Relative Roles of the Hypothalamus, Pituitary and Photoperiod on Fetal Sheep Prolactin

Daniel Charles Houghton

Submitted for partial fulfilment of the requirements for the degree of

Doctor of Philosophy

THE UNIVERSITY OF ADELAIDE
AUSTRALIA

The University of Adelaide
School of Molecular and Biomedical Science
Centre for the Early Origins of Adult Health
Discipline of Physiology
Adelaide, Australia

December 2004

TABLE OF CONTENTS

ABSTRACT

Prolactin has a multiplicity of homeostatic, endocrine and reproductive functions in mammals. Photoperiod induced changes in fetal sheep prolactin may represent seasonal preparation for postnatal demands. Physiological changes required for survival and reproductive success are relatively slow and therefore an animal's ability to 'predict' the impending environment is advantageous. The mechanism by which seasonal changes in photoperiod can influence fetal prolactin concentrations are poorly understood. This thesis investigated the role of the hypothalamus and pituitary on photoperiod induced changes and daily rhythms of prolactin in fetal sheep. The first paper investigated the effect of surgical disconnection of the fetal hypothalamus and pituitary on the fetal prolactin response to different photoperiods. Fetal sheep underwent either a sham operation or hypothalamo-pituitary disconnection (HPD) and were placed in either a long (16h light: 8h dark) or short photoperiod (8h light: 16h dark). Mean fetal prolactin concentrations were significantly higher under long photoperiod than short photoperiod in both intact and HPD animals. This demonstrated that the functionally isolated fetal pituitary can provide a prolactin response to external photoperiod.

The second paper investigated whether the fetal sheep can construct and express a photoperiodic history *in utero* and the role of the fetal hypothalamus in the generation of a photoperiodic history. Ewes carrying intact or HPD fetal sheep were placed in an intermediate (12h light: 12h dark) photoperiod after prior exposure to long or short photoperiod. In the HPD group previously exposed to long photoperiod fetal prolactin concentrations decreased significantly during exposure to an intermediate photoperiod, whilst fetal prolactin levels significantly

increased in those animals previously exposed to short photoperiod. This demonstrates different fetal prolactin responses to the same ambient photoperiod depending on prior photoperiod exposure.

The final paper for this thesis examined the impact of fetal HPD on the generation of a daily rhythm in fetal sheep prolactin concentrations under long and short photoperiods. The daily fetal prolactin rhythm was abolished in fetal sheep that had undergone HPD. Intact fetal sheep demonstrated a daily prolactin rhythm in long and short photoperiods. Thus the fetal hypothalamus is essential for the generation of a daily fetal prolactin rhythm.

These studies were the first demonstration that the fetal sheep pituitary can produce prolactin responses to ambient photoperiod independently of the hypothalamus. Prolactin responses are determined by the prior photoperiod exposure and this effect of photoperiodic history can also occur in the functionally isolated pituitary. However, the hypothalamus appears to be essential for the generation of a daily prolactin rhythm in the fetal sheep.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

December 2004

Daniel Houghton

Acknowledgements

I am extremely grateful to my supervisor Professor Caroline McMillen for giving me the opportunity to perform my postgraduate studies in her laboratory. Completion of this thesis would not have been possible without her unwavering enthusiasm, encouragement and support.

I would also like to thank Dr Ross Young for his surgical expertise, support and advice. I will never forget his enthusiastic approach to all aspects of life.

To past and present members of the laboratory, animal house and Physiology Department I thank you for your services and the friendly, collaborative atmosphere that helped me complete this PhD.

To my family, I am grateful for your ongoing understanding and continual support of my academic and private life.

Finally, to my wife Lisa, I thank you for all your understanding, advice and support. This thesis would not have been completed without your love and patience. I dedicate this thesis to you.

1. SEASONAL RHYTHMS AND PROLACTIN

1.1 INTRODUCTION

Temperate regions of the world exhibit a marked seasonal fluctuation in average daily temperature (Larcher, 1980), food availability (de Wilde, 1962; Larcher, 1980) and day length (Downs & Helmers, 1975) and the ability of organisms to cope with such seasonal fluctuations in environmental conditions depends on their ability to anticipate the impending season. Given that some of the physiological changes required for reproductive success and survival are relatively slow, it is important that organisms can predict imminent environment change rather than just react to the prevailing environmental conditions. A variety of seasonally breeding animals living in these temperate areas display important seasonal variations in reproductive behaviour (Baker, 1938), body weight (Bartness & Wade, 1984), pelage (Hoffman, 1973), gonad size (Reiter et al, 1974) and circulating hormone concentrations (Lincoln, 1990). Prolactin (PRL), a hormone synthesised and secreted by lactotrophs in the anterior pituitary is one hormone which demonstrates seasonal fluctuations in circulating concentrations in seasonally breeding animals (Curlewis 1992; Lincoln 1990).

PRL has been shown to exist in all vertebrates examined thus far and has more actions than all other hormones combined with more than 300 separate functions identified (Bole-Feysot et al, 1998). An in-depth discussion of the numerous functions of PRL is beyond the scope of this thesis; however a brief synopsis with a couple of examples of the effect and target organ of PRL is appropriate. The actions of PRL fall into six broad and overlapping categories:

1) Water and electrolyte balance – PRL has been shown to reduce renal sodium and potassium excretion in mammals (Richardson, 1973), reduce sodium loss (Maetz, 1970) and water uptake (Ogawa et al, 1973) in the gills of fish and increases secretion of salt from the nasal salt gland of birds (Peaker et al, 1970).
2) Growth and development – PRL increases postnatal body growth in mammals (Rynikova et al, 1988), matures fetal lung (Quirk et al, 1982) and increases testis weight in hypophysectomized rats (Dombrowicz et al, 1992).
3) Endocrinology and metabolism – PRL increases steroidogenesis, androgen and cortisol secretion from mammalian adrenal glands (Glasow et al, 1996; Higuchi et al, 1984), increases insulin secretion from mammalian pancreas (Nielsen, 1982) and increases phospholipid synthesis in mammalian fetal lung (Hamosh and Hamosh, 1977).
4) Brain and behaviour – PRL is involved in the regulation of nesting behaviour in birds (Horseman and Buntin, 1995), maternal behaviour of mammals (Bridge et al, 1985) and is important in the maturation of the neonatal neuroendocrine system (Shyr et al, 1986).
5) Reproduction – PRL is best known as the hormone primarily responsible for the induction and maintenance of milk production (Bole-Feysot et al, 1998), but also has luteolytic and luteotropic actions on the ovary (Ota et al, 1985) and stimulates testicular functions in most mammals (Bole-Feysot et al, 1998).
6) Immunoregulation and protection – PRL increases cellular (Nagy and Berczi, 1981) and hormonal (Berczi and Nagy, 1982) immunity in

lymphocytes, increases the weight of spleen and thymus (Berczi et al, 1991) and the activation of macrophages is stimulated by PRL and mediated by the PRL-receptor (Bernton et al, 1988).

The seasonal cycle in plasma PRL concentrations appears to be regulated by seasonal alterations in day length, or photoperiod. In a number of species, including pigs (Ravault et al, 1982), roe deer (Schams & Barth, 1982), rhesus monkey (Beck & Wuttke, 1979), blue fox (Mondain-Monval et al, 1985), mink (Martinet, 1982), hamsters (Bex et al, 1978), dogs (Kreeger & Seal, 1992), rats (Wong et al, 1983), cows (Buttle & Forsyth, 1976), mares (Johnson, 1986) and bears (Tsubota et al, 1995), summer months and long photoperiod are associated with elevated plasma levels of PRL, and winter months, or short day lengths, are associated with low plasma PRL levels (Curlewis 1992). In rams (Lincoln et al, 1978; Barrell & Lapwood, 1979), lambs (Brown & Forbes, 1980), ewes and their fetuses (Bassett et al, 1989) in a range of breeds of wild and domesticated sheep, elevated plasma levels of PRL in long photoperiods and low PRL levels in short photoperiods have been reported.

Seasonal changes in concentrations of fetal hormones (eg PRL) in sheep may represent in utero preparation for postnatal demands. The seasonal rhythms in fetal PRL are an expression of the photoperiodic information being relayed to the fetus about the ongoing season and may provide information on the predicted nature of the season after birth. Such seasonal fluctuations in PRL may in turn alter several endocrine and metabolic functions and allow animals to adapt more effectively to their postnatal environment. In utero reception of photoperiodic information may determine postnatal behaviour by allowing discrimination of the

postnatal photoperiod and the ability to initiate an appropriate physiological response.

In deer (Adam et al, 1992) and hamsters (Stetson, 1986) photoperiodic information acquired in utero determines the rate of postnatal development in order for the newborn to reach puberty at an optimal time of year. In sheep, prenatal photoperiod can influence onset of puberty (Helliwell et al, 1992), whilst the growth and sexual maturation of voles is also dependent upon the time of year they are born. Voles born after the summer solstice do not achieve full maturation until the following spring when approximately six months old, whereas spring born cohorts reach full maturity by two months of age (Lee et al, 1987). Thus information acquired before birth prepares the fetus for the metabolic demands of the season to be encountered after birth.

Photoperiod and the light-dark cycle also drive the circadian rhythm generating system of many mammals. Diurnal variations in plasma hormone concentrations (McMillen et al, 1987) and behaviour (McMillen and Walker, 1991) have been observed in ewes and their fetuses. The timing of these rhythms is similar in ewe and fetus and it may be that the fetus is responding directly or indirectly to environmental cues in order to synchronise its physiological functions with that of its mother and the external environment. The synchronisation of prenatal circadian rhythms in behaviour and in endocrine functions may be important in ensuring that there is a ready and mature circadian rhythm generating system in place at birth and that the responses of this system will allow adaptation to maternal patterns of feeding and to daily fluctuations in the external temperature.

Thus it can be argued that there is evidence in seasonal breeders that the fetus is not sequestered from either seasonal or circadian information and that this is important in allowing short and long-term physiological adaptations to occur to maximise likelihood of survival during the perinatal period. This literature review will summarise the mechanisms of seasonal and photoperiodic regulation of PRL in adult and fetal animals, the role of the hypothalamus and pars tuberalis in PRL secretion, the development and expression of a photoperiodic history and finally the regulation of the diurnal PRL rhythm.

1.1.1 Role of photoperiod

Photoperiodic information about the length of day is transduced by the pineal gland into an endocrine signal. The pineal gland synthesises and secretes melatonin at night and pineal synthesis of melatonin is regulated by daylength via an indirect and multisynaptic inhibitory pathway (Moore, 1978). This pathway includes a retinohypothalamic projection from ganglion cells of the retina to the suprachiasmatic nucleus (SCN). The SCN (widely regarded as the mammalian circadian pacemaker) projects to the pineal gland via the paraventricular nucleus of the hypothalamus, intermediolateral cell column of the upper thoracic spinal cord and superior cervical ganglion of the sympathetic system arising from the upper thoracic spinal cord (Moore, 1996). The SCN provides a rhythmic inhibitory signal during the day resulting in low production of melatonin during daylight. High levels of nighttime neural activity, in components of the pathway from the paraventricular nucleus to the sympathetic innervation of the pineal, result in a high level of melatonin production at night. (Moore, 1996)

The duration of the nocturnal melatonin signal provides humoral information about photoperiod (Kennaway et al, 1982; Poulton et al, 1986; Bassett et al 1989). Long days have short nights and consequently the duration of the nocturnal melatonin signal is short. Conversely during long winter nights, the duration of the nocturnal melatonin signal is extended.

Lincoln and co-workers (1978) reported that plasma PRL levels decreased when rams were moved from long (16h light: 8h dark) to short (8h light: 16h dark) days and that transition from short to long photoperiods had the opposite effect. Barrell and Lapwood (1979) confirmed that circulating levels of PRL were influenced by the lighting schedule in rams exposed to a range of artificial photoperiods and Brown and Forbes (1980) demonstrated that plasma PRL concentrations were consistently higher in growing lambs exposed to long compared to short photoperiods. Finally, Bassett and co-workers (1989) reported that plasma PRL levels in fetal sheep were high when the ewe was exposed to long photoperiods and low when the ewe was exposed to short photoperiods. Not surprisingly given the relationship between circulating PRL and the external photoperiod it has been suggested that the pineal indole amine, melatonin, plays a major role in the modulation of PRL secretion (Bassett et al, 1989).

1.1.2 Role of melatonin

Kennaway et al (1982) found that when ewes were held under 16h of daily light, oral administration of melatonin reduced circulating PRL levels. During long summer photoperiods, melatonin feeding to mimic winter melatonin levels has also been found to reduce PRL levels to that observed during the winter photoperiod in sheep (Symons et al 1983).

Subcutaneous melatonin implants also reduce plasma PRL in ewes (Poulton et al 1986) and rams (Lincoln & Ebling, 1985) and melatonin implants in various brain regions including the mediobasal hypothalamus (Lincoln 1992) and pars tuberalis (Lincoln 1994) also reduced circulating PRL levels. Interestingly, however, melatonin implants near the lateral septum or the pars distalis had no effect on circulating PRL levels (Lincoln 1994).

Bassett and co-workers (1989) investigated the effect of intravenous infusion of melatonin for 14h per day in pregnant ewes exposed to a 16h photoperiod. They found that circulating PRL levels in both the ewes receiving melatonin and their fetuses were decreased relative to control animals in late summer pregnancies.

The role of melatonin in regulating circulating PRL was also investigated by removing the pineal gland (pinealectomy) in sheep. Barrell and Lapwood (1979) observed that pinealectomized adult rams had elevated PRL levels compared with sham-operated animals. There was also a reduction in the pinealectomized group in the photoperiod related changes in circulating PRL.

Brown and Forbes (1980) also found that the effect of daylength on plasma levels of PRL was blocked in lambs although, a diurnal variation with a peak in plasma PRL at dusk remained after removal of the pineal gland. Other authors have also reported similar effects. McMillen and co-workers (1991) found that in pregnant ewes a diurnal variation of PRL persisted after pinealectomy. In this study whilst there was a trend for pinealectomized ewes to have higher PRL levels, there was no significant difference in plasma PRL between pinealectomized and non-pinealectomized ewes. Interestingly, the fetal sheep of

pinealectomized ewes had significantly elevated PRL compared with fetal sheep of sham-operated ewes.

Surgical ablation or interruption of the neural pathway between the retina and the pineal gland abolishes the diurnal rhythm in plasma concentrations of melatonin (Lincoln et al, 1981; Maeda et al, 1986) and can also alter circulating PRL. Bilateral superior cervical ganglionectomy (sympathetic denervation) has been shown to interfere with the PRL response to a change in daylength in rams (Lincoln 1979), ewes (Maxwell et al, 1989) and goats (Maeda et al, 1986). In all these studies, ganglionectomised animals failed to maintain or produce elevated plasma concentrations of PRL in response to a long photoperiod compared with sham-operated animals.

Therefore melatonin plays a pivotal, but not exclusive role in the transduction of photoperiodic information and in the response of PRL to changes in daylengths in seasonally responsive mammals.

1.1.3 Role of the hypothalamus

Prolactin secretion from the pituitary gland is influenced by a number of factors. Increases in PRL secretion occur with increasing photoperiod, hyperthermia and in the presence of environmental stressors. Prolactin secretion is also stimulated by a range of neuropeptides and neurotransmitters including thyrotrophin releasing hormone (TRH), vasoactive intestinal peptide (VIP), serotonin, β-endorphin, met & leu-enkephalin, angiotensin II, vasopressin, substance P, estradiol, epidermal & fibroblast growth factors and cholecystokinin (Leong et al, 1983). The physiological role of each of these substances in PRL secretion has not been fully elucidated.

It is well established that PRL secretion by the anterior pituitary is inhibited by the hypothalamus. (Leong et al, 1983; Ben-Jonathan, 1980). This effect is mediated by dopamine secreted from tuberoinfundibular neurons. Dopamine exerts its effect on dopamine D2 receptors (Sibley et al, 1982) located on lactotrophs in the adenohypophysis after secretion into hypophysial portal blood (Reymond and Porter, 1985).

Section of the pituitary stalk results in increased circulating PRL levels in a variety of species including man, monkeys, rats and pigs (Thomas et al, 1986). In ewes a transient increase in PRL levels has been reported in response to surgical disconnection of the hypothalamus and pituitary (HPD) (Clarke et al, 1983; Thomas et al, 1986). Similar transient PRL elevations after HPD have also been observed in rams housed in long photoperiods (16h light per day) with plasma PRL concentrations returning to levels observed in control rams after 24 days (Lincoln and Clarke 1994).

Interestingly a PRL response to a change in photoperiod was still present after HPD in ewes housed in natural photoperiods (Thomas et al, 1986) or in rams housed in artificial photoperiods (Lincoln & Clarke, 1994). This implied that there was an extrahypothalamic site for photoperiodic regulation of PRL secretion in the adult sheep.

1.1.4 Response of fetal prolactin to the external photoperiod

Although the fetus is sequestered in utero away from the direct effects of photoperiod, seasonal rhythms in plasma PRL levels have been measured in sheep fetuses in late gestation (Bassett et al, 1988; Seron-Ferre et al, 1989). As PRL has been demonstrated not to cross the ovine placenta (Alexander et al,

1973), this seasonal PRL rhythm must be endogenously generated in the sheep fetus ie. the sheep fetus is able to respond to the prevailing photoperiod.

As in the adult, fetal PRL levels are high during summer and low during winter. This effect of photoperiod occurs under both natural and artificial lighting conditions (Bassett et al 1989). When pregnant ewes were moved from winter photoperiods (8h light: 16h dark) to summer photoperiods (16h light: 8h dark) there was an increase in the fetal plasma PRL concentrations. This effect occurred in synchrony with maternal changes in PRL and within three weeks of the abrupt change in photoperiod (Bassett et al, 1989).

A diurnal rhythm in plasma melatonin concentrations has also been demonstrated in fetal sheep in late gestation (Zemdegs et al, 1988; Yellon & Longo, 1987). In fetal sheep there was a typical nocturnal rise in fetal melatonin concentrations that coincided with lights off and with the melatonin rhythm of the pregnant ewe. Melatonin was also shown to cross the ovine placenta in both the maternal-fetal direction using tritiated melatonin (Zemdegs et al, 1988) and the fetal-maternal direction (Yellon and Longo 1988). Finally, maternal pinealectomy abolished the nocturnal rhythm of melatonin in both the ewe and the fetus (McMillen & Nowak, 1989; Yellon & Longo, 1988). Thus, the maternal pineal gland is the source of the melatonin rhythm in fetal sheep, and the melatonin crossing the placenta provides the fetus with humoral information about the duration of the night and hence the prevailing photoperiod.

When the melatonin rhythm was abolished by maternal pinealectomy, McMillen and co-workers (1991) observed that fetal PRL levels were higher than those in control animals. This elevated PRL response to maternal pinealectomy suggested that cessation of the melatonin rhythm was being interpreted as a long,

or summer photoperiod. Similar to the ewe, fetal PRL levels also respond to exogenous melatonin. When ewes were kept in long photoperiods fetal PRL concentrations were lower when melatonin was infused for 14hours per day (Bassett et al, 1989). Continual exposure to melatonin via subcutaneous implants in the ewe also lowers fetal PRL concentrations (Serron-Ferre et al, 1989).

These experiments demonstrated that the external photoperiod regulates circulating PRL in the fetal sheep via melatonin. The mechanism and location of transduction of the melatonin signal in the adult and fetal hypothalamo-pituitary axis have also been investigated.

1.1.5 Melatonin receptors and the pars tuberalis

The identity of melatonin receptors, their regional localisation and cellular functions have been investigated using autoradiographic binding techniques. Melatonin receptors are located in a number of areas including the suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), and Reuniers nucleus (Krause & Dubocovich, 1990). However, the pars tuberalis exhibits the highest concentrations of iodomelatonin binding sites with the pars distalis having minimal iodomelatonin binding (Morgan et al, 1989; de Reviers et al, 1989; Bittman and Weaver, 1990). In the developing sheep fetus, iodomelatonin binding sites have been identified on the pars tuberalis from 30 days gestation (Helliwell & Williams, 1994).

The pars tuberalis is a structurally distinct region of the mammalian adenohypophysis. It surrounds the hypophyseal stalk as a highly vascular 'tube' of cells. Three phenotypes of cells have been identified:

1. pars tuberalis-specific cells which are ultrastructurally distinct from any pars distalis cells. These cells contain Golgi bodies, rough endoplasmic reticulum and are very sparsely granulated. In the ovine pars tuberalis they represent 90% of the glandular (secretory) cells (Morgan et al 1991).
2. pars distalis-like cells which are immunocytochemically similar to the trophic cells of the pars distalis. These are a mixture of thyrotrophs and gonadotrophs, and have abundant dense core vesicles. In the ovine pars tuberalis they represent 10% of the remaining glandular cells.
3. Follicular (non-glandular) cells are similar to folliculo-stellate cells of the pars distalis. They are smaller than the pars tuberalis-specific cells or pars-distalis-like cells and have no morphological characteristics of secretory activity (Morgan & Williams, 1996).

Of the three types of cells identified the agranular pars tuberalis-specific secretory cells are the melatonin responsive cell type in the pars tuberalis of the sheep (Morgan et al 1991).

With the identification of these extra-hypothalamic melatonin binding sites it was therefore hypothesised that the seasonal or photoperiodic regulation of PRL secretion may be independent of the hypothalamus. To investigate this hypothesis we have investigated whether the fetal PRL response to different photoperiods is dependent on a functionally intact hypothalamo-pituitary axis.

1.2 PHOTOPERIODIC HISTORY

1.2.1 Impact of photoperiodic history on PRL secretion

For many species there is no 'rigid' response to photoperiod. Rather the response to an intermediate photoperiod (ie between the extremes of long ~16hours light/day and short ~8hours light/day) is dependent upon prior photoperiod exposure (ie photoperiodic history). Plasma concentrations of PRL in sheep are determined by the ambient photoperiod (Barrell and Lapwood, 1979), but the same photoperiod is capable of eliciting different PRL concentrations depending upon the prior photoperiodic exposure in ewes (Sweeney et al, 1999), lambs (Ebling et al, 1989), newborn hamsters (Shaw and Goldman, 1995) and deer calves (Adam et al, 1992). The varying responses to the same photoperiod indicate the flexibility of the photoperiod interpreting system and may be important in adaptations required for survival or reproduction.

1.2.2 Photoperiodic history in adults

Hoffman and colleagues (1986) investigated the reproductive response of adult Djungarian hamsters to an intermediate photoperiod (14h L: 10h D). Male hamsters were exposed to either a long daylength (16h L: 8h D) or a short daylength (8h L: 16h D) for 3-5 months. They were then moved to an intermediate photoperiod for 10 weeks and the testes were weighed. Testis weight was significantly greater in those animals that had moved from short to intermediate photoperiod when compared with those which had moved from a long to the intermediate photoperiod. Thus the hamsters were interpreting the same intermediate photoperiod differently depending upon their prior photoperiod.

Those from long daylength were interpreting the intermediate photoperiod as a short daylength, and those from a short daylength were interpreting the intermediate photoperiod as a long daylength. Thus photoperiodic history is important in the reproductive response to the current photoperiod.

An effect of photoperiodic history on the reproductive neuroendocrine response of the ewe has been demonstrated by Robinson and Karsch (1987). They measured plasma LH levels in sexually mature ovariectomized ewes exposed to an intermediate photoperiod of 13h L: 11h D after prior exposure to either long (16h L: 8h D) or short (10h L: 14h D) photoperiods for 74 days. Thus the ewes were either reproductively suppressed or stimulated prior to exposure to the intermediate photoperiod. The ewes were then placed in the intermediate photoperiod, 13h L: 11h D, for 80 days and plasma LH levels were determined. The intermediate photoperiod had either stimulatory or inhibitory effects on circulating LH depending upon the photoperiodic history of the ewe. A 3h decrease in total daylength stimulated plasma LH concentrations after 40 days, whereas a 3h increase in daylength resulted in a decline in circulating LH. These authors concluded that the duration of the nocturnal melatonin signal relative to the preceding melatonin pattern was the key to determining the reproductive outcome in the ewe.

Sweeney et al (1999) also exposed ewes to 35 long light days (18h L: 6h D) at different times of the year after prior exposure to natural photoperiods varying between summer and winter daylength. They observed that the ewes had elevated plasma PRL concentrations during the 35 long day challenge only after natural daylength had dropped from summer levels. If ewes were moved to the long day challenge when the natural photoperiod was still relatively high, there

was no stimulation of PRL secretion. The PRL response to the 35 long days was therefore dependent upon the photoperiodic history of the ewe prior to the long day challenge

1.2.3 Photoperiodic history and the neonate

Horton (1984a, 1984b, 1985) investigated the influence of photoperiodic history on gonadal enlargement in the pre-pubertal male montane vole. Parents of male voles were maintained in either long (16h L: 8h D) or short (8h L: 16h D) photoperiods during pregnancy. At birth, male voles born in to a specific photoperiod were retained in that photoperiod until weaning (age 18.5 days), when they were placed in an intermediate photoperiod (14h L: 10h D). Gonadal responses were measured at 74 days of age. Testicular weight was significantly greater in those animals gestated and raised in a short photoperiod compared with those raised in a long photoperiod. The gonadal response of the vole to an intermediate photoperiod depended upon photoperiodic exposure prior to weaning. However, these results did not allow a differentiation between the effects of prenatal (or in utero) or postnatal exposure to different photoperiods on the gonadal responses of the vole.

Horton (1984b) then investigated the gonadal response in male voles transferred to an intermediate photoperiod on the day of birth. The parents of the offspring were maintained in either long or short photoperiods and then transferred with their litters to an intermediate photoperiod on the day of delivery. Testis weight was greater in those animals whose mother had gestated in short photoperiods and smaller in those animals whose mothers had gestated in long photoperiods. The male neonatal voles therefore interpreted the intermediate

photoperiod differently dependent upon the prior photoperiod exposure during pregnancy. These results suggested that the fetal vontane mole could construct a photoperiodic history in utero. However, the possible effects of different maternal care and factors in the milk provided by the mother who had experienced different photoperiodic histories needed to be taken into account.

To this end, Horton (1985) investigated gonadal development in male voles exposed to short or long photoperiods during gestation that were raised by foster mothers who had been exposed to different photoperiods during their pregnancy. Gestating voles were exposed to either long or short photoperiods throughout pregnancy until the day of delivery. On this day, half the male voles from each litter were transferred to a foster mother that had been exposed to the same photoperiod as the natural mother throughout pregnancy. The remaining half was transferred to a foster mother, which had undergone pregnancy in the alternative photoperiod. All animals were then housed (from day of birth) in an intermediate photoperiod. Gonadal responses were measured at 74 days of age.

Prenatal exposure of mothers to short photoperiod resulted in large testes of male offspring exposed to an intermediate photoperiod. Long photoperiod exposure during gestation resulted in smaller testes when male voles were placed in an intermediate photoperiod post partum. These effects were not influenced by transfer to different foster mothers but were only dependent on the gestational photoperiod. Similar results have been found in other seasonally breeding animals, including Djungarian hamsters (Stetson et al, 1986) and Siberian hamsters (Elliot and Goldman, 1989).

Stetson et al (1986) also investigated the effect of photoperiodic history on neonatal gonadal development in male Djungarian hamsters. Pregnant hamsters

were placed in long, intermediate or short photoperiods. On the day of parturition parents and litters from each photoperiod were transferred to the other two photoperiods, while some animals stayed in the same photoperiod to act as controls. For those animals raised in the same pre and postnatal photoperiod, testes weight was large, intermediate or small at 28 and 34 days of age if they had been exposed to long, intermediate or short photoperiods respectively. Thus long daylength stimulated testicular growth whilst short daylength inhibited growth. In hamsters exposed to an intermediate photoperiod in postnatal life after exposure to a long photoperiod prenatally, testicular growth was impaired to the same extent as in animals maintained in a short pre and postnatal photoperiod. In hamsters exposed to an intermediate photoperiod postnatally after a prenatal short photoperiod, testicular growth was stimulated.

The gonadal response of the pre-pubertal hamsters to an intermediate photoperiod was therefore dependent upon the previous photoperiodic history. Thus the photoperiodic history is established in utero with information about the photoperiod experienced by the mother being transferred to the fetus and this 'information' subsequently determines the gonadal responses to the ambient photoperiod during postnatal life.

1.2.4 Photoperiodic history and the pineal gland

Eliot and Goldman (1989) investigated the mechanism of transfer of photoperiodic information from mother to fetus. Adult female hamsters were either pinealectomized or sham-operated, then placed in either long or short photoperiod during pregnancy and then moved with their litters to an intermediate photoperiod on the day of birth. The testes of offspring born to sham-operated mothers varied

according to the prenatal or gestational photoperiod. Similar to previous investigations, those gestated in short photoperiod had large testes and those gestated in long photoperiod had small testes when placed in an intermediate photoperiod at birth for 30 days (Elliot and Goldman, 1989). In contrast, the testes of offspring born to pinealectomized mothers were small irrespective of the gestational photoperiod. Pinealectomy of the mother had therefore disrupted transfer of photoperiodic information from mother to fetus. The authors interpreted this as being due to impairment of the nocturnal melatonin signal. As discussed above, the melatonin signal has been demonstrated to be important in regulating circulating PRL levels (Barrell & Lapwood, 1979; Brown & Forbes, 1980; Maxwell et al, 1989) and may also be important in the impact of the photoperiodic history on PRL secretion.

1.2.5 Photoperiodic history and PRL

Shaw and Goldman (1995) examined prenatal photoperiod effects on postnatal plasma PRL levels in Siberian hamsters. Adult hamsters were placed in long (16h L: 8h D) or short (10h L: 14h D) photoperiods during pregnancy. On the day of parturition, parents and litters were transferred to an intermediate photoperiod. PRL levels were measured between 18 and 62 days of age. These authors observed that circulating PRL levels fell in animals gestated in long photoperiod and increased in animals gestated in short photoperiod. These effects were only evident between 27 and 52 days of exposure to an intermediate photoperiod. The interpretation of the intermediate photoperiod by the newborn hamsters was therefore influenced by their gestational photoperiod. Thus the PRL

responses to the relative change in ambient photoperiod were influenced by the prior photoperiod.

Adam et al (1992) also investigated prenatal photoperiod effects on postnatal PRL secretion in red deer. Pregnant red deer hinds were kept in long (18h L: 6h D) or short (6h L: 18h D) photoperiods for approximately 33 weeks until term. On the day of birth, mothers and newborn calves were placed in an intermediate (12h L: 12h D) photoperiod and PRL levels were monitored until weaning at 14 weeks of age. Newborn calves gestated in long photoperiod were born with a high circulating PRL level and PRL concentrations decreased for 8 weeks before stabilising at a relatively low level. In contrast, calves gestated in a short photoperiod were born with low circulating PRL levels that gradually increased over 8-12 weeks to levels measured in newborn animals gestated in long photoperiods. Thus the calves gestated in long days were interpreting the intermediate photoperiod as 'short days', whereas calves gestated in short days were interpreting the intermediate photoperiod as long days in terms of the circulating PRL response. These authors concluded that the deer fetus can respond to photoperiodic information and can acquire a photoperiodic history in utero, which then influences the postnatal PRL responses to ambient photoperiod.

Similar effects of the prenatal photoperiod on postnatal PRL have been measured in lambs (Ebling et al, 1989). Pregnant ewes were exposed to either a long (16h L: 8h D) or short (8h L: 16h D) photoperiod, approximately 42 days before term. On the day of parturition, newborn lambs and their mothers were transferred to an intermediate (12h L: 12h D) photoperiod and plasma PRL levels monitored for 28 days. Lambs born to mothers kept in long days during late pregnancy had initially high plasma PRL levels that then decreased and stabilised

at low levels after 28 days. Lambs born to mothers kept in short days during late pregnancy initially had low plasma PRL levels that gradually started to increase to become similar to levels (after 28 days) measured in newborn lambs gestated in long days. Thus plasma PRL concentrations in the lambs did not reach levels that reflected the ambient photoperiod, rather, lambs gestated in different photoperiods constructed different photoperiodic histories in utero, and interpreted the same postnatal photoperiod differently, leading to different PRL levels at 28 days of age.

Thus the Montane vole, Siberian hamster, red deer and sheep can each develop a photoperiodic history whilst in utero as the neonatal 'interpretation' of ambient photoperiod is influenced by the gestational photoperiod, with evidence for an effect of photoperiodic history on LH and PRL secretion and testis weight. All these studies have investigated the *neonatal* response to ambient photoperiod after construction of a photoperiodic history as a fetus.

There have been no investigations that have determined whether the fetus can respond differently to an intermediate photoperiod depending upon its prior photoperiodic exposure. We therefore tested for the first time whether the fetal sheep can construct a photoperiodic history and respond to an intermediate photoperiod in utero depending upon the prior photoperiodic exposure. Furthermore, we have investigated the role of the fetal hypothalamus in determining the PRL responses to photoperiodic history.

1.3 DIURNAL RHYTHMS

1.3.1 Daily melatonin rhythm

In the sheep, as in many mammalian species there is a diurnal variation in plasma melatonin (MEL) concentrations. The pattern of MEL secretion is clearly regulated by the external light: dark cycle with elevated levels of MEL measured during the dark phase. As previously discussed, MEL crosses the placenta and fetus is therefore exposed to the diurnal maternal MEL rhythm (Yellon and Longo, 1987; Zemdegs et al, 1988). Removal of the maternal pineal gland abolishes the diurnal MEL rhythm in both the ewe and fetus, which indicates that the maternal pineal gland is the predominant source of the fetal MEL rhythm (McMillen and Nowak, 1989; Yellon and Longo, 1988).

As discussed above, in seasonally responsive species, the diurnal MEL rhythm provides the fetus with neurohumoral information about the prevailing daylength and hence time of year. It has also been suggested that this diurnal MEL pattern acts as a circadian signal providing the fetus with information about time of day and that this may regulate diurnal patterns of fetal behaviour or hormone concentrations.

A diurnal rhythm in fetal breathing movements (FBM) had been observed by a number of investigators (Boddy et al, 1973; Dawes and Robinson, 1976; Patrick and Richardson, 1985), which appeared to occur independent of diurnal changes in plasma glucose or prostaglandin concentrations (Callea et al, 1990).

The effects of different 24h lighting regimes on the diurnal FBM rhythm were investigated by McMillen and Walker (1991). Pregnant ewes were exposed to a 12hL: 12hD lighting regime with one group experiencing lights off at 1900h

and a second group experiencing lights off at 1100h. Plasma concentrations of MEL increased after lights were turned off in maternal and fetal sheep. It was reported that the diurnal FBM rhythm in each group showed a peak in FBM activity at the time of lights off suggesting the daily rhythm in MEL may play a role in the regulation of the FBM rhythm.

McMillen and co-workers (1990) then investigated the effect of removing the MEL signal on the diurnal variation in FBM. Ewes were either pinealectomized or sham operated and placed in 12hL: 12hD. These authors reported that there was a different 24h pattern of FBM in the two groups. In pineal-intact ewes, FBM peaked between 1900-2000, coinciding with the time of 'lights off' and the associated increase in maternal plasma MEL concentrations. In the pinealectomized ewes the peak incidence of FBM occurred between 1200-1300h. These authors therefore suggested that the maternal MEL signal is required to entrain the timing of the diurnal FBM rhythm.

To examine the relative roles of the light/dark cycle and the MEL diurnal rhythm Houghton et al (1993) pinealectomized pregnant ewes and infused MEL or saline in a different phase to the light/dark cycle. One group of ewes were exposed to 12h L: 12h D with lights off at 1900 and were infused for 12h with MEL or saline from 1100. The second group of ewes were exposed to 12h L: 12h D with lights off at 1100 and the MEL or saline infusion from 1900 for 12h. This provided the ewe and fetus with both a 12h diurnal MEL rhythm and a light/dark cycle but at different clock times. The saline infused animals provided the controls. The peak incidence of FBM coincided with onset of MEL infusion, not lights off, in both lighting regimes. This indicated that MEL generates or entrains the diurnal FBM rhythm and that MEL acts as a circadian signal. The mechanism

by which MEL exerts its influence remains unclear. It is possible that MEL acts at the fetal suprachiasmatic nuclei to influence FBM and other fetal rhythms.

1.3.2 Daily PRL rhythm

A diurnal PRL rhythm has been demonstrated in a variety of species including humans (Sassin et al, 1972), monkeys (Spies et al, 1979), pregnant sheep and their fetuses (McMillen et al, 1987). The acrophase in a 12hL: 12hD lighting regime occurs around the time of 'lights off' in both ewe and fetus. The acrophase changes with the season in the fetus (Serron-Ferre et al, 1989), occurring around dusk during summer and near midnight during autumn. In the pregnant ewe, whilst a change in the time of 'lights off' alters the acrophase of the daily PRL rhythm, it did not shift the timing of the acrophase of the fetal PRL rhythm (McMillen and Walker, 1991). Fetal sheep plasma PRL concentrations were highest between 1700h and 0100h irrespective of lights off at either 1900h or 1100h (McMillen and Walker, 1991). These results contrasted significantly with the findings of Serron-Ferre et al (1989) and suggested that the diurnal fetal PRL rhythm occurred independently of the maternally derived MEL rhythm.

Although one study in rams found that pinealectomy abolishes the nocturnal surge in PRL (Barrell and Lapwood, 1978), a number of subsequent studies have found that the daily PRL rhythm persists in sheep after pinealectomy or sympathetic denervation of the pineal gland (Brown and Forbes, 1980; Brinklow and Forbes, 1984; Poulton et al, 1989, Lincoln, 1979). Interestingly, pinealectomy of the pregnant ewe did not abolish the daily PRL rhythm in either the ewe or fetus, with both control and pinealectomized groups having a similar acrophase in the daily PRL rhythm close to onset of darkness (McMillen and Walker, 1991

Houghton et al (1993) investigated the effect of separately altering the time of dark onset and the timing of melatonin replacement on daily maternal and fetal PRL rhythms. Pinealectomized ewes were housed in one of two lighting regimes and then infused with either MEL or saline. When the lights were turned off between 1900h and 0700h either MEL or saline was infused between 1100h and 2300h. When the lights were turned off between 1100h and 2300h, either MEL or saline was infused between 1900h and 0700h. They observed that a diurnal PRL rhythm persisted in both ewe and fetus after maternal pinealectomy. In the ewe, the timing of the acrophase of the daily PRL rhythm changed with time of dark onset and was independent of the timing of MEL infusion. This suggested that the daily PRL profile in the ewe was regulated by the effects of the light: dark cycle and not by the timing of the daily MEL rhythm. Interestingly in the fetus, sequestered away from the light: dark cycle in the uterus, a similar effect was observed. The timing of the daily fetal PRL rhythm peak altered with change in time of dark onset and this occurred independently of the timing of the MEL infusion. Thus there appears to be either a direct effect of the light: dark cycle or an unknown factor that transmits information about the external environment to the fetal hypothalamo-pituitary axis, which regulates the daily rhythm in plasma PRL concentrations in the sheep fetus.

It has been shown that light of varying wavelengths can penetrate into the uterus of pregnant rats, guinea pigs (Jacques, 1987) and pregnant sheep (Parraguez, 1998). In pregnant sheep exposed to outdoor sunlight, a daily variation in the amount of light present in the uterus was observed and the amount of light penetrating the uterus increased with gestational age (Parraguez et al, 1998). The retino-hypothalamic tract, which is necessary for retina-mediated

effects of light on circadian rhythms, may function prenatally. In the fetus of the spiny mouse, the retino-hypothalamic tract is present within the SCN on the day of birth and is thought to be present antenatally, whilst metabolic activity of the SCN in the spiny mouse fetus can be stimulated by exposure of pregnant mice to bright light at night (Weaver and Reppert, 1989). In this study the dams had been enucleated prior to bright light exposure and thus bright light did not stimulate maternal SCN activity. This demonstrated that fetal SCN activity could be independent of maternal SCN activity and suggested that the fetus could use its retinohypothalamic tract to perceive and convey information about the environmental lighting to the SCN (Weaver and Reppert, 1989). In these experiments however, there was no effect of prenatal light on circadian rhythmicity. In fetal sheep, the development of the retino-hypothalamic tract occurs several weeks before birth suggesting that exposure to light in utero may be important in the development and/or entrainment of fetal circadian behavioural and hormonal rhythms, including the daily rhythm in plasma PRL concentrations (Torrealba et al, 1993). Interestingly, ocular enucleation of fetal sheep reduced plasma PRL concentrations five-fold but a diurnal PRL rhythm persisted with a robust acrophase occurring around lights off (Vergara et al, 1992). This suggested that the retino-hypothalamic tract might be important in the regulation of plasma PRL concentrations but is not necessary for the generation and entrainment of the diurnal PRL rhythm in the sheep fetus. In the absence of fetal light perception the maternally derived MEL rhythm may have some role in entrainment of fetal PRL, but is not involved in the generation of the PRL rhythm.

The role of the hypothalamus in the generation of a daily PRL rhythm has been investigated in rhesus monkeys (Spies et al, 1979) and rams (Lincoln and

Clarke, 1994). Pituitary stalk section in rhesus monkeys abolished the diurnal rhythm in plasma PRL concentrations (Spies et al, 1979), whilst the same effect occurred in adult rams after hypothalamo-pituitary disconnection (Lincoln and Clarke, 1994). These studies suggested that in the adult, the isolated pituitary was unable to produce a daily rhythm in PRL and that the generation of this rhythm was therefore dependent upon the hypothalamus.

To investigate the role of the hypothalamus on the generation of the daily fetal PRL rhythm we disconnected the hypothalamus from the pituitary in fetal sheep in late gestation. We then observed plasma PRL and MEL levels in long and short photoperiods. We determined whether the daily fetal PRL rhythm is dependent upon an intact hypothalamo-pituitary axis.

Therefore the following Chapters of this thesis will report on a series of studies designed to test hypotheses related to the role of the external photoperiod and the fetal hypothalamus in the generation of photoperiod and circadian induced changes in circulating prolactin in the fetal sheep during late gestation.

1.4 AIMS AND HYPOTHESES

Chapter 2 – Response of prolactin to different photoperiods after surgical disconnection of the hypothalamus and pituitary in sheep fetuses

Chapter 2 – Hypothesis

That the circulating PRL response to different photoperiods is not dependent on a functionally intact hypothalamo-pituitary axis in the sheep fetus.

Chapter 2- Experimental Aims

1. To determine whether fetal plasma concentrations of PRL are different in ewes held in long or short photoperiod.
2. To determine whether there are differences in fetal sheep plasma concentrations of PRL in ewes held in long and short photoperiod after surgical disconnection of the fetal hypothalamus and pituitary.
3. To determine whether the fetal PRL response to a thyrotrophin releasing hormone stimulus is different in ewes held in long or short photoperiod after surgical disconnection of the fetal hypothalamus and pituitary.
4. To determine whether plasma concentrations of PRL in pregnant ewes are different in long and short photoperiods and whether there is any the effect of fetal hypothalamo-pituitary disconnection on maternal prolactin concentrations.

Chapter 3 – Photoperiodic history and hypothalamic control of prolactin secretion before birth

Chapter 3 – Hypothesis

That the fetal sheep can construct a photoperiodic history and respond to an intermediate photoperiod in utero depending on the prior photoperiodic exposure, and that this response is not dependent on a functionally intact hypothalamo-pituitary axis.

Chapter 3 – Experimental Aims

1. To determine whether the fetal sheep plasma concentrations of PRL in an intermediate photoperiod are different depending on the pregnant ewes prior photoperiodic exposure to either long or short photoperiod.
2. To determine whether the fetal sheep PRL responses in an intermediate photoperiod, after exposure to an external long or short photoperiod, is dependent upon an intact fetal hypothalamo-pituitary axis.
3. To determine whether the fetal PRL response to a thyrotrophin releasing hormone stimulus is different depending on the pregnant ewes prior photoperiodic exposure to long or short photoperiod or fetal hypothalamo-pituitary disconnection.
4. To determine whether maternal plasma concentrations of PRL in an intermediate photoperiod are different after prior photoperiodic exposure to an external long or short photoperiod.

Chapter 4 – Evidence for hypothalamic control of the diurnal rhythms in prolactin and melatonin in the fetal sheep during late gestation

Chapter 4 – Hypothesis

The daily fetal MEL and PRL rhythm is dependent on an intact fetal hypothalamo-pituitary axis.

Chapter 4 – Experimental Aims

1. To determine the effect of fetal hypothalamo-pituitary disconnection on the daily rhythm of fetal plasma concentrations of PRL in long and short photoperiod.
2. To determine the effect of fetal hypothalamo-pituitary disconnection on the daily rhythm of maternal plasma concentrations of PRL in long and short photoperiod.
3. To determine the effect of fetal hypothalamo-pituitary disconnection on the daily rhythm of fetal plasma concentrations of MEL in long and short photoperiod.
4. To determine the effect of fetal hypothalamo-pituitary disconnection on the daily rhythm of maternal plasma concentrations of MEL in long and short photoperiod.

1.5 OVERVIEW OF THESIS AND LINKS BETWEEN PUBLICATIONS 1-3

Paper 1: Response of prolactin to different photoperiods after surgical disconnection of the hypothalamus and pituitary in sheep fetuses

Fetal sheep plasma PRL is low in short photoperiod and high in long photoperiod. Photoperiodic information is provided to the sheep fetus via the maternally derived nocturnal duration of elevated plasma levels of MEL. The high concentration of iodomelatonin binding at the pars tuberalis indicates that photoperiodic regulation of PRL may occur at an extra-hypothalamic site. Therefore the aim of this study was to determine whether the fetal sheep PRL response to different photoperiods was dependent upon an intact fetal hypothalamo-pituitary axis. This study demonstrated for the first time that the functionally isolated fetal sheep pituitary could produce different plasma concentrations of PRL in long and short photoperiods. This supports the notion that photoperiodic information via maternal nocturnal MEL secretion can regulate plasma PRL through an effect at the fetal pars tuberalis. The next step was to investigate whether the fetal sheep plasma PRL level was determined only by the ambient photoperiod, or whether the plasma PRL level is determined by the ambient photoperiod relative to the previous photoperiod.

Paper 2: Photoperiodic History and Hypothalamic Control of Prolactin Secretion Before Birth

The neonatal interpretation of ambient photoperiod is influenced by photoperiodic history in utero in sheep. Newborn lamb plasma PRL responses to an intermediate photoperiod (12h L: 12h D) are different depending on whether they were exposed to a long or short photoperiod during gestation. In this paper

we investigate the development and expression of a photoperiodic history on plasma PRL levels in fetal sheep in utero. This paper also investigates the role of the fetal hypothalamus in determining the PRL response to photoperiodic history. We demonstrated for the first time that the PRL response to an intermediate photoperiod was determined by the photoperiodic history in fetal sheep that had undergone hypothalamo-pituitary disconnection. Interestingly, in intact fetal sheep no effect of photoperiodic history was evident on the gestational age increase in PRL. Thus the isolated fetal sheep pituitary PRL response to ambient photoperiod is determined by the preceding photoperiod and this effect is unmasked when hypothalamic influences on the pituitary are removed. This study provided further support that MEL exerts its effect via the pars tuberalis to regulate photoperiodic history and expression of PRL in fetal sheep. The diurnal MEL rhythm has also been proposed to be influential on the diurnal PRL rhythm and therefore the next stage was to investigate the hypothalamic control of the diurnal PRL rhythm.

Paper 3: Evidence for Hypothalamic Control of the Diurnal Rhythms in Prolactin and Melatonin in the Fetal Sheep during Late Gestation

The fetal sheep diurnal MEL rhythm has been demonstrated to be derived from the maternal pineal gland. This MEL rhythm is reliably elevated during darkness and low during the day. The duration of the nocturnal MEL exposure is well demonstrated to regulate PRL levels in the fetus. It has been suggested that this rhythm also acts as a circadian signal and provides the fetus with information about time of day that may then influence the diurnal fetal PRL rhythm. As the first two papers demonstrated the functionally isolated pituitary can regulate plasma PRL levels, this paper focussed on the hypothalamic control of the diurnal PRL

rhythm. We investigated the effect of fetal hypothalamo-pituitary disconnection (HPD) on the daily rhythm of PRL and MEL under long and short photoperiods. This study demonstrated for the first time that the isolated fetal pituitary gland is unable to produce a daily rhythm in plasma PRL concentrations. Interestingly, there was no fetal MEL rhythm in HPD fetal sheep under long photoperiod, suggesting that the fetal hypothalamus may contribute to daily MEL rhythm in the fetus. The hypothalamus therefore has a significant role in the generation of the daily fetal PRL rhythm and may be important in contributing to the diurnal fetal MEL rhythm.

Taken together, these studies demonstrate that the fetal sheep is not only aware, but can respond to the external environment. Fetal sheep plasma PRL levels are regulated by the ambient photoperiod relative to the preceding photoperiod. The diurnal MEL rhythm derived from the maternal pineal gland crosses the placenta and provides the humoral information that can influence fetal PRL levels by acting at the pars tuberalis (Figure 1). The fetal hypothalamus is not necessary for a PRL response to different photoperiod, with the functionally isolated pituitary and pars tuberalis producing different plasma PRL levels in different photoperiods in fetal sheep. The PRL response to different photoperiods is dependent upon the prior photoperiodic history in fetal sheep that had undergone surgical disconnection of the hypothalamus and pituitary. In intact fetal sheep gestational factors acting at the hypothalamus may mask any effect of photoperiodic history on PRL levels. Finally, the hypothalamus is vital for the generation and expression of a daily fetal PRL rhythm and may be involved in the diurnal MEL rhythm. Connection between the fetal SCN and pituitary is therefore required for development of the daily fetal PRL rhythm.

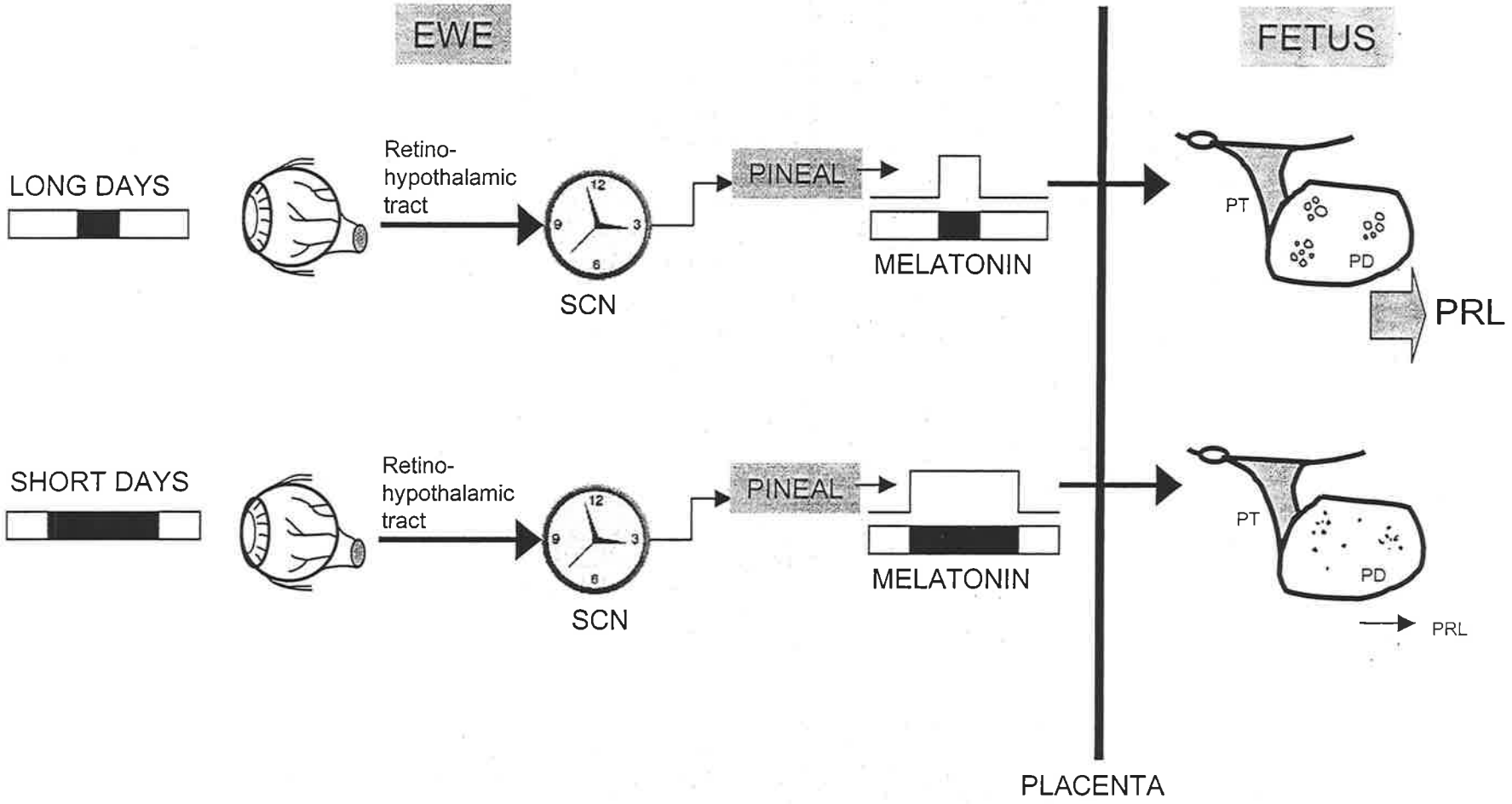

Figure 1. Schematic model of photoperiod regulation of PRL secretion in fetal sheep.

2. Response of Prolactin to Different Photoperiods After Surgical Disconnection of the Hypothalamus and Pituitary in Sheep Fetuses

D.C. Houghton[1], I.R. Young[2] and I.C. McMillen[1]

[1]Department of Physiology, The University of Adelaide, Adelaide SA 5005, Australia
[2]Department of Physiology, Monash University, Clayton 3168, Australia

Journal of Reproduction and Fertility (1995) 104, 199-206.

STATEMENT OF AUTHORSHIP

Response of prolactin to different photoperiods after surgical disconnection of the hypothalamus and pituitary in sheep fetuses

Journal of Reproduction and Fertility (1995) 104, 199-206.

HOUGHTON, D.C. (Candidate)

Established and maintained chronically catheterised fetal sheep preparations, collected and performed analysis on all samples, data analysis and interpretation, wrote manuscript and acted as corresponding author.

Signed.. .....*Date*...5/10/04

YOUNG, I.R.

Performed surgery on HPD sheep, data interpretation and manuscript evaluation.

Signed..... .....*Date*...12-10-04

MCMILLEN, I.C.

Supervised development of work, helped in data interpretation and manuscript evaluation.

Signed.. .....*Date*...8/11/04

Houghton, D.C., Young, I.R. and McMillen, I.C. (1995) Response of prolactin to different photoperiods after surgical disconnection of the hypothalamus and pituitary in sheep foetuses.
Journal of Reproduction and Fertility, v. 104 (2), pp. 199-206, July 1995

3. Photoperiodic History and Hypothalamic Control of Prolactin Before Birth

D.C. Houghton[1], I.R. Young[2] and I.C. McMillen[1]

[1]*Department of Physiology, The University of Adelaide, Adelaide SA 5005, Australia*
[2]*Department of Physiology, Monash University, Clayton 3168, Australia*

Endocrinology (1997) 138, 1506-1511.

STATEMENT OF AUTHORSHIP

Photoperiodic History and Hypothalamic Control of Prolactin Secretion Before Birth

Endocrinology (1997) 138, 1506-1511.

HOUGHTON, D.C. (Candidate)

Established and maintained chronically catheterised fetal sheep preparations, collected and performed analysis on all samples, data analysis and interpretation, wrote manuscript and acted as corresponding author.

Signed. .....*Date*.....5/10/04.....

YOUNG, I.R.

Performed surgery on HPD sheep, data interpretation and manuscript evaluation.

Signed..... .....*Date*.....12-10-04

MCMILLEN, I.C.

Supervised development of work, helped in data interpretation and manuscript evaluation.

Signed. .....*Date*.....6/11/04

0013-7227/97/$03.00/0
Endocrinology

Vol. 138, No. 4
Printed in U.S.A.

Photoperiodic History and Hypothalamic Control of Prolactin Secretion Before Birth*

D. C. HOUGHTON†, I. R. YOUNG, AND I. C. MCMILLEN

Department of Physiology, The University of Adelaide (D.C.H., I.C. McM.), Adelaide SA 5005; and Department of Physiology, Monash University (I.R.Y.), Clayton, Victoria 3168, Australia

ABSTRACT

We investigated whether the fetal lamb can construct a photoperiodic history *in utero*. We measured the fetal PRL response to a 12-h photoperiod in intact fetal sheep and in fetal sheep after hypothalamo-pituitary disconnection (HPD), following exposure of the ewe to either a long (16 h L) or short (8 h L) photoperiod for 50 days in early pregnancy. Ewes were maintained on either a long light (LL, n = 20) or a short light (SL, n = 19) regimen from 57 days gestation until fetal HPD (pre-LL, n = 7; pre-SL, n = 7) or sham surgery (pre-LL, n = 13; pre-SL, n = 12) was performed at 99–113 days gestation. All ewes were housed in a 12-h photoperiod from surgery until 140 days gestation. In HPD fetal sheep previously exposed to SL, fetal PRL concentrations were significantly higher ($P < 0.05$) after 20 days in the 12-h L regimen than previously (0–5 days, 3.2 ± 0.6 ng/ml; 21–25 days, 5.6 ± 1.4 ng/ml). In the HPD fetal sheep previously exposed to LL, however, fetal PRL concentrations significantly decreased ($P < 0.05$) after 5 days exposure to the 12-h L regimen (6.7 ± 2.9 ng/ml) and remained low throughout the remaining study period (31–35 days, 1.7 ± 0.5 ng/ml). In contrast, in the sham group there was no effect of photoperiodic history on the gestational age profile of fetal PRL, and PRL concentrations increased significantly ($F = 22.4$, $P < 0.001$) in fetal sheep previously exposed to either SL or LL. Fetal PRL concentrations were significantly higher ($P < 0.05$) after 121 days gestation in the 12-h L regimen in all sham fetal sheep (<110 days, pre-SL 6.4 ± 0.3 ng/ml, pre-LL 12.0 ± 3.3 ng/ml; 121–125 days, pre-SL 20.0 ± 3.9 ng/ml, pre-LL 25.9 ± 4.4 ng/ml). TRH (50 μg) was administered iv to all fetal sheep at 130–134 days gestation. There was a significant fetal PRL response to TRH in both the HPD ($F = 20.9$, $P < 0.001$) and sham ($F = 31.3$, $P < 0.001$) groups. There was no difference, however, in the PRL response to TRH in fetal sheep previously exposed to SL or LL in either the HPD or sham groups. The maximum percentage changes in PRL occurred at +10 min after TRH administration in the HPD (pre-SL, 421 ± 75%; pre-LL, 555 ± 76%) and sham groups (pre-SL, 394 ± 68%; pre-LL, 369 ± 59%). In summary, therefore, we have demonstrated that there is an effect of photoperiodic history on the PRL response to an intermediate photoperiod *in utero* in HPD fetal sheep. It appears, however, that the effect of photoperiodic history on PRL secretion in intact fetal sheep is either masked or suppressed by the stimulatory effect of factors associated with an increase in gestational age acting at the fetal hypothalamus. (*Endocrinology* **138:** 1506–1511, 1997)

ALTHOUGH FETAL SHEEP are not directly exposed to light *in utero*, changes in the length of the external photoperiod are associated with changes in the fetal plasma concentrations of PRL (1–3). Fetal PRL concentrations are higher when pregnant ewes are maintained in long compared with short photoperiods (1–3). Because PRL does not cross the ovine placenta, this suggests that the fetal hypothalamo-pituitary axis is sensitive to changes in the external photoperiod. It has also been shown that melatonin crosses the placenta in the sheep, and that pinealectomy of the pregnant ewe abolishes the daily rhythm in maternal and fetal melatonin concentrations in late gestation (4–6). Fetal PRL concentrations are increased after maternal pinealectomy (7) and decreased when melatonin is infused into pregnant ewes during summer pregnancies to simulate the winter duration of the nocturnal melatonin increase (8). Similarly, plasma PRL concentrations are higher in lambs born to ewes exposed to a long photoperiod [16 h light (L), 8 h dark (D)] during late gestation when compared with lambs born to ewes exposed to a short photoperiod (8 h L, 16 h D) (9). It appears, therefore that the duration of the maternal melatonin signal provides the fetal lamb with information about the length of the prevailing photoperiod in the sheep.

Ebling and co-workers (9) have also shown that the PRL response in the newborn lamb to an intermediate photoperiod of 12 h L, 12 h D depends on the photoperiodic history of the ewe. In lambs born to mothers that had been maintained in long photoperiods (16 h L) from 100 days gestation (term = 145 ± 3 days gestation), PRL concentrations were high at birth and then decreased rapidly within 14 days of exposure to 12 h L, 12 h D and remained low thereafter. In contrast, PRL concentrations in lambs born to mothers maintained on short photoperiods (8 h L), were low at birth and then gradually increased in the postnatal 12 h L, 12 h D photoperiod to exceed those in the group exposed to long photoperiods *in utero* (9). Similarly, studies in the juvenile male Siberian hamsters and other seasonal breeders have demonstrated that serum PRL concentrations and the reproductive response to an intermediate photoperiod are also dependent on the photoperiod experienced by the mother during late gestation (10–14). It has been inferred from such findings that in seasonal breeders, the fetus receives and responds to information about day length *in utero* and begins to develop a photoperiodic history before birth.

In the present study, we tested for the first time whether the lamb can construct a photoperiodic history *in utero*. We measured the fetal PRL response to an intermediate photo-

Received September 6, 1996.
Address all correspondence and requests for reprints to: I.C. McMillen, Department of Physiology, The University of Adelaide, Adelaide SA 5005, Australia. E-mail: cmcmillen@physiol.adelaide.edu.au.
* This work was supported by the Australian Research Council.
† Recipient of a Commonwealth Postgraduate Research Award during the conduct of this research.

period (12 h L) in late gestation after exposure of the ewe to either a long (16 h L) or short (8 h L) photoperiod for 50 days in early pregnancy. We also investigated the relative roles of the fetal hypothalamus and pituitary in the measurement of photoperiodic history before birth. We previously have shown that the difference in fetal PRL concentrations in long and short photoperiods persists after surgical disconnection of the fetal hypothalamus and pituitary in late gestation (3). We have argued therefore that the effects of external photoperiod and maternal melatonin on fetal PRL secretion may be mediated at such extrahypothalamic sites as the melatonin-responsive cells of the pars tuberalis (3). In the present study, we investigated the effect of fetal hypothalamo-pituitary disconnection on the PRL response of the sheep fetus to a 12-h L photoperiod after prior exposure of the pregnant ewe to either a long or short photoperiod.

Materials and Methods

Animals and photoperiodic history

All experimental procedures in this study received approval from the University of Adelaide Standing Committee on Ethics in Animal Experimentation. Thirty nine pregnant Merino ewes were used, and the study was carried out between March and September (*i.e.* between autumn and early spring in the southern hemisphere) in each of 2 yr (1994 and 1995). All ewes were held in a central animal holding facility and were maintained on either a long light (LL: 16 h light, 8 h dark; lights on at 0700 h; n = 20) or a short light (SL: 8 h light, 16 h dark; lights on at 0700 h; n = 19) regimen from 57 days gestation until surgery at between 99–113 days gestation. At surgery, hypothalamo-pituitary disconnection (HPD) or a sham fetal operation was performed in one fetus in each ewe as described below. The mean time spent by pregnant ewes in the LL regimen before surgery was 50.5 days (HPD pre-LL group,51 days, n = 7; sham pre-LL group; 50 days, n = 13), and the mean time spent by pregnant ewes in the SL regimen before surgery was 50 days (HPD pre-SL group: 51 days, n = 7; sham pre-SL group; 49 days, n = 12). After surgery, all ewes were housed in a 12 h L, 12 h D light cycle (lights on at 0700 h) for the remainder of the experimental period.

Surgery

Surgery was performed between 99–113 days gestation under general anesthesia using halothane (0.5–4.0%) and N_2O:O_2 (50:50 vol/vol) with aseptic techniques. HPD was performed in the fetal sheep of 14 ewes as described in full previously (3, 15). A midline incision was made in the fetal nose and the nasal bone opened just left of the intranasal septum. The fetal ethmoid and presphenoid bones were drilled to form a paramedian tunnel beneath the anterior cranial fossa. The optic chiasm was located and exposed to allow access to the median eminence. The neural tissue of both internal and external laminae of the median eminence were removed using gentle suction. A small piece of gelfoam soaked in thrombin (Thrombostat: Parke-Davis, Caringbah, New South Wales, Australia) and penicillin (Depomycin: Intervet, Lane Cove, New South Wales, Australia) was introduced to separate the remaining hypothalamic tissue from the pituitary. A sham procedure was carried out in 25 fetal sheep in which either limited or no cranial dissection was performed, but in which fetal vascular and amniotic catheters were inserted (sham group). Catheters were inserted into a fetal and maternal carotid artery and jugular vein, and into the amniotic cavity in all ewes. All catheters were filled with heparinized saline, and the fetal catheters were exteriorized via an incision in the ewes flank.

Blood sampling protocols after surgery

After surgery, all ewes were housed in metabolic cages and fed alfalfa chaff (1 kg) once a day between 0900–1100 h, with water available *ad libitum*. In 34 ewes, daily fetal (2.5 ml) and maternal (5 ml) arterial blood samples were collected from the first day after surgery until the end of the experimental period. In the remaining 5 ewes, fetal and maternal arterial blood samples were collected on every alternate day from day 6 or 7 after surgery. A total of 920 fetal and 884 maternal blood samples were collected from the 39 pregnant ewes for PRL assay in this part of the study. Blood samples were collected into heparinized tubes that were centrifuged for 10 min at 1100 × *g* before separation and storage of plasma at −20 C for assay. Fetal arterial blood samples (0.6 ml) were also collected on each occasion for blood gas and pH analysis with an ABL 330 acid base analyzer and OSM 2 hemoximeter (both from Radiometer, Copenhagen, Denmark).

TRH

TRH (50 μg; 15–25 μg/kg) was administered iv to fetal sheep between 130–134 days gestation. Fetal arterial or venous blood (1.5 ml) samples were collected at −30, −5, +10, +20, +40, +60, and +120 min relative to the time of TRH administration. All blood samples were collected into heparinized tubes that were centrifuged for 10 min at 1100 × *g* before separation and storage of plasma at −20 C for assay.

Confirmation of HPD

Disconnection of the hypothalamus was confirmed in all HPD fetal sheep as previously described (3) on the basis of the PRL responses to intravascular chlorpromazine and on the basis of macroscopic examination of the lesion site at postmortem. Chlorpromazine (CPZ, 12.5 mg; 4–6 mg/kg) was administered iv to fetal sheep between 132–140 days gestation. In the sham groups, fetal PRL concentrations were 50 ± 9 ng/ml (−5 min) and 116 ± 12 ng/ml (CPZ, +120 min) (pre-LL) and 47 ± 13 ng/ml (−5 min) and 81 ± 16 ng/ml (CPZ, +120 min) (pre-SL). In the HPD groups, however, fetal PRL concentrations were 2.3 ± 0.9 ng/ml (−5 min) and 2.8 ± 1.1 ng/ml (CPZ, +120 min) in the pre-LL group and 6.8 ± 1.8 ng/ml (−5 min) and 8.5 ± 2.3 ng/ml (CPZ, +120 min) in the pre-SL group. Macroscopic examination of the lesion site postmortem confirmed the completeness of the HPD procedure in all HPD fetal sheep.

PRL RIA

Plasma PRL was measured using rabbit antiovine PRL (Antiserum batch number AFP 973269, generously donated by the National Hormone and Pituitary Program, NIDDK, Baltimore, MD), and an assay that was previously described and validated (3, 16). The sensitivity (defined as the dose required to produce 10% displacement) of the assay was 0.1 ng/tube and the inter- and intraassay coefficients of variation were <20% and <10%, respectively.

Postmortem

Thirty six ewes were killed between 136–144 days gestation using an overdose of sodium pentobarbitone. Four ewes were killed between 115–127 days gestation after sudden fetal death.

Statistical analyses

All results are expressed as the mean ± SEM. Where the Cochrans and Bartlett-Box tests identified significant heterogeneity of variance, maternal and fetal hormone concentrations were logarithmically transformed before further statistical analysis. The data were analyzed using the Statistical Package for Social Scientists (SPSS Inc., Chicago, IL) and a VAX mainframe computer using multifactorial ANOVA with repeated measures and photoperiodic history (*i.e.* either pre-LL or pre-SL), treatment (*i.e.* HPD or sham group), and the number of days spent in the 12-h L regimen (in 5-day blocks) or gestational age (in 5-day blocks) as the specified variables. PRL responses to TRH were also expressed as a percentage change from baseline, (baseline values were taken as the mean of the concentrations in the 30-min period before administration of TRH). ANOVAs were used to determine whether there were significant changes in PRL concentrations or in the percentage change from baseline in the TRH experiments with photoperiodic history, treatment group, time (−30, −5, +10, +20, +40, +60, and +120 min relative to time of drug administration), and animal as the specified variables. Where the multifactorial ANOVAs identified significant interactions between major factors, the data were split on the basis of the interaction and rean-

alyzed. When the ANOVAs indicated there were differences between groups, the Duncan's post hoc test was used to identify significant differences between mean values. A probability of 5% (*i.e.* $P \leq 0.05$) was taken to be significant.

Results

Fetal outcome

The mean body weights of the catheterized fetal sheep at 140–145 days gestation were 3.97 ± 0.14 kg (HPD pre-LL: 5 twins, 2 singletons), 4.69 ± 0.21 kg (sham pre-LL: 3 twins, 7 singletons), 4.29 ± 0.38 kg (HPD pre-SL: 5 twins, 2 singletons) and 4.15 ± 0.17 kg (sham pre-SL: 5 twins, 5 singletons).

Photoperiodic history: impact on fetal PRL response to a 12-h photoperiod

Fetal PRL data were first analyzed to determine the effects of HPD and photoperiodic history (*i.e.* pre-SL or LL) on fetal PRL concentrations in a 12-h L regimen. The mean fetal PRL concentrations were significantly lower ($F = 20.1$, $P < 0.001$) in the HPD group (3.4 ± 1.0 ng/ml, n = 14 fetal sheep) than in the sham fetal sheep (22.3 ± 4.0 ng/ml, n = 25 fetal sheep) throughout the entire study period, *i.e.* between 105–144 days gestation. There was also a significant interaction ($F = 11.5$, $P < 0.001$) between the surgical treatment and the effects of time spent in the 12-h L regimen, *i.e.* the HPD and sham groups responded differently to the 12-h L regimen. The data were split on the basis of surgical treatment group and reanalyzed.

In the HPD group, there was a significant interaction ($F = 6.3$, $P < 0.001$) between the effects of previous photoperiod and the time spent in the 12-h L photoperiod on fetal PRL concentrations. In HPD fetal sheep previously exposed to SL, fetal PRL concentrations were significantly higher ($P < 0.05$) after 20 days in the 12-h L regimen than previously (0–5 days, 3.2 ± 0.6 ng/ml; 21–25 days, 5.6 ± 1.4 ng/ml). In the HPD fetal sheep previously exposed to LL, however, fetal PRL concentrations significantly decreased ($P < 0.05$) during the first 5 days exposure to the 12-h L regimen (6.7 ± 2.9 ng/ml) and remained low throughout the remaining study period (31–35 days, 1.7 ± 0.5 ng/ml) (Fig. 1a).

In the sham group, however, there was no effect of photoperiodic history on the subsequent PRL response to the 12-h L regimen. Plasma concentrations of PRL increased significantly ($F = 23.4$, $P < 0.001$) in fetal sheep previously exposed to either SL or LL in early gestation. In the sham-operated fetal sheep, PRL concentrations were significantly higher in both the pre-SL and pre-LL groups with increasing time spent in the 12-h L regimen (0–5 days, 13.3 ± 2.9 ng/ml *vs.* 16–20 days, 23.9 ± 2.94 ng/ml; $P < 0.05$) (Fig. 1b).

Photoperiodic history: impact on gestational age profile of fetal PRL

The fetal PRL data from the HPD and sham fetal sheep were also analyzed on the basis of gestational age. There was a significant interaction ($F = 11.9$, $P < 0.001$) between the effects of surgical treatment and gestational age. The data were split on the basis of treatment group and reanalyzed.

In the HPD group there was a significant interaction ($F = 6.9$, $P < 0.001$) between the effects of previous photoperiod and the impact of gestational age on fetal PRL concentrations. In HPD fetal sheep after exposure to SL, fetal PRL concentrations were significantly higher ($P < 0.05$) after 130 days gestation (6.7 ± 1.2 ng/ml) than earlier in gestation (< 110 days, 2.3 ± 0.4 ng/ml) (Fig. 1c). In HPD fetal sheep previously exposed to LL, however, fetal PRL concentrations did not change significantly with increasing gestational age (<110 days, 2.1 ± 0.5 ng/ml; 136–144 days, 2.5 ± 0.7 ng/ml) (Fig. 1c).

In the sham group, there was no effect of photoperiodic history on the gestational age profile of fetal PRL, and PRL concentrations increased significantly ($F = 22.4$, $P < 0.001$) in fetal sheep previously exposed to either SL or LL. Fetal

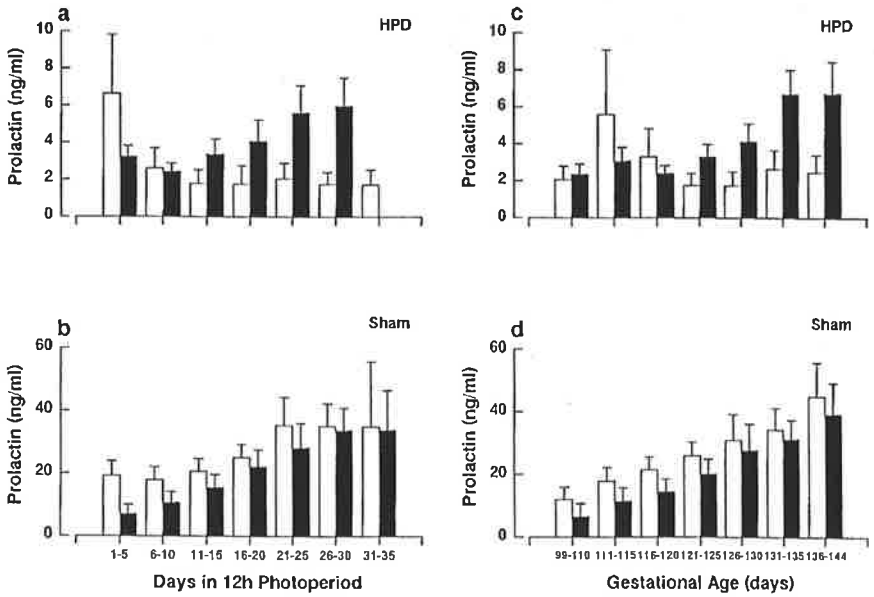

FIG. 1. Plasma PRL concentrations (mean ± SEM) in HPD fetal sheep (a) and sham fetal sheep previously exposed to LL (b) (*open histograms*) and SL (*closed histograms*) during 35 days exposure to a 12-h photoperiod. No mean value is presented for HPD group at 31–35 days exposure because only three samples were available for this group in this range. Plasma PRL concentrations (mean ± SEM) are also shown for HPD (c) and sham (d) groups exposed to LL (*open histograms*) and SL (*closed histograms*) in relation to gestational age range of fetal sheep.

PRL concentrations were significantly higher ($P < 0.05$) after 121 days gestation in the 12-h L regimen in all sham fetal sheep (<110 days: pre-SL 6.4 ± 0.3 ng/ml, pre-LL, 12.0 ± 3.3 ng/ml; 121–125 days: pre-SL 20.0 ± 3.9 ng/ml, pre-LL 25.9 ± 4.4 ng/ml) (Fig. 1d).

Photoperiodic history and fetal PRL response to TRH

There was a significant fetal PRL response to TRH in both the HPD ($F = 20.9$, $P < 0.001$) and sham ($F = 31.3$, $P < 0.001$) groups (Fig. 2). There was no difference, however, in the PRL response to TRH in fetal sheep previously exposed to SL or LL in either the HPD or sham groups (Fig. 2). The maximum percentage changes in PRL occurred at +10 min after TRH administration in the HPD (pre-SL, 421 ± 75%; pre-LL, 555 ± 76%) and sham groups (pre-SL, 394 ± 68%; pre-LL, 369 ± 59%).

Photoperiodic history: impact on maternal PRL

There was no significant interaction between the effects of previous photoperiod exposure and surgical treatment of the fetus on maternal PRL when PRL concentrations were grouped in relation to either time spent in the 12-h L regimen or in relation to gestational age. The maternal PRL concentrations in the HPD and sham groups were therefore combined. PRL concentrations were higher in ewes previously exposed to LL compared with SL at 99–110 days (pre-LL, 52 ± 7 ng/ml; pre-SL, 20 ± 3 ng/ml). There was an increase, however, in maternal PRL concentrations in all groups with increasing time in the 12-h L photoperiod ($F = 14.4$, $P < 0.001$) and with increasing gestational age ($F = 16.4$, $P < 0.001$). Maternal PRL concentrations increased to a maximum at 136–144 days gestation (pre-SL, 116 ± 13 ng/ml; pre-LL, 97 ± 11 ng/ml) (Fig. 3).

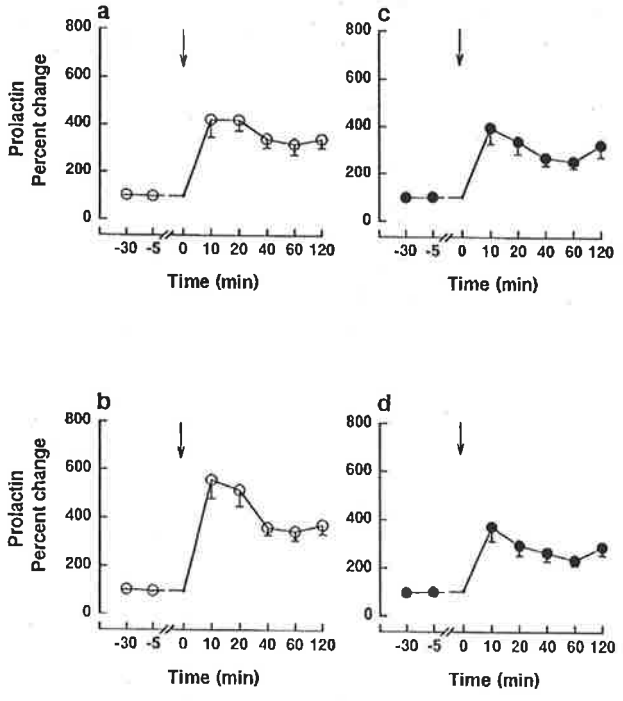

FIG. 2. Percentage increase in fetal PRL concentrations (mean ± SEM) after administration of 50 μg TRH to HPD fetal sheep (○) previously exposed to SL photoperiod (a) and LL photoperiod (b) and to sham fetal sheep (●) previously exposed to SL photoperiod (c) and LL photoperiod (d).

Discussion

We demonstrated that photoperiodic history determined the PRL response to an intermediate photoperiod after surgical disconnection of the fetal hypothalamus and pituitary in the sheep fetus during late gestation. In intact fetal sheep, however, there was no evidence that photoperiodic history altered the pattern of the gestational age increase in circulating PRL concentrations.

In this and in previous studies we found that fetal PRL concentrations are lower in HPD than in intact fetal sheep, regardless of the prevailing photoperiod (3, 17). Surgical disconnection of the fetal pituitary from the hypothalamus does not alter the morphology, distribution, or proportion of the lactotrophs in the pars distalis (15). Furthermore, we showed that the proportional changes in fetal PRL from 10 min after TRH administration are similar in HPD and intact fetal sheep, which indicate that the lactotrophs in the surgically isolated pituitary are functional and responsive to external stimulation. It therefore appears that after HPD in fetal sheep, there is a loss of a predominantly stimulatory drive to PRL secretion. This loss may be a consequence of the lack of a hypothalamic PRL releasing factor after HPD. Alternatively there may be a loss of a placental signal that normally stimulates PRL secretion from the fetal pituitary and that depends on the presence of an intact hypothalamo-pituitary axis.

The findings of the present study indicate that the control of PRL secretion by the surgically isolated pituitary can be influenced by the photoperiodic history of the sheep fetus. Fetal PRL concentrations increased after 3–4 weeks exposure to an intermediate photoperiod (12 h L) in HPD sheep previously maintained in a short photoperiod (8 h L) regimen.

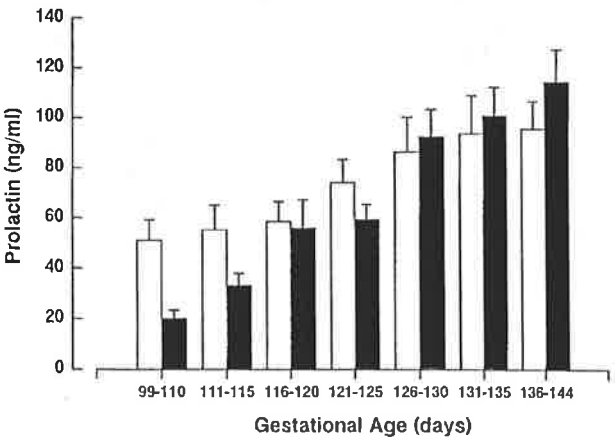

FIG. 3. Plasma concentrations of PRL (mean ± SEM) in pregnant ewes (n = 38) previously exposed to LL (*open histograms*) and SL (*closed histograms*) in relation to gestation length.

Conversely, fetal PRL concentrations decreased within 6–10 days exposure to the intermediate photoperiod in HPD sheep previously maintained in a long photoperiod (16 h L) regimen. After 21–30 days exposure to the 12-h L regimen, PRL concentrations were around 3–5 ng/ml higher in the pre-SL than in the pre-LL HPD fetal sheep. If the HPD fetus simply responded to the absolute photoperiod, then the same pattern of PRL response to 12 h L might have been expected in the pre-SL and pre-LL groups. This did not appear to be the case, however, because the HPD fetal sheep maintained in pre-SL conditions responded to the 12 h L as a long day, whereas the pre-LL group responded to the 12 h L as a short day. In contrast, there was no measurable effect of photoperiodic history on the plasma PRL response to an intermediate photoperiod in the sham groups. In the sham animals, however, there was a consistent increase in the fetal plasma concentrations of PRL with increasing gestational age in both the pre-SL and pre-LL groups.

Although the effect of photoperiodic history on fetal PRL concentrations in the HPD groups was significant, it was relatively small, *i.e.* the greatest difference between the pre-SL and pre-LL groups was around 3–5 ng/ml after 21–30 days exposure to the intermediate photoperiod. It could be argued, therefore, that the effect of photoperiodic history on the PRL response to the intermediate photoperiod is present in the sham groups but is masked by the relatively greater stimulation due to factors acting on the fetal hypothalamus that are associated with increasing gestational age. It is important to note, however, that we found no evidence for a greater effect of photoperiodic history on the fetal PRL response to the intermediate photoperiod in the intact fetal sheep when compared with the HPD group. This implies that the effect of photoperiodic history on the fetal PRL response to an intermediate photoperiod is predominantly exerted through the fetal pituitary rather than the fetal hypothalamus.

In a recent study we demonstrated that plasma concentrations of PRL were higher in intact and HPD sheep fetuses exposed to long photoperiods (16 h L) compared with those exposed to short photoperiods (8 h L). These results are in agreement with those of Lincoln and Clarke (17) who demonstrated that there was photoperiodic regulation of PRL secretion in HPD rams in which the pituitary gland was functionally isolated from the brain. These authors also demonstrated that administration of melatonin to HPD rams under long days suppressed circulating PRL to values observed in HPD rams under short days (17). We concluded therefore, that in the sheep, differences in the prevailing external photoperiod and in the duration of the nocturnal increase in melatonin concentrations regulate PRL secretion by a pituitary dependent mechanism before and after birth. The present study also provides direct evidence that the surgically isolated pituitary has the capacity to generate a fetal PRL response that is influenced by the photoperiodic history of the ewe. It is likely that the reading of the photoperiodic history relies at least in part on the change in the duration of the nocturnal melatonin increase, which occurs on transition between the long or short days to the intermediate photoperiod. One possible extrahypothalamic site of action of melatonin is the pars tuberalis of the fetal pituitary, which remains intact after HPD. There is considerable indirect evidence that the pars tuberalis may be important in mediating seasonal reproductive responses to melatonin. In all mammalian groups, the pars tuberalis, unlike the pars distalis contains exceptionally high concentrations of iodomelatonin binding sites (18). Most types of secretory cells in the ovine pars tuberalis are agranular, and it appears that these cells are melatonin responsive (19). One possibility is that the measurement of a photoperiodic history by these cells requires the stimulation or inhibition of the synthesis of a lactotrophic factor during exposure to long or short photoperiods, respectively, which is followed by a long-term decrease or increase in its secretion on transition to an intermediate photoperiod.

Ebling and co-workers (9) have previously demonstrated that prenatal photoperiod influences PRL secretion in the newborn lamb. In lambs born to ewes maintained on long days (16 h L) from 100 days gestation, circulating PRL concentrations were high for the first few days after birth, but fell rapidly to low levels within 14 days postnatally in 12 h L. Conversely, lambs born to ewes maintained on short days (8 h L) from 100 days gestation, had low PRL concentrations at birth but these gradually increased above 150 ng/ml by 30 days exposure to the 12-h L photoperiod. It is interesting that the difference between PRL concentrations in the pre-SL and pre-LL groups was greater (>100 ng/ml) in this latter study in the newborn lamb than that measured in our HPD or intact group of fetal lambs. There are a number of possible explanations for such a difference. The first may relate to the timing of exposure of the fetal lambs to the long and short photoperiods in these two studies. In the newborn lamb study, ewes were maintained in the long or short day regimens from around 100 days gestation until birth, whereas in our study, the ewes were maintained in similar regimens for around 50–57 days gestation. It has been established that plasma PRL concentrations are higher in 16-h L than in 8-h L regimens in intact fetal sheep after 100 days gestation (3, 20), but it is unknown whether this effect is present from as early as around 57 days gestation. Thus exposure to the different photoperiod regimens in early gestation does not necessarily imply that the fetal neuroendocrine system is able to monitor the photoperiodic environment from the time of exposure. It may also be that the greater apparent effect of photoperiodic history on neonatal PRL concentrations is simply related to a higher basal secretion of PRL in the newborn compared with the fetal lamb at the time of exposure to the intermediate photoperiod. Finally, direct exposure to light may amplify the effects of the intermediate photoperiod on the neuroendocrine system after birth compared with indirect exposure to the effects of the same photoperiod *in utero*.

Although this is the first study to investigate the neuroendocrine measurement of photoperiodic history directly *in utero*, there are a range of studies in other species, including the montane vole and Siberian hamster, that have shown that exposure to different prenatal photoperiods can influence the PRL and reproductive responses to intermediate day lengths postnatally (10–14). Studies in Siberian hamsters have shown that melatonin from the mothers' pineal gland is involved in the prenatal transmission of the photoperiodic message to the pups, and that the message is related to the duration of

the nocturnal melatonin increase in the mother (13). Interestingly, Shaw and Goldman (14) recently demonstrated that in the male Siberian hamster there is an influence of the prenatal photoperiod on the melatonin rhythm generating system in postnatal life, and they have suggested that this may contribute to the effect of prenatal photoperiodic history on postnatal secretion of FSH and PRL.

It is clear in the present study that there was a consistent effect of increasing gestational age on the fetal plasma concentrations of PRL in the sham group that was independent of the effects of prior photoperiodic history. We have previously demonstrated that there is an increase in PRL messenger RNA levels in the anterior pituitary of the sheep fetus between 130–141 days gestation (21). Further, the recent study of Houghton *et al.* (3) also demonstrated that fetal plasma concentrations of PRL increased progressively in late gestation with increasing exposure to either a long or a short photoperiod, which again provides evidence for a separate influence of gestational age on PRL secretion. Factors that may be important in the stimulation of PRL in late gestation include estrogens derived from the placenta. Estrogens stimulate PRL gene expression in pituitary lactotrophs in the adult sheep (22), and there is an increase in circulating oestrogens in the fetal sheep in late pregnancy (23). We have demonstrated that there was a significant effect of gestational length on maternal PRL concentrations that was also independent of the effects of prior photoperiod. The gestational increase in maternal PRL may also be related to the influence of placental estrogens in late pregnancy.

In summary, we demonstrated that there is an effect of photoperiodic history on the PRL response to an intermediate photoperiod *in utero* that does not require an intact and functional fetal hypothalamus. This effect, however, is either masked or suppressed by the stimulatory effect of factors associated with an increase in gestational age acting on the fetal hypothalamus. It appears from this study that although the fetal neuroendocrine system can construct a photoperiodic history, the prevailing photoperiod and gestational age exert relatively greater influences on fetal PRL secretion. Recent studies in the rat showed that PRL receptor messenger RNA is present in a range of key fetal organs, including the fetal liver, spleen, kidney, and muscle (24, 25). Definition of the factors that regulate the PRL axis *in utero* would enhance our understanding of the role that circulating PRL plays in fetal organ growth and metabolism in species that are responsive to changes in the external photoperiod.

Acknowledgments

We are particularly grateful to F. Carbone, S. Fielke, and A. Jurisevic for expert assistance with the surgical and RIA procedures associated with these studies.

References

1. **Bassett JM, Bomford J, Mott JC** 1988 Photoperiod: an important regulator of plasma prolactin concentrations in fetal lambs during later gestation. Q J Exp Physiol 73:241–244
2. **Seron-Ferre M, Vergara M, Parraguez VH, Riquelme R, Llanos AJ** 1989 The circadian variation of prolactin in fetal sheep is affected by the seasons. Endocrinology 125:1613–1616
3. **Houghton DC, Young IR, McMillen IC** 1995 Response of prolactin to different photoperiods after surgical disconnection of the hypothalamus and pituitary in sheep fetuses. J Reprod Fertil 104:199–206
4. **Yellon SM, Longo LD** 1988 Effect of maternal pinealectomy and reverse photoperiod on the circadian melatonin rhythm in the sheep and fetus during the last trimester of pregnancy. Biol Reprod 39:1093–1099
5. **Zemdegs IZ, McMillen IC, Walker DW, Thorburn GD, Nowak R** 1988 Diurnal rhythms in plasma melatonin concentrations in the fetal sheep and pregnant ewe during late gestation. Endocrinology 123:284–289
6. **McMillen IC, Nowak R** 1989 Maternal pinealectomy abolishes the diurnal rhythm in plasma melatonin concentrations in the fetal sheep and pregnant ewe during late gestation. J Endocrinol 120:459–464
7. **McMillen IC, Walker DW, Young IR, Nowak R** 1991 A daily prolactin rhythm persists in the ewe, foetus and newborn lamb after maternal pinealectomy in late gestation. J Neuroendocrinol 3:369–374
8. **Bassett JM, Curtis N, Hanson C, Weeding CM** 1989 Effects of altered photoperiod or maternal melatonin administration on plasma prolactin concentrations in fetal lambs. J Endocrinol 122:633–643
9. **Ebling FJP, Wood RI, Suttie JM, Tovaghgol Adel E, Foster DL** 1989 Prenatal photoperiod influences neonatal prolactin secretion in the sheep. Endocrinology 125:384–391
10. **Horton TH** 1984 Growth and reproductive development of male *Microtus montanus* is affected by the prenatal photoperiod. Biol Reprod 31:499–504
11. **Stetson MH, Elliott JA, Goldman BD** 1986 Maternal transfer of photoperiodic information influences the photoperiodic response of the prepubertal Djungarian hamsters (*Phodopus sungorus sungorus*). Biol Reprod 34:664–669
12. **Lee TM, Smale L, Zucker I, Dark J** 1987 Influence of daylength experienced by dams on postnatal development of young meadow voles (*Microtus pennsylvanicus*). J Reprod Fertil 81:337–342
13. **Elliott JA, Goldman BD** 1989 Reception of photoperiodic information by fetal Siberian hamsters: role of the mother's pineal gland. J Exp Zool 252:237–244
14. **Shaw D, Goldman BD** 1995 Gender differences in influence of prenatal photoperiods on postnatal pineal melatonin rhythms and serum prolactin and follicle stimulating hormone in the Siberian hamster (*Phodopus sungorus*). Endocrinology 136:4237–4246
15. **Antolovich GC, Clarke IJ, McMillen IC, Perry RA, Robinson PM, Silver M, Young IR** 1990 Hypothalamo-pituitary disconnection in the fetal sheep. Neuroendocrinology 51:1–10
16. **McMillen IC, Thorburn GD, Walker DW** 1987 Diurnal variations in plasma concentrations of cortisol, prolactin, growth hormone and glucose in the fetal sheep and pregnant ewe during late gestation. J Endocrinol 114:65–72
17. **Lincoln GA, Clarke IJ** 1994 Photoperiodically induced cycles in the secretion of prolactin in the hypothalamo-pituitary disconnected rams: evidence for translation of the melatonin signal in the pituitary gland. J Neuroendocrinol 6:251–260
18. **Krause DN, Dubucovich ML** 1990 Regulatory sites in the melatonin system of mammals. Trends Neurosci 13:464–470
19. **Morgan PJ, King TP, Lawson W, Slater D, Davidson G** 1991 Ultrastructure of melatonin responsive cells in the ovine pars tuberalis. Cell Tissue Res 263:529–534
20. **Houghton DC, Young IR, McMillen IC** 1995 Evidence for hypothalamic control of the diurnal rhythms in prolactin and melatonin in the fetal sheep during late gestation. Endocrinology 136:218–223
21. **Merei JJ, Rao A, Clarke IJ, McMillen IC** 1993 Proopiomelanocortin, prolactin and growth hormone messenger ribonucleic acid levels in the fetal sheep pituitary during late gestation. Acta Endocrinol (Copenh) 128:263–267
22. **Vician L, Shupnik MA, Gorski J** 1979 Effects of oestrogen on primary ovine pituitary cell cultures: stimulation of prolactin secretion, synthesis and prep-rolactin mRNA activity. Endocrinology 104:736–743
23. **Nathanielsz PW, Elsner C, Magyer D, Frishal Freeman A, Buster JE** 1982 Time trend analysis of plasma unconjugated and sulfoconjugated oestrone and 3 beta-delta 5 steroids in fetal and maternal sheep plasma in relation to spontaneous parturition at term. Endocrinology 110:1402–1407
24. **Anthony RV, Smith GW, Duong A, Pratt SL, Smith MF** 1995 Two forms of the prolactin receptor messenger ribonucleic acid are present in ovine fetal liver and adult ovary. Endocrine 3:291–295
25. **Freemark MF, Nagano M, Edery M, Kelly PA** 1995 Prolactin receptor gene expression in the fetal rat. J Endocrinol 144:285–292

4. Evidence for Hypothalamic Control of the Diurnal Rhythms in Prolactin and Melatonin in the Fetal Sheep during Late Gestation

D.C. Houghton[1], I.R. Young[2] and I.C. McMillen[1]

[1]*Department of Physiology, The University of Adelaide, Adelaide SA 5005, Australia*
[2]*Department of Physiology, Monash University, Clayton 3168, Australia*

Endocrinology (1995) 136, 218-223.

STATEMENT OF AUTHORSHIP

Evidence for Hypothalamic Control of the Diurnal Rhythms in Prolactin and Melatonin in the Fetal Sheep during Late Gestation

Endocrinology (1995) 136, 218-223.

HOUGHTON, D.C. (Candidate)

Established and maintained chronically catheterised fetal sheep preparations, collected and performed analysis on all samples, data analysis and interpretation, wrote manuscript and acted as corresponding author.

Signed.. .....*Date*...5/10/04..

YOUNG, I.R.

Performed surgery on HPD sheep, data interpretation and manuscript evaluation.

Signed..... .....*Date*...12-10-04..

MCMILLEN, I.C.

Supervised development of work, helped in data interpretation and manuscript evaluation.

Signed... .....*Date*.....8/11/04

0013-7227/95/$03.00/0
Endocrinology

Vol. 136, No. 1
Printed in U.S.A.

Evidence for Hypothalamic Control of the Diurnal Rhythms in Prolactin and Melatonin in the Fetal Sheep during Late Gestation*

D. C. HOUGHTON†, I. R. YOUNG, AND I. C. McMILLEN

Department of Physiology, University of Adelaide (D.C.H., I.C.M.), Adelaide, South Australia 5005; and the Department of Physiology, Monash University (I.R.Y.), Clayton, Victoria 3168, Australia

ABSTRACT

We have investigated the effect of surgical disconnection of the fetal hypothalamus and pituitary (HPD) on generation of the daily rhythm in fetal plasma melatonin and PRL concentrations under long and short photoperiods. Fetal HPD or a sham operation was carried out at around 110 days gestation. Ewes carrying either HPD fetal sheep (n = 10) or intact fetal sheep (n = 12) were exposed to a long light (LL; 16 h of light and 8 h of darkness) or a short light (SL; 8 h of light and 16 h of darkness) regimen for the remainder of gestation. All ewes were subjected to a 24-h blood-sampling experiment (13 samples collected between 0900–0900 h the following day) between 135–140 days gestation, and fetal and maternal plasma melatonin and PRL concentrations were measured using specific RIAs. The hormonal data were analyzed using multifactorial analysis of variance and cosinor analysis. There was an increase in maternal melatonin concentrations during the dark phase in each lighting regimen in ewes carrying HPD or intact fetal sheep. In the SL regimen, there was also a significant increase in fetal melatonin concentrations during the dark phase in the HPD and intact groups. Under LL conditions, however, fetal melatonin concentrations were only consistently increased during the dark phase in the intact, not the HPD, group. The 24-h mean fetal plasma concentrations of PRL were significantly higher ($P < 0.001$) in both intact and HPD fetuses in the LL (intact, 111.0 ± 22.0 pg/ml; HPD, 37.6 ± 7.3 pg/ml) than in the SL regimen (intact, 37.8 ± 18.4 pg/ml; HPD, 6.7 ± 4.3 pg/ml). There was also a significant interaction ($P < 0.001$) between the effects of fetal surgical treatment and time of day on fetal PRL concentrations. In the intact group, fetal PRL concentrations were significantly higher ($P < 0.05$) at 1300 and 1700 h than between 0300–0700 h in both lighting conditions. Cosinor analysis also identified a significant rhythm in 8 of the 12 fetal PRL profiles in the intact group. In contrast, in the HPD group, there was no significant effect of time of day on fetal PRL in either the LL or SL regimen, and cosinor analysis only identified a significant rhythm in 2 of the 10 fetal PRL profiles in this group. We have, therefore, demonstrated that in the fetal sheep, HPD resulted in abolition of the diurnal melatonin rhythm under LL conditions and in the loss of the diurnal PRL rhythm under LL and SL conditions. These results provide direct evidence for a fetal hypothalamic role in generation of the diurnal rhythms in melatonin and PRL before birth in the sheep. (*Endocrinology* **136:** 218–223, 1995)

THERE IS a diurnal variation in plasma concentrations of PRL and melatonin in the fetal sheep and pregnant ewe during late gestation (1–3). The daily rhythm in circulating melatonin is abolished in the fetus and the pregnant ewe after removal of the maternal pineal gland, and it has, therefore, been concluded that the maternal pineal is the source of the fetal melatonin rhythm (4, 5). It has also been demonstrated that there is a seasonal variation in the fetal and maternal plasma concentrations of PRL and in the timing of the acrophase of the daily PRL rhythm in the sheep before birth (6, 7). Fetal PRL concentrations increase after pinealectomy of the pregnant ewe (8), and infusion of melatonin into ewes during summer pregnancies results in a decrease in the maternal and fetal plasma concentrations of PRL (9). Although the seasonal variation in circulating PRL concentrations in the fetus and ewe may depend on the duration of the nocturnal increase in maternal and fetal melatonin concentrations, pinealectomy of the pregnant ewe does not abolish the diurnal rhythm in fetal or maternal PRL concentrations (8). We have also previously studied the effects of independently altering the time of dark onset and the phase of the daily melatonin rhythm during a 12-h photoperiod on the daily rhythm in fetal and maternal PRL concentrations in pinealectomized ewes (10). We found that the timing of the daily peak in fetal and maternal PRL concentrations was related to changes in the external light-dark cycle rather than to changes in the phase of the daily melatonin rhythm (10). The source and the control mechanisms that underlie the timing of the daily PRL rhythm before or after birth are unknown.

In the present study we investigated the effect of surgical disconnection of the fetal hypothalamus and pituitary on generation of the daily rhythm in fetal plasma PRL and melatonin concentrations under long and short photoperiods. We demonstrated that an intact and functional fetal hypothalamus is essential for generation of the daily PRL rhythm under both lighting regimens. We have also shown that the daily rhythm in fetal melatonin concentrations in long light conditions is abolished after fetal hypothalamopituitary disconnection (HPD). This provides direct evidence for a fetal hypothalamic role in the control of melatonin secretion, which is unmasked during long light conditions before birth.

Received June 28, 1994.
Address all correspondence and requests for reprints to: Prof. I. C. McMillen, Department of Physiology, University of Adelaide, Adelaide, South Australia 5005, Australia.
* This work was supported by the Australian Research Council.
† Recipient of a Commonwealth Postgraduate Research Award during the conduct of these studies.

Materials and Methods

Animals and surgery

All experimental procedures in this study received approval from the University of Adelaide Standing Committee on Ethics in Animal Experimentation. Twenty-two pregnant Merino ewes were used, and the study was carried out between April and September (*i.e.* between autumn and early spring in the southern hemisphere). All ewes were housed in a central animal holding facility and were maintained under a 12-h light, 12-h dark cycle (lights off at 1900 h) from at least 70 days gestation (calculated from the date of mating) until surgery. Surgery was performed between 106–120 days gestation under general anesthesia using halothane (0.5–4.0%) and N_2O-O_2 (50:50, vol/vol) with aseptic techniques. HPD was performed in the fetuses of 10 ewes (HPD group), as described in full previously (11). A midline incision was made in the fetal nose, and the nasal bone was opened just left of the intranasal septum. The optic chiasm was located and exposed to allow access to the median eminence. The neural tissue of both internal and external laminae of the median eminence were removed using gentle suction. A small piece of gel foam soaked in thrombin (Thrombostat, Parke-Davis, Caringbah, Australia) and penicillin (Depomycin, Intervet, Lane Cove, Australia) was introduced to separate the remaining hypothalamic tissue from the pituitary. A sham procedure was carried out in 12 fetal sheep (intact group), in which either cranial dissection was performed but no neural tissue was removed (25% of the group) or no cranial dissection was carried out (75% of the group). Catheters were inserted into a fetal and a maternal carotid artery and jugular vein and into the amniotic cavity in all ewes. All catheters were filled with heparinized saline, and the fetal catheters were exteriorized via an incision in the ewes flank.

Experimental design

After surgery, all ewes were housed in metabolic cages and fed alfalfa chaff (1 kg) once a day between 0900–1100 h, with water available *ad libitum*. Six ewes in the HPD group and six ewes in the intact group were moved immediately after surgery into a long lighting regimen (LL; 16 h of light and 8 h of darkness; lights on at 0700 h) until 143 days gestation or term. Four ewes in the HPD group and six ewes in the intact group were moved immediately after surgery into a short lighting regimen (SL; 8 h of light and 16 h of darkness; lights on at 0700 h) until 143 days gestation or term. All ewes were killed at the end of the experiment using an overdose of sodium pentobarbitone, and the completeness of the disconnection of the hypothalamus and pituitary was confirmed at post mortem in all HPD fetal sheep.

Blood sampling protocol

There was at least a 14-day period between surgery and the first 24-h blood sampling experiment. All 22 ewes were subjected to a 24-h blood-sampling experiment between 135–140 days gestation. During the 24-h experiments, maternal (5 ml) and fetal (2 ml) arterial or venous blood samples were collected every 2 h from 0900 h to 0900 h the following day. During the hours of darkness, blood samples were collected with the aid of a dim red light. All blood samples were collected into heparinized tubes, which were centrifuged for 10 min at 1100 × *g* before separation and storage of the plasma at −20 C for RIA.

Melatonin RIA

Plasma melatonin was measured using sheep melatonin antiserum generously donated by Dr. Andrew Foldes (CSIRO, Division of Animal Production, Blacktown, Australia) and a RIA that has been previously validated and described (3). The sensitivity of the assay was 35.7 pmol/liter, and the inter- and intraassay coefficients of variation were always less than 20%.

PRL RIA

Plasma PRL was measured using rabbit antiovine PRL (antiserum batch AFP 973269, generously donated by the National Hormone and Pituitary Program, NIDDK, Baltimore, MD) and a validated RIA, which has been previously described (1). The sensitivity of the assay was 0.1 ng/tube, and the inter- and intraassay coefficients of variation were less than 20% and less than 10% respectively.

Statistics

All results are expressed as the mean ± SEM. Where the Cochrans and Bartlett-Box tests identified significant heterogeneity of variance, maternal and fetal hormone concentrations were logarithmically transformed before further statistical analysis. The data were analyzed, using the Statistical Package for Social Scientists (SPSS, Chicago, IL) and a VAX mainframe computer, by multifactorial analysis of variance (ANOVA), with treatment (*i.e.* HPD or intact group), environment (*i.e.* long or short photoperiod), time of day, and animal as the specified variables. Where the multifactorial ANOVAs identified significant interactions between major factors, the data were split on the basis of the interaction and reanalyzed. Where a significant effect of time of day ($P < 0.05$) was identified by the ANOVA, Duncan's multiple range test was then used to test for significant differences ($P < 0.05$) in maternal and fetal plasma hormone concentrations during any 24-h period.

The fetal melatonin data were also split on the basis of the lighting regimen and analyzed by ANOVA, with treatment, lighting (*i.e.* light or dark phase), and animal as the specified variables.

Data were also analyzed using a cosinor treatment program (12), which determined whether a sine curve could be significantly fitted ($P < 0.05$) to the 24-h hormonal profiles. The acrophase (*i.e.* the time of day at which the maximum value in the rhythm occurs) of the sine curve was determined for each identified rhythm. A probability of 5% (*i.e.* $P \leq 0.05$) was taken to be significant.

Results

Maternal and fetal melatonin concentrations

There was no significant effect of fetal surgical treatment alone or any interaction between the effects of fetal surgical treatment and time of day on the maternal plasma concentrations of melatonin. There was, however, a significant interaction ($P < 0.001$) between the effects of time of day and the external lighting regimen on plasma melatonin concentrations in the ewes. In the LL regimen (*i.e.* lights off at 2300 h), plasma melatonin concentrations were significantly higher ($P < 0.05$) between 0100–0700 h than between 0900–2300 h in ewes carrying HPD and intact fetal sheep (Fig. 1). In the SL regimen (*i.e.* lights off at 1500 h), plasma melatonin concentrations were significantly higher ($P < 0.05$) between 1900–0100 h than between 0700–1500 h in the ewes in each treatment group (Fig. 1).

There was a significant interaction between the effects of fetal surgical treatment and the lighting regimen *vs.* time of day on the fetal plasma concentrations of melatonin. When each lighting regimen was considered separately, there was still a significant interaction between the effects of surgical treatment and time of day on fetal melatonin concentrations. In the intact fetal sheep under the SL regimen (lights off 1500 h), melatonin concentrations were higher ($P < 0.05$) between 2100–0100 h than between 0900–1700 h (Fig. 1). In the HPD fetal sheep under SL, melatonin concentrations were higher between 1700–0500 h than between 0900–1500 h (Fig. 1). Under LL conditions, there were more marked differences between the 24-h profiles of fetal melatonin in the HPD and intact groups. In the intact fetal sheep under LL (lights off 2300 h), fetal melatonin concentrations were higher ($P < 0.05$) between 0300–0700 h than between either 0900–1100 h or

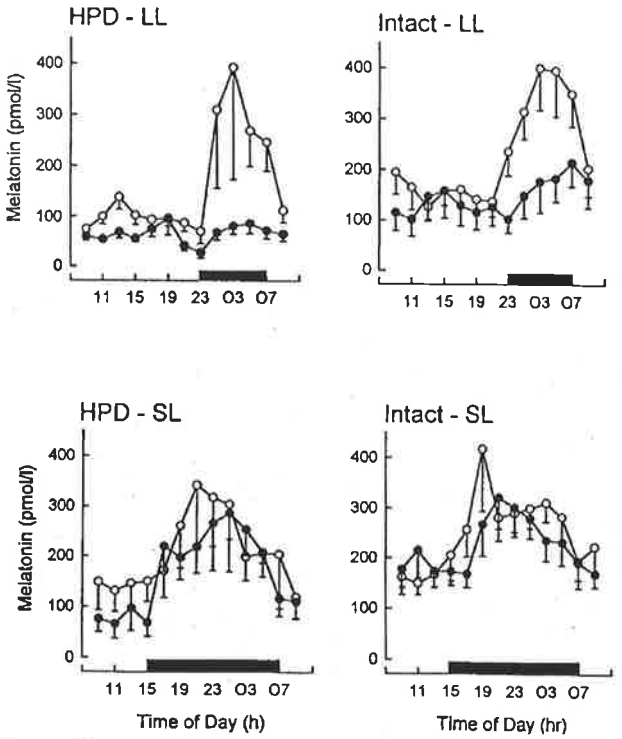

FIG. 1. Plasma melatonin concentrations (mean ± SEM) in pregnant ewes (○) and fetal sheep (●) during 24-h periods under LL (*top graphs*) and SL (*bottom graphs*) conditions. The *horizontal shaded bar* on the time axis represents the period of darkness in each lighting regimen. The data from the HPD and intact treatment groups are presented separately in the *left and right panels*, respectively.

1700–2300 h. In the HPD group under long light, however, fetal melatonin was only higher at two separate time points, *i.e.* at 1900 and 0500 h than at 2100 and 2300 h, *i.e.* there was no consistent nocturnal increase in melatonin in this group (Fig. 1).

In the SL regimen, there was no difference between the treatment groups in the mean plasma concentrations of melatonin in the dark phase (intact, 241.1 ± 47.1 pmol/liter; HPD, 204.3 ± 51.5 pmol/liter). In the LL regimen, however, the mean plasma melatonin concentrations during the dark phase were significantly higher $P < 0.05$) in intact fetal sheep (165.7 ± 46.2 pmol/liter) than in the HPD group (66.9 ± 17.3 pmol/liter).

There was no significant difference between the two treatment groups in the mean plasma melatonin concentrations during the light phase with either the SL regimen (intact, 184.1 ± 37.7 pmol/liter; HPD, 87.3 ± 32.4 pmol/liter; $P = 0.07$) or the LL regimen (intact, 134.3 ± 40.5 pmol/liter; HPD, 64.4 ± 15.8 pmol/liter; $P = 0.1$).

Fetal PRL concentrations

The mean fetal plasma concentrations of PRL were significantly lower ($P < 0.05$) in the HPD fetal sheep than in the intact group, and this effect was present in both lighting regimens. The 24-h mean plasma PRL concentrations were significantly higher ($P < 0.001$) in both treatment groups in the LL regimen (intact, 111.0 ± 22.1 pg/ml; HPD, 37.6 ± 7.3 pg/ml) than in the SL regimen (intact, 37.8 ± 18.4 pg/ml; HPD, 6.7 ± 4.3 pg/ml).

There was also a significant interaction ($P < 0.001$) between the effects of fetal surgical treatment and time of day on fetal PRL (expressed as either absolute concentrations or a percentage of the daily mean value). In the intact group, there was a significant variation ($P < 0.001$) in fetal PRL with respect to time of day, which was similar in each lighting regimen (Fig. 2). In this group, fetal PRL concentrations were significantly higher ($P < 0.05$) at 1300 and 1700 h than between 0300–0700 h (Fig. 2). In contrast, in the HPD fetal sheep, there was no significant effect of time of day on fetal PRL concentrations in either the LL or SL regimen when the PRL data were analyzed using ANOVA (Fig. 2).

Cosinor analysis was also performed on the data from each fetus to determine whether a sine curve significantly fitted the fetal 24-h plasma PRL profiles. The cosinor analysis identified a significant rhythm in 8 of the 12 fetal PRL profiles in the intact group. In this group under LL conditions, 4 of the 6 fetal PRL profiles had a significant rhythm ($P < 0.05$) with acrophase values ranging between 0706–1748 h. Under SL conditions, the cosinor analysis identified a significant rhythm in 4 of the 6 fetal PRL profiles with acrophase values between 1252–0018 h. In contrast, there were only 2 of the 10 24h PRL profiles from the HPD group that were identified as having a significant rhythm. The acrophase values for these rhythms were 0336 and 0356 h, and both of these fetal sheep were maintained under LL conditions.

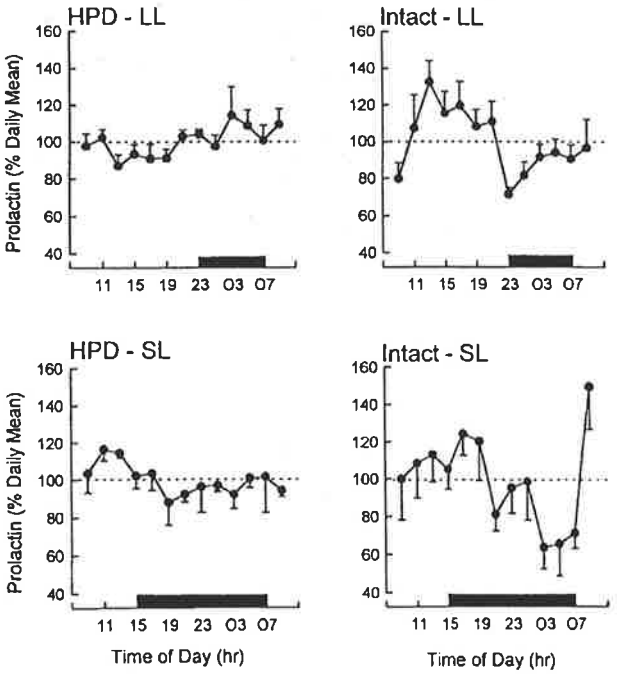

FIG. 2. The percentage of the daily mean plasma PRL concentration (mean ± SEM) in HPD (*left panel*) and intact (*right panel*) fetal sheep during 24-h sampling periods under LL (*top graphs*) and SL (*bottom graphs*) conditions. The *horizontal shaded bar* on the time axis represents the period of darkness in each lighting regimen.

Maternal PRL concentrations

There was no difference between the mean maternal plasma concentrations of PRL in the ewes carrying HPD or intact fetuses. The 24-h mean PRL concentrations were significantly higher in the LL than in the SL regimen in the ewes carrying HPD (LL, 95.0 ± 45.0 pg/ml; SL, 31.8 ± 12.2 pg/ml) and intact fetuses (LL, 121.4 ± 48.2 pg/ml; SL, 32.4 ± 11.0 pg/ml).

In the ewes carrying intact fetuses, there was no significant variation in maternal plasma PRL with respect to time of day in either light regimen when the group hormonal data were analyzed by ANOVA (Fig. 3). When cosinor analysis was performed on the maternal PRL data, however, a significant daily PRL rhythm was identified in 6 of the 13 ewes carrying intact fetal sheep (acrophase values between 1609–0013 h in LL and between 2217–0151 h in SL).

There was a significant variation ($P < 0.001$) in maternal PRL with respect to time of day in the ewes carrying HPD fetuses, and this effect was similar in each lighting regimen (Fig. 3). In these ewes, PRL concentrations (expressed as either absolute values or a percentage of the daily mean value) were significantly higher ($P < 0.05$) between 1300–1900 h compared to those between 0500–0700 h. When cosinor analysis was performed on the PRL data, a significant PRL rhythm was identified in 6 of the 10 ewes carrying HPD fetal sheep (acrophase values between 1131–1743 h in LL and between 1406–1744 h in SL).

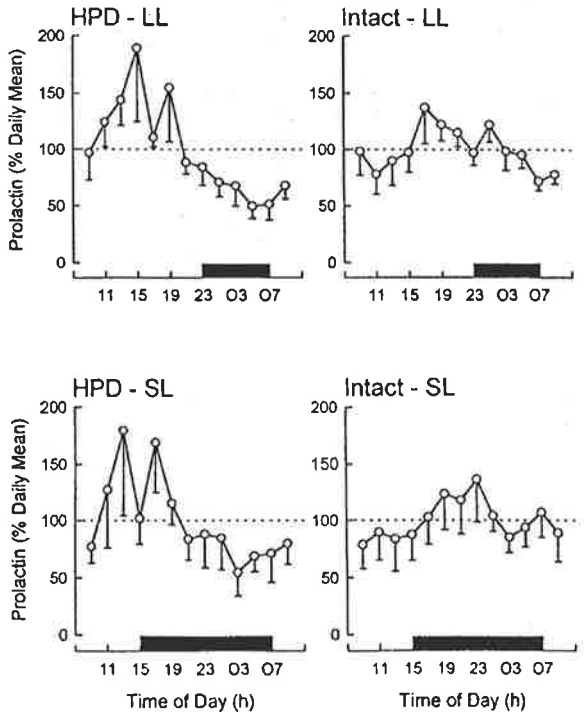

FIG. 3. The percentage of the daily mean plasma PRL concentration (mean ± SEM) in pregnant ewes carrying HPD (*left panel*) and intact (*right panel*) fetuses during 24-h sampling periods under LL (*top graphs*) and SL (*bottom graphs*) conditions. The *horizontal shaded bar* on the time axis represents the period of darkness in each lighting regimen.

Discussion

We have demonstrated that in fetal sheep, the HPD procedure resulted in abolition of the diurnal melatonin rhythm under LL conditions and the loss of the diurnal PRL rhythm under LL and SL conditions. This provides direct evidence for a role for the fetal hypothalamus in the generation of diurnal rhythms in melatonin and PRL before birth in the sheep.

We and others have previously shown that there is a diurnal rhythm in plasma melatonin concentrations in pregnant ewes and their fetal lambs during late gestation (2, 3). Melatonin rapidly crosses the sheep placenta, and pinealectomy of the pregnant ewe abolishes the daily rhythm in both maternal and fetal melatonin concentrations (3–5). Furthermore, a clear nocturnal increase in melatonin concentrations does not emerge until after the first week of life in the newborn lamb (13). In light of these findings, it has been concluded that the maternal pineal is the major source of the diurnal rhythm in maternal and fetal plasma melatonin concentrations and that the emergence of an endogenous melatonin rhythm is a postnatal event in the sheep. We did note, however, in a previous study that maternal pinealectomy did not remove all of the immunoreactive melatonin from the maternal and fetal plasma in late gestation, and that in the fetuses of pinealectomized ewes, there was a steady increase in melatonin concentrations during the last 2–3 weeks of gestation (3). In the present study, there was a significant diurnal rhythm in circulating melatonin concentrations in pregnant ewes and their HPD or intact fetuses during the SL conditions. Although it was interesting that in the SL regimen, the mean fetal plasma concentrations of melatonin appeared lower during the light phase in the HPD than in the intact group, this difference did not reach significance. In the LL conditions, although the time profiles of maternal melatonin were similar in the ewes carrying HPD or intact fetuses, there were clear differences between the two treatment groups in the fetal melatonin profiles. In the intact fetal sheep under LL conditions, melatonin concentrations were higher at the end of the dark phase than at the end of the light phase. In the HPD group under LL, however, there was no consistent increase in melatonin during the dark phase compared with that during the light phase. Furthermore, under LL conditions, the mean plasma melatonin concentrations during the entire dark phase were significantly lower in the HPD than in the intact fetal sheep. These data indicate that the fetal HPD procedure results in the loss of a source of circulating fetal melatonin during the dark phase in LL.

One possibility is that the fetal HPD procedure resulted in changes in the characteristics of the transplacental transfer of melatonin from the ewe to the fetus. In the SL conditions, however, we found that there was no significant difference between the HPD and intact fetuses in the mean melatonin concentrations during the dark phase; therefore, it seems unlikely that the HPD procedure has a major effect on the placental transfer of melatonin.

During the HPD procedure, the optic chiasm is separated, and the fetal suprachiasmatic nuclei (SCN) are destroyed. In the adult, the circadian rhythm in the synthesis and secretion of melatonin by the pineal gland is controlled by the circa-

dian pacemaker activity of the hypothalamic SCN (14). In the present study, loss of the nocturnal increase in fetal melatonin during the LL conditions after HPD may have been a consequence of ablation of the fetal circadian pacemakers. We have previously demonstrated in an *in vitro* study that the fetal lamb pineal gland has the capacity to synthesize and secrete melatonin in response to β-adrenergic stimulation during the last 10 days of gestation (15). If there is a fetal source of melatonin, then it is difficult to explain the lack of effect of fetal HPD on fetal melatonin concentrations under the SL conditions. Perhaps the sustained nocturnal increase in maternal melatonin during the 16-h dark phase effectively masks or suppresses any fetal source of melatonin. As discussed above, we have previously found that maternal pinealectomy abolishes fetal melatonin rhythms during 12-h photoperiodic conditions throughout late gestation (3), and it is, therefore, necessary to postulate that any fetal hypothalamic control of melatonin secretion is, in turn, dependent on a maternal melatonin signal. There is evidence from other species that there is a circadian variation in the metabolic activity of neurons within the fetal SCN (16), and melatonin-binding sites have been identified in the SCN of adult and fetal hypothalami (17). There is also evidence in the sheep that the maternal melatonin rhythm acts to entrain the daily rhythm of fetal breathing movements, although the site of action of melatonin in the fetal brain is unknown (10, 18). Further experiments are required to define the contribution of the fetal pineal gland to the daily rhythm in fetal melatonin concentrations during LL and SL regimens.

In the present study, the circulating PRL concentrations were significantly lower in the fetal sheep after HPD. This is in contrast to the findings of previous studies that pituitary stalk section consistently increases PRL secretion in the adult rat (19), monkey (20, 21), and man (22). Our results, however, agree with those of Thomas *et al.* (23), who demonstrated that there was a transient elevation in circulating PRL concentrations in the first 7 days after HPD in the adult ewe and that PRL levels gradually declined thereafter. These researchers suggested that the decrease in PRL after HPD was unlikely to be due to pituitary gland infarction or depletion of PRL stores because the PRL responses to TRH 190 days after HPD were similar to those in control ewes. These researchers concluded that pituitary PRL secretion in the sheep may be under the control of both hypothalamic releasing and inhibitory factors (23). We have previously shown that disconnection of the fetal pituitary from the hypothalamus at 108–112 days gestation did not alter the morphology, distribution, or proportion of lactotrophs in the anterior pituitary at 135–138 days gestation (11). It, therefore, appears likely that, as in the adult sheep, fetal PRL secretion is under the predominant control of hypothalamic releasing factors. There exists the additional possibility, however, in the fetus that circulating factors derived from the placenta, such as estrogens, are no longer able to stimulate PRL secretion via the hypothalamus after HPD.

It has been previously demonstrated that there is an effect of season or of a change in the time of dark onset on the timing of the peak in the daily fetal PRL rhythm (6, 10). In the present study there were no consistent differences between the acrophases of the PRL rhythm under LL or SL conditions in the intact group of fetal sheep, perhaps because of the relatively short period of exposure (3–4 weeks) of the ewes to the light conditions used in this study. It was interesting, however, that there was no significant daily rhythm in fetal PRL concentrations in the HPD group of fetal sheep. The lack of PRL rhythmicity in this group was probably not due to a lower level of PRL secretion from the surgically isolated pituitaries because the PRL concentrations in the HPD fetal sheep under LL conditions were similar to those measured in the intact fetal sheep under SL conditions.

There are very few studies that have investigated the source and control of the daily PRL rhythm in either the adult or fetal sheep. Although the results from the present study indicate that the presence of a daily PRL rhythm is dependent on a functional hypothalamus, Lau and Jackson (24) previously reported that frontal hypothalamic deafferentation did not alter the photoperiod-induced changes in the 24-h profiles of PRL in the adult ewe. These researchers concluded that the direct neural pathways between the suprachiasmatic-preoptic region and the mediobasal hypothalamus must play a relatively minor role in the generation of the daily PRL rhythm. In previous studies we have shown that the daily PRL rhythm persists in the maternal and fetal circulation after maternal pinealectomy (8), and that the timing of the daily acrophase in the fetal and maternal PRL rhythm was related to the time of dark onset and not to changes in the phase of the daily melatonin rhythm (10). The hypothalamic site of the generation of the daily PRL rhythm in the fetus and ewe and the nature of the inputs to this site, therefore, remain to be established.

In this study we have also confirmed our previous findings in this group of animals that there is a fetal PRL response to photoperiods of different lengths and that this response is maintained after surgical disconnection of the hypothalamus and pituitary (25). It has been demonstrated that when melatonin is infused into pregnant ewes during summer pregnancies to simulate the winter duration of the nocturnal melatonin increase, there is an associated reduction in the fetal and maternal plasma concentrations of PRL (9). It appears, therefore, that in the fetal sheep, as in the adult, the PRL response to photoperiod length is dependent on the duration of the nocturnal melatonin signal. We have postulated that a possible extrahypothalamic site of action of maternal melatonin is the pars tuberalis of the fetal pituitary. The pars tuberalis contains exceptionally high concentrations of iodomelatonin-binding sites (26), and the secretory cell types of the pars tuberalis of the adult sheep pituitary seem to be melatonin responsive (27). Our present study, therefore, demonstrates that photoperiod length, transduced as the phase and duration of the nocturnal melatonin signal and the time of day, control fetal PRL secretion at separate neuroendocrine sites.

There was a significant daily rhythm in PRL concentrations in most, but not all, of the ewes in this study. The acrophase values of the maternal PRL rhythms were consistently between 1100–1800 h in the ewes carrying HPD fetuses and between 1600–0200 h in the ewes carrying intact fetal sheep. There are a number of factors other than time of day, such as circulating estrogen concentrations, that may influence maternal PRL secretion during late gestation, and the

effect of fetal HPD on placental estrogen output remains to be determined.

In summary, we have provided direct evidence for a fetal hypothalamic role in the control of fetal melatonin secretion during long photoperiods. We have also demonstrated that an intact and functional fetal hypothalamus is essential for generation of the daily PRL rhythm under both lighting regimens. These data in combination with our previous findings suggest that there are different neuroendocrine sites of control of the seasonal and diurnal variations in PRL in the sheep before birth.

Acknowledgments

We are also grateful to Simon Fielke and Anne Jurisevic for their expert assistance with the surgical and RIA procedures associated with these studies.

References

1. **McMillen IC, Thorburn GD, Walker DW** 1987 Diurnal variations in plasma concentrations of cortisol, prolactin, growth hormone and glucose in the fetal sheep and pregnant ewe during late gestation. J Endocrinol 114:65–72
2. **Yellon SM, Longo LD** 1987 Melatonin rhythms in fetal and maternal circulation during regnancy in sheep. Am J Physiol 252:E799–E802
3. **Zemdegs IZ, McMillen IC, Walker DW, Thorburn GD, Nowak R** 1988 Diurnal rhythms in plasma melatonin concentrations in the fetal sheep and pregnant ewe during late gestation. Endocrinology 123:284–289
4. **Yellon SM, Longo LD** 1988 Effect of maternal pinealectomy and reverse photoperiod on the circadian melatonin rhythm in the sheep and fetus during the last trimester of pregnancy. Biol Reprod 39: 1093–1099
5. **McMillen IC, Nowak R** 1989 Maternal pinealectomy abolishes the diurnal rhythm in plasma melatonin concentrations in the fetal sheep and pregnant ewe during late gestation. J Endocrinol 120: 459–464
6. **Bassett JM, Bomford J, Mott JC** 1988 Photoperiod: an important regulator of plasma prolactin concentrations in fetal lambs during later gestation. Q J Exp Physiol 73:241–244
7. **Seron-Ferre M, Vergara M, Parraguez VH, Riquelme R, Llanos AJ** 1989 The circadian variation of prolactin in fetal sheep is affected by the seasons. Endocrinology 125:1613–1616
8. **McMillen IC, Walker DW, Young IR, Nowak R** 1991 A daily prolactin rhythm persists in the ewe, foetus and newborn lamb after maternal pinealectomy in late gestation. J Neuroendocrinol 3:369–374
9. **Bassett JM, Curtis N, Hanson C, Weeding CM** 1989 Effects of altered photoperiod on maternal melatonin administration on plasma prolactin concentrations in fetal lambs. J Endocrinol 122: 633–643
10. **Houghton DC, Walker DW, Young IR, McMillen IC** 1993 Melatonin and the light/dark cycle separately influence daily behavioural and hormonal rhythms in the pregnant ewe and sheep fetus. Endocrinology 133:90–98
11. **Antolovich GC, Clarke IJ, McMillen IC, Perry RA, Robinson PM, Silver M, Young IR** 1990 Hypothalamo-pituitary disconnection in the fetal sheep. Neuroendocrinology 51:1–10
12. **Vokac M** 1984 A comprehensive system of cosinor treatment programs written for the Apple II microcomputer. Chronobiol Int 1:87–92
13. **Nowak R, Young IR, McMillen IC** 1990 The emergence of diurnal rhythms in plasma melatonin concentrations in lambs delivered to intact or pinealectomised ewes. J Endocrinol 125:97–102
14. **Moore RY** 1983 Organization and function of a central nervous system circadian oscillator: the suprachiasmatic hypothalamic nucleus. Fed Proc 42:2783–2789
15. **McMillen IC, Parkington HC, McCance IC** 1989 The effect of isoprenaline on the melatonin output from the fetal and newborn lamb pineal gland *in vitro*. Acta Endocrinol (Copenh) 121:773–776
16. **Reppert SM, Schwartz WJ** 1984 The suprachiasmatic nuclei of the fetal rat. Characterization of a functional circadian clock using ^{14}C-labelled deoxyglucose. J Neurosci 6:2724–2729
17. **Reppert SM, Weaver DR, Rivkees SA, Stopa EG** 1988 Putative melatonin receptors in a human biological clock. Science 242:78–81
18. **McMillen IC, Nowak R, Walker DW, Young IR** 1990 Maternal pinealectomy alters the daily pattern of fetal breathing in sheep. Am J Physiol 258:R284–R287
19. **Kanematsu S, Kishi K, Mihami S** 1979 Rise of prolactin and changes in fine structure of the anterior hypophysis after pituitary stalk section in rats. Endocrinology 105:427–430
20. **Butler WR, Krey LC, Lu K-H, Peckham WD, Knobil E** 1975 Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. IV. Prolactin secretion. Endocrinology 90:1099–1105
21. **Diefenbach WP, Carmel PW, Frantz AG, Ferin M** 1976 Suppression of prolactin secretion by L-Dopa in the stalk sectioned rhesus monkey. J Clin Endocrinol Metab 43:638–642
22. **Turkington RW, Underwood LE, Van Wyk JJ** 1971 Elevated serum prolactin levels after pituitary-stalk section in man. N Engl J Med 285:707–710
23. **Thomas GB, Cummins JT, Cavanagh L, Clarke IJ** 1986 Transient increase in prolactin secretion following hypothalamo-pituitary disconnection in ewes during anoestrus and the breeding season. J Endocrinol 111:425–431
24. **Lau K-YP, Jackson GL** 1984 Effect of frontal hypothalamic deafferentation on photoperiod induced changes in the secretion of prolactin in the ewe. Endocrinology 115:1663–1671
25. **Houghton DC, Young IR, McMillen IC,** Response of prolactin to different photoperiods after surgical disconnection of the hypothalamus and pituitary in the sheep fetus. Program of the 37th Annual Scientific Meeting of the Endocrine Society of Australia, 1994 (Abstract 65)
26. **Krause DN, Dubocovich ML** 1990 Regulatory sites in the melatonin system of mammals. Trends Neurosci. 13:464–470
27. **Morgan PJ, King TP, Lawson W, Slater D, Davidson G** 1991 Ultrastructure of melatonin responsive cells in the ovine pars tuberalis. Cell Tissue Res 263:529–534

5. DISCUSSION

5.1 Photoperiod effects on PRL

The first original finding of this thesis was that a fetal sheep plasma PRL response to different photoperiods occurred irrespective of whether the fetal hypothalamo-pituitary axis was intact or surgically disconnected. A similar finding had been only just been found in adult rams (Lincoln and Clarke, 1994) but the animals were directly exposed to the ambient photoperiod. The research for this thesis was focussed on the fetal situation in utero and without direct influences of the light: dark cycle allowed postulation about possible mechanisms of varying PRL concentrations under different photoperiods.

It is well established that the duration of elevated concentrations of nocturnal MEL mediates photoperiodic effects on plasma PRL concentrations in the ewe and sheep fetus (Brown and Forbes, 1980; McMillen et al, 1991; Maxwell et al, 1989). That the fetal sheep in this thesis could produce different plasma PRL concentrations depending on the ambient photoperiod strongly implicated the nocturnal MEL rhythm, derived from the maternal pineal gland (McMillen & Nowak, 1989; Yellon & Longo, 1988), as mediator of photoperiodic information. An extra-hypothalamic site for MEL to exert its photoperiodic effects is supported by the PRL response of hypothalamo-pituitary disconnected (HPD) fetal sheep under different lighting regimes. The functionally isolated pituitary in the sheep fetus can respond to the external photoperiod (and thus the maternally derived nocturnal MEL rhythm) and adjust PRL production and secretion.

There is growing indirect evidence that MEL may exert its effect on seasonal expression of PRL via the pars tuberalis. The pars tuberalis is a distinct 'tube' of cells that surrounds the pituitary stalk and adjoins the pars distalis. Very

high concentrations of iodomelatonin binding have been demonstrated in the sheep fetus from as early as 30 days gestation. This thesis suggests that the pars tuberalis may to be functional before birth and is important in mediating PRL secretion in response to ambient photoperiod. This may allow the fetus to prepare for the upcoming season after birth by adjusting short and long-term physiological responses.

The first study of this thesis also demonstrated that fetal sheep that have undergone HPD have lower plasma PRL concentrations than sham operated animals. This suggests that in fetal sheep PRL release may be under tonic stimulation by a hypothalamic PRL releasing factor rather than the widely accepted adult situation where PRL secretion is thought to be regulated by inhibitory dopamine. It is also possible that oestrogen or placental factors that are released during gestation may stimulate PRL secretion but require an intact hypothalamo-pituitary axis. Alternatively HPD may alter the oestrogenic profile of the fetus and influence PRL secretion through gestation.

Interestingly the association of increasing PRL levels with increasing gestational age was not seen in HPD fetal sheep held in a short photoperiod. This indicated that an effect of increasing gestational age that increases PRL production and secretion depends on an intact hypothalamo-pituitary axis. Effects of increasing exposure to long photoperiod may mask the lack of a gestational effect under long photoperiod in HPD fetuses. Increasing levels of circulating oestrogen during late gestation have been suggested to stimulate PRL secretion (Gluckman et al, 1981) and this thesis suggests that an oestrogenic effect may act via the hypothalamus rather than on the pituitary directly to influence the gestational profile of PRL.

Finally the first study in this thesis found that the proportional PRL response of fetal sheep to a TRH bolus was greater in short photoperiod than long photoperiod. This indicated that the potential pool of PRL available for secretion is greater when secretion is low. However, the absolute PRL response was greater in long photoperiod than short photoperiod suggesting that greater production and secretion could occur in both HPD and intact fetal sheep. The isolated pituitary could therefore alter its reserve of PRL without hypothalamic influence

The problems encountered with the first study of this thesis were mainly technical limitations imposed by working with fetal sheep. HPD surgery could not be performed before 105 days gestation, as the fetus would be too small. Performing surgery at this stage in gestation limited exposure to a change in photoperiod and provided a limited window of opportunity for blood sampling after 7 days recovery from surgery and term (~145 days gestation). There were also limited blood sample sizes and quantities in fetal sheep available for assay and analysis of fetal well being.

Future research could focus on the role of the pars tuberalis on PRL production and secretion. Potential prolactin releasing factors from the pars tuberalis and their role in photoperiod regulation of PRL release could be investigated. The role of MEL by infusion on plasma PRL concentrations in HPD fetal sheep in long photoperiod could provide further support for a role of MEL at the pars tuberalis. Finally, investigating any possible photoperiodic changes in PRL mRNA in the isolated pituitary could provide evidence for a role of photoperiod in PRL gene expression.

5.2 Effect of photoperiodic history on PRL

In the second study of this thesis the principle original finding was that photoperiodic history determines the plasma PRL concentrations in fetal sheep after HPD but not in intact fetal sheep. Therefore it is necessary to remove the hypothalamic influences on the pituitary to reveal effects of photoperiodic history on the plasma PRL response to an intermediate photoperiod. The first study revealed that the isolated pituitary could respond to the prevailing photoperiod, whilst this second study demonstrates that the PRL response is dependent on prior photoperiodic history in the HPD fetal sheep. Thus the interpretation of a change in photoperiod from short or long photoperiod to intermediate photoperiod occurs at the pituitary. It is likely that this effect is mediated by MEL acting at the pars tuberalis that then effects PRL secretion from lactotrophs in the pars distalis. This is the first finding of an *in utero* effect of photoperiodic history and provides evidence that nocturnal MEL exposure can determine the generation and expression of photoperiodic history without the direct effects of the light: dark cycle.

Interestingly, no effect of photoperiodic history on the gestational age increase in PRL was seen in the intact fetal sheep. The gestational age PRL increase in intact animals potentially masks any small difference of photoperiodic history seen in HPD animals. The influence of the hypothalamus and a potential PRL releasing factor have a significant impact on PRL in sham animals as gestation progresses. Oestrogen or a factor from the placenta could act at the hypothalamus to stimulate PRL secretion as gestation progresses, as there is no gestational age PRL increase in the functionally isolated pituitary in animals previously exposed to long photoperiod. It is impossible to differentiate between

the effects of a gestational age increase and an effect of photoperiodic history in HPD animals previously exposed to short photoperiod. The lack of a gestational age PRL rise in the HPD animals from a long photoperiod suggests this is a photoperiodic history effect.

Similar to the first study, the second study also found that HPD animals had lower plasma PRL concentrations. This supported the contention that a PRL releasing factor(s) from the hypothalamus can influence PRL levels in intact but not HPD animals. Oestrogen or a placental factor may be stimulating PRL secretion via the hypothalamus in intact animals.

The second study also demonstrated that the proportional PRL increase after a TRH bolus in the intermediate photoperiod is the same after pre-exposure to short or long photoperiod in both intact and HPD fetal sheep. This indicates that the potential pool of PRL for secretion is heavily influenced by the ambient photoperiod and not the photoperiodic history. In conjunction with the findings of the first study this suggests regulation of a pool of PRL for secretion occurs at the pituitary. The isolated pituitary is thus able to store a relative pool of PRL that reflects the ambient photoperiod and at the same time secrete different amounts of PRL depending upon the photoperiodic history. This suggests potentially different regulating mechanisms for manufacture, storage and secretion of PRL in response to different photoperiods.

Similar to the first study, the second was limited by technical aspects of working with fetal sheep. This included gestational limitations on time of surgery, the duration of the photoperiod exposure and limited size and quantity of blood samples for analysis.

Future directions for research could include an investigation of the HPD adult sheep to display varying PRL responses to an intermediate photoperiod depending on prior photoperiodic history. An investigation of any differences in pars tuberalis cells depending on photoperiodic history may provide information about a mechanism by which photoperiodic history can be expressed in the isolated pituitary. Finally, examination of PRL mRNA may reveal information about PRL manufacture and storage in intermediate photoperiods after pre exposure to long or short photoperiods.

5.3 Diurnal rhythms in PRL and MEL

The studies presented in Chapter 4 demonstrated that HPD abolished the diurnal plasma PRL rhythm in fetal sheep held in short and long photoperiods. This was the first demonstration that a functionally isolated pituitary in the fetal sheep cannot generate a diurnal rhythm in plasma PRL concentrations. Connections between the hypothalamus (and potentially the SCN) and the pituitary are therefore necessary for generation of a diurnal PRL rhythm in the fetus. This is similar to the neuroendocrine control of the diurnal PRL rhythm in the adult ram (Lincoln and Clarke, 1994). As the SCN (the circadian pacemaker) is destroyed in HPD animals' one possibility may be that it is the SCN which generates and/or regulates the diurnal rhythm of PRL secretion from the lactotrophs in the anterior pituitary.

Surprisingly, HPD also abolished the diurnal fetal MEL rhythm in long photoperiods. Conventional wisdom has it that the nocturnal rise in fetal MEL is derived from the maternal pineal and that fetal pineal does not contribute significantly to the MEL rhythm until around three weeks after birth. The lack of a

nocturnal MEL rise in HPD fetal sheep held in long photoperiods suggests the fetal hypothalamus and/or SCN may be involved in the generation of fetal MEL and a diurnal MEL rhythm before birth. There was no evidence for this under short photoperiods in HPD animals but this effect may only occur when exposure to the dark period (and maternally derived MEL) is relatively short.

Plasma PRL levels were again lower in the HPD fetal sheep confirming the earlier findings in Chapters 2 and 3 of this thesis, again implicating a possible PRL releasing factor in the regulation of fetal PRL. Fetal sheep held in long photoperiods had higher levels of PRL than those in short photoperiods in intact and HPD animals. This confirms the original finding that photoperiodic information may be transduced via the duration of nocturnal MEL to mediate PRL secretion and production directly at the pituitary. Thus photoperiodic information acts at an intra pituitary site, most likely the pars tuberalis, whilst the diurnal expression of PRL is derived from a site within the hypothalamus, most likely the SCN.

Similar limitations as the previous two chapters were encountered with limited time for photoperiod exposure and entrainment, limited size of the fetal sheep reducing the amount of blood and plasma that can be sampled and the constraints of gestation.

Future areas that could be investigated include determination of the hypothalamic site of the diurnal PRL rhythm and exclusion of a direct role of the light: dark cycle through the abdominal wall of the mother on the diurnal PRL rhythm in the fetal sheep by blindfolding.

Subsequent research in this field has focussed on the role of the pars tuberalis in the regulation of PRL and also on the expression of the PRL receptor and its variants in a range of tissues and on intracellular signalling through

activation of this receptor. The major findings in these areas are summarised below.

5.4 Pars tuberalis

At the time of my experimental studies that are presented in this thesis, the evidence for a role of the pars tuberalis in the regulation of synthesis and secretion of PRL from the pars distalis was circumstantial. It was known that the pars tuberalis was structurally distinct from the pars distalis (Morgan & Williams, 1996) and that there were iodomelatonin binding sites on the pars tuberalis (Krause & Dubocovich, 1990). The cells of the pars tuberalis had been demonstrated to be responsive to MEL (Morgan et al, 1991), whilst photoperiod could change the exocytic activity (Mercks et al, 1993) and cytology (Wittkowski et al, 1984) of cells of the pars tuberalis in Djungarian hamsters.

Since publication of my thesis papers, subsequent research has focussed on the identification of a factor from the pars tuberalis that influences the pars distalis and the demonstration of photoperiodic regulation of PRL synthesis and secretion via the pars tuberalis. Further support for MEL as the humoral representation of photoperiod was provided by the demonstration that MEL injections into Djungarian hamsters under long photoperiod caused similar cytological changes to pars tuberalis cells as those animals kept under short photoperiod (Bockers et al, 1995). Thus photoperiod and MEL administration can change the morphology, exocytic activity and immunoreactivity of pars tuberalis cells in the Djungarian hamster (Wittkowski, 1999). Exposure to short photoperiods leads to decreased secretory activity and long photoperiod increases secretory activity of pars tuberalis cells.

Pars tuberalis cells have been found to secrete a peptide(s) that stimulates PRL secretion from pars distalis cells (Hazlerigg et al, 1996; Morgan et al, 1996). Termed 'tuberalins', the molecular identity of these factors was investigated by raising antibodies against proteins secreted into the culture medium of pars tuberalis explants (Guerra and Rodriguez, 2001). They found a 72kDa protein (tuberalin I) and a 21kDa protein (tuberalin II) in variably sized secretory granules of bovine, rat and hamster pars tuberalis cells that were not present in pars distalis cells. Further evaluation of the tuberalin II protein suggested it would correspond with the ß chain of a specific glycoprotein of pars tuberalis specific cells (Guerra and Rodriguez, 2001). It is possible that the tuberalin proteins play a role in the regulation of PRL secretion.

PRL synthesis has been demonstrated to depend on photoperiodic modulation of a pars tuberalis derived factor, whilst short photoperiods inhibit PRL gene expression and shift the distribution of PRL mRNA expression per lactotroph in hamsters (Stirland et al, 2001). It was also demonstrated that the pars tuberalis releases a factor that can stimulate gene transcription in lactotrophs with pars tuberalis conditioned medium increasing PRL mRNA and PRL promoter activity in pars distalis cells (Stirland et al, 2001). MEL inhibits this effect of pars tuberalis conditioned medium on PRL mRNA expression suggesting photoperiod via MEL may regulate PRL mRNA expression in the lactotroph. Interestingly, pars tuberalis fragments from hamsters which had been exposed to long photoperiods had greater PRL promoter activity than pars tuberalis fragments from hamsters exposed to short photoperiods (Stirland et al, 2001). The authors concluded that photoperiod, via MEL, regulates the PRL axis by acting at the pars tuberalis to modulate secretion of a PRL secretagogue.

5.5 PRL receptor

Since publication of my research there has been much work investigating the localisation and expression of the PRL-receptor (PRL-R), its variant isoforms, distribution in mammalian tissues and on ligand activation of this receptor and consequent signal transduction. I have included a brief summary of this contemporary information about the PRL-R and the mechanism of its activation below.

The PRL-R belongs to class 1 of the cytokine superfamily and is a single pass membrane bound protein that contains an extracellular, transmembrane and intracellular domain (Kelly et al, 1991; Bole-Feysot et al, 1998). PRL-R isoforms vary in the length and composition of their cytoplasmic domains with long and short PRL-R's described in the sheep (Bignon et al, 1997). Given that PRL has over 300 separate biological activities it is not surprising that there is widespread distribution of the PRL-R. Predictably the PRL-R is found in the mammary gland and ovary. Interestingly, PRL-R mRNA has also been found in rat central nervous system including choroid plexus, amygdala, thalamus, hypothalamus, stria terminalis, cerebral cortex, central grey of the midbrain, area postrema and the olfactory bulb. Peripheral organs and tissue also exhibit the PRL-R including the pituitary gland, adrenal gland, brown adipose tissue, kidney, spleen, pancreas, liver, thymus, heart, lung, uterus, skeletal muscle and skin (Nagano & Kelly, 1994; Freeman et al, 2000)

Activation of the PRL-R occurs when binding site 'one' of the PRL ligand interacts with the extracellular domain of the PRL-R molecule. The formation of an initial ligand-receptor complex induces the interaction of binding site 'two' on the same PRL molecule with a second PRL-R (Fig 2). The second PRL-R must also

contain a conserved region termed 'Box 1' and have identical homology of the intracellular domain as the first PRL-R to activate a tyrosine kinase termed Janus Kinase 2 (JAK2). JAK2 is constitutively associated with the membrane proximal region of the intracellular domain of the PRL-R. JAK2 is not induced by ligand binding as demonstrated for the Growth Hormone Receptor (GHR). Activation of JAK2 occurs by transphosphorylation of tyrosines during ligand induced dimerization when the two JAK2 kinases are close to each other. Tyrosine residues of the PRL-R itself are also phosphorylated and JAK2 kinases are involved in this process (Freeman et al, 2000; Bole-Feysot et al, 1998).

Signal transduction is initiated when a tyrosine residue of the PRL-R interacts with a Signal Transducer and Activator of Transcription (STAT) protein. Eight STAT proteins have been identified of which STAT1, STAT3, STAT5a and STAT5b are transducer molecules of the PRL-R. STAT5 is the most important transducer of the long and intermediate isoforms of the PRL-R. STAT, whilst interacting with the tyrosine residue of the PRL-R, is phosphorylated by the receptors JAK2. The STAT then dissociates from the receptor, dimerizes with another phosphorylated STAT molecule and translocates to the nucleus, where it regulates transcription by binding to specific DNA sequences in the promoters of target genes (Freeman et al, 2000).

PRL induced signal transduction is regulated to avoid excessive stimulation by Cytokine-Inducible SH2-containing proteins (CIS) and Suppressors Of Cytokine Signalling (SOCS) proteins (Krebs & Hilton, 2001). PRL induces expression of SOCS-1 and SOCS-3, which inhibit the activity of JAK2 and activation of STAT proteins. SOCS-1 binds to JAK and inhibits catalytic activity, SOCS-3 binds to JAK-proximal sites on the PRL-R and inhibits JAK activity, whilst

CIS proteins compete with and block the binding of STAT to docking sites on the PRL-R (Krebs & Hilton, 2001). The SOCS proteins therefore act as part of a negative feedback loop that regulates PRL induced transcription.

The studies for this thesis have demonstrated that the functionally isolated fetal sheep pituitary can alter fetal plasma concentrations of PRL to changes in ambient photoperiod, that functional isolation of the fetal pituitary gland is necessary for expression of a photoperiodic history in intermediate photoperiods and that maintenance of a diurnal PRL rhythm in the fetal sheep is dependent on an intact hypothalamo-pituitary axis. This photoperiodic regulation of fetal PRL may be transduced by the PRL-R pathway to play a role in the growth and development of the fetus through its uniquely varied actions in a wide variety of tissues.

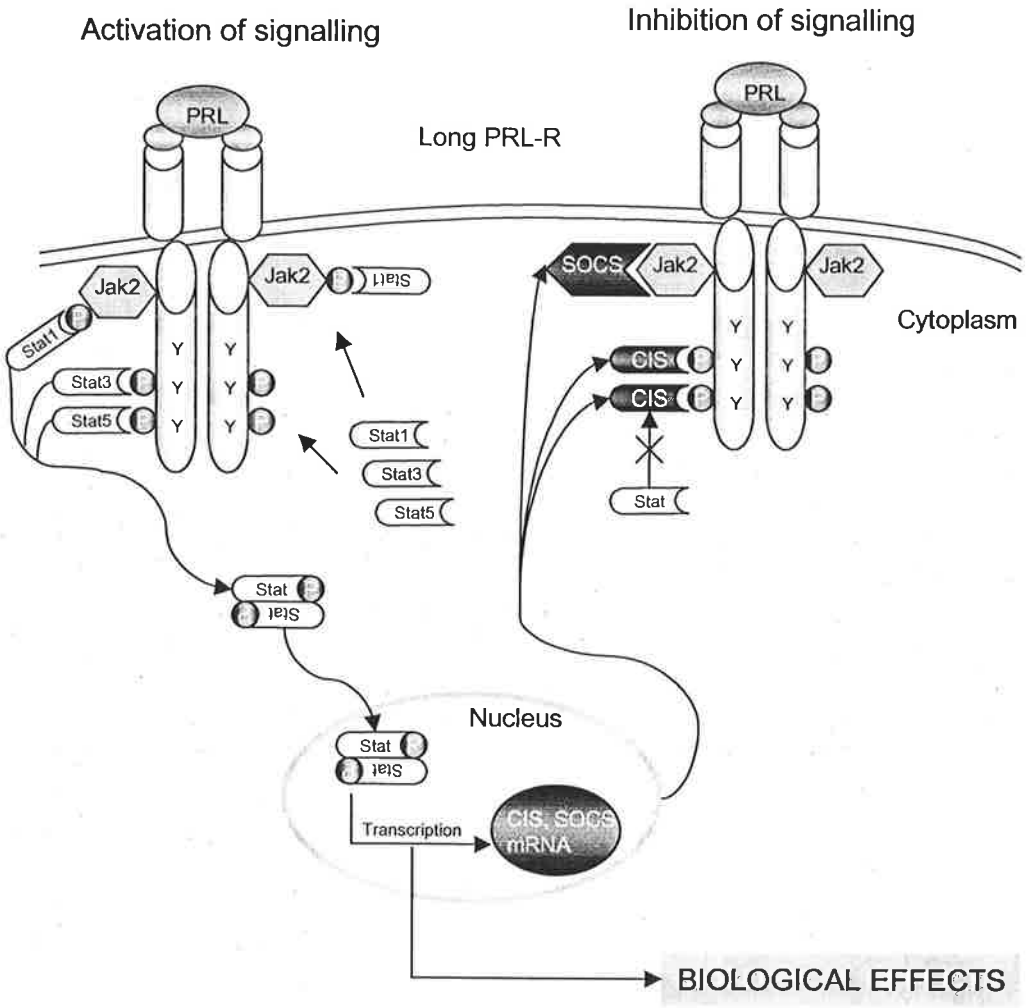

Figure 2. Schematic representation of the PRL-R signal transduction pathways initiated by binding of PRL to the PRL-R. Jak2 – Janus Kinase 2, STAT – Signal Transducer and Activator of Transcription, CIS – Cytokine Inducible SH2 Containing protein, SOCS – Suppressors of Cytokine Signalling, P – Phosphotyrosine. Diagram based on figures in Bole-Feysot et al (1998) and Krebs & Hilton (2001).

5.6 SUMMARY

This thesis demonstrates for the first time that changes in the ambient photoperiod result in changes in fetal plasma concentrations of PRL through mechanisms which reside within the functionally isolated fetal sheep pituitary. Furthermore, the secretion of fetal PRL in an intermediate photoperiod is dependent on prior photoperiodic history and this effect is only revealed after hypothalamo-pituitary disconnection in the fetal sheep. Finally, the expression of a diurnal plasma PRL rhythm is dependent upon an intact fetal hypothalamo-pituitary axis.

BIBLIOGRAPHY

Adam CL, Kyle CE, Young P (1992) Influence of prenatal photoperiod on postnatal prolactin secretion in red deer *(Cervus elaphus) Journal of reproduction and fertility* 95 959-964.

Adam CL, Kyle CE, Young P (1994) Influence of prenatal photoperiod on postnatal reproductive development in male red deer *(Cervus elaphus) Journal of reproduction and fertility* 100 607-611.

Alexander DP, Britton HG (1973) Impermeability of the sheep placenta towards endogenous prolactin *Research in veterinary science* 14 271-272.

Baker JR (1938) The evolution of breeding seasons. In *Evolution*, J DeBeer, ed, pp161-177, Clarendon, Oxford.

Barrell GK, Lapwood KR (1978) Effects of pinealectomy of rams on secretory profile of luteinizing hormone, testosterone, prolactin and cortisol *Neuroendocrinology* 27 216-227.

Barrell GK, Lapwood KR (1979) Effects of pinealectomy on the secretion of luteinizing hormone, testosterone and prolactin in rams exposed to various lighting regimes *Journal of endocrinology* 80 397-405.

Bartness TJ, Wade GN (1984) Photoperiodic control of bodyweight and energy metabolism in Syrian hamsters *(Mesocricetus auratus)*: role of pineal gland, melatonin, gonads, and diet *Endocrinology* 114 492-498.

Bassett JM, Bomford J, Mott JC (1988) Photoperiod: an important regulator of plasma prolactin concentration in fetal lambs during late gestation *Quarterly journal of experimental physiology* 73 241-244.

Basett JM, Curtis N, Hanson C, Weeding CM (1989) Effects of altered photoperiod or maternal melatonin administration on plasma prolactin concentrations in fetal lambs *Journal of endocrinology* 122 633-643.

Beck W, Wuttke W (1979) Annual rhythms of luteinizing hormone, follicle-stimulating hormone, prolactin and testosterone in the serum of male rhesus monkeys *Journal of endocrinology* 83 131-139.

Ben-Jonathan N (1980) Catecholamines and pituitary prolactin release *Journal of reproduction and fertility* 58 501-512.

Berczi I, Nagy E (1982) A possible role of prolactin in adjuvant arthritis *Arthritis and rheumatology* 25 591-594.

Berczi I, Nagy E, de Takado SM, Matusik RJ, Frieson HG (1991) Pituitary hormones regulate c-myc and DNA synthesis in lymphoid tissue *Journal of immunology* 146 2201-2206.

Bernton EW, Meltzer MS, Holaday JW (1988) Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice *Science* 239 401-404.

Bex F, Bartke A, Goldman BD, Dalterio S (1978) Prolactin, growth hormone luteinizing hormone receptors, and seasonal change in testicular activity in the golden hamster *Endocrinology* 103 2069-2080.

Bignon C, Daniel N, Belair L, Djiane J (1999) In vitro expression of long and short ovine prolactin receptors: activation of Jak2/STAT5 pathway is not sufficient to account for prolactin signal transduction to the ovine beta-lactoglobulin gene promoter *Journal of Molecular Endocrinology* 23 125-136.

Bittman EL, Weaver DR (1990) The distribution of melatonin binding sites in neuroendocrine tissues of the ewe *Biology of reproduction* 43 986-993.

Bockers TM, Niklowitz P, Bockmann J, Fauteck JD, Wittkowski W, Kreutz MR Daily melatonin injections induce cytological changes in pars tuberalis-specific cells similar to short photoperiod *Journal of Neuroendocrinology* 7 607-613.

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA (1998) Prolactin (PRL) and Its Receptor: Actions, Signal Transduction Pathways and Phenotypes Observed in PRL Receptor Knockout Mice *Endocrine Reviews* 19 225-268.

Bridges RS, Dibiase R, Loundes DD, Doherty PC (1985) Prolactin stimulation of maternal behaviour in female rats *Science* 227 782-784.

Brinklow BR, Forbes JM (1984) Effect of pinealectomy on the plasma concentrations of prolactin, cortisol and testosterone in sheep in short and skeleton long photoperiods *Journal of endocrinology* 100 287-294.

Brown WB, Forbes JM (1980) Diurnal variations of plasma prolactin in growing sheep under two lighting regimes and the effect of pinealectomy *Journal of endocrinology* 84 91-99.

Buttle HL, Forsyth IA (1976) Placental lactogen in the cow *Journal of endocrinology* 68 141-146.

Callea J, McMillen IC, Walker DW (1990) Effect of feeding regimen on diurnal variation of breathing movements in late-gestation fetal sheep *Journal of applied physiology* 68 1786-1792.

Clarke IJ, Cummins JT, de Kretser DM (1983) Pituitary gland function after disconnection from direct hypothalamic influences in the sheep *Neuroendocrinology* 36 376-384.

Curlewis JD (1992) Seasonal prolactin secretion and its role in seasonal reproduction: a review *Reproduction, fertility and development* 4 1-23.

de Reviers MM, Ravault JP, Tillet Y, Pelletier J (1989) Melatonin binding sites in the sheep pars tuberalis *Neuroscience letters* 100 89-93.

Dombrowicz D, Sente B, Closset J, Hennen G (1992) Dose dependent effects of human prolactin on the immature hypophysectomized rat testis *Endocrinology* 130 695-700.

Downs RJ, Helmers H (1975) Environmental control of plant growth. London, New York: Academic Press.

Ebling FJP, Wood RI, Suttie JM, Adel TE, Foster DL (1989) Prenatal photoperiod influences neonatal prolactin secretion in the sheep *Endocrinology* 125 384-391.

Elliot JA, Goldman BD (1989) Reception of photoperiodic information by fetal Siberian hamsters: role of the mothers pineal gland *The journal of experimental zoology* 252 237-244.

Freeman ME, Kanyicska B, Lerant A, Nagy G (2000) Prolactin: Structure, Function and Regulation of Secretion. *Physiological Reviews* 80 1523-1631.

Glasow A, Breidert M, Haidan A, Anderregg U, Kelly PA, Bornstein S (1996) Functional aspects of prolactin (PRL) on adrenal steroidogenesis and distribution of the PRL receptor in the human adrenal gland *Journal of clinical endocrinology and metabolism* 81 3103-3111.

Gluckman PD, Marti-Henneberg C, Kaplan SL, Grumbach MM (1983) Hormone Ontogeny in the Ovine Fetus: XIV. The Effect of 17β-Estradiol Infusion on Fetal Plasma Gonadotropins and Prolactin and the Maturation of Sex Steroid-Dependent Negative Feedback *Endocrinology* 112 1618-1623.

Guerra M, Rodriguez EM (2001) Identification, cellular and subcellular distribution of 21 and 72 kDA proteins (tuberalins?) secreted by specific cells of the pars tuberalis *Journal of endocrinology* 168 363-379.

Hamosh M, Hamosh P (1977) The effect of prolactin on the lecithin content of fetal rabbit lung *Journal of clinical investigation* 59 1002-1005.

Hazlerigg D, Hastings M, Morgan P (1996) Production of a prolactin releasing factor by the ovine pars tuberalis. *Journal of Neuroendocrinology* 8 489-492.

Helliwell RJA, Wallace JM, Aitken RP, Robinson JJ (1992) Prenatal photoperiod times the onset of puberty in ewe lambs *Journal of reproduction and fertility, abstract series* 10, abstract 71, p 38.

Helliwell RJ, Williams LM (1994) The development of melatonin-binding sites in the ovine fetus *Journal of endocrinology* 142 475-484.

Higuchi K, Nawata H, Maki T, Higashima M, Kato K, Ibayashi H (1984) Prolactin has a direct effect on adrenal androgen secretion *Journal of clinical endocrinology and metabolism* 59 714-718.

Hoffmann K (1973) The influence of photoperiod and melatonin on testes size, body weight, and pelage colour in the Djungarian hamster *(Phodopus sungorus)* 95 267-282.

Hoffman K, Illnerova H, Vanecek J (1986) Change in duration of the nighttime melatonin peak may be a signal driving photoperiodic responses in the Djungarian hamster *(Phodopus sungorus)* *Neuroscience Letters* 67 68-72.

Horseman ND, Buntin JD (1995) Regulation of pigeon crop milk secretion and parental behaviours by prolactin *Annual review of nutrition* 15 213-218.

Horton TH (1984a) Growth and maturation in *Microtus montanus*: effects of photoperiods before and after weaning *Canadian journal of zoology* 62 1741-1746.

Horton TH (1984b) Growth and reproductive development of male *Microtus montanus* is affected by the prenatal photoperiod *Biology of reproduction* 31 499-504.

Horton TH (1985) Cross-fostering of voles demonstrates in utero effect of photoperiod *Biology of reproduction* 33 934-939.

Houghton DC, Walker DW, Young IR, McMillen IC (1993) Melatonin and the light-dark cycle separately influence daily behavioural and hormonal rhythms in the pregnant ewe and sheep fetus *Endocrinology* 133 90-98.

Jacques SL, Weaver DR, Reppert SM (1987) Penetration of light into the uterus of pregnant mammals *Photochemistry and photobiology* 45 637-641.

Johnson AL (1986) Serum concentrations of prolactin, thyroxine and triiodothyronine relative to season and the estrous cycle in the mare *Journal of animal science* 62 1012-1020.

Kelly PA, Djiane J, Postel-Vinay MC, Edery M (1991) The prolactin/growth hormone receptor family *Endocrine Reviews* 12 235-251.

Kennaway DJ, Gilmore TA, Seamark RF (1982) Effect of melatonin feeding on serum prolactin and gonadotropin levels and the onset of seasonal estrous cyclicity in sheep *Endocrinology* 110 1766-1772.

Krause DN, Dubocovich ML (1990) Regulatory sites in the melatonin system of mammals *Trends in neurosciences* 13 464-470.

Krebs DL, Hilton DJ (2001) SOCS Proteins: Negative Regulators of Cytokine Signaling *Stem Cells* 19 378-387.

Kreeger TJ, Seal US (1992) Circannual prolactin rhythm in intact dogs housed outdoors *Chronobiologia* 19 1-8.

Larcher W (1980) Physiological plant ecology. Berlin, Heidelberg, New York: Springer-Verlag.

Leong DA, Frawley LS, Neill JD (1983) Neuroendocrine control of prolactin secretion *Annual review of physiology* 45 109-127.

Lee TM, Smale L, Zucker I, Dark J (1987) Influence of daylength experienced by dams on post-natal development of young meadow voles *(Microtus pennsylvanicus) Journal of reproduction and fertility* 81 337-342.

Lincoln GA (1979) Light induced rhythms of prolactin secretion in the ram and the effect of cranial sympathectomy *Acta endocrinologica* 91 421-427.

Lincoln GA (1990) Correlation with changes in horns and pelage, but not reproduction, of seasonal cycles in the secretion of prolactin in rams of wild, feral and domesticated breeds of sheep *Journal of reproduction and fertility* 90 285-296.

Lincoln GA (1992) Administration of melatonin into the mediobasal hypothalamus as a continuous or intermittent signal affects the secretion of follicle stimulating hormone and prolactin in the ram *Journal of pineal research* 12 135-144.

Lincoln GA (1994) Effects of placing micro-implants of melatonin in the pars tuberalis, pars distalis and the lateral septum of the forebrain on the secretion of FSH and prolactin, and testicular size in rams *Journal of endocrinology* 142 267-276.

Lincoln GA, Clarke IJ (1994) Photoperiodically-induced cycles in the secretion of prolactin in hypothalamo-pituitary disconnected rams: evidence for translation of the melatonin signal in the pituitary gland *Journal of neuroendocrinology* 6 251-260.

Lincoln GA, Ebling FJP (1985) Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams *Journal of reproduction and fertility* 73 241-253.

Lincoln GA, Almeida OFX, Arendt J (1981) Role of melatonin and circadian rhythms in seasonal reproduction in rams *Journal of reproduction and fertility Supplement* 30 23-31.

Lincoln GA, McNeilly AS, Cameron CL (1978) The effects of a sudden decrease or increase in daylength on prolactin secretion in the ram *Journal of reproduction and fertility* 52 305-311.

Maeda KI, Mori Y, Kano Y (1986) Superior cervical ganglionectomy prevents gonadal regression and increased plasma prolactin concentrations induced by long days in goats *Journal of endocrinology* 110 137-144.

Maetz J (1970) Mechanisms of salt and water transfer across membranes in teleosts in relation to the aquatic environment. *Membrane society endocrinology* 18 3-29.

Martinet L, Ravault JP, Meunier M (1982) Seasonal variations in mink *(Mustela vison)* plasma prolactin measured by heterologous radioimmunoassay *General and comparative endocrinology* 48 71-75.

Maxwell CA, Rintoul AJ, Foldes A, Downings JA, Scaramuzzi RJ, Carter NB (1989) Seasonal modification of ovine pineal function *Neuroendocrinology* 50 274-279.

McMillen IC, Nowak R (1989) Maternal pinealectomy abolishes the diurnal rhythm in plasma melatonin concentrations in the fetal sheep and pregnant ewe during late gestation *Journal of endocrinology* 120 459-464.

McMillen IC, Nowak R (1989) The pre- and postnatal development of hormonal circadian rhythms *Baillieres clinical endocrinology and metabolism* 3 707-721.

McMillen IC, Nowak R, Walker DW, Young IR (1990) Maternal pinealectomy alters the daily pattern of fetal breathing in sheep *American journal of physiology* 258 R284-R287.

McMillen IC, Thorburn GD, Walker DW (1987) Diurnal variations in plasma concentrations of cortisol, prolactin, growth hormone and glucose in the fetal sheep and pregnant ewe during late gestation *Journal of endocrinology* 114 65-72.

McMillen IC, Walker DW (1991) Effects of different lighting regimes on daily hormonal and behavioural rhythms in the pregnant ewe and sheep fetus *Journal of physiology* 442 465-476.

McMillen IC, Walker DW, Young IR, Nowak R (1991) A daily prolactin rhythm persists in the ewe, foetus and newborn lamb after maternal pinealectomy in late gestation *Journal of neuroendocrinology* 3 369-374.

Merks T, Schulze-Bonhage A, Wittkowski W (1993) Photoperiod-dependent changes in exocytic activity in the hypophyseal pars tuberalis of the Djungarian hamster, *Phodopus sungorus*. *Cell and Tissue Research* 273 287-291.

Mirarchi RE, Howland BE, Scanlon PF, Kirkpatrick RL, Sanford LM (1978) Seasonal variation in plasma LH, FSH, prolactin, and testosterone concentrations in adult male white-tailed deer *Canadian journal of zoology* 56 121-127.

Mondain-Monval M, Moller OM, Smith AJ, McNeilly AS, Scholler R (1985) Seasonal variations of plasma prolactin and LH concentrations in the female blue fox *(Alopex lagopus) Journal of reproduction and fertility* 74 439-448.

Moore RY (1978) Neural control of pineal function in mammals and birds *Journal of neural transmission. Supplementum* 13 47-58.

Moore RY (1996) Neural control of the pineal gland *Behavioural brain research* 73 125-130.

Morgan PJ, Williams LM, Davidson G, Lawson W, Howell E (1989) Melatonin receptors on ovine pars tuberalis: characterization and autoradiographic localization *Journal of neuroendocrinology* 1 1-4.

Morgan PJ, King TP, Lawson W, Slater D, Davidson G (1991) Ultrastructure of melatonin-responsive cells in the ovine pars tuberalis *Cell and tissue research* 263 529-534.

Morgan PJ, Webster C, Mercer J, Ross A, Hazlerigg D, MacLean A, Barrett P (1996) The ovine pars tuberalis secretes a factor(s) that regulates gene expression in both lactotropic and nonlactotropic pituitary cells. *Endocrinology* 137 4018-4026.

Morgan PJ, Williams LM (1996) The pars tuberalis of the pituitary: a gateway for neuroendocrine output *Reviews of reproduction* 1 153-161.

Nagy E, Berczi I (1981) Prolactin and contact sensitivity *Allergy* 36 429-431.

Nagano M, Kelly PA (1994) Tissue distribution and regulation of rat prolactin receptor gene expression. Quantitative analysis by polymerase chain reaction *Journal of Biological Chemistry* 269 13337-13345.

Nielsen JH (1982) Effects of growth hormone, prolactin and placental lactogen on insulin content and release, and deoxyribonucleic acid synthesis in cultured pancreatic islets *Endocrinology* 110 600-606.

Ogawa M, Yagasaki M, Yamazaki, F (1973) The effect of prolactin on water influx in isolated gills of the goldfish, *Carrassius auratus*. *Lessons in comprehensive biochemistry and physiology A* 44 1177-1183.

Ota H, Wakizaka A, Fukushima M, Maki M (1985) Dual regulation of rat ovarian LH-receptor by the administration of prolactin or sulpiride *IRCS journal of medical science* 63 257-264.

Parraguez VH, Sales F, Valenzuela GJ, Vergara M, Catalan L, Seron-Ferre M (1998) Diurnal changes in light intensity inside the pregnant uterus in sheep *Animal reproduction science* 52 123-130.

Peaker MJ, Phillips JG, Wright A (1970) The effect of prolactin on the secretory activity of the nasal salt-gland of the domestic duck (*Anas platythyncas*). *Journal of endocrinology* 47 123-127.

Poulton AL, English J, Symons AM, Arendt J (1986) Effects of various melatonin treatments on plasma prolactin concentrations in the ewe *Journal of endocrinology* 108 287-292.

Poulton AL, English J, Symons AM, Arendt J (1989) Plasma prolactin concentrations in pinealectomized ewes receiving melatonin treatment and in pineal intact ewes maintained under a non-24-hour photoperiod *Journal of pineal research* 6 243-252.

Quirk Jr JG, MacDonald PC, Johnston JM (1982) Role of fetal pituitary prolactin in fetal lung maturation *Seminars in perinatology* 6 238-245.

Ravault JP (1976) Prolactin in the ram: seasonal variations in the concentration of blood plasma from birth until three years old *Acta Endocrinologica (Copenhagen)* 83 720-725.

Ravault JP, Martinat-Botte F, Mauget R, Martinat N, Locatelli A, Bariteau F (1982) Influence of the duration of daylight on prolactin secretion in the pig: hourly rhythm in ovariectomized females, monthly variation in domestic (male and female) and wild strains during the year *Biology of reproduction* 27 1084-1089.

Reiter RJ, Vaughan MK, Blask DE, Johnson LY (1974) Melatonin: its inhibition of pineal antigonadatrophic activity in male hamsters *Science* 185 1169-1171.

Reymond MJ, Porter JC (1985) Involvement of hypothalamic dopamine in the regulation of prolactin secretion *Hormone Research* 22 142-152.

Richardson BP (1973) Evidence for a physiological role of prolactin in osmoregulation inhibited by 2-bromo-a-ergokryptine. *British journal of pharmacology* 47 623P-624P.

Robinson JE, Karsch FJ (1987) Photoperiodic history and a changing melatonin pattern can determine the neuroendocrine response of the ewe to daylength *Journal of reproduction and fertility* 80 159-165.

Rynikova A, Koppel J, Kuchor S, Cikas S, Mazes S (1988) Effects of ovine prolactin in infant rats *Experiments in clinical endocrinology* 92 241-244.

Sassin JF, Frantz AG, Weitzman ED, Kapen S (1972) Human prolactin: 24-hour pattern with increased release during sleep *Science* 177 1205-1207.

Schams D, Barth D (1982) Annual profiles of reproductive hormones in peripheral plasma of the male roe deer *(Capreolus capreolus) Journal of reproduction and fertility* 66 463-468.

Seron-Ferre M, Vergara M, Parraguez VH, Riquelme R, Llanos AJ (1989) The circadian variation of prolactin in fetal sheep is affected by the seasons *Endocrinology* 125 1613-1616.

Shaw D, Goldman BD (1995) Gender differences in influence of prenatal photoperiods on postnatal pineal melatonin rhythms and serum prolactin and follicle-stimulating hormone in the Siberian hamster *(Phodopus sungorus) Endocrinology* 136 4237-4246.

Shyr SW, Crowley WR, Grosvenor CE (1986) Effect of neonatal prolactin deficiency on pre-pubertal tuberoinfundibuler and tuberohypophyseal dopaminergic neuronal activity *Endocrinology* 119 1217-1221.

Sibley DR, De Lean A, Creese I (1982) Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor *Journal of biological chemistry* 257 6351-6361.

Spies HG, Norman RL, Buhl AE (1979) Twenty-four-hour patterns in serum prolactin and cortisol after partial and complete isolation of the hypothalamic-pituitary unit in rhesus monkeys *Endocrinology* 105 1361-1368.

Stetson MH, Elliot JA, Goldman BD (1986) Maternal transfer of photoperiodic information influences the photoperiodic response of prepubertal Djungarian hamsters *(Phodopus sungorus sungorus) Biology of reproduction* 34 664-669.

Stirland JA, Johnston JD, Cagampang FRA, Morgan PJ, Castro MG, White MRH, Davis JRE, Loudon ASI (2001) Photoperiodic regulation of prolactin gene expression in the Syrian hamster by a pars tuberalis-derived factor *Journal of Neuroendocrinology* 13 147-157.

Sweeney T, Kelly G, O'Callaghan D (1999) Seasonal variation in long-day stimulation of prolactin in ewes *Biology of reproduction* 60 128-133.

Symons AM, Arendt J, Laud CA (1983) Melatonin feeding decreases prolactin levels in the ewe *Journal of endocrinology* 99 41-46.

Thomas GB, Cummins JT, Cavanagh L, Clarke IJ (1986) Transient increase in prolactin secretion following hypothalamo-pituitary disconnection in ewes during anoestrus and the breeding season *Journal of endocrinology* 111 425-431.

Torrealba F, Parraguez VH, Reyes T, Valenzuela G, Seron-Ferre M (1993) Prenatal development of the retinohypothalamic pathway and the suprachiasmatic nucleus in the sheep *Journal of comparative neurology* 338 304-316.

Tsubota T, Nelson RA, Thulin JD, Howell L, Bahr JM (1995) Annual changes in serum concentrations of prolactin in captive male black bears *(Ursus americanus) Journal of reproduction and fertility* 104 187-191.

Vergara M, Parraguez VH, Recabarren S, Riquelme R, Garay F, Valenzuela G, Seron-Ferre M (1992) The retino-hypothalamic tract is involved in prolactin regulation in fetal sheep *Journal of Developmental Physiology* 18 19-23.

Weaver DR, Reppert SM (1989) Direct in utero perception of light by the mammalian fetus *Developmental brain research* 47 151-155.

Wittkowski W, Hewing M, Hoffmann K, Bergmann M, Fechner J (1984) Influence of photoperiod on the ultrastructure of the hypophyseal pars tuberalis of the Djungarian hamster, *Phodopus sungorus*. *Cell and Tissue Research* 238 213-216.

Wittkowski W, Bockmann J, Kreutz MR, Bockers TM (1999) Cell and molecular biology of the pars tuberalis of the pituitary *International Review of Cytology* 185 157-194.

Wong CC, Dohler KD, Atkinson MJ, Geerlings H, Hesch RD, von zur Muhlen A (1983) Circannual variations in serum concentrations of pituitary, thyroid, parathyroid, gonadal and adrenal hormones in male laboratory rats *Journal of endocrinology* 97 179-185.

Yellon SM, Longo LD (1987) Melatonin rhythms in fetal and maternal circulation during pregnancy in sheep *American journal of physiology* 252 E799-E802.

Yellon SM, Longo LD (1988) Effect of maternal pinealectomy and reverse photoperiod on the circadian melatonin rhythm in the sheep and fetus during the last trimester of pregnancy *Biology of reproduction* 39 1093-1099.

Zemdegs IZ, McMillen IC, Walker DW, Thorburn GD and Nowak R (1988) Diurnal rhythms in plasma melatonin concentrations in the fetal sheep and pregnant ewe during late gestation *Endocrinology* 123 284-289.