperature causes affected animals to rapidly utilise their body energy stores, which may already be reduced in a small lamb. If these energy resources are not replaced by suckling, the animal will eventually starve to death, usually within about three days of birth (McCutcheon *et al.*, 1981; Mellor, 1983).

So far, there is no conclusive report of differences in mortality rates between autumn- and spring-born lambs (McQueen, 1986; McNeal, 1978; Reid *et al.*, 1988). However, autumn-born lambs, which have lowered birth weights, would more likely be susceptible to fatal hypothermia than spring-born lambs, either because their shivering and non-shivering thermogenic (brown fat) mechanisms have not matured fully or are partly inhibited (Alexander *et al.*, 1973), or because the fetal nutrient deficiency has so depleted their energy reserves that a high rate of heat production cannot be sustained after birth (Eales *et al.*, 1982).

2.4. Growth rate to weaning

It is well established that postnatal growth rate of lambs during the first few weeks is correlated positively with birth weight (Mellor, 1983). Pre-weaning growth rates of autumn lambs have been reported to be similar to, or slightly lower than, those of spring lambs. They were: 230 and 235 g/day (Andrewes and Taylor, 1986); 167 and 179 g/day (McQueen, 1986); 233 and 276 g/day (Cruickshank and Smith, 1989); and 184 and 227g/d (Peterson *et al.*, 1990), for autumn and spring lambs respectively.

The lower growth rates in autumn-born lambs are likely to be influenced by many other factors, such as changes in photoperiod, changes in ambient temperature and the availability of superior quality herbage (Cruickshank and Smith, 1989), in addition to the effect of their low birth weights. However, it is found that milk intake and quality are the main determinants of lamb growth rates (Lawlor and Hopkins, 1981).

With regard to photoperiodic effects, lamb growth rates have been significantly influenced by seasonal changes in photoperiod and it is thought that prolactin may be involved in regulating this effect (Forbes *et al.*, 1979) since alteration in photoperiod also affects maternal plasma prolactin concentrations. Prolactin, and also growth hormone and placental lactogens, exhibit the properties of homeorhetic hormones. They direct the flow of nutrients to the process of highest priority, partly by coordinating nutrient utilisation by competing tissues (Bauman *et al.*, 1982). In contrast, a later study (Eisemann *et al.*, 1984) showed that there was no definitive support for a role for prolactin in growth or in mediating the photoperiod-induced growth response in sheep. Nor was there any effect of long photoperiod during late pregnancy on either birth weight or subsequent post-natal growth (Ebling *et al.*, 1989). However, most of those later studies are related to effects of prolactin on fetal growth during late pregnancy, or on lamb growth *post partum*. Thus, seasonal prolactin effects on fetal or subsequent lamb growth during early pregnancy might be different from those reported in previous studies.

2.5. Milk production and udder size

As with fetal growth and birth weight, there is conflicting evidence as to the effect of a long-day photoperiod on mammary gland development in the ewe. Long photoperiod may increase the rate of udder development in the ewe. However, the magnitude of the effect on udder size was less than that due to increased fetal number (Bassett, 1992). Mammary gland weights were recently reported to be higher in spring-lambing ewes than in autumn-lambing ewes, despite the two groups having similar mammary gland dimensions (Jenkinson, 1994).

Milk production from ewes kept in a long day photoperiod, from 6 weeks before to 8 weeks after lambing, may be 30-50% higher during early lactation than in ewes experiencing a short-day photoperiod (Bocquier *et al.*, 1986). Milk production in autumn-lambing ewes was also 33-42% lower during the first 7 days of lactation than that of spring-lambing ewes fed on a diet of either pasture or meadow hay and sheep nuts (Peterson *et al.*, 1990). This lowered milk production has been associated with

seasonal effects of daylength duration and of maternal plasma prolactin concentrations during pregnancy and lactation (Ortavant *et al.*, 1988; Peterson *et al.*, 1990). In contrast to those results, Bassett (1992) reported that long photoperiod seems unlikely to have effects in increasing milk production.

Studies in the goat (Forsyth *et al.*, 1985) showed that suppression of prolactin release throughout much of pregnancy led to some delay in mammary development in does with a single fetus, but the ultimate mammary size and milk production were not reduced. It was suggested that during such periods, the loss of prolactin secretion was compensated by the production of placental lactogens. A later study (Peterson *et al.*, 1994) also showed that administration of *ovine* prolactin (oPRL) by the intramammary or subcutaneous routes in autumn-lambing ewes around the time of parturition did not increase milk yields, or change the composition of milk, compared to controls, indicating that the circulating and intramammary concentrations of prolactin in autumn-lambing ewes are not limiting lactogenesis. Hence it is not clear that large changes in plasma prolactin concentration, due to photoperiod effects during pregnancy, would necessarily alter udder development and subsequent milk production.

However, it is likely that udder development cannot be solely measured by using the changes in mammary size since there is an increase in mammary weight without a change in mammary size in spring-lambing ewes (Jenkinson, 1994), and milk production is higher in spring-lambing ewes with naturally higher plasma prolactin concentrations during the time around parturition compared with that of autumn-lambing ewes (Peterson *et al.*, 1990). Thus, it may be true that the udder development that will determine subsequent milk production has been determined since early pregnancy. This udder development, during early to mid gestation, may involve prolactin and placental lactogen, whereas high prolactin concentrations during late pregnancy will have an additional effect, to that of the prevailing capacity of milk production of the udder, in increasing milk production in spring-lambing ewes.

2.6. Wool growth

Wool growth rate is a function of mean fibre length growth rate, mean fibre cross-sectional area, density of active follicles within the skin and the specific gravity of the fibre produced (Sumner *et al.*, 1994). It has long been known that sheep possess an inherent seasonal cycle of wool growth which is entrained by annual photoperiod (reviewed by Sumner and Bigham, 1993). The cycle has a maximum growth rate in the winter with the amplitude varying between breed (Ferguson, 1954; Bigham *et al.*, 1978). Wool growth in sheep is also directly influenced by the dietary supply of nutrients (Wynn, 1982) and indirectly by seasonal climatic effects on pasture quantity and quality (Sumner *et al.*, 1994).

Seasonal changes in plasma prolactin concentration have also been closely associated with the seasonal cycle of wool, horn and hair growth in sheep (Lincoln, 1990). High plasma prolactin concentrations are necessary for reactivation of hair follicles in Khasmir goats and administration of prolactin can advance the onset of moulting, while blockade of its secretion with bromocriptine delays it (Lynch and Russel, 1990).

In contrast with those previous studies, it has been recently found that high seasonal prolactin concentrations suppress both primary and secondary wool growth (Craven *et al.*, 1994; Pearson *et al.*, 1996). Natural and experimental increases in daylength have been reported to have a short-term inhibitory effect on growing wool follicles which could be mediated through rising plasma concentrations of prolactin (Pearson *et al.*, 1996). Exposure of NZ Wiltshire sheep to either 1, 2 or 3 months of long-day photoperiod decreased primary and secondary follicle activities. These decreased primary and secondary follicle activities occurred following the increase of prolactin concentrations induced by increasing daylength period (Craven *et al.*, 1994). The optimal time frame to achieve an effective synchronised follicle regression was between 2 and 3 months of long-day treatment (Craven *et al.*, 1994).

3. SEASONAL EFFECTS ON PROLACTIN SECRETION

3.1. Seasonal prolactin secretion

Maternal circulating plasma prolactin concentrations in the majority of seasonally breeding mammals, including ewes, are strongly influenced by season (Ravault *et al.*, 1982; Bassett, 1992; Curlewis, 1992), being higher in spring or summer than in autumn or winter. Like prolactin concentrations in ewes, plasma prolactin concentrations in fetuses also depend almost totally on season (Ravault *et al.*, 1982). Over 80% of the variation in prolactin concentrations between fetuses was associated with variation in the natural daily photoperiod to which the ewes were exposed during pregnancy (Ravault *et al.*, 1982). In the anoestrous season, prolactin concentrations were higher than in the breeding season in sheep (Buys *et al.*, 1990). A study by Jenkinson *et al.* (1994) also showed that there is a different pattern of plasma prolactin concentrations between autumn- and spring-lambing ewes. Plasma prolactin concentrations were higher in autumn-lambing ewes at day 70 of gestation and in spring-lambing ewes at day 100 of gestation, but did not differ significantly between autumn- and spring-lambing ewes at day 140 of gestation, showing there was a significant stage of gestation by season interaction. Placental formation in autumn-lambing ewes occurs in summer when plasma prolactin concentrations are likely to be at the highest level.

3.2. Control of seasonal prolactin secretion

3.2.1. Melatonin

Photoperiod is the major environmental factor controlling the seasonal pattern of prolactin secretion, with long days increasing prolactin concentrations and short days having the opposite effect (Pelletier 1973; Lincoln *et al.*, 1978). As with the seasonal control of gonadotrophin secretion, photoperiod influences prolactin secretion via its effects on the secretion of melatonin, which acts as a transducer to convert the neural

information into an endocrine signal (Curlewis, 1992). Melatonin is the pineal hormone deriving from tryptophan via a route which involves the neurotransmitter substance serotonin as an intermediary (Wurtman, 1976),

The role of melatonin in prolactin secretion has been shown in several experiments. Denervation of the pineal disrupted the ability of daylength to influence prolactin secretion (Lincoln *et al.*, 1989), and continuous treatment with melatonin or administration of a short-day melatonin pattern inhibited prolactin secretion (Kennaway *et al.*, 1982; Poulton *et al.*, 1987). The site at which melatonin acts to cause these effects on prolactin secretion has not been identified. Although direct confirmation is still required, it appears possible that melatonin influences prolactin secretion by acting within the anterior hypothalamus or another site which projects to this region, and the differences in response to photoperiod may be due to differences in the processing and/or interpretation of the melatonin signal. (Curlewis, 1992).

3.2.2. Dopamine

Unlike the photoperiodic control of gonadotrophin, which is mediated, in part, by the effect of a change in sensitivity to oestradiol long-loop negative feedback on pulsatile gonadotrophin releasing hormone (GnRH) secretion in the hypothalamus (Karsch *et al.*, 1985), the seasonal changes in prolactin secretion are not due to changes in the sensitivity of a feedback loop, since such a loop does not exist (Curlewis, 1992). Thus, they are most likely to be due to direct effects of photoperiod on the hypothalamic pathways that control prolactin secretion. These pathways involve alteration of prolactin inhibitory factor (PIF) and prolactin releasing factor (PRF) release, which, in turn, modulate prolactin secretion from the pituitary lactotroph (Lamberts and MacLeod, 1990). The major PIF is generally considered to be dopamine, and, in seasonally breeding species, dopamine antagonists increase prolactin concentrations during both summer and winter, indicating that there is dopaminergic tone at each time of year. There is currently no evidence for an involvement of PRFs in the seasonal change in prolactin concentrations (Curlewis, 1992).

High prolactin concentrations increase dopamine turnover in the mediobasal hypothalamus, resulting in increased concentrations of dopamine in the hypophyseal portal blood which then suppress prolactin secretion (Moore, 1987). This prolactin short-loop feedback also operates in seasonally breeding sheep (Curlewis and McNeilly, 1991). Removal of the PIF by hypothalamo-pituitary disconnection in winter results in a ten-fold increase in prolactin concentration, whereas in summer a much smaller increase (twofold) occurs (Thomas *et al.*, 1986). Hence, it is unlikely that the decrease in prolactin concentration in winter is due to the effects of increased concentrations of dopamine in the portal vessels. Other experimental results also showed that dopamine turnover in the arcuate nucleus and median eminence of the hypothalamus decreases or remains unchanged under short daylength (Steger *et al.*, 1982; Glass *et al.*, 1988; Thiery, 1991; Zinn *et al.*, 1991), and measurements of secretion of dopamine in hypophyseal portal blood during this time also have led to some controversial results. A study by Thomas *et al.* (1989), in sheep and rats, has reported that the dopamine concentrations in the long portal vessels are below the limit of detection during short days. There is, however, evidence for increased pituitary sensitivity to dopamine under short days, so increased dopamine concentrations may not be required for suppression of prolactin secretion at this time. In addition to the diminished secretion of prolactin under short days, the rate of prolactin synthesis and pituitary content of prolactin also decline, although the mechanisms that regulate these changes are poorly understood (Curlewis, 1992).

However, the dopaminergic control of prolactin secretion in adult mammals, including sheep, is well established (Lowe *et al.*, 1979; Levy and Lightman, 1988; Curlewis *et al.*, 1991). Several experiments have shown that administration of dopaminergic agonists, such as the ergot alkaloid 2- α - bromoergocryptine (CB154) and apomorphine, were followed by a rapid and pronounced decline in fetal and maternal plasma prolactin concentrations within 24 hours of the commencement of infusion (Lowe *et al.*, 1979; Buys, 1990). In the ovine fetus, short term administration of the dopaminergic agonist apomorphine leads to suppression of prolactin, whereas administration of a dopaminergic blocking agent (dopaminergic antagonist) such as haloperidol elevates ovine fetal prolactin secretion (Lowe *et al.*, 1979). It is suggested

that the primary action of dopaminergic agonists, including CB154, in inhibition of prolactin release is by direct stimulation of dopamine receptors on the pituitary. This mechanism was also exhibited in *in vitro* experiments (Fluckiger, 1978). However, the dopaminergic agonist may also act at the level of the hypothalamus (Fluckiger, 1978). Prolactin concentrations were persistently suppressed throughout the infusion of CB154 with no evidence of a compensatory mechanism that comes into action to overcome the dopamine-induced inhibition of prolactin secretion (Lowe *et al.*, 1979).

In addition, the continuous infusion of CB154 into either sheep fetuses (days 116-129 of gestation) or pregnant ewes (days 118-137 of gestation) was followed by pronounced ultrastructural changes in the binucleate (BN) cells of the ovine chorionic epithelium (Lowe *et al.*, 1979), which are the source of placental lactogens (PL) and placental prolactin-related protein (Lowe *et al.*, 1979; Anthony *et al.*, 1995). Although infusion of CB154 in the ewes significantly depressed maternal plasma PL concentrations, the infusion of CB154 to the fetus did not lower fetal plasma PL concentration or affect the duration of gestation (Lowe *et al.*, 1979).

3.2.3 Other factors

In sheep, prolactin secretion is also influenced by an underlying circannual rhythm which, under natural photoperiod, is probably entrained by the annual change in daylength. Expression of this rhythm has been demonstrated in both pineal-intact and pinealectomised animals under constant short, equatorial and long daylength (Howles *et al.*, 1982; Karsch *et al.*, 1989; Jackson and Jansen, 1991). The circannual rhythms of plasma prolactin concentrations persist under both fluctuating and constant environmental temperatures (Karsch *et al.*, 1989). As would be expected for a circannual rhythm, exposure to constant photoperiod leads to changes in the period and phase of the prolactin rhythm (Howles *et al.*, 1982; Karsch *et al.*, 1989; Jackson and Jansen, 1991). Under constant photoperiod and temperature, the rhythm is of lower amplitude than under natural environmental conditions, becomes less pronounced with time and, is even completely absent from some individuals. It seems,

therefore, that in addition to entraining this circannual rhythm, changing photoperiod is essential for full expression of the seasonal prolactin profile (Curlewis, 1992).

The level of nutrition also changes on a seasonal basis and can influence plasma prolactin concentrations (Rhind *et al.*, 1985). However, feeding *ad libitum* or restricted food did not show any effects on the seasonal pattern of prolactin (Loudon *et al.*, 1989; Suttie and Kay, 1985), suggesting that the seasonal changes in level of nutrition are unlikely to be the cause of seasonal changes in plasma prolactin concentrations.

High ambient temperature has been shown to increase plasma prolactin concentrations (Schillo *et al.*, 1978) and could therefore be involved in the increase in prolactin concentration which occurs during summer. Indeed, in animals maintained under natural environmental conditions, ambient temperature and daylength correlate equally well with prolactin concentrations (Schams and Reinhardt, 1974). However, it is likely that photoperiod can override the effects of temperature on prolactin secretion (Brown *et al.*, 1979; Lincoln, 1979), although it remains possible that, under natural environmental conditions, temperature might interact with photoperiod to influence plasma prolactin concentration (Curlewis, 1992).

Prolactin secretion is also influenced by steroid hormones and, in particular, the ability of oestradiol to cause prolactin release is well known (Lamberts and MacLeod, 1990). A recent study (Hu and Lawson, 1996) in rats also showed that steroids modulate responses of the hypophyseal lactotrophs to dopamine in stimulating or withdrawing prolactin secretion. However, gonadectomy or adrenalectomy has no effect on the overall seasonal pattern of plasma prolactin concentration, indicating that changes in steroid hormones are not mediators of the seasonal prolactin pattern (Schillo *et al.*, 1988).

4. REGULATION OF FETAL GROWTH

It has been well established that nutritional supply to the fetus is the major regulator of fetal growth. However, the direct supply of nutrients to provide building blocks for tissue growth is likely to be only a minor component of this regulation. It has been suggested that indirect effects of nutrition may have a more important role in the regulation of fetal growth. The indirect effects of nutrition on fetal growth are likely to be exerted through its effects on fetal endocrine and metabolic status, and on the interaction between the fetus, placenta and mother, all of which must be coordinated to allow normal fetal growth (Harding and Johnston, 1995).

The intrauterine removal of the key hormones known to influence postnatal growth has indicated that some of these hormones are also critical for normal fetal development (Fowden, 1989). Therefore, it seems that hormones have an important role in regulation of fetal growth.

The placenta is responsible for directly mediating or modulating the maternal environment required for maintenance of normal fetal growth and development. Besides being the site of nutrient and waste transfer between the mother and fetus, it also serves as a barrier against pathogens and the maternal immune system, as well as functioning as an active endocrine organ (Anthony *et al.*, 1995).

Hence, it is likely that endocrine and/or paracrine/autocrine regulation of fetal growth will involve the endocrine and/or paracrine/autocrine regulation which originally comes from the placental endocrine system as well as from maternal and fetal endocrine systems.

4.1. Placental development

The placenta is formed by the apposition and fusion of tissues derived from the mother and the conceptus, particularly the extra-embryonic tissue. This usually takes place inside the uterus and, in some species, is confined to specialised structures such as the

endometrial caruncles of the sheep or the mesometrial endometrium in the mouse (Robinson *et al.*, 1995).

In general, placental development involves three stages: implantation, growth and maturation (Battaglia and Meschia, 1986). Implantation is a critical step in the progress of pregnancy, during which the conceptus acquires a fixed position within the uterine lumen, and leads to the establishment of the placental structures. This process implies some cellular modifications of both the uterine epithelium and the trophoblast to ensure cell adhesion between the two tissues (Guillomot, 1995). In ruminants, the implantation process is characterised by three main steps: a long pre-attachment period lasting 2-3 weeks, during which the conceptus elongates considerably; an apposition stage when cellular contacts are established between the trophoblast and the uterine epithelium; and an adhesion stage which ends the process and gives rise to the cellular structure of an epithelio-chorial placenta. In sheep, trophoblast apposition begins in the vicinity of the embryo by day 15, and is followed by differentiation of the binucleate cells (adhesion) at day 16 (Guillomot, 1995).

Based on studies of different species, it is apparent that the potential implantation sites or “caruncles” can be identified prior to implantation. These caruncles are, for example, present in the sheep uterus but less obvious in non-ruminant species (e.g., pigs).

4.1.1. Placental size

As described by Alexander (1974), the non-pregnant uterus contains about 60 to 150 caruncles (endometrial thickenings). The number varies between breeds and strains of sheep, and there is even a wide range in number within an apparently homogeneous group of ewes. The events occurring at the time of implantation have been reported to play a role in determining the ultimate size attained by the placenta and hence the fetus, and may also alter the length of gestation (Battaglia and Meschia, 1986; Walker *et al.*, 1992).

The chorionic attachment to caruncles (the fusion of the fetal cotyledons and the maternal caruncles) begins at about 15 days after conception, and usually 70-80% of caruncles are available for chorionic attachment, depending on litter size and other factors (Robinson, 1982). The point of attachment develops into a button-shaped structure called the "placentome". The lower the number of caruncles that are available for the formation of placental cotyledons, the smaller the placental weight at term (Alexander, 1964; Alexander, 1974). However, there is an inverse relationship between the average cotyledonary weight and the number of implantation sites (caruncles), indicating that there is some compensatory growth of individual cotyledons when their total number is reduced (Alexander, 1964; Alexander, 1974). This implies that the total weight of placentomes may be a better index of placental size than placentome number (Mellor *et al.*, 1977). The proportion of caruncle occupancy is also widely variable but tends to decline with increasing age of the ewe and to be about 10% greater in placentae of male than female fetuses. Individual twin fetuses tend to have considerably fewer placentomes than most single fetuses (Alexander, 1974).

The implantation phase in sheep is complete by approximately day 50 of gestation and the total cotyledonary number has reached its maximum by that time. The placenta then enters a rapid growth phase, which is completed at approximately day 90 of gestation. Thereafter until term (day 145 of gestation), the placental weight and DNA content do not increase and, the placental weight actually decreases, while the fetal weight continues its exponential increase. Thus, the fetal:placental weight ratio increases as gestation progresses (Battaglia and Meschia, 1986). However, the progressive decrease of the placental weight after day 90 of gestation does not mean that the ability or the function of the placenta in supporting fetal growth declines. It is suggested that during the later phase, the placenta is maturing. This maturation process consists of the continued growth of the surface area of the placental villi, which is concomitant with the decrease of the thickness of the placental barrier. It appears that the maturation process facilitates an increase in maternal and fetal placental blood flows, thus satisfying the increased fetal metabolic demands (Battaglia and Meschia, 1986).

Overall, it is likely that there is a relationship and an interdependence between fetal and placental weight. A small placenta, which causes either a deficiency of placental function (e.g., by carunclectomy, by perturbation of the implantation process) or an imbalance between placental function and fetal metabolic demand (e.g., by perturbation of placental growth process), can restrain fetal growth (Alexander, 1964; Alexander, 1974). In a fetus with a small placenta, the area of maternal-fetal exchange is small in relation to fetal metabolic demands. Thus, it is hypothesised that fetal growth retardation can be secondary to stunted placental growth (Robinson *et al.*, 1995).

4.1.2. Regulation of placental metabolism by glucose supply

The placenta is an organ with a high metabolic demand. It normally consumes approximately two-thirds of the oxygen and half of the glucose delivered from the uterine circulation (Gu *et al.*, 1987; Owens *et al.*, 1989). Glucose is supplied to the placenta and fetus from the maternal plasma according to concentration-dependent mechanisms (Simon *et al.*, 1979). This glucose uptake and transport by the placenta are mediated by facilitative transporter proteins on both the maternal-facing microvillus and fetal-facing basal trophoblast membranes (Hay, 1995). Placental glucose transport to the fetus requires a net maternal-to-fetal plasma glucose concentration gradient that is determined by placental as well as fetal glucose consumption. Fetal plasma glucose concentration, independent of maternal glucose concentration, regulates the partition of placental glucose uptake into the fetus and consumption by the placenta. This placental transport capacity increases with advancing gestation. Placental glucose consumption contributes to most or all of placental lactate and fructose production and to other less well defined non-oxidative pathways of carbon metabolism. Placental glucose consumption accounts for at least 50% of placental oxygen consumption which remains independent of short-term or long-term changes in placental glucose supply, thus requiring varying amounts of other carbon substrates. Hence, placental glucose supply plays a key role in regulating

placental glucose metabolism and placental carbon balance, and interacts reciprocally with other carbon substrates to maintain placental oxidative metabolism (Hay, 1995).

4.1.3 Endocrine control of placental growth

Even though the placenta is known to function as an active endocrine organ which produces several hormones regulating fetal growth and development, its growth and development are also regulated by a number of systemic and local hormones or growth factors. The critical phases of placental growth and development are likely to take place during the implantation and growth phases (early gestation up to day 90 of gestation). Therefore, the endocrine control of placental growth may be exerted mainly during these periods.

It was reported that administration of exogenous progesterone in pregnant ewes for the first six days of pregnancy resulted in enhancement of fetal and placental growth (Kleeman *et al.*, 1994). Exogenous oestrogen has also been reported to increase embryonic survival and lead to the birth of larger piglets (Robinson *et al.*, 1995). Thus, it is likely that progesterone has an important role in both fetal and placental growth, especially during the early stage of pregnancy. In addition, progesterone and oestrogen are suggested to play a role in modulating the expression of a number of peptide growth factors and cytokines in uterine tissues (Schultz *et al.*, 1993, Robertson *et al.*, 1994).

Growth factors and cytokines have been reported to act locally to regulate placental and fetal growth and differentiation (Han and Fowden, 1994). They seem also to play a major role in placental and fetal development by providing a signalling system exchange between the mother and the conceptus. These hormones originally come from the uterine epithelium, and their expression and production are modulated by the ovarian hormones, progesterone and oestrogen (Schultz *et al.*, 1993, Robertson *et al.*, 1994).

The most likely growth factors predominantly involved in placental and fetal growth and development are IGF-I and IGF-II. Both IGF-I and IGF-II and their mRNA are present in most fetal and placental tissues as early as the preimplantation stages of embryonic development (De Groot and Hochberg, 1993; Schultz *et al.*, 1993). They seem to act either locally, or in an endocrine manner, on placental and fetal tissues (De Groot and Hochberg, 1993; Schultz *et al.*, 1993). However, it is suggested that IGF-I correlates better with fetal growth and acts via the type I IGF receptor, whereas placental growth appears to be dependent only on IGF-II, the influence of which is mediated by unidentified receptors (Baker *et al.*, 1993).

Recently it was found that the expression of IGF-II genes also plays a key role in the interaction between the fetus, placenta and mother. IGF-II is normally expressed from the paternally-inherited chromosome and the maternally-inherited gene is repressed (imprinted). On the other hand, the IGF type II receptor is paternally imprinted and only the maternally-inherited gene is expressed. The absence of the IGF type II receptor gene leads to early death of the mouse embryo on day 17 of pregnancy unless the IGF-II gene is also absent (Li *et al.*, 1993). It has been suggested that the IGF type II receptor gene is necessary to prevent over abundance of the IGF-II gene. This reciprocal balance between the expression of the IGF-II gene and IGF type II receptor gene is necessary for normal placental development (Haig and Graham, 1991; Willison, 1991). Errors of this process may account for part of the overgrowth of the human fetus and its placenta in the Beckwith-Weideman syndrome, a condition that is associated with abnormal placental development (Henry *et al.*, 1991).

Since placental growth is also affected by nutritional status, it is likely that the restriction on placental growth is caused by the restriction of substrate supply. The reduced concentrations of the substrate in placental and fetal blood largely account for the endocrine changes that reduce the trophic drive to growth in the placenta and fetus (Owens and Robinson, 1988).

4.2. Fetal development

4.2.1. Fetal growth

Fetal growth involves both the accretion and differentiation of tissues and requires tight coordination between these two processes if development is to proceed normally. Abnormalities in either process or in their coordination will alter the pattern of intrauterine growth and may have adverse effects on neonatal viability as a consequence (Silver, 1992). Poor prenatal growth has also been associated with a failure to thrive after birth in domestic animals, and with an increased risk of cardiovascular and other diseases in later life in humans (Barker, 1992).

Fetal weight, which represents an achievement of fetal growth, is reported to increase exponentially for at least part of gestation. In sheep, a semilogarithmic plot of fetal weights versus gestational age yields a curve with convexity toward the weight axis, indicating that the fractional rate of weight gain is greater in early pregnancy. However, such equations derived from the fetal growth curve do not seem to have a precise, theoretical meaning because of the complex changes in fetal body composition during pregnancy (Koong *et al.*, 1975; Battaglia and Meschia, 1986). At the end of gestation, fetal mass varies considerably, reflecting differences in both fetal growth rate and gestation length (Battaglia and Meschia, 1986).

It was also reported that postembryonic fetal growth through mid-pregnancy proceeds at a rapid but slowly diminishing relative rate, which in sheep varies from about 15%/day at 40 days to about 6%/day at 100 days gestation. Until that time, fetal growth appears to be largely insensitive to variations in maternal nutrition or other potential constraints, suggesting that it occurs at a rate close to genetic potential (Bell, 1992). This early fetal growth characteristic may provide the reason why the restriction of post-implantation embryonic growth in sheep by severe maternal undernutrition in early pregnancy had no residual effect on lamb birth weight if ewes were later fed normally (Parr *et al.*, 1986). However, even though fetal growth during early to mid pregnancy may not be affected by nutrient supply, fetuses of ewes

undernourished before pregnancy and in early pregnancy grew more slowly in late pregnancy and showed much less change in their growth rate when ewes were exposed to further undernutrition. Thus it is likely that, in early pregnancy, nutrition has an important role in the 'programming' of fetal response to later insults (Harding and Johnston, 1995).

In addition, it was also reported that weight-specific rates of umbilical uptake of oxygen, glucose and amino acids, and of whole-body protein synthesis, in fetal sheep were much greater in mid than in late gestation (Bell *et al.*, 1986; Bell *et al.*, 1989; Kennaugh *et al.*, 1987). Similar gestational declines in fetal metabolic rates also occur in cattle (Reynold *et al.*, 1986). Therefore, this physiological characteristic of nutrient supply may also explain that in normal conditions fetal metabolism and growth in mid-pregnancy is not restricted by nutrient supply, and that fetal metabolism and growth are normally constrained during late pregnancy (Bell, 1992). Thus, it is likely that fetal growth retardation during early to mid pregnancy may be just as a consequence of a placental retardation caused by maternal undernutrition or other factors such as maternal heat stress (Everitt, 1968; Alexander *et al.*, 1987). The retarded placenta causes the reduction in functional capacity of the placenta, which in turn mediates a slowing fetal growth (Bell, 1992).

On the other hand, fetal growth retardation in late gestation seems to be predominantly affected by placental restriction of fetal nutrient supply, even in very well-fed dams (Gluckman, 1986; Bell *et al.*, 1987). It was suggested that the aspects of placental functional capacity which may be limiting for fetal growth are associated with reduced placental transport of oxygen and glucose. Thus, fetal hypoxaemia and hypoglycaemia are well correlated with reduction in placental weight (Bell, 1992). However, in normal placentae, it is suggested that placental capacity of glucose transport is a more likely limiting factor of fetal growth (Bell, 1992).

In vivo experiments in pregnant ewes have shown that glucose transport capacity is greater on the fetal (cotyledonary) than on the maternal (caruncular) surface of the placenta (Hay *et al.*, 1990). Therefore, it is suggested that the maternal capacity for

uterine uptake and placental transport of glucose is a component of placental insufficiency during late pregnancy (Bell, 1992).

It is then suggested that the declining rate of fetal growth during late pregnancy must involve interplay between nutrient supply and both extracellular (endocrine) and local (autocrine/paracrine) trophic factors (Bell, 1992). However, this does not mean that early fetal growth is unregulated by extracellular factors, even though the mechanism by which these factors may regulate or coordinate the extremely dynamic phase of this fetal growth remain to be fully understood.

4.2.2 Placental control of fetal growth and development

The role of the placenta in controlling fetal growth is exerted through its effects on the growth of the fetus from the beginning of pregnancy via metabolic and endocrine mechanisms. To achieve this, the placenta exchanges a wide array of nutrients, endocrine signals, cytokines and growth factors with the mother and the fetus. These exchanges, in turn, will modulate or programme fetal growth and development (Robinson *et al.*, 1995).

Maternal nutritional and hormonal state, from as early as the first few days after fertilisation, can influence the growth rate of the placenta and fetus, and also the length of gestation. Influences on placental development and their consequences will clearly have an impact on the placental control of fetal growth (Robinson *et al.*, 1995).

4.3. Hormonal regulation of fetal growth

Endocrine regulation of fetal growth, particularly at a tissue or cellular level, is less well understood than the endocrine regulation of postnatal growth. A study involving intrauterine removal of the key hormones known to control postnatal growth showed that these hormones also have a critical role in normal fetal development (Fowden, 1989). *In vivo* studies summarised by Fowden (1995), showed that the ablation of the fetal pancreas, thyroid, pituitary and adrenal all affect fetal development, although the

extent and precise nature of the abnormalities in growth depend on the particular endocrine deficiency, its severity and duration.

Hence, it is apparent that hormones have an important role in the control of fetal growth throughout gestation, particularly during the last trimester of gestation. They seem to act on both tissue accretion and differentiation (Fowden, 1995). Several hormones, such as insulin, thyroid hormones, glucocorticoids, some growth factors and perhaps pituitary growth hormone and prolactin, and also placental hormones, have been found to have an important role in fetal growth.

4.3.1. Growth hormone (GH)

There is a marked increase in plasma GH concentration during fetal life, and a subsequent decline during the postnatal period (Gluckman *et al.*, 1987; Breier *et al.*, 1994). However, it has been well established that growth during the fetal and early postnatal periods is largely independent of pituitary GH (Gluckman *et al.*, 1983). This GH-independent growth is suggested to be related to the appearance of GH receptors, in which the number of GH receptors is relatively low during the fetal and early neonatal periods, but begins to increase soon after birth (Gluckman *et al.*, 1983).

In contrast, GH has been reported to have a role in adipocyte differentiation and islet cell replication, and has also been shown to be lipolytic and diabetogenic in fetuses of several species (Gluckman and Breier, 1989). Thus, it seems that the ontogenesis of GH receptors maybe not necessarily be parallel in all tissues. However, several recent studies appear to confirm the relative independence of fetal growth from GH regulatory effects.

It was reported that congenital absence of GH in the human infant and specific manipulation of fetal GH concentrations in experimental animals (rat, sheep, monkey, pig) are not associated with major changes in fetal growth (Gluckman, 1986; Milner, 1988). GH treatment of the hypophysectomized sheep fetus was also reported to have little, if any, effect on body weight at term (Stevens and Alexander, 1986).

Based on studies summarised by Fowden (1995), it was found that the pattern of body growth observed after hypophysectomy of the sheep fetus is asymmetrical and closely resembles that seen in thyroidectomised fetuses with disproportionate shortening of the long bones and a greater reduction in body weight than in CRL (Fowden, 1995). The maturation of individual fetal tissues is also adversely affected by fetal hypophysectomy in a manner similar to that seen in thyroidectomised and adrenalectomised sheep fetuses. Treatment with T₄ (thyroxine), ACTH or cortisol in hypophysectomised sheep fetuses was reported to be able to correct most of the abnormalities in tissue development caused by hypophysectomy (Barnes *et al.*, 1978; Mesiano *et al.*, 1987; Silver, 1990). It was also reported that treatment with T₄ restored normal body weight but not CRL or limb lengths in the hypophysectomized sheep fetus at term (Steven and Alexander, 1986; Mesiano *et al.*, 1987; Fowden and Silver, 1995).

Hence, it was suggested that the intrauterine growth retardation due to the total deficiency of all pituitary hormones is caused primarily by the lack of thyroid-stimulating hormone (TSH) and adrenocorticotrophic hormone (ACTH), but not by GH deficiency *per se* (Fowden, 1995).

However, fetal hypophysectomy has also been reported to have specific effects on fetal body composition. It leads to increased subcutaneous fat deposits, which are not found in either thyroidectomized or adrenalectomized fetuses (Hopkins and Thornburn, 1972; Barnes *et al.*, 1978; Steven and Alexander, 1986). Similar increases in lipogenesis were also reported to be observed in fetal pigs after hypophysectomy (Latimer *et al.*, 1993). Furthermore, the increase in fat accumulation of fetal sheep after hypophysectomy is prevented by GH treatment but not by T₃ or ACTH treatments (Steven and Alexander, 1986). These studies suggest that GH may be involved in fetal lipid metabolism. This finding may also support the study reporting that GH can exert its lipolytic and diabetogenic effects in fetuses of different species (Gluckman and Breier, 1989). However, it has recently been well established that neither lipolytic or diabetogenic effects of GH are an intrinsic property of GH, and that GH has been known to antagonise the action of insulin *in utero* (Parkes and Bassett,

1985). Thus, it is more likely that the role of GH in fetal lipid metabolism may not be direct, but indirect, and mediated through changes in the effective insulin concentration (Fowden, 1995).

Overall, GH does not seem to have a major role in fetal growth, except perhaps in its indirect effects on fetal lipid metabolism. In addition, even though the lack of pituitary hormones has resulted in a reduction in IGF-I (but not IGF-II) concentrations in fetal sheep and pigs (Thorburn *et al.*, 1988; Mesiano *et al.*, 1989; Latimer *et al.*, 1993), GH does not also seem to modulate endocrine or autocrine/paracrine actions of IGFs on fetal growth (Latimer *et al.*, 1993).

In addition, recently it is reported that bGH receptor transcripts have been shown to be widespread in fetal and placental tissues at mid pregnancy (Scott *et al.*, 1992). However, despite high circulating concentrations of GH, there is no specific bGH-GH receptor binding found during that period. Since it was known that placental lactogens have the ability to bind GH receptors with a higher affinity of binding than GH itself, the presence of GH receptor transcripts, which is likely to be translated into a certain type of GH receptors, will enable PL to bind to these receptors (Scott *et al.*, 1992). Therefore, the role of GH during fetal life may be represented by a part of the paracrine/autocrine role of placental lactogen on fetal growth and development.

4.3.2. Insulin-like growth factors (IGFs)

Insulin-like growth factors (IGFs) have been reported to have metabolic, mitogenic and differentiative activities, all of which play a major role in promoting growth before and after birth (Sara and Hall, 1990). However, IGFs' production and their controls seem to be different in pre- and postnatal animals. In contrast to adult animals, the IGF-II gene is expressed in a wide variety of tissues in the fetus (Gilmour *et al.*, 1992). Tissue abundance of IGF-II mRNA is therefore high in the fetus compared with postnatal values, although downregulation of the gene does occur in certain fetal tissues in the period immediately before birth (Delhanty and Han, 1993; Li *et al.*, 1993). This down regulation of the IGF-II gene expression is likely under the control

of the negative feedback mechanism regulated by IGF-I, IGF-II and high concentrations of insulin via IGF type I receptors (Magri *et al.*, 1994).

Circulating IGF-II concentrations in the fetus are 5-6 fold greater than postnatal values in most species. There is also a prepartum decrease in plasma IGF-II concentration in the sheep fetus which closely parallels the decrease in tissue IGF-II abundance observed in the fetal liver and adrenal (Delhanty and Han, 1993; Li *et al.*, 1993). In contrast, circulating concentrations of IGF-I and its tissue expression are low *in utero* but rise rapidly after birth (Sara and Hall, 1990). Thus, as discussed previously, it is IGF-II that is more well known to have a role in fetal development than IGF-I. However recent studies show that IGF-I is also recognised to have an important role in fetal development, perhaps more important than that of IGF-II, particularly in domestic animals.

Both fetal IGF-I and IGF-II production are controlled by hormonal and nutritional factors. It was reported that both IGF-I and IGF-II actions in fetal growth are likely to be regulated by hormones such as insulin, T₄ and cortisol (glucocorticoid), but do not appear to involve GH as occurs in postnatal growth (Fowden, 1995). Thus, the actions of IGFs in the fetus may be more paracrine than endocrine, although changes in circulating IGFs concentrations do occur in response to physiological stimuli and with increasing gestational age (Han and Fowden, 1994).

It was reported that circulating concentrations of IGF-I and IGF-II closely parallel the fetal glucose concentration during manipulations of the maternal nutritional state (Gluckman, 1986; Oliver *et al.*, 1993). In sheep, mean fetal IGF-I concentrations are reduced by maternal fasting and are restored to normal values by fetal or maternal glucose infusion but not by fetal amino acid administration (Oliver *et al.*, 1993). It was also reported that IGF-I concentrations can be altered by insulin independently of the fetal glucose concentration (Fowden, 1989). Thus, it is likely that changes in IGF-I concentrations may be due, in part, to the concomitant changes in fetal plasma insulin concentrations (Fowden, 1995), and that fetal IGF-I concentrations are therefore directly related to plasma insulin concentrations over the range of concentrations

observed *in utero* (Gluckman *et al.*, 1987). Hence, it has been suggested that it is the cellular availability of glucose, which is controlled by insulin, which is the critical factor in regulating fetal IGF-I production (Fowden, 1995).

On the other hand, fetal IGF-II concentrations have been reported to be more closely correlated with the concentration of fetal glucose than fetal insulin throughout the various endocrine and nutritional manipulations (Fowden, 1989). Since it **was known** that cortisol has effects on IGF-II gene expression in several fetal tissues, and that cortisol concentration rises in response to fetal hypoglycaemia during late gestation, it was suggested that changes in fetal cortisol may account, in part, for the relationship between plasma IGF-II concentrations and glucose concentrations (Silver, 1990; Li *et al.*, 1993; Lu *et al.*, 1994).

Other hormones such as T_4 are also known to affect fetal IGF-I production and may influence fetal IGF-II gene expression in certain species (Mesiano *et al.*, 1989; Latimer *et al.*, 1993). It was suggested that cortisol and T_4 act by altering IGFs gene transcription, but the mechanisms whereby nutrients such as glucose regulate the IGF gene remain unknown (Fowden, 1995).

Hence, it is apparent that both IGF-I and IGF-II production are regulated by the same nutritional factor, glucose, but by slightly different hormonal factors. IGF-I production is governed by insulin and T_4 , whereas IGF-II production is governed by cortisol and perhaps T_4 . These regulatory factors are interdependent in affecting IGFs production and in turn, they stimulate IGFs actions on fetal growth and development (Figure 1).

The IGFs are known to be anabolic and stimulate fetal growth by both metabolic and non-metabolic mechanisms. Both IGFs have been reported to increase protein and glycogen synthesis in fetal tissues and cell lines maintained in culture (Sara and Carlson-Skwirut, 1988). They were also reported to act as progression factors in the cell cycle, and increase DNA synthesis and cell differentiation in a variety of different fetal cells *in vitro* (Hill *et al.*, 1987).

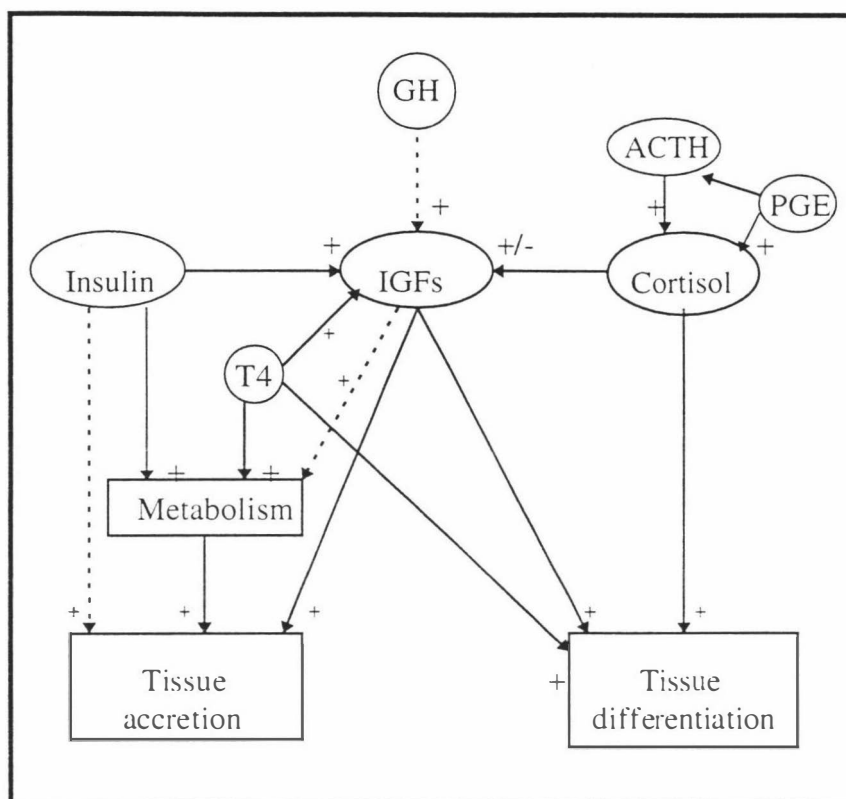


Figure 1. Schematic diagram to show the endocrine control of fetal growth and development: known pathways (—); possible pathway (---); stimulatory effects (+); inhibitory effects (-). GH, growth hormone; ACTH, adrenocorticotrophic hormone; PGE, prostaglandin E; IGFs, insulin-like growth factors (Fowden, 1995).

Hence, the effect of the classical hormones (pituitary hormones) on fetal growth may be mediated, in part, via the IGFs or their receptors as outlined in Figure 1. Insulin, T₄, and cortisol have all been shown to affect circulating or tissue IGF concentrations and their effects on tissue accretion and differentiation are similar to those of IGFs. In addition, since insulin, and T₄ enhance fetal IGF-I production, these effects may all be mediated via the type I IGF receptor which preferentially binds IGF-I (Han and Fowden, 1994). However, the actions of insulin and IGF-I on fetal glucose metabolism appear to be more distinct. In the sheep fetus, infusion of IGF-I increases placental in preference to fetal glucose consumption, whereas insulin infusion increases fetal, but not placental, glucose utilisation (Hay, 1991; Owens, 1991).

The effects and the relative importance of the two IGFs in stimulating cell growth *in vivo* remain unclear. In rodents, IGF-II is essential for cell proliferation. Deletion of the IGF-II gene in fetal mice retards fetal growth by 40% whereas IGF-II administration to 10-day-old rat embryos accelerates their growth (Liu *et al.*, 1989; De Chiara *et al.*, 1990). In other species (human, sheep, guinea-pig, pig), fetal body weight is more closely correlated with the serum concentrations of IGF-I than IGF-II (Hill *et al.*, 1987; Sara and Hall, 1990). In addition, conditions which lead to fetal growth retardation, such as fasting, hypoxia and placental insufficiency, have more pronounced effects on tissue IGF-I mRNA abundance than on IGF-II mRNA abundance, even in the rat (Owens, 1991; Han and Fowden, 1994). Thus, it was suggested that fetal IGF-I has a more prominent role than fetal IGF-II in modulating cell proliferation in relation to the specific endocrine and nutritional conditions prevailing *in utero*. Fetal IGF-II is suggested to provide a general stimulus for cell growth *in utero* and may also be responsible for developmental and tissue-specific changes in cell differentiation (Fowden, 1995). It has also been reported that IGF-II potentiates and enhances the effect of IGF-I on cell growth regulation independent of direct IGF-II interaction with IGF type I or II receptors. This enhanced cell responsiveness to IGF-I appears to be due to IGF-II-induced changes in pericellular IGF binding protein-3 (IGFBP-3) and IGFBP-4. These IGFBPs barriers at the cell surface are disrupted by IGF-II, so increasing IGF-I availability for IGF type I receptor interaction (Conover *et al.*, 1994).

Thus, differential effects of the two IGFs on fetal growth observed between species and during different intrauterine conditions may be due, in part, to changes in the production of the IGF-binding proteins (IGFBPs) (Fowden, 1995). It has been established that IGFBPs are a major determinant of IGFs concentrations in the blood and substantially modify the biological activities of IGFs (Sara and Carlsson-Skwirut, 1988). Among the six IGFBPs that are present in blood circulation or extravascular fluid, IGFBP-1, IGFBP-2 and IGFBP-5 are IGFBPs that are more likely to be involved in normal fetal growth and development, whereas IGFBP-3 is related to fetal growth retardation (Barrett *et al.*, 1994; Lassarre and Binoux, 1994; Beanland *et al.*, 1995).

4.3.3. Prolactin (PRL) and PRL/GH gene family

So far there are no conclusive studies that support the possible role of pituitary prolactin in regulation of growth or in mediating the photoperiod-induced growth response in both fetal and postnatal ruminants (Eisemann *et al.*, 1984; Bauman and McCutcheon, 1986). However, a high concentration of prolactin, up to 100-fold higher than in maternal serum, was found in amniotic fluid during human pregnancy (Riddick, 1985; Wu *et al.*, 1993). It was previously presumed that amniotic prolactin originated from either the maternal or the fetal pituitary gland (Riddick, 1985). Finally it was found that the prolactin was produced specifically by the decidualised stromal cells in the endometrium of the utero-placental unit (Wu *et al.*, 1993). It has also been recently reported that mRNA for prolactin has been produced in decidual stromal cells of human utero-placental tissues. Prolactin gene expression increases throughout gestation and is correlated with an increase in decidual prolactin secretion as well as in decidual cell size. This decidual prolactin has been suggested to be directly related to the process of decidualization that continues throughout human pregnancy (Wu *et al.*, 1995).

Recently it has also been recognised that hormones related to the pituitary hormones PRL and GH are produced by the utero-placental unit of many species (Kessler *et al.*, 1991; Wu *et al.*, 1993; Anthony *et al.*, 1995). These hormones are suggested to be involved in the process of communication between embryo and mother in order to develop and maintain an appropriate environment for development. They play various roles in this process, including modulating the uterine environment (Kessler *et al.*, 1991).

Hormones such as placental lactogens (PLs), prolactin-related proteins (PRPs) and placental growth hormone (GH-V) are hormones that are members of the GH/PRL gene family and are produced by the placenta (Robert and Anthony, 1994) particularly the chorionic binucleate cells of the placentome (Anthony *et al.*, 1995). Ruminant placenta has been reported to produce at least two distinct subclasses of the GH/PRL gene family, PLs and PRPs (Milosavijevic *et al.*, 1992; Anthony *et al.*, 1995).

Furthermore, bovine and ovine placental lactogens are structurally more similar to PRL than they are to GH, and they have ability to bind to both PRL and GH receptors (Kessler *et al.*, 1991). The binding affinity of PLs to PRL and GH receptors are higher than for PRL and GH themselves, and yet, in many ways, PL behaves like a powerful somatogen (Emane *et al.*, 1986). PRPs appear also to have the ability to bind PRL receptors as well as their own receptors (Scott *et al.*, 1992).

In the pig and rabbit, PRL receptors have been identified in the uterine endometrium, and PRL has been reported to cause hypertrophy and glandular differentiation and to alter uterine secretions (Young and Bazer, 1989; Young *et al.*, 1989; Randall *et al.*, 1991). In cattle, PRL and GH receptor transcripts are widespread in fetal and placental tissues at mid-pregnancy (Scott *et al.*, 1992). It was suggested that PLs exert their action via PRL and GH receptors as well as via their own receptors. Thus, physiologically, placental lactogens will compete with other available ligands.

In addition, fetal GH secretion begins relatively early, and circulating bPL and bGH concentrations are relatively equal in the bovine fetus during the second trimester (Oxender, 1971). On the other hand, PRL secretion begins later, positioning bPL as the primary ligand for the PRL receptors in the fetus at mid pregnancy. However, in the sheep fetuses the ability of oPL to compete for binding to PRL receptors only occurs when the oPL concentration is 5-fold higher than oPRL concentrations (Scott *et al.*, 1992).

Hence, it is likely that the role of PRL in fetal or placental development may be mediated by PL or PRP actions via their interactions to PRL receptors, and that prolactin concentrations can modify the affinity of the PL/PRP binding to PRL receptors.

Ruminant placental lactogens, particularly ovine PLs, are suggested to play a role in modifying maternal and fetal intermediary metabolism to provide energy substrates to the fetus, repartitioning maternal nutrition to the fetus. It has been proposed that the

possible effects of ovine PL on regulation of fetal metabolism are exerted, in part, via its effects on upregulating IGFs, especially IGF-I, production (Anthony *et al.*, 1995).

Prolactin has been reported to cause a diminished flux of water from the amniotic side of fetal membranes which is proportionate to the amount of prolactin present in the bathing media and is not reproducible by hPL or hGH. This effect is inhibited by antiprolactin in the bathing medium *in vitro* (Riddick, 1985). Therefore, besides PRL acting via PRL receptors binding PL, and hence its actions being mediated by PL actions, PRL or PRPs are also suggested to be involved directly in the regulation-of electrolyte and fluid balance between the maternal and fetus systems (Riddick, 1985). The action of PRL via PL actions is likely to be exerted in different stages of gestation from that of its direct actions.

4.3.4. Other hormones

As outlined in Figure 1, there are several other hormones, apart from GH, that seem to have an important role in regulation of fetal growth. These hormones are insulin, thyroid hormones and glucocorticoids. They act directly at the cellular level to promote fetal growth and development, and indirectly through their regulatory effects on paracrine/autocrine actions of IGFs, which in turn promote fetal growth and development.

4.3.4.1. Insulin

Insulin has been recognised to primarily promote tissue accretion and cell proliferation of the fetus, but to have little effect on tissue differentiation and prepartum maturation of the fetal tissues (Fowden, 1995). Insulin promotes fetal growth mainly through its anabolic actions on fetal metabolism (Hay, 1991). Insulin stimulates not only a general increase in tissue accretion but also a specific accumulation of adipose tissue in the fetus (Fowden *et al.*, 1986). In addition to increasing fat deposition, exogenous infusion of insulin into the fetus increases the uptake, utilisation and oxidation of glucose by fetal tissues (Hay, 1991). In sheep fetuses, it was suggested that insulin acts

in the fetus to depress amino acid catabolism, thus altering amino acid extraction and uptake. Depressed protein catabolism with or without enhanced amino acid uptake would have the theoretical effect of stimulating net protein synthesis with a shift toward use of nonprotein substrates for energy purposes (Philipps *et al.*, 1990).

Insulin is also required throughout late gestation for normal fetal growth. Its deficiency *in utero* leads to fetal growth retardation, whereas excessive insulin secretion can lead to enhanced body weight at term (Fowden, 1989). Both in experimental conditions with manipulated insulin concentrations in the sheep fetus, and in normal conditions in fetuses of some species (sheep, rat, rabbits, and human infant), it is apparent that fetal growth rate, measured as CRL increment or fetal body weight, is positively correlated with the insulin concentrations *in utero* (Fowden, 1989).

4.3.4.2. Thyroid hormones

Thyroid hormones, particularly T₄, have been reported to have an important role in both tissue accretion and differentiation in the fetus (Fowden, 1995; Polk, 1995). However, their specific actions in utero seem to differ between species and with gestational age. Deficiency of thyroid hormones is associated with growth retardation in certain tissues (Browne and Thornburn, 1989). The specific effects that thyroid hormones have on cell differentiation appear to depend on the stage of development of the fetus. In sheep fetuses, thyroidectomy only prevents wool growth when it is performed before 90 days of gestation (Thornburn, 1974). Thereafter, it has little effect on the primary wool follicles although body size and bodyweight are still reduced at term (Fowden, 1995). Thyroid hormones stimulate fetal growth by both metabolic and non-metabolic mechanisms. The main metabolic action of T₄ that is important in controlling fetal growth is the stimulation of O₂ utilisation by the fetal tissues. As a consequence of a low O₂ uptake, the fetus will be less able to use energy for growth (Fowden, 1995).

In addition, thyroid hormones probably affect fetal growth by altering tissue production and circulating concentrations of various fetal growth factors (e.g. IGFs, EGF) and their binding proteins (Thorburn *et al.*, 1981; Sara and Hall, 1990; Han and Fowden, 1994).

4.3.4.3. Glucocorticoids

Glucocorticoids are known to inhibit postnatal growth but are recognised to have a role in fetal tissue accretion *in utero* (Fowden, 1995). They have been also reported to have major effects on the differentiation and prepartum maturation of many fetal tissues in the period immediately before birth (Silver, 1990). In fetal sheep, cortisol is mainly of maternal origin and its concentrations are low for most of gestation, and then rise gradually from 10 to 15 days before term with final rapid increase in the last 3-5 days before birth (Silver and Fowden, 1988). This later increased cortisol concentration is derived mainly from the fetal adrenal (Wintour *et al.*, 1985). Hence, it may suggest that effects on fetal tissue accretion and differentiation are exerted dominantly in the late period of gestation.

However, it is likely that normal, *in vivo* fetal growth and development will be affected by a number of different hormones and/or growth factors acting simultaneously and interacting with each other at both the metabolic and molecular level to ensure that the rate and the pattern of growth is appropriate for the specific conditions prevailing *in utero* (Fowden, 1995).

5. PURPOSE AND SCOPE OF STUDY

The substantially lower birth weights in lambs born in autumn or winter compared with those born in spring were previously proposed to be due to a direct seasonal effect on impaired placental development rather than to effects of maternal nutrition. This seasonal effect is likely to be exhibited during the early to mid gestational period (by

days 84-100 of gestation), which is during the summer period for ewes due to lamb in the autumn, and is suggested to be mediated by increased seasonal prolactin concentrations during that period.

Plasma prolactin concentrations are very high in autumn-lambing ewes (December-mated ewes) during the early to mid gestation period due to a seasonal (primarily daylength) effect (December-February period). Although, there is no conclusive evidence of role for prolactin in regulation of seasonal-induced placental and/or fetal development, and hence of birth weight, it is apparent that there is a negative relationship between birth weights of autumn-born lambs and seasonal plasma prolactin concentrations in the ewes and in the conceptus (utero-placenta plus fetus) during the early to mid gestation. Hence, there may be an endocrine mechanism that directly or indirectly involves the seasonal prolactin patterns in the regulation of placental and fetal growth and development, particularly during the early to mid gestational period.

If the seasonal prolactin pattern is important in the regulation of placental and fetal growth and development, reducing prolactin concentrations at that time could be expected to improve placental and fetal growth and development, and hence increase birth weight. The objective of this study was therefore to determine whether reducing seasonal prolactin concentrations in the dams and/or in the fetuses, by bromocriptine and melatonin treatments during early to mid gestation, would improve placental and fetal growth near term, or increase birth weights, of autumn-born lambs.

CHAPTER TWO

EFFECTS OF MATERNAL BROMOCRIPTINE AND MELATONIN TREATMENTS ON FETAL DEVELOPMENT

1. ABSTRACT

Lowered birth weights of autumn (May)-born lambs compared with those of spring (August)-born lambs have been suggested to be due to a direct seasonal effect on impaired placental development, which in turn mediates a slower fetal growth. The effect of season on placental and fetal development is established early in pregnancy (by day 84 of gestation), and is suggested to be mediated by higher summer circulating prolactin concentrations in the dams and/or fetuses. This study attempted to determine whether reducing circulating prolactin concentrations in the dams and/or in the fetuses, by bromocriptine (a dopaminergic agonist) and melatonin treatments, during early to mid gestation, would improve placental and fetal growth near term, and/or increase birth weights of autumn-born lambs.

Pregnant Romney ewes (aged 3-6 years) were randomly allocated to 3 treatment groups, balanced for age, live weight and source flocks, at day 15 of mating. Ewes in the first group (n=20 with mean LWT of 58.3 ± 6.7 kg) were injected with Parlodel (Bromocriptine mesilate), the second group (n=20 with mean LWT of 58.6 ± 6.6 kg) were administered Regulin implants containing melatonin and the third group (n=21 with mean LWT of 58.5 ± 5.8 kg) were injected with physiological saline (0.9% NaCl) and used as the control group. On day 139 of gestation, ewes were balanced first for treatment group, source farm, live weight, age and litter size, and were then randomly allocated into two subgroups: a "Slaughter group" (n=30 with mean LWT of 61.2 ± 5.6 kg) and a "Live birth group" (n=31 with mean LWT of 60.0 ± 7.1 kg).

Maternal plasma prolactin concentrations were significantly reduced by both Parlodel (control vs Parlodel): 48.28 ± 9.24 vs 0.69 ± 9.92 ng/ml ($P < 0.001$) at day 19 of gestation, and 82.64 ± 8.46 vs 4.51 ± 9.08 ng/ml ($P < 0.001$) at day 40 of gestation, and Regulin treatments (control vs Regulin): 82.64 ± 8.46 vs 18.39 ± 9.47 ng/ml ($P < 0.001$) at day 40 of gestation, and 16.84 ± 3.74 vs 2.93 ± 4.19 ng/ml ($P < 0.05$) at day 60 of gestation. Fetal plasma prolactin concentrations were not affected by Parlodel or Regulin treatments (control vs Parlodel vs Regulin, respectively): 32.4 ± 18.3 vs 32.4 ± 19.1 vs 59.8 ± 16.4 ng /ml.

At day 140 of gestation, measures of fetal growth and placental development, adjusted for sex and litter size, were (control vs Parlodel vs Regulin groups): individual fetal weight (5.1 ± 0.2 vs 5.0 ± 0.2 vs 4.9 ± 0.2 kg); fetal CRL (59.0 ± 0.7 vs 57.8 ± 0.8 vs 57.4 ± 0.7 cm); individual occupied caruncle weight (2.6 ± 0.3 vs 2.5 ± 0.3 vs 2.0 ± 0.3 g); individual cotyledon weight (3.3 ± 0.4 vs 3.1 ± 0.4 vs 3.0 ± 0.4 g); total caruncle number (121.0 ± 7.7 vs 114.1 ± 7.7 vs 123.1 ± 7.7); placentome number (92.4 ± 7.1 vs 92.0 ± 7.1 vs 99.2 ± 7.1); caruncle occupancy, i.e., number of placentomes/number of caruncles (76.9 ± 4.1 vs 80.4 ± 4.1 vs 81.3 ± 4.1 %); and total placentome weight (447.7 ± 28.9 vs 473.6 ± 25.5 vs 434.0 ± 27.2 g). Lamb birth weights were similar in both control and treated groups (4.3 ± 0.2 ; 4.4 ± 0.2 ; and 4.2 ± 0.2 kg for control, Parlodel and Regulin groups, respectively).

Despite both Parlodel and Regulin treatments having successfully reduced maternal plasma prolactin concentrations in the autumn-lambing ewes, these results indicate that neither Parlodel nor Regulin affects placental and fetal growth, suggesting that high prolactin concentrations in early pregnancy are not responsible for the depressed placental and fetal growth observed in autumn-lambing ewes.

Keywords Parlodel; Regulin; prolactin; birth weight; placental development; fetal growth; autumn-lambing ewes

2. INTRODUCTION

Relying only on a traditional lambing system (March - May mating) has created a highly seasonal killing pattern and consequent marketing difficulties for the New Zealand sheep industry. Extending the normal lambing pattern may overcome these problems. The use of hormones to induce oestrus in anoestrous ewes ("out-of-season breeding") has enabled farmers to extend the normal breeding season and permitted mating of ewes earlier than normal (Robinson, 1954; Welch and Tervit, 1970; Welch, 1985). The application of both normal season and out-of-season lamb production has given opportunities to provide a year-round supply of chilled lamb, especially for overseas supermarkets. However, the system of out-of-season lamb production still has a problem relating to birth weights. Most experimental results have shown that lambs born to autumn-lambing ewes were up to 25-30% lighter at birth than those born to spring-lambing ewes (Reid *et al.*, 1988; Peterson *et al.*, 1990; Morris, 1993). Given that low lamb birth weight has an important impact, both on the neonatal survival of lambs (Dalton *et al.*, 1980; McCutcheon *et al.*, 1981), and on their subsequent growth to weaning (Schinckel and Short, 1961), finding a possible way of controlling fetal growth is important in the context of sheep production.

A previous study (Jenkinson *et al.*, 1994) proposed that lowered birth weights of autumn (May)-born lambs compared with those of spring (August)-born lambs are due to a direct seasonal effect on impaired placental development, rather than to effects of maternal nutrition, which in turn mediates a slower fetal growth. The effect of season on placental and fetal development is established early in pregnancy (by day 84 of gestation), and is suggested to be mediated by seasonal differences in circulating prolactin concentrations in the dams and/or fetuses (Jenkinson *et al.*, 1994; McCoard *et al.*, 1996).

Plasma prolactin concentrations are likely to be very high in autumn-lambing ewes (December-mated ewes) during the early- to mid-gestation period (December-February) due to a seasonal (primarily daylength) effect (Bassett, 1992; Pearson *et al.*,

1993; Pearson *et al.*, 1996). Although there is lack of evidence that leads to a conclusive role for prolactin in regulation of seasonally-induced placental and/or fetal development, and hence of birth weight, it is apparent that there is a negative relationship between birth weights of autumn-born lambs and seasonal plasma prolactin concentrations in the ewes and in the conceptus (utero-placenta plus fetus) during early to mid gestation. Hence, there may be an endocrine mechanism that directly or indirectly involves the changes in seasonal prolactin concentrations in the regulation of placental and fetal growth and development, particularly during the early to mid gestational period. If the seasonal prolactin concentration is important in the regulation of placental and fetal growth and development, reducing prolactin concentrations at the appropriate time might be expected to improve placental and fetal growth and development, and hence increase birth weight.

In addition, declining circulating prolactin levels around the time of the summer solstice are thought to be involved in the regulation of seasonal changes in wool growth (Lincoln, 1990). Changes in maternal plasma prolactin concentrations due to seasonal (primarily daylength) effects have also been associated with udder development and milk production in sheep (Ortavant *et al.*, 1988; Peterson *et al.*, 1990; Bassett, 1992).

The primary objective of this study was therefore to determine whether reduced seasonal prolactin concentrations in the dams and/or in the fetuses, induced by bromocriptine (a dopaminergic agonist) and melatonin treatments during early to mid gestation, would improve placental and fetal growth near term, and/or increase birth weights and weaning weights, of autumn-born lambs. A secondary objective was to determine whether the treatments affected wool growth or mammary development in the ewes.

3. MATERIALS AND METHODS

3.1. Animals and treatments

This study involved 117 Romney ewes aged 3-6 years, obtained from two commercial flocks on the Sheep and Beef Cattle Research Unit (SBCRU), Massey University. The trial was conducted on the Terrace Block, SBCRU. All ewes had lambed in the previous spring, and were weaned in early December. The ewes were shorn on 27-28 November 1994 (i.e., a few weeks prior to mating)

Mating was accomplished using a combination of progesterone-impregnated controlled internal drug releasers (EAZI-breed CIDR Type G, Carter Holt Harvey Plastic Products, Hamilton, New Zealand) and pregnant mare serum gonadotrophin (PMSG Folligon, Intervet, Chemavet Division Pharmaco (NZ) Ltd, Auckland) to induce ovulation outside the normal breeding season, and a high ram to ewe ratio (Knight *et al.*, 1989). The CIDRs, each containing 0.3 g progesterone, were inserted intravaginally for 10 days. PMSG (400 IU/ewe) was injected intramuscularly on the day before CIDR removal. Border Leicester rams, at a ratio of 1 ram to 10 ewes, were introduced about 24 hours after CIDR removal.

Rams were harnessed with mating crayons (Radford *et al.*, 1960), and crayon marks were recorded on a daily basis (between 0900 and 1800 h). Mating was for one cycle only. Crayon colour was changed on day 7 and the rams were removed on day 15 of the mating period. The second day after CIDR removal (the day after the rams were joined) was the mean mating date (18 December=day 0 of gestation).

At day 15 of mating, 105 ewes had been mated. Their average live weight (LWT) was 58.5 ± 6.4 kg. On this day, the ewes were randomly allocated to 3 treatment groups balanced for age, previous live weight and source flock: group I with mean LWT of 58.3 ± 6.7 kg, group II with mean LWT of 58.6 ± 6.6 kg, and group III with mean LWT of 58.5 ± 5.8 kg.

Parlodel LA[®] (Bromocriptine mesilate) and Regulin[®] implants were administered to modulate plasma prolactin and melatonin concentrations during early gestation. To avoid any effects of treatments on the endogenous signal of a viable embryo (13 days after conception) for the maintenance of the corpus luteum and establishment of pregnancy (Smith, 1982), treatments commenced at day 15 of gestation (about 18 days after ovulation or about 13 days after the embryo passes into the uterus)(Wimsatt, 1975).

Each ewe in group I (Parlodel group) was injected intramuscularly with Parlodel LA[®] (Sandoz Pharma Ltd, Basle, Switzerland), a long-acting inhibitor of prolactin secretion, containing 50 mg bromocriptine mesilate in 2 ml vehicle (dextran 70 in 0.9% NaCl).

Each ewe in group II (Regulin group) was implanted with Regulin[®] (Regulin Limited, South Melbourne, Vic., Australia), a slow-release implant of melatonin. The implants were biodegradable (approx. length 4 mm, diameter 2 mm) and contained 18 mg melatonin. They were inserted subcutaneously at the base of the ear and were designed to release melatonin at a constant rate for about 30 to 40 days (Handbook, Regulin Limited).

Each ewe in group III (Control group) was injected intramuscularly in the neck area with 2 ml physiological saline (0.9% NaCl).

To assist in balancing treatment groups for litter size, pregnancy was diagnosed using ultrasound pregnancy detection at day 80 of gestation. Pregnancy diagnosis revealed 39 dry ewes and 66 ewes pregnant to the first oestrus after CIDR removal. Non-pregnant ewes were then removed from the trial.

On day 139 of gestation, the 61 pregnant ewes remaining were balanced first for treatment group, source flock, live weight, age and expected litter size, and then were randomly allocated into two subgroups: a "Slaughter group" (n = 30 with mean LWT = 61.2 ± 5.6 kg) and a "Live birth group" (n = 31 with mean LWT = 60.0 ± 7.1 kg).

The “Slaughter group” was then divided into 3 groups (balanced for treatment and litter size) to be slaughtered on days 140, 141 and 142 of gestation, whereas the “Live birth group” was maintained in one group and monitored for any changes in live weight until their lambs were of weaning age (about 8 weeks of age).

3.2. Grazing management and weighing

Ewes of all groups were grazed together at pasture until day 139 of gestation, on the Massey University Sheep and Beef Cattle Research Unit (SBCRU), and managed to maintain similar live weights. Ewes were weighed on electronic scales straight off pasture at twenty-day intervals to monitor any changes in live weight. Lambs from ewes of the “Live birth group” were weighed on the day they were born (using a simple spring balance) and thereafter on the same day as their dams were weighed (using electronic laboratory bench scales).

3.3. Blood sampling

Blood samples were collected by jugular venipuncture, to determine circulating concentrations of prolactin, immediately off pasture (between 1000 h and 1600 h) at regular intervals of about 20 days. The first blood sample was taken 3 days before expected conception (just before PMSG injection) and samples followed at days 19, 40, 60, 80, 100, 120, 140 of gestation for both “Slaughter” and “Live birth” groups, and continued every 20 days for the “Live birth” group until their lambs were of weaning age (56-70 days after parturition). At day 140, 141 and 142 of gestation, ewes of the “Slaughter group” were blood sampled immediately prior to slaughter, and foetuses were blood sampled by cardiac puncture immediately prior to euthanasia using an overdose of pentobarbitone sodium. Samples (about 8 ml) were withdrawn into vacutainers (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey) containing sodium heparin as the anticoagulant and immediately placed on ice. Within 1 to 3 hours the samples were centrifuged at 3000 g and 4° C for 20 minutes. Plasma was pipetted into duplicate vials and stored at -20°C until assayed.

3.4. Wool sampling

Wool growth during pregnancy was monitored every forty days up to day 120 of gestation, by clipping midside wool samples taken from the right midside of the ewes (Bigham, 1974). An initial patch measuring 20 cm x 20 cm was cleared of wool using Oster or Sunbeam clippers on day -3 of gestation, and subsequently an area of about 100 cm² (10 cm x 10 cm) was sampled within this patch. The dimensions of the patch were measured and the area calculated at each sampling time. The midside samples were then conditioned and scoured to obtain the clean sample weights and the clean wool growth rates calculated for each 40-day period. Scoured, conditioned fibres, cut to lengths between 0.4 and 0.8 mm, were spread on a slide in a circle and placed on the movable stage of a projection microscope (Teasdale, 1988), and then mean fibre diameter (MFD) of the samples was measured using an optical fibre diameter analyser (OFDA) (IWTO-47-95).

3.5. Slaughtering procedures

Thirty ewes were slaughtered immediately off pasture, commencing at 0930 h on day 140 (10 ewes), 141 (10 ewes) and 142 (10 ewes) of gestation, by stunning with a captive bolt pistol and exsanguination.

Immediately prior to slaughter, the dimensions of the mammary gland of each ewe were measured as described by Mellor and Murray (1985). Three duplicate measurements were taken. One measurement was made from the posterior margin to the anterior margin of the udder (i.e., along the midline), and one on each side of the midline, parallel to the first measurement and immediately medial to each teat. The measurements were then summed and the mean taken. Immediately after slaughter, the mammary gland was removed and trimmed of skin, fat, and connective tissue before being weighed.

Next, the abdominal cavity was opened and the uterus removed. A ligature was tied at the junction of the cervix and uterus, and the cervix, vagina and associated tissue removed. The uterus was then weighed (total gravid uterus weight). The foetuses were removed from the uterus, through an incision made along the greater curvature of the pregnant horn, and the umbilical cord ligated at the abdomen before being cut. Foetuses were then blood sampled by cardiac puncture. Foetal number, weight and sex were recorded. The allantoic and amniotic fluids were removed without being weighed, and placentomes were dissected from the uterus, separated into their maternal (caruncle) and foetal (cotyledon) components, counted, and their individual weights recorded. The myoendometrium was then weighed.

The liver, spleen, heart, kidneys, lungs and thyroid glands of the ewes were dissected out, blotted dry and their fresh weights recorded (combined weights of bilateral organs). Sections of the alimentary tract, comprising rumino-reticulum, omasum, abomasum, small intestine, caecum and large intestine were removed, flushed of their contents, blotted dry and their fresh weights recorded.

Foetal crown-rump length and foetal girths were measured (Mellor and Matheson 1979). Foetal liver, spleen, heart, kidneys, lungs, brain, thyroid and thymus were removed, blotted dry and weighed (combined weights of bilateral organs).

3.6. Assays

The prolactin assay was a standard (homologous) double-antibody competitive binding radioimmunoassay (RIA) based upon the method of van Landeghem and van de Weil (1978). Standard ovine prolactin, NIADDK-oPRL-18 (AFP-8277E, 30 IU/mg) from National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA, and supplied through National Hormone and Pituitary Program, University of Maryland School of Medicine, was used at working concentrations of 1, 10, 25, 50, 100, 150, 200, 400, 600, 800 and 1200 ng/ml with a linear range of approximately 10-800 ng/ml. The first antibody,

NIADDK-anti-oPRL-2 (AFP-C358106) rabbit-anti-ovine prolactin, donated by the National Hormone and Pituitary Program, University of Maryland School of Medicine, was used at a working dilution of 1: 30,000 (final dilution 1: 550,000). The second antibody, donkey anti-rabbit-serum (Lot No. 24761, IDS, Washington, Tyne and Wear, England), was used at working dilution of 1:40. Assay binding was typically 50-65% and the mean assay sensitivity was 0.41 ng/ml. Ovine plasma samples, assayed neat or at stepwise serial dilutions up to final dilution of 1: 128, 1: 256 and 1: 512, exhibited parallelism with the standards. Plasma samples were assayed in triplicate. The mean intra-assay coefficient of variation (CV) calculated over 6 assays was 8.3% and the mean inter-assay CV was 12.8% for 5 reference plasma samples corresponding with the linear portion of the standard curve.

3.7. Statistical analysis

Analysis of variance for a 3x2x2 factorial design was carried out to compare the effects of the three treatments (control, Parlodel and Regulin treatments), litter size (single and twin) and sex (male and female) on maternal and foetal factors. All data were analysed using the GLM procedures of the Statistical Analysis System computer package (SAS, 1988). Maternal organ weights, uterine components, foetal weights, foetal organ weights, foetal plasma prolactin concentrations and lamb birth weights were analysed using analysis of covariance or variance. Any significant differences between treatments were then tested using the LSM-test (SAS,1988).

Clean wool growth rates, mean fibre diameters, sequential weights of lambs and ewes, and maternal plasma prolactin concentrations were analysed using multivariate (repeated measures) analysis of covariance or variance (SAS, 1988), to determine any effects of treatments on those parameters (adjusted to a common litter size and sex for lamb weights). Data are expressed in terms of least square means and standard errors (Lsmeans \pm s.e.m.).

4. RESULTS

4.1. Maternal traits

Figure 2 shows the changes in ewe live weight from day -3 of gestation (around mating day) through to day 140 of gestation of both the “slaughter” and the “live birth” ewes, and to day 60 postpartum of the “live birth” ewes. The mean live weights of Parlodel- and Regulin-treated ewes did not differ significantly from that of control ewes at mating, at the initial commencement of treatment, or at slaughter. The maximum difference in live weight occurred between Regulin and control groups (2.92 kg or 4.93 %) at day 100 of gestation (Table 2). However, this difference was not significant.

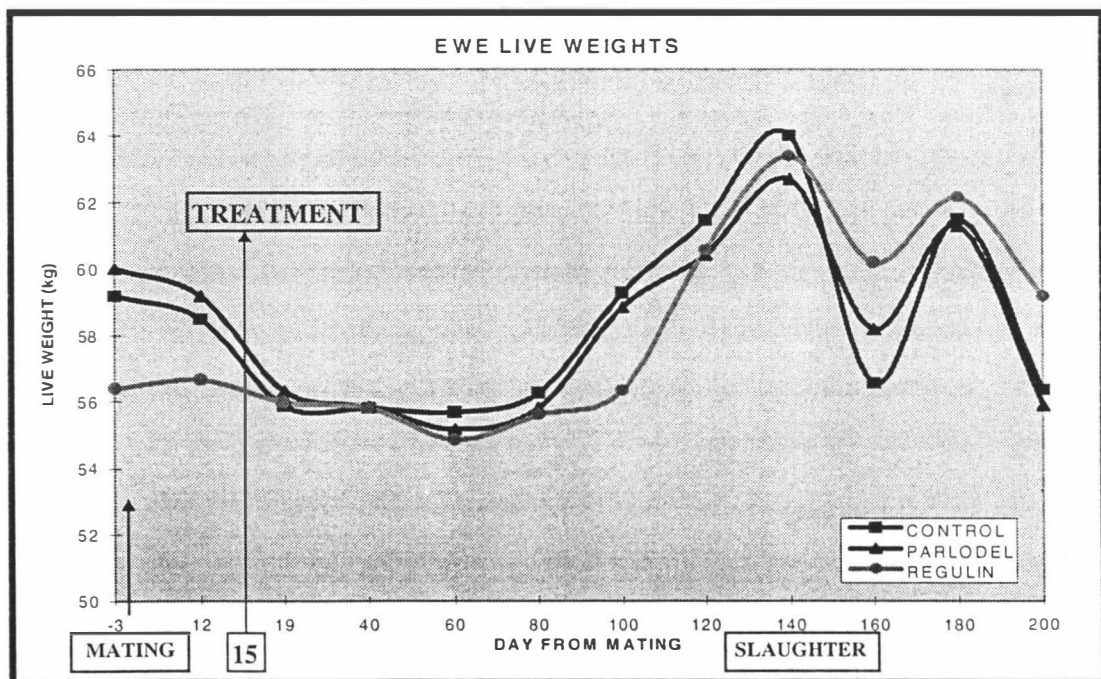


Figure 2. Live weights of Control (n=21), Parlodel-(n= 20) and Regulin-(n=20) treated ewes in the “slaughter” (until day 140) and the “live birth” groups.

Table 2. Live weights (kg) of Control, Parlodel- and Regulin-treated ewes from 3 d prior to, until 200 d after, conception (Lsmeans \pm s.e.m.). Data include means for both the “slaughter” and the “live birth” groups up to and including day 140, and subsequently for only the “live birth group”.

DAY	n	CONTROL	PARLODEL	REGULIN
-3	61	59.18 \pm 1.26	60.00 \pm 1.32	56.39 \pm 1.39
12	61	58.48 \pm 1.28	59.18 \pm 1.35	56.66 \pm 1.38
19	61	55.86 \pm 0.51	56.31 \pm 0.56	55.97 \pm 0.56
40 ⁻	61	55.81 \pm 0.59	55.84 \pm 0.59	55.81 \pm 0.59
60	61	55.68 \pm 0.69	55.18 \pm 0.76	54.86 \pm 0.76
80	61	56.26 \pm 0.60	55.81 \pm 0.65	55.59 \pm 0.65
100	61	59.26 \pm 1.03	58.83 \pm 1.12	56.34 \pm 1.13
120	61	61.44 \pm 0.74	60.41 \pm 0.81	60.56 \pm 0.81
140	61	64.00 \pm 0.76	62.68 \pm 0.83	63.37 \pm 0.84
160	31	56.55 \pm 1.74	58.19 \pm 2.21	60.18 \pm 2.17
180	31	61.48 \pm 1.76	61.28 \pm 2.24	62.14 \pm 2.20
200	31	56.36 \pm 1.61	55.90 \pm 2.05	59.16 \pm 2.02

Figure 3 shows the changes in mean maternal circulating concentrations of prolactin in control, Parlodel- and Regulin-treated ewes from day -3 to 140 of gestation, and through to 60 days postpartum. Parlodel treatment significantly lowered circulating concentrations of prolactin almost immediately after the treatment commenced, i.e., from day 15 of gestation, until about day 50 of gestation ($P < 0.001$), whereas Regulin treatment lowered prolactin concentrations from about day 30 until day 60 of gestation. The maximum difference occurred at day 40 of gestation ($P < 0.001$).

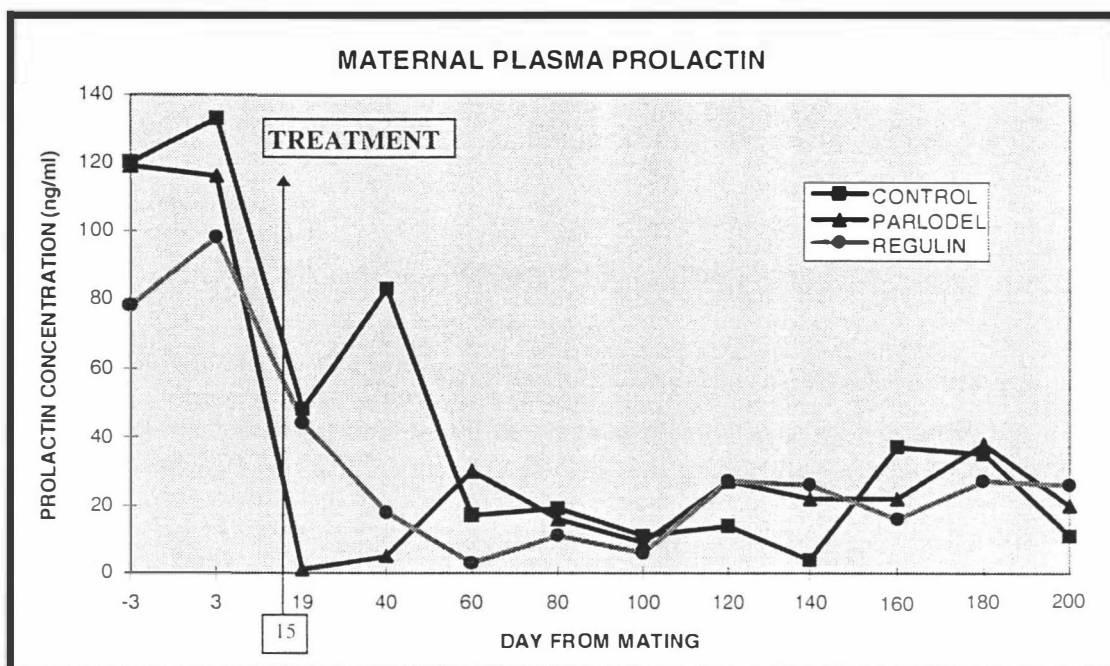


Figure 3. Maternal plasma prolactin concentrations in Control (n=21), Parlodel- (n=20) and Regulin- (n=20) treated ewes of both the “slaughter” and the “live birth” groups from day -3 to day 140 of gestation, and through to 60 days postpartum of the “live birth” ewes (n=11, 10 and 10 for control, Parlodel and Regulin groups, respectively).

Table 3. Maternal plasma concentrations (ng/ml) of prolactin from day -3 to day 140 of gestation and through to 60 days postpartum in Control, Parlodel-and Regulin-treated ewes (Lsmeans ± s.e.m.).

DAY	n	CONTROL	PARLODEL	REGULIN
-3	61	119.78 ± 12.40	118.85 ± 13.45	77.81 ± 13.86
3	61	132.93 ± 15.17	115.90 ± 16.46	98.22 ± 16.96
19	61	48.28 ± 9.24	0.69 ± 9.92 ^{***}	43.63 ± 10.35
40	61	82.64 ± 8.46	4.51 ± 9.08 ^{***}	18.39 ± 9.47 ^{***}
60	61	16.84 ± 3.74	29.72 ± 4.01 [*]	2.93 ± 4.19 [*]
80	61	19.29 ± 5.50	15.87 ± 5.90	10.89 ± 6.15
100	61	10.66 ± 2.51	8.74 ± 2.70	6.47 ± 2.81
120	61	13.95 ± 6.44	26.90 ± 6.91	27.18 ± 7.21
140	61	3.66 ± 6.94	22.31 ± 7.45	26.25 ± 7.77 [*]
160	31	37.11 ± 15.83	22.05 ± 19.38	15.90 ± 21.23
180	31	34.73 ± 14.13	37.58 ± 17.31	26.58 ± 18.96
200	31	10.91 ± 8.02	19.95 ± 9.82	26.42 ± 10.76

* P < 0.05 ** P < 0.01 *** P < 0.001 (comparison with control group)

Live weights, carcass and pelt weights, organ weights, and lower digestive tract segment weights of control, Parlodel- and Regulin-treated ewes are presented in Table 4. None of these parameters was significantly affected by either Parlodel or Regulin treatments.

Table 4. Effect of Parlodel and Regulin treatments on live weights, carcass and pelt weights, organ and lower digestive tract segment weights of treated ewes compared to control ewes at day 140 of gestation (Lsmeans \pm s.e.m.).

	CONTROL	PARLODEL	REGULIN
n	10	10	10
Final live weight (kg)	64.0 \pm 0.8	62.7 \pm 0.8	63.4 \pm 0.8
Carcass weight (kg)	24.4 \pm 0.8	23.9 \pm 0.8	24.2 \pm 0.8
Pelt weight (kg)	6.2 \pm 0.6	5.5 \pm 0.6	5.6 \pm 0.6
<u>Organ weights (g) :</u>			
Liver	923.6 \pm 39.0	938.8 \pm 37.6	941.2 \pm 40.2
Heart	250.5 \pm 7.2	265.3 \pm 6.9	264.9 \pm 7.4
Spleen	81.6 \pm 6.7	84.0 \pm 6.4	91.8 \pm 6.9
Kidneys	153.7 \pm 4.7	153.0 \pm 4.5	150.5 \pm 4.8
Lungs	479.5 \pm 15.6	457.8 \pm 15.0	471.3 \pm 16.1
Thyroid	7.3 \pm 0.8	8.4 \pm 0.7	6.3 \pm 0.8
<u>Lower digestive tract (g) :</u>			
Reticulo-rumen	1129.3 \pm 48.3	1137.4 \pm 47.9	1150.5 \pm 48.6
Omasum	108.1 \pm 7.8	122.9 \pm 7.8	120.4 \pm 7.9
Abomasum	498.4 \pm 40.6	549.0 \pm 40.3	455.0 \pm 40.9
Small intestine	1089.5 \pm 54.3	1134.3 \pm 53.8	1173.4 \pm 54.6
Caecum	103.7 \pm 17.6	114.6 \pm 17.4	112.6 \pm 17.7
Large intestine	963.3 \pm 48.0	941.9 \pm 47.5	897.8 \pm 48.3

Neither weight nor dimension of the mammary gland was significantly different between the treatment groups (Table 5).

Table 5. Effect of Parlodel and Regulín treatments on mammary gland weights and dimensions of treated ewes compared to control ewes at day 140 of gestation (Lsmeans \pm s.e.m.).

	CONTROL	PARLODEL	REGULIN
<u>Mammary Gland :</u>			
Weight (g)	709.6 \pm 68.9	696.9 \pm 68.9	811.9 \pm 68.9
Dimension (cm)	45.6 \pm 2.0	43.4 \pm 2.0	46.8 \pm 2.1

Table 6 presents the mean weights of the uterus and uterine components of control, Parlodel- and Regulín-treated ewes. There were no significant differences in these parameters between control and treated groups. However, it was noticed that there was a large variation in the size of placental components (e.g., 2.78 \pm 1.05 g and 2.68 \pm 1.06 g for caruncle and cotyledone weights, respectively) within individual placenta, suggesting that there were some undeveloped placentomes as well as very large placentomes within a placenta.

Table 6. Uterine components of Control, Parlodel- and Regulín-treated ewes at day 140 of gestation (Lsmean \pm s.e.m.)

	CONTROL	PARLODEL	REGULIN
n	10	10	10
<u>Weight (kg) of:</u>			
Gravid uterus	11.4 \pm 0.4	10.8 \pm 0.4	10.9 \pm 0.4
Foetus (individual)	5.1 \pm 0.2	5.0 \pm 0.2	4.9 \pm 0.2
<u>Weight (g) of:</u>			
Myoendometrium	704.6 \pm 21.9	674.8 \pm 21.9	672.0 \pm 22.1
Foetal membranes	275.0 \pm 11.6	276.7 \pm 11.6	277.6 \pm 11.7
Occ. caruncle (individual)	2.6 \pm 0.3	2.5 \pm 0.3	2.0 \pm 0.3
Cotyledon (individual)	3.3 \pm 0.4	3.1 \pm 0.4	3.0 \pm 0.4
Placentome (individual)	5.9 \pm 0.5	5.8 \pm 0.5	4.6 \pm 0.5
Total placentome	447.7 \pm 28.9	473.6 \pm 25.	434.0 \pm 27.2
<u>Number of</u>			
Placentomes	92.4 \pm 7.1	92.0 \pm 7.1	99.2 \pm 7.1
U.O. caruncles	27.6 \pm 5.3	19.1 \pm 5.3	23.9 \pm 5.3
Total caruncles	121.0 \pm 7.7	114.1 \pm 7.7	123.1 \pm 7.7
Caruncle occupancy (%)	76.9 \pm 4.1	80.4 \pm 4.1	81.3 \pm 4.1

Occ. : occupied

U.O. : unoccupied

Clean wool growth rate and fibre diameter were not significantly affected by either Parlodel or Regulin treatments (Table 7).

Table 7. Clean wool growth rate (mg/mm²/d) and fibre diameter (μm) of Control, Parlodel- and Regulin-treated ewes (Lsmean ± s.e.m.).

Stage of Gestation (days)	CONTROL	PARLODEL	REGULIN
n	21	20	20
<i>Wool growth rate (mg/mm²/d):</i>			
-3 - 40	0.0117 ± 0.0007	0.0120 ± 0.0007	0.0123 ± 0.0007
40 - 80	0.0102 ± 0.0007	0.0097 ± 0.0007	0.0097 ± 0.0007
80 - 120	0.0090 ± 0.0005	0.0085 ± 0.0005	0.0081 ± 0.0006
<i>Fibre diameter(μm):</i>			
-3 - 40	41.68 ± 0.64	41.41 ± 0.67	42.25 ± 0.68
40 - 80	39.07 ± 0.66	37.82 ± 0.70	38.56 ± 0.71
80 - 120	37.52 ± 0.67	37.19 ± 0.70	37.30 ± 0.72

4.2. Fetal traits

Fetuses from Parlodel- and Regulin-treated ewes had a similar body weight to those of control ewes (Table 8). Heart and thyroid weights were significantly ($P < 0.05$) higher in fetuses from Parlodel treated-ewes than in those from Control ewes, whereas the thymus weight in fetuses from the Regulin group was significantly ($P < 0.05$) lower than that of the control group. Crown-rump length (CRL) and weights of other fetal organs were not significantly different between fetuses from control and treated ewes.

Table 8. Effect of maternal Parlodel and Regulin treatments on CRL, girth, body and organ weights of sheep fetuses compared to fetuses of control ewes at day 140 of gestation (Lsmeans \pm s.e.m.).

	CONTROL	PARLODEL	REGULIN
n	13	11	15
CRL (cm)	59.0 \pm 0.7	57.8 \pm 0.8	57.4 \pm 0.7
Girth (cm)	36.8 \pm 0.5	37.3 \pm 0.6	36.2 \pm 0.5
Body weight (kg)	5.1 \pm 0.2	5.0 \pm 0.2	4.9 \pm 0.2
<u>Weights (g) of :</u>			
Brain	44.9 \pm 1.0	45.8 \pm 1.0	45.4 \pm 0.9
Liver	112.8 \pm 3.6	105.9 \pm 3.8	110.3 \pm 3.3
Heart	34.1 \pm 1.1	37.7 \pm 1.2*	33.8 \pm 1.0
Spleen	5.5 \pm 0.5	6.0 \pm 0.5	5.7 \pm 0.4
Kidneys	22.8 \pm 0.9	23.9 \pm 0.9	22.3 \pm 0.8
Lungs	144.7 \pm 7.2	139.4 \pm 7.5	133.7 \pm 6.5
Thyroid	2.0 \pm 0.3	3.0 \pm 0.3*	2.3 \pm 0.0
Thymus	20.7 \pm 1.2	20.7 \pm 1.2	16.5 \pm 1.1*

* P < 0.05 (compared with control group)

Fetuses from both Parlodel- and Regulin-treated ewes had similar circulating concentrations of prolactin to those of fetuses from control ewes (Table 9). However, the mean fetal prolactin concentration in the Regulin group tended to be higher than those of the control and Parlodel groups.

Table 9. Circulating concentration of prolactin in fetuses from Control, Parlodel- and Regulin-treated ewes at day 140 of gestation (Lsmean \pm s.e.m.).

	CONTROL	PARLODEL	REGULIN
n	13	11	15
Prolactin (ng/ml)	32.4 \pm 18.3	32.4 \pm 19.1	59.8 \pm 16.4

4.3. Lamb traits

There was no significant difference in birth weights of lambs from control and treated ewes (Table 10).

Table 10. Effect of maternal Parlodel and Regulin treatments on birth weights of autumn-born lambs (Lsmeans \pm s.e.m.).

	CONTROL	PARLODEL	REGULIN
n	15	13	11
Birth weight (kg)	4.3 \pm 0.2	4.4 \pm 0.2	4.2 \pm 0.2

Figure 4 shows the changes in lamb live weight from birth until day 60 postpartum. Lambs from Parlodel- and Regulin-treated ewes had a similar live weights ($P > 0.10$) to those of control ewes.

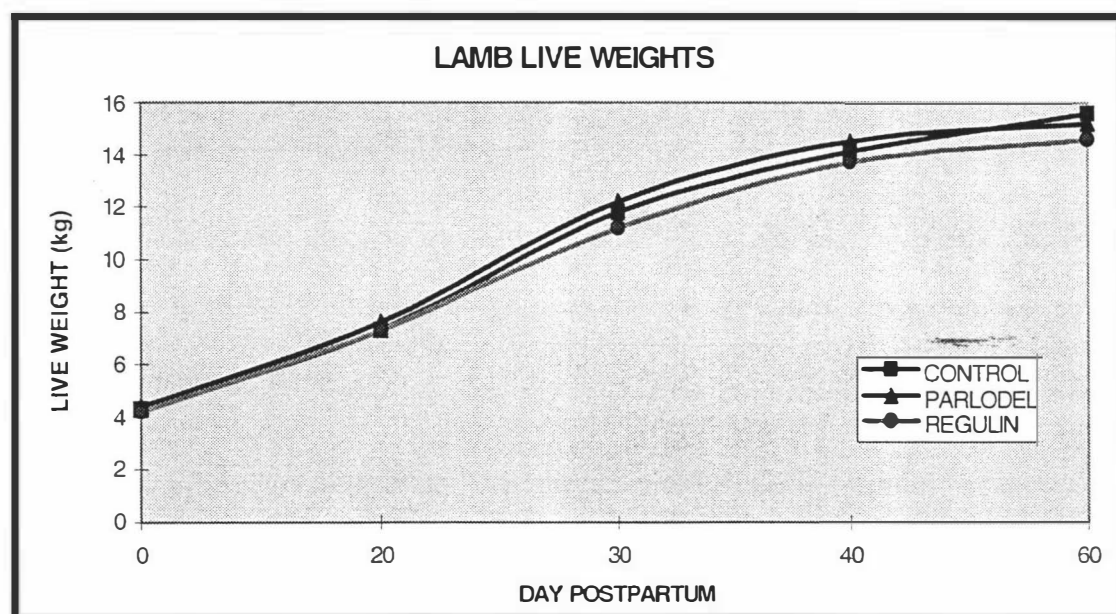


Figure 4. Live weights of lambs from control (n= 15), Parlodel (n= 13) and Regulin (n= 11) groups from birth until 60 days postpartum.

5. DISCUSSION

There were no significant differences in the live weights of control, Parlodel- and Regulin-treated ewes during the trial period (December-July period), suggesting that maternal live weights were not affected either by treatment effects, or by possible stress effects due to the application of Parlodel injection or of Regulin implantation. Although Regulin treatment did not seem to affect maternal live weights, it appeared to delay the late gestation increase in live weight compared with that of control ewes (though differences in live weight were not significant).

Both Parlodel and Regulin administration significantly reduced maternal plasma prolactin concentrations, for a period of about 30 days, but did not affect fetal plasma prolactin concentrations. Although both treatments commenced on the same day (i.e., day 15 of gestation), Parlodel reduced maternal plasma prolactin concentrations to almost zero by day 19 of gestation, and concentrations remained very low until about day 50 of gestation, whereas Regulin reduced maternal plasma prolactin concentrations more gradually, not reaching minimal levels until day 60 of gestation. The different onset in the reduction of maternal plasma prolactin concentrations induced by these treatments seems to have a similar pattern to the late gestation increase in live weights in the treated ewes, i.e., later suppression of maternal plasma prolactin concentrations by Regulin treatment also resulted in a trend towards a delay in the increase in maternal live weights (Figure 2) compared with that in the Parlodel group.

Maternal organ, carcass and pelt weights, wool growth, wool fibre diameter, mammary gland dimensions and mammary gland weights were not affected by Parlodel or Regulin treatments, suggesting that there were no effects of either treatment on body composition of treated ewes. However, while increased wool growth rates were observed in ewes, under natural daylength from early summer to late autumn, in the study of Pearson *et al.* (1996), Parlodel- and Regulin-treated ewes in this study had declining wool growth rates as well as fibre diameters from early pregnancy until the second half of pregnancy. The average wool growth rate observed in this study was

higher (1.002 vs 0.27 mg/cm²/d) than that of Pearson *et al.* (1996). The differences are probably due to breed and nutrition differences. The reduction in maternal plasma prolactin concentrations apparently did not affect the pattern of wool growth rates in the treated ewes, since the same patterns of wool growth rates were also found in the control group (Table 7).

Suppression of maternal plasma prolactin concentrations during early pregnancy by both treatments did not have significant effects on mammary gland weights and dimensions, indicating that prolactin may not be essential for promoting mammary gland development during early pregnancy. In contrast, a study by Forsyth *et al.* (1985) showed that suppression of prolactin release throughout much of pregnancy led to some delay in mammary development in does, without affecting the ultimate mammary size and milk production. It was suggested that during such periods, the loss of prolactin secretion was compensated for by the production of placental lactogen.

It is also possible that since the treatments were commenced when the prolactin concentrations in the control group was declining, so the reduced prolactin concentrations, induced by the treatments, might not have effects on both wool growth rates and mammary gland development in the treated ewes.

Individual fetal weights, fetal crown-rump lengths (CRLs), fetal girths and lamb birth weights (adjusted for litter size and sex) were not significantly affected by either Parlodel or Regulin treatments. The lower birth weight of the lambs compared with the weight of the fetuses from both Parlodel and Regulin groups, may be due to the wet condition of the fetuses soon after being removed from the uterus, or the discrepancy may be due to different calibration of the simple spring scales used to weigh newborn lambs in the field, compared to the electronic laboratory scales used to weigh the fetuses.

Treatment with Parlodel resulted in significantly greater weights of the fetal hearts and thyroids (Table 8). An increase in an organ weight is usually associated with an increased activity of the organ, hence it is likely that increased weights of the fetal

hearts and thyroids in Parlodel-treated ewes indicated increased activities of those organs in the regulation of fetal growth and metabolism. An increased fetal heart weight may indicate an increase in blood flow in the fetus, which is very important for facilitating transport of nutrients and O₂ from the placenta to the fetus, and also transport of the metabolic waste from the fetus to the placenta (Robinson *et al.*, 1995). Since thyroids are the sites of thyroid hormone production, the higher weight of the thyroids may be an indicator of greater thyroid hormone production. Thyroid hormone is very important in upregulating the production of various fetal growth factors, including IGFs (Sara and Hall, 1990; Han and Fowden, 1994), as well as in stimulating O₂ utilisation by the fetal tissues (Fowden, 1995).

Parlodel and Regulin treatments had no significant effects on uterine components. Jenkinson *et al.* (1994) suggested that impaired placental growth in autumn-lambing ewes had a strong relationship with very high summer plasma prolactin concentrations during early to mid pregnancy. However in this study, reduced plasma prolactin concentrations during early pregnancy in autumn-lambing ewes, caused either by Parlodel or Regulin treatment, did not improve placental development assessed in terms of placentome formation, including total placentome weight, number of placentomes and percentage of caruncles occupied. This result leads to the conclusion that seasonal differences in prolactin concentrations are not responsible for the seasonal difference in placental and fetal development, and hence lamb birth weights, in autumn- and spring-lambing ewes.

The efficiency of the placenta, in terms of fetal weight per gram of cotyledons, decreases as placental weight increases (Alexander, 1974; Mellor, 1983), and there is a compensatory increase in cotyledon weights as caruncle number declines (Alexander, 1964). Also, cotyledons appear to be nourished by glucose from the fetal circulation rather than by glucose in transit from the mother, and this utilisation of the glucose accounts for virtually all of the glucose consumed by the uteroplacental tissues (Hay, 1991; Bassett, 1992). Furthermore, a reduction in birth weights of human infants has been well associated with a reduction in the functional surface area for exchange within the placenta (Robinson *et al.*, 1995). Hence, it is likely that the proportional

weight of cotyledons in the placentome formation is essential for facilitating efficient nutrient and oxygen transport from the mother to the fetus as well as nutrition partitioning between fetus and the placenta. The use of parameters such as total weight of the placentomes, the number of placentomes and the percentage of caruncles occupied, seems insufficient for explaining the importance of placental size in determining the fetal growth or birth weight. The percentage of cotyledons and placentomes within the placenta that are of optimal size may be an important parameter, since the cotyledonary proportion may have important effects associated with placental efficiency.

The reduction of maternal plasma prolactin concentrations during a period of about 30 days was the only marked effect induced by administration of Parlodel and Regulin. However, Parlodel and Regulin treatments did not affect fetal plasma prolactin concentrations.

Data from the control group, i.e., December-mated control ewes, indicate that the profile of prolactin release observed in this study was consistent with the profile of prolactin release under natural photoperiod, observed in studies by Craven *et al.* (1994) and Pearson *et al.* (1993; 1996), i.e., plasma prolactin concentrations were high during early to mid December, then declined slightly during the end of the month, and rose again during the period of January-February, reaching its summer peak in late January. Thereafter, plasma prolactin concentrations declined to levels similar to previous spring levels. This profile of prolactin secretion in New Zealand ewes seems slightly different with the classic profile observed in the northern hemisphere (Ravault *et al.*, 1982; Bassett, 1992). Using the classic profile of seasonal plasma prolactin concentrations presented by Ravault *et al.* (1982) and Bassett (1992), it can be seen that plasma prolactin concentrations in pregnant ewes are low during the winter period and begin to increase during the spring period. Hence, it is likely that there is an inverse profile of maternal, and maybe also fetal, plasma prolactin concentrations in summer and winter periods of gestation, and only slight differences in variation of maternal, and perhaps fetal, plasma prolactin concentrations during the autumn and spring period of gestation in ewes. The inverse profile in maternal plasma prolactin

concentrations is, in part, also observed in December- and March-mated ewes by day 100 of gestation, during which their gestation periods are in summer and winter, respectively (Jenkinson, 1994). In that study, there were no significant differences found between plasma prolactin concentrations of those autumn- and spring-lambing ewes at day 140 of gestation. This may be because, at day 140 of gestation, both spring- and autumn-lambing ewes were in early spring and autumn respectively, during which there were no marked differences in daylength periods. Later, from 1 or 2 days prior to lambing until the first week of lactation, there are markedly higher seasonal plasma prolactin concentrations in spring-lambing ewes than in autumn-lambing ewes, as observed by Peterson *et al.* (1990). However, this cannot account for differences in fetal weights observed at day 140 of gestation.

During early to mid pregnancy, higher summer maternal and fetal plasma prolactin concentrations in autumn-lambing ewes, appear to be detrimental to fetal growth, and may lead to lower birth weights of their lambs compared those of spring-lambing ewes which, when they are in the same period of gestation, are likely to have lower maternal and fetal plasma prolactin concentrations. Prolactin seems to have a negative effect on fetal growth, when it has been actually, but also controversially, proposed as a somatogenic and homeorhetic hormone both in prenatal and postnatal life (Bauman *et al.*, 1982). The results of the present study could not confirm the role of seasonal prolactin in regulating fetal growth either. Although the apparent inverse relationships suggest a possible role of prolactin as proposed previously, the role of prolactin during fetal life may be mediated by a different mechanism, and might involve an interplay between prolactin and other hormones such as placental lactogen.

The prolactin/GH gene family produced by the utero-placental tissues, particularly placental lactogen (PL) and prolactin related protein (PRP), have been recognised to have major effects in regulation of fetal growth and metabolism (Anthony, 1995). These placental hormones have a stronger affinity to bind prolactin and GH receptors than prolactin and GH themselves (Emane *et al.*, 1986; Kessler *et al.*, 1991). However, the ability of placental lactogens to bind prolactin receptors in the ovine fetal tissues is exerted only when their concentrations are at least 5-fold higher than

plasma concentrations of fetal prolactin (Scott *et al.*, 1992). Furthermore, it was reported that utero-placental prolactin is normally secreted later than PL secretion, positioning PL as the main ligand for prolactin receptors in the fetal tissues during early to mid gestation (Scott *et al.*, 1992). Hence, it is strongly suggested that the somatogenic as well as homeorhetic effects of prolactin during early, and perhaps mid-, pregnancy are taken over by placental lactogen effects. The effects of placental lactogen in promoting growth and regulating fetal metabolism may be coupled by its ability to recognise more than one receptor, including its ability to bind to GH receptors. Measuring fetal plasma placental lactogen concentrations would have been valuable in this trial.

The original purpose of this study was to attempt to mimic the naturally low winter plasma prolactin concentrations in spring-lambing ewes. Although the reduced maternal plasma prolactin concentrations caused by Parlodel and Regulin treatments in this study did not significantly affect placental and fetal growth, and hence lamb birth weight, of autumn-lambing ewes, perhaps it is too early to conclude that seasonal prolactin change does not have any roles in regulation of fetal growth. It is possible that the period of reduced plasma prolactin concentration produced by both Parlodel and Regulin treatment carried out in this study was inadequate to mimic the period of low winter plasma concentrations of prolactin in spring-lambing ewes.

In conclusion, further studies are required to determine a suitable timing and length of treatment period using Parlodel and/or Regulin administration, which can mimic the length period of low winter maternal, and perhaps fetal, plasma prolactin concentrations. As the 30-day treatments carried out in this study appear insufficient to imitate the period of naturally low winter plasma concentrations of prolactin in spring-lambing ewes, perhaps it is necessary to prolong the period of treatment used to reduce maternal plasma prolactin concentrations for a period of 60 to 80 days.

With the possibility that placental lactogen may be involved in the regulation of placental and fetal growth during early to mid pregnancy, it may be useful to measure fetal plasma concentrations of placental lactogen, in addition to prolactin

concentration, to obtain prolactin-placental lactogen ratios during early to mid gestation.

For further studies, it is important to consider the cotyledon-caruncle ratio, or optimal size of placentomes, in addition to those of total placentome weight, number of placentomes and caruncle occupancy for assessing the importance of placental size in determining fetal growth and lamb birth weights. What size of these ratios might be considered as being optimal, perhaps, can be obtained by calculating the correlation between the cotyledon-caruncle ratio and the fetal weight from all available raw data of these placental-fetal components.

CHAPTER THREE

GENERAL DISCUSSION

1. EVALUATION OF THE PRESENT STUDY

Both Parlodel and Regulin treatments successfully reduced plasma prolactin concentrations of autumn-lambing ewes during early pregnancy to approximately basal plasma concentrations of prolactin for periods of about 30 days. However, this study has not been able to confirm any role of prolactin in regulating seasonal differences in placental and fetal growth.

Considering the natural profile of seasonal prolactin secretion in pregnant ewes mated in the breeding season or out-of-season (Ravault *et al.*, 1982; Bassett, 1992; Pearson *et al.*, 1993; Craven *et al.*, 1994; Jenkinson *et al.*, 1994; Pearson *et al.*, 1996), it is apparent that there is an inverse relationship between the profile of prolactin secretions during early to mid pregnancy and the fetal weight or the lamb birth weight in both March-mated (season breeding) and December-mated (out-of season breeding) ewes.

Significantly lower winter plasma concentrations of prolactin in March-mated ewes, compared with summer plasma concentrations of prolactin in December-mated ewes (Ravault *et al.*, 1988; Bassett, 1992), have been associated with 25-30 % heavier lambs than those born to autumn-lambing ewes (Reid *et al.*, 1988; Peterson *et al.*, 1990; Morris *et al.*, 1993). This marked inverse relationship suggests that seasonal prolactin secretions are important in directly or indirectly controlling fetal growth and lamb birth weight. Such a negative effect produced by high plasma prolactin concentrations on fetal growth of autumn-born lambs, positions prolactin as an anti somatogenic and/or anti homeorhetic hormone, when in fact, it is proposed as a somatogenic and homeorhetic hormone (Bauman *et al.*, 1982). A recent development in hormonal assay techniques, has revealed an understanding of the ontogeny of

prolactin receptors (Scott *et al.*, 1992) and its relationship with utero-placental hormones from the prolactin/GH gene family (Kessler *et al.*, 1991; Wu *et al.*, 1993; Anthony *et al.*, 1995). This new understanding has recognised the possibility of placental lactogen, a feto-placental hormone which has a higher affinity to bind prolactin or GH receptors than that of prolactin or GH themselves (Emane *et al.*, 1986; Kessler *et al.*, 1991; Scott *et al.*, 1992), being nominated as a mediator of prolactin's role during the period of placental and fetal growth. However, this assumption needs to be elucidated in further studies.

This study suggests that reduced plasma prolactin concentrations induced by either Parlodel or Regulin treatment for approximately a period of 30 days during the early gestation, were insufficient to produce a similar effect to that produced by a longer period of low winter plasma concentrations of prolactin in regulating placental and fetal growth in spring-lambing ewes. It was also noticed that the treatments were commenced when prolactin concentrations in the control group were declining. Based on the profile of prolactin release observed in the control group in this study, it is likely that the treatments were carried out in the period during which the prolactin concentrations were not at the highest level. Thus, until the manipulation of prolactin concentrations in autumn-lambing ewes can mimic those of spring-lambing ewes, a conclusion regarding the role of changing seasonal prolactin concentrations might not be able to be drawn correctly.

A previous study has associated lower birth weight of autumn-born lamb, than those born to spring lambing ewes with retarded growth of the placenta due to higher summer plasma prolactin concentrations from the early to mid pregnancy in autumn-lambing ewes compared with those of spring-lambing ewes. The present study could not find any significant relationship between reduced maternal plasma prolactin concentrations, due to either Parlodel or Regulin treatment, with the uterine components of autumn-lambing ewes.

Classic fetal physiology holds that placentome number is fixed at implantation (Alexander, 1964). However, Jenkinson *et al.* (1994) could not confirm whether the

seasonal difference in placentome number occurred at implantation, or whether there was a differential loss/gain of placentomes as the pregnancy progressed in ewes of each seasonal group. Most of the previous studies associated the importance of placental size in determining fetal growth in terms of parameters such as total placentome weight, number of placentomes or percentage of caruncles occupied, parameters that are likely to be insufficient to describe placental efficiency in terms of its capacity for both transporting nutrient and oxygen as well as in producing placental hormones.

In fact, this study found that there was a large variation in placentome sizes within utero-placental tissues, implying that there were some placentomes that might not be developed well and might therefore be unable to function, and some placentomes that become very large, either to compensate for other undeveloped placentomes, or as a response of their own growth capacity. Both undeveloped placentomes and very large placentomes are likely to reduce the placental efficiency. Thus, total number or total weight of placentomes might not be able to explain the whole situation associated with the role of the placenta in controlling fetal growth. Therefore, it seems important to consider the number of optimal size placentomes within the total number of placentomes as a critical parameter for associating placental size with fetal growth. The correlation between the number of certain placentome sizes and fetal weight from all available raw data of these placentomes could indicate the size of the placentomes that might be optimal.

2. FUTURE RESEARCH

It is possible that the period of lowered plasma prolactin concentrations carried out in this study was inadequate to imitate the period of naturally low winter plasma prolactin concentrations in spring-lambing ewes. Perhaps it is necessary to prolong the period of lowered prolactin concentration using Parlodel or Regulin, or a combination of Parlodel and Regulin treatments, for a period of 60 to 80 days.

Since it is found that the profile of prolactin secretion in New Zealand ewes appears different with that of the classic profile, hence prolonging the period of lowered prolactin concentrations in autumn-lambing ewes, for 60 to 80 days, is necessary to be carried out only when the ewes are also mated, at least, 30 days earlier than December.

Reducing plasma prolactin concentrations for a period of 60 to 80 days, can be carried out using two injections of Parlodel with a 30-day interval between treatments, or two Regulin implants with a 30-day interval, or using a combination of Parlodel injection and Regulin implantation. All types of treatments should be commenced at day 15 of gestation or later, but not before day 15 of gestation. This timing of trial commencement is important to ensure that the maternal recognition of established pregnancy is not interrupted.

With the possibility that placental lactogen may be involved in the regulation of placental and fetal growth during early to mid pregnancy, it may be useful to measure fetal plasma concentrations of placental lactogen, in addition to prolactin measurement, to obtain prolactin-placental lactogen ratios during early to mid gestation.

For further studies, it is important to consider the cotyledon-caruncle ratio, or optimal size of placentomes, in addition to those of total placentome weight, number of placentomes and caruncle occupancy for assessing the importance of placental size in determining fetal growth and lamb birth weights. The proportion of optimal cotyledon-caruncle ratios within the fetoplacental tissues may have an important role in determining the efficiency of the placenta in facilitating nutrient and oxygen transport from the dam to the fetus, and hence it is important in determining fetal growth.

3. APPLICATIONS TO THE ANIMAL INDUSTRY

The New Zealand sheep industry loses about 6 million lambs per annum, or about 15% of all lambs born (McCutcheon *et al.*, 1981). Mortality rates of lambs are greatest at birth weight extremes, particularly at the lower extreme, and lowest at intermediate birth weights ((Robinson *et al.*, 1979), so that some reduction in losses could be achieved by compressing the birth weight range. Since fetal growth is a very complex mechanism contributed by many factors, therefore, it is very difficult to control fetal growth and hence lamb birth weight.

It has been well established that nutritional supply to the fetus is the major regulator of fetal growth. However, the direct supply of nutrients to provide building blocks for tissue growth is likely to be only a minor component of this regulation (Harding and Johnston, 1995). Besides, it is also well established that there is the mother's ability to buffer the fetus against changes in nutrition (Bell, 1992). Hence, to control birth weight through management is difficult.

It is well known that hormones have an important role in the regulation of fetal growth, coordinating the nutrient supply from the mother to the fetus, and modulating fetal growth and hence lamb birth weight (Fowden, 1989; Fowden, 1995). Manipulation of hormones that are suspected to be potentially involved in regulation of placental and fetal growth have been carried out in several studies but have not produced significant effects in increasing birth weight of lambs. These hormonal treatments include the use of recombinant growth hormone preparations (Stelwagen *et al.*, 1994; Min *et al.*, 1996). An attempt to manipulate maternal prolactin concentrations, using either Parlodel or Regulin carried out in this study, could not increase birth weight of lambs either.

However, considering a possibility that the effect of prolactin in regulating placental and fetal growth is mediated by placental lactogen, and a possibility that the recent

study was designed inadequately to produce a longer period of reduced maternal plasma prolactin concentrations, compared to that of the naturally low winter plasma prolactin concentrations, a future study is necessary to be carried out to justify the assumption.

If a future study could demonstrate that prolonging either Parlodel or Regulin treatment, for about 60 to 80 days, would improve placental and fetal growth, and increase birth weight of autumn-born lambs, the finding would be very important. The use of a dopamine agonist, an inhibitor of prolactin secretion (such as bromocryptine) for reducing plasma prolactin concentrations, implicating the possible use of the dopamine antagonist for producing the opposite effect, may give a control for both increasing and reducing birth weight of lambs, which could be used to reduce mortality rates due to birth weight extremes.

4. CONCLUSION

It is tentatively concluded that differences in maternal circulating prolactin concentrations cannot be associated with the difference in placental formation, and hence, seasonal prolactin secretions might not have direct effects on regulation of fetal growth and birth weight of autumn-born lambs.

A future study needs to be carried out to confirm a more conclusive role of seasonal prolactin secretions in association with seasonal differences in birth weights between spring- and autumn-born lambs.

Establishing an optimal size of the placentomes, or an optimal ratio of the caruncle and cotyledon weights, is necessary, since this parameter would be very important for describing the importance of the placental size, in terms of placental capacity and efficiency, in determining fetal growth, and hence birth weights.

APPENDIX

CALENDAR OF EVENTS FOR AUTUMN-LAMBING EWES (CONTROL, PARLODEL & REGULIN GROUPS)

Event	Autumn-lambing ewes
<ul style="list-style-type: none"> • Shearing 	<ul style="list-style-type: none"> • 27 - 28 November 1994
<ul style="list-style-type: none"> • CIDRs in 	<ul style="list-style-type: none"> • 6 December 1994
<ul style="list-style-type: none"> • Day -3 blood sample & weigh ewes Midside clear PMSG injected 	<ul style="list-style-type: none"> • 15 December 1994
<ul style="list-style-type: none"> • CIDRs out 	<ul style="list-style-type: none"> • 16 December 1994
<ul style="list-style-type: none"> • Rams in 	<ul style="list-style-type: none"> • 17 December 1994
<ul style="list-style-type: none"> • Expected mean mating date (Day 0 of gestation). 	<ul style="list-style-type: none"> • 18 December 1994
<ul style="list-style-type: none"> • Day 3 blood sample & weigh ewes 	<ul style="list-style-type: none"> • 21 December 1994
<ul style="list-style-type: none"> • Day of the commencement of treatments (Parlodel, Regulin & control groups) 	<ul style="list-style-type: none"> • 2 January 1995
<ul style="list-style-type: none"> • Day 19 blood sample & weigh ewes 	<ul style="list-style-type: none"> • 6 January 1995
<ul style="list-style-type: none"> • Day 40 blood sample, weigh ewes & wool sample 	<ul style="list-style-type: none"> • 27 January 1995
<ul style="list-style-type: none"> • Day 60 blood sample & weigh ewes Pregnancy diagnosis 	<ul style="list-style-type: none"> • 16 February 1995
<ul style="list-style-type: none"> • Day 80 blood sample, weigh ewes & wool sample 	<ul style="list-style-type: none"> • 8 March 1995
<ul style="list-style-type: none"> • Day 100 blood sample & weigh ewes 	<ul style="list-style-type: none"> • 28 March 1995
<ul style="list-style-type: none"> • Day 120 blood sample, weigh & wool sample 	<ul style="list-style-type: none"> • 17 April 1995
<ul style="list-style-type: none"> • Day 140 blood sample & weigh ewes Days of slaughter 	<ul style="list-style-type: none"> • 8 - 10 May 1995
<ul style="list-style-type: none"> • Lambing 	<ul style="list-style-type: none"> • 8 - 17 May 1995

<ul style="list-style-type: none">• Day 160 blood sample & weigh ewes Day 10 weigh lambs• Day 180 blood sample & weigh ewes Day 20 weigh lambs• Day 200 blood sample & weigh ewes Day 40 weigh lambs	<ul style="list-style-type: none">• 27 May 1995• 16 June 1995• 6 July 1995
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