Photoperiodism in Pigs

Studies on timing of male puberty and melatonin

Håkan Andersson
Department of Clinical Chemistry
Department of Animal Breeding and Genetics

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Abstract

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The routine castration of male piglets, which is performed in many countries to avoid boar taint in meat, is a cause for great concern in terms of animal welfare. Castrates also have reduced feed efficiency and more fat than do entire males. More of the ingested energy therefore goes to fat tissue than to muscle tissue. The European wild boar is a seasonal short-day breeder and although domestic pigs breed all year round there are indications that they remain responsive to photoperiod. Since boar taint is closely associated with male sexual maturation, the use of artificial light regimens to delay puberty could be a non-invasive method of reducing boar taint in entire males. Therefore, a series of experiments were performed in order to study photoperiodism in young pigs.

In two experiments, matched winter-born siblings of crossbred males were allocated after weaning to either one of two light-sealed rooms with high-intensity light regimens or a conventional stable environment. In the first study, groups subjected to an 'artificial autumn' treatment and an 'artificial spring' treatment were compared with a 'natural spring' group. Animals in the 'artificial autumn' group were less sexually mature at the time of slaughter than were those of the 'natural spring' group, which probably was due to the abrupt and large increase in photoperiod for the 'artificial autumn' group.

At the start of the second experiment, animals were transferred from 6.5 h of natural light to 12 h of artificial light. Thereafter, the 'artificial winter' group were exposed to short days, whereas the 'artificial summer' group had long days and both were compared with a 'natural spring' group. At slaughter, the 'artificial summer' group showed immature spermatogenesis and less boar taint than did the 'artificial winter' group. An interesting observation was that all three groups which were exposed to an abrupt shift in photoperiod at the start of the experiment ('artificial autumn', 'winter' and 'summer') showed a deviation from the normally parallel secretion of testicular steroids In these pigs, testosterone increased while oestrone-sulphate remained low. In contrast to wild pigs, a photoperiod-induced seasonal pattern in prolactin secretion has not been demonstrated for domestic pigs. Although both experiments showed tendencies of higher prolactin concentrations during long days, the results of these studies are not conclusive.

Together, the two studies show that photoperiod influences the timing of puberty in boars. Short days stimulate puberty and long days delay sexual maturation and reduce boar taint. However, the pre-weaning photoperiod can influence the response to the subsequent photoperiod.

In mammals, seasonal changes in photoperiod are mediated through the pineal hormone melatonin. Melatonin secretion is typically high during the dark hours and negligible during the day, and thus reflects the period of darkness. In pigs,

there have been considerable difficulties to demonstrate a typical melatonin profile, which has led to speculation that impaired melatonin production accounts for the lack of a seasonal breeding pattern in domestic pigs.

Two assays for human melatonin were compared in plasma from pigs that had been exposed to either standard stable lighting or artificial short days or long days. According to conventional laboratory evaluating procedures both assays performed well, but only one assay measured a typical circadian plasma melatonin profile entrained by the photoperiod for all individuals. The measured increase in melatonin secretion during the dark phase was low compared with that in other species. However, the variation in melatonin levels between animals was high and there were indications of a genetic background to this.

To investigate whether the pineal melatonin synthesis has been impaired by domestication, European wild boars of both sexes and domestic gilts were cannulated and sampled for 48 h in four seasons. A circadian melatonin profile entrained by the photoperiod of the season was observed in all animals and there was no difference was seen between wild and domestic pigs.

To further study the great variation between animals in the increase of nocturnal melatonin, blood from 48 piglets and their parents was sampled during the day and night. Although the sampling method occasionally appeared to cause overestimated values, differences between litters in nocturnal melatonin concentrations strengthen the hypothesis that the observed variation between animals of nocturnal melatonin secretion in pigs is genetically based.

Key words: swine, photoperiod, melatonin, puberty, spermatogenesis, pig endocrinology, light regimen

Author's address: Håkan Andesson, Department of Clinical Chemistry, SLU, P.O. Box 7038, SE-750 07 Uppsala, Sweden.

Mbwene shundwa na mbuzi wachandamana pamoya
Na kuku mke na kozi wanawao wachilea
Na mtu msi maozi akionya watu ndia
Hayano sikusikia, niwene kwa mato yangu.

Jag har sett en hyena och en get hålla varandra sällskap
Och en höna och en hök föda upp sina ungar
Och en blind visa vägen för andra
Detta har jag inte hört utan sett med egna ögon.

Muyaka bin Hajji (1776-1837)

To Berit and Ingvar, my parents

Contents

Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I Andersson H, Rydhmer L, Lundström K, Wallgren M, Andersson K and Forsberg M. (1998) Influence of artificial light regimens on sexual maturation and boar taint factors in entire male pigs. *Animal Reproduction Science 51,* 31-43.

II Andersson H, Wallgren M, Rydhmer L, Lundström K, Andersson K and Forsberg M. (1998) Photoperiodic effects on pubertal maturation of spermatogenesis, pituitary responsiveness to exogenous GnRH, and expression of boar taint in crossbred boars. *Animal Reproduction Science 54,* 121-137.

III Andersson H, Lillpers K, Rydhmer L and Forsberg M. Influence of light environment and photoperiod on plasma melatonin and cortisol profiles in young domestic boars, comparing two commercial melatonin assays. *Domestic Animal Endocrinology.* (In press)

IV Tast A, Hälli O, Ahlström S, Andersson H, Love RJ and Peltoniemi OAT. Seasonal alterations in circadian melatonin rhythms of the European wild boar and domestic gilt. *Journal of Pineal Research.* (In press)

V Andersson H. Plasma melatonin levels in relation to the light-dark cycle, gender and parental background in domestic pigs. (Submitted for publication)

Introduction

Domesticated animals have lived in a world of secured food supply and protection from predators and competitors for thousands of generations, yet physiological responses to environmental factors bear witness to an adaptation to a life in a more variable milieu. The interaction between endogenous rhythms and the light-dark cycle enables animals not only to adapt to seasonal variations, but also, to predict these changes and adjust their metabolism, immune functions and reproductive activities accordingly.

Photoperiodism

To time the most energy-demanding stages of reproduction with the best environmental conditions for survival, photoperiod is widely used as a noise-free cue among seasonally breeding animals outside the equatorial region of the tropics. The spring is generally the most favourable time of the year for mammals to give birth and the length of the gestation period - including any delayed implantation or embryonic diapause - therefore determines when mating occurs. Based on the photoperiodic conditions at the time of the initiation of the mating, seasonally breeding mammals are traditionally referred to as 'long-day' (i.e. with long or increasing photoperiod) or 'short-day' (with short or decreasing photoperiod) breeders. This classification, however, does not always correspond to the photoperiodic signals that time reproductive events under natural conditions. For instance, in the short-day breeding ewe, it is the long days of spring, rather than the decreasing day length of autumn, that time the start of the ovulatory period (Malpaux et al., 1989; Woodfill et al., 1994; Barrell et al., 2000).

Endogenously generated circannual rhythms have been demonstrated for breeding activity (Ducker et al., 1973), hibernation (Pengelley and Fischer, 1957), and migration (see Gwinner, 1995), as well as for various hormones (androgens, Michael and Bonsall, 1977; gonadotropins, Karsch et al., 1989; Concannon et al., 1998; thyroid hormones, Concannon et al., 1999; prolactin, Howels et al., 1982; Karsch et al., 1989; Jackson and Jansen, 1991). In a constant environment, the circadian rhythm of melatonin secretion shows circannual changes (Thrun et al., 1995). In the most illustrative examples, some animals put in a constant environment may undergo several periods of reproductive activity with intermediate periods during which they are sexually quiescent (e.g. 9 years in the East African stonechat, Gwinner, 1995), whereas other species such as the hamster require photoperiodic input in order to generate an additional complete reproductive cycle (see Gorman and Zucker, 1998). The ability of circannual rhythms to run free in a constant environment varies. Some circannual rhythms are only expressed under certain constant photoperiodic conditions and not under others (e.g. antler growth in the Sika deer, Goss, 1984), whereas in some species, a free-running pattern is observed in only certain individuals (e.g. ovulatory period in ewes; Jansen and Jackson, 1993). Except in North American ground squirrels and marmots, the duration of endogenous circannual rhythms typically is

shorter than 12 months (Turek and Van Cuter, 1994) and the role of photoperiod is to synchronize these rhythms to the geophysical year.

The interaction between circannual rhythms and the light-dark cycle is dynamic and dependent on changes in day length as well as phases of sensitivity to photoperiod (Barrell et al., 2000). Animals that are exposed over a prolonged period of time to a fixed photoperiod of time will become unresponsive to the photoperiodic effect and proceed with the next phase of the reproductive cycle (also termed 'photorefractoriness', Turek et al., 1975; Almeida and Lincoln, 1984; Robinson and Karsch, 1984; Robinson et al., 1985; Karsch et al., 1986; Malpaux et al., 1987). Periods of photorefractoriness enable birds to migrate between the hemispheres and mammals to hibernate without being affected reproductively by the photoperiod of the winter residence or the darkness in the den (see Gwinner, 1986).

The effect of a photoperiod signal is dependent on previously experienced photoperiod. A certain photoperiod can induce a long-day or a short-day response, depending on whether the previous photoperiod was shorter or longer (Hoffman et al., 1986; Robinson and Karsch, 1987; Stetson et al., 1989; Niklowitz et al., 1994; Gorman and Zucker, 1997a). Such a photoperiodic history allows a greater refinement in predicting seasonal changes. In the uterus, information about the prevailing photoperiodic conditions is conducted to the foetus through maternal melatonin during late gestation. Thus, once the newborn can perceive photoperiod on its own it already has a photoperiodic history, which can influence the reproductive response to the post-natal photoperiod (Stetson et al., 1989).

Whether it results in total azoospermia or merely in mild oligospermia, the circannual variation in activity of the hypothalamic-pituitary-testicular axis drives the seasonal reproductive changes. Circannual variation (e.g. in pulse frequency or basal levels) in luteinising hormone (LH) secretion and follicle-stimulating hormone (FSH) has been demonstrated and for at least for some species, the feedback of gonadal steroids on the hypothalamus or pituitary gland is central to expression of seasonality (e.g. horse, Irvine and Alexander, 1982; rams, Lincoln, 1984). Since neither melatonin nor gonadal steroids act directly on gonadotropin-releasing hormone (GnRH) neurones *in vivo* (Herbison and Theodosis, 1992), afferent neural pathways must be involved in the regulation of seasonal variations in GnRH secretion. This conclusion is further supported by morphological observations of a seasonal plasticity in the ovine GnRH neuroendocrine system, with more synaptic input during the breeding season (Xiong et al., 1997).

The investigations of possible neurotransmitters that may mediate photoperiodic effects on GnRH neurones have shown an opoidergic (i.e. ß-endorphin) inhibition of LH during autumn and winter in both the short-day breeding ram (Lincoln et al., 1987) and the long-day breeding stallion (Aurich et al, 1994a). This opoidergic inhibition of LH release requires the presence of gonads in both species (horse, Aurich et al., 1994a; Aurich et al., 1994b; ram, Ebling and Lincoln, 1985; and Lincoln et al., 1987). In sheep, dopaminergic pathways inhibit gonadotropin release during long days but not during short days

(Meyer and Goodman, 1985; Tortonese and Lincoln, 1994). The dopaminergic system further inactivates opoidergic effects on gonadotropins during the non-breeding season, which may explain the lack of opoidergic regulation of gonadotropins during long days in sheep (see Gerlach and Aurich, 2000). Also, Gamma-aminobutyric acid (GABA) appears to inhibit the gonadotropin release during the non-breeding season in ewes (Scott and Clarke, 1993). As no single pharmacological manipulation has been able to fully mimic the seasonal changes in gonadotropin secretion or sensitivity to steroid feedback (see Hileman and Jackson, 1999), photoperiodic effects on GnRH activity probably involve a complex of several neurotransmitter systems.

Many mammals show a clear seasonal pattern in prolactin secretion, with a summer peak and a winter nadir. The spring increase in prolactin is luteotrophic and terminates embryonic diapause in the mink (Martinet et al., 1981; Murphy et al., 1981) and western spotted skunk (Berria et al., 1989). Also, the moult from winter to summer coat is induced by the spring rise in prolactin in several species including the blue fox (Smith et al., 1987) and red deer hind (Curlewis et al., 1988), but for many species, any significance of the seasonal variations in prolactin has not been demonstrated (Curlewis, 1992). Photoperiod affects the circannual rhythm in prolactin rapidly (Viguié et al., 1997) and independently of photoperiodic history (Hastings et al., 1989), via the melatonin profile. Melatonin binds to receptors in the pars tuberalis cells that secrete a factor 'tuberalin', which controls prolactin release from the lactotrophs in the pars distalis (see Morgan and Williams, 1996). The melatonin effect on prolactin occurs also in the absence of an intact hypothalamic-pituitary axis (Lincoln and Clarke, 1994), and therefore, independently of any reproductive effect of melatonin. New data suggest that the circannual rhythm of prolactin is generated within the pituitary gland itself (Lincoln and Clarke, 2000). European wild boars show a typical seasonal pattern in prolactin secretion, with a summer peak (Ravault et al., 1982; Mauget, 1985), whereas a clear prolactin rhythm has not been demonstrated for domestic pigs (Ravault et al., 1982).

In some seasonal breeders, thyroid hormones and their binding proteins show seasonal variations, reflecting circannual changes in metabolism (Concannon et al., 1999). The presence of thyroid hormones is necessary for the termination of the breeding season in both birds and mammals (Nicholls et al., 1988), perhaps because they act permissively to a photoperiod-induced reorganization of the GnRH-regulating system (Dahl et al., 1995; Thrun et al., 1997).

Melatonin

The crucial role of the pineal hormone melatonin in conveying photoperiodic information in an endocrine signal which can synchronize circannual rhythms in mammals is well established (e.g. Hoffman and Reiter, 1965; Bittman et al., 1983; Yellon and Foster, 1986; Hiebert et al., 2000) and appears to be applicable to all mammalian species. Melatonin-binding sites have been identified in various parts of the central nervous system (CNS), with some species differences;

however, the anatomical connection between the melatonin receptors in the CNS and the GnRH-controlling neurones is yet to be elucidated.

The indolamine melatonin acts as a signal for the duration of the dark period and during the light hours of the day, the pineal secretion is typically negligible. Melatonin (*N*-acetyl-5-methoxytryptamine) is synthesized from L-tryptophan via serotonin by 5-hyroxylation, decarboxylation, *N*-acetylation (the rate-limiting step) and 5-methylation by the corresponding enzymes in the pineal gland. The suprachiasmatic nucleus (SCN), the mammalian pacemaker of circadian rhythms in the hypothalamus, also termed the 'biological clock', generates the rhythm of melatonin secretion by regulating the activity of pineal *N*-acetyltransferase (NAT) (see Klein et al., 1997).

The circadian melatonin rhythm is synchronized by the light-dark cycle to be in tune with the environment. Light signals are transmitted from photoreceptors in the retina via a neural pathway to the SCN (see Moore, 1996), where the circadian rhythm of pineal melatonin synthesis is entrained (see Figure 1). Light can also directly inhibit pineal NAT production and thus decrease melatonin release (Klein et al., 1997), and the shape of the melatonin profile as measured in peripheral blood is a result of an interaction between the entraining and suppressive effects of light (Picazo and Lincoln, 1995).

Even though small amounts of extra-pineal melatonin are synthesized (Heuther, 1993; Bubenik et al., 1999), levels of melatonin in body fluids, such as blood, cerebrospinal fluid, saliva and - with some time delay - the urinary metabolite 6-sulphatoxymelatonin, directly reflect the pineal production since melatonin is released immediately after synthesis (see Pang et al., 1993). The duration of the nocturnal increase in melatonin secretion is considered to be the essential signal of the melatonin profile (Bittman and Karsch, 1984; Yellon et al., 1985; Hoffman et al., 1986; Bartness et al., 1993; Gorman and Zucker, 1997b). If the melatonin level is kept constantly elevated by exogenous administration it will induce a short-day response (sheep, Kennaway et al., 1982; Lincoln and Ebling, 1985; O'Callaghan et al., 1991; rat, Kennaway and Rowe, 1997). In addition to seasonal effects, maternal melatonin provides circadian information to the developing brain, as the retina-SCN pathway in the foetus is not yet functional (Davis and Mannion, 1988).

Melatonin-binding sites in the pars tuberalis of the pituitary, the hypothalamus, and other parts of the brain have been identified in mammals (see Morgan et al., 1994). In neonatal pigs, there are functional melatonin-binding sites in the pars tuberalis, as well as in many other sites within the brain, but very little specific binding in the hypothalamus (Williams et al., 1999), which has also been noticed in seasonally breeding mustelids (Weaver and Reppert, 1990; Boission-Agasse et al., 1992; Duncan and Mead, 1992; Bonneford et al., 1993). Two membrane-bound G protein (guanine nucleotide-binding protein)-coupled melatonin receptors, Mel_{1a} and Mel_{1b}, have been demonstrated in mammals (Reppert, 1997), and there is evidence that melatonin exerts its effect on reproduction through Mel_{1a} (Weaver et al., 1996; Pelletier et al., 2000). In hamsters, the melatonin receptors are co-localized with androgen receptors, and an influence of

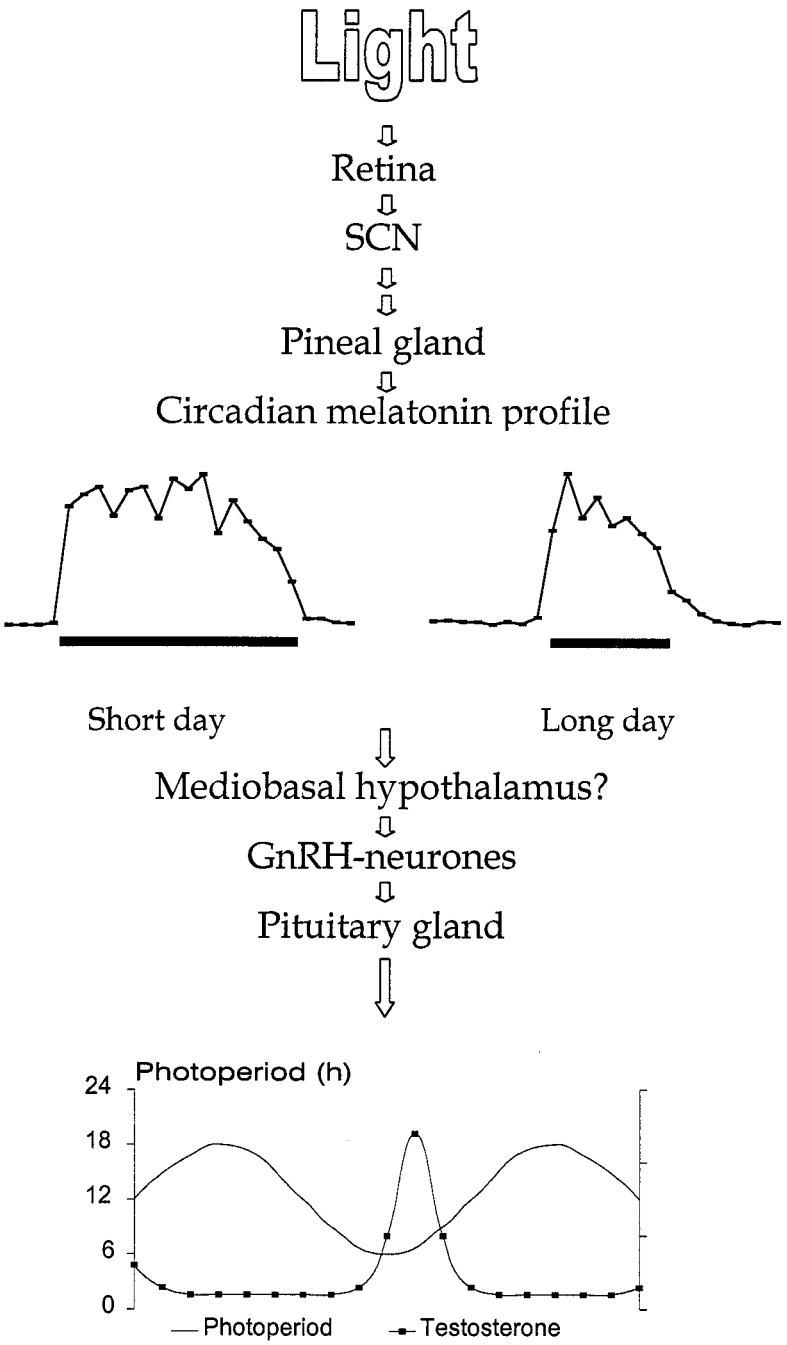

Figure 1. Schematic drawing of the retinal-pineal-hypothalamic pathway in mammals (SCN = Suprachiasmatic nucleus).

melatonin on androgen feedback on GnRH release has been suggested (Maywood et al., 1995). No melatonin-binding sites on GnRH neurones have, however, been found.

Melatonin-containing micro-implants have revealed the mediobasal hypothalamus as a likely neural region site where melatonin executes its effect on the reproductive system in male (Lincoln and Maeda, 1992) and female (Malpaux et al., 1993) sheep as well as in hamsters (Maywood and Hastings, 1995). Though in contrast to the adjacent pars tuberalis, the density of melatonin-binding sites is very low in the mediobasal hypothalamus.

In pigs, there have been considerable difficulties in demonstrating a circadian melatonin pattern resembling that of other mammals (see Reiter, 1993) and several research reports have been published in which no clear circadian melatonin pattern could be found (McConnell and Ellendorff, 1987; Reiter et al., 1987; Minton et al., 1989; Minton and Cash, 1990; Peacock et al., 1991, and 1995; Diekman et al., 1992; Griffith and Minton, 1992; Green et al., 1996, and 1999; Bassett et al., 1996; Bollinger et al., 1997; Diekman and Green, 1997; Lewczuk and Przybylska-Gornowicz, 1997; Bubenik et al., 2000). Klupiec et al. (1997) point out that this contradictory information has probably been created by methodological problems. Although the molecule structure of melatonin does not differ between species, some non-specific disturbance appears to be present in porcine blood, causing an overestimation of melatonin content in some assays. Pre-assay extraction is a common approach to overcome problems with unspecific disturbance and is important also for analysing porcine melatonin.

The difficulties in demonstrating a typical circadian rhythm of porcine melatonin have led to the hypothesis that pineal melatonin secretion is impaired in domestic pigs and accounts for the lack of a strict seasonal breeding pattern in domestic pigs (Green et al., 1996; Skinner et al., 1999). Besides, exogenous administration of melatonin has shown varied results in pigs. In three independent studies, melatonin administered orally but not by implants have advanced puberty in gilts kept under long-day conditions (Diekman et al., 1991 and 1997; Paterson et al., 1992a).

Puberty

A general goal of domestication is to maximize reproductive performance and thus, to accelerate the onset of fertility. Selection for greater body weight has probably advanced puberty, as sufficient body condition is a threshold for allowing sexual maturation (Foster et al., 1985). Modern animal husbandry, including regular food supply and protection from a harsh environment, probably has downgraded or masked the environmental influence for many domesticated species. The environmental conditions of animal husbandry vary and, consequently, the responsiveness to environmental factors such as photoperiod may vary, depending on the breed (e.g. rams, Lincoln et al., 1990).

Puberty in boars has been clearly advanced by domestication (Mauget and Boissin, 1987). The Meishan boar reaches puberty at almost half the age of European breeds (Lunstra et al., 1997) and crossbred boars reach puberty earlier

than do purebred boars (Wilson et al., 1977). In Swedish crossbred boars, the germ cells begin to develop at around 115 days of age. This development is followed by a very rapid increase in the number of sperm cells, which continues until the cellular organization of the seminiferous tubules is sexually mature, with functional spermatogenesis, at approximately 180 days of age. A low concentration of spermatozoa can be found in the cauda epididymis at 125 days of age; thereafter, the quantity and quality (i.e. reduced abnormalities) improve over time (Malmgren et al., 1996). Although functional spermatogenesis has been established by 6 months of age in the domestic boar, sperm production does not reach its maximum until approximately 24 months of age (Kennedy and Wilkins, 1984).

Puberty can be viewed either as a period of accelerated reproductive development culminating in functional fertility or as the first time when an animal is capable of conception. Regardless of definition, puberty is the result of a series of progressive maturational changes in the central hypothalamic-pituitary-gonadal axis. The role of photoperiod in timing of reproduction varies not only between species but also between sexes and maturational stage. For instance, the Syrian hamster shows a seasonal photoperiod-entrained breeding pattern among adult animals but photoperiod has no effect on early sexual development (Gaston and Menaker, 1967; Darrow et al., 1980; Donham et al., 1994). The opposite applies to the domestic rat, in which pre-natal and early post-natal photoperiod influences the male sexual maturation (Jarrige et al., 1992), whereas reproduction in adults is unresponsive to photoperiod (Jarrige and Boucher, 1992).

Sheep show a sexual dimorphism in the significance of photoperiod for pubertal timing. The female lamb requires a combination of the long days of summer and the decreasing day length of autumn to correctly time first ovulation with the breeding season (Yellon and Foster, 1985), whereas photoperiod has only a modifying influence on pubertal timing in ram lambs (Wood et al., 1991). The reproductive pattern of adult rams and ewes is seasonal, but there is a sexual differentiation of the neuroendocrine mechanisms regulating the seasonal changes of LH secretion (Lubbers and Jackson, 1993). The physiological mechanisms of the seasonal resumption of reproductive activity in adult animals are proposed to resemble puberty (e.g. Lincoln, 1981). In gilts, artificial short days, in contrast to long days, have been shown to advance first ovulation although the stimulating effect of boar contact can mask the photoperiodic effects (Paterson and Pearce, 1990). A comparison of male piglets kept under natural photoperiods suggests a similar effect on puberty in boars (Claus and Weiler, 1985). Earlier studies have, however, indicated that supplemental light during decreasing photoperiod can advance puberty in boars (Berger et al., 1980).

Pre-natal photoperiodic conditions can also be involved in timing puberty. Information to the foetus concerning the external photoperiod is mediated via maternal melatonin, which readily passes through the placenta (Klein, 1972; Reppert et al., 1979). A sensitive period for reception of a pre-natal melatonin signal which can provide the foetus with a photoperiodic memory has been defined in the Siberian hamster (Weaver et al., 1987). The significance of the pre-

natal photoperiod for pubertal timing can be sexually differentiated. In two such different species as the small, long day-breeding Siberian hamster and the large, short day-breeding red deer, there is a stronger influence of pre-natal photoperiod on male puberty (Siberian hamster, Shaw and Goldman, 1995a; Shaw and Goldman, 1995b; red deer, Adams et al., 1994; Adams et al, 1995).

In the pubertal boar, there is a parallel secretion of a wide range of testicular steroids, reflecting the high activity of steroid synthesis by the Leydig cells in the interstitial testicular tissue. A pre-pubertal peak of steroid products in male piglets occurs at 2-4 weeks of age, followed by low concentrations until a marked increase around puberty (Schwarzenberger et al., 1993). Although the testicular volume percentage of Leydig cells decrease during pubertal development owing to the immense growth of the testes, the total number of Leydig cells in the porcine testis increase (Allrich et al., 1983). A dramatic and transient increase in the size of the Leydig cells occurs at the onset of puberty (Lunstra et al., 1986).

Oestrogens, predominantly oestrone-sulphate (E_1-SO_4), are present in high concentrations in boar blood. The aromatization of androgens to oestrogens in the boar takes place mainly in the testes, but also in peripheral tissue (Booth, 1980), as it does in male humans (Lieberman et al., 1984). The aromatization of testosterone to oestrogens in neural tissue is a prerequisite to expression of reproductive behaviour in both males and females. Other functions of oestrogens in boars that have been described, are support of the development of accessory sex organs (Booth, 1983) and, through oestrogens in semen, stimulation of the female reproductive tract at mating (see Claus, 1990).

Hormones that are not part of the central endocrine axis, such as prolactin and thyroxine, have a broad metabolic influence, and effects on production. For instance, in the rat, prolactin stimulates testicular function during puberty (Dombrowicz et al., 1992). After high neonatal levels in pigs, prolactin decreases and remains low except for peaks around 10-16 weeks of age and at puberty (Meijer et al., 1988). Thyroxin is required for foetal and neonatal neural development (Granholm, 1985), is typically high in the neonatal and decreases with age (Irvine, 1984; Reimers et al., 1990). A thyroidectomy has been shown to advance pubertal testicular growth in the ram (Parkinson et al., 1995).

Pig reproduction

Through intensive management and breeding efforts, the great reproductive potential of pigs has been successfully utilized to make them reproduce all year round. More in-depth studies of reproductive performance over the year, however, have shown that although litters are born in all seasons the reproductive success rate varies. Together, observations from both hemispheres show that periods of decreased reproductive performance occur during summer and early autumn (Claus and Weiler, 1985).

The European wild boar (*Sus scrofa)* mates from late autumn to early winter and gives birth in late winter or early spring. Nutrition plays an important part in the reproduction of wild boars and if high-quality food is available in early autumn, mating and birth occurs earlier than in other years and a second litter

may be born in summer. Despite these opportunistic characteristics, all females are in anoestrus during summer and early autumn (Mauget, 1985) and male wild boars show a prominent seasonal variation in testis weight and testosterone, with peak values in mid-winter (Mauget and Boissin, 1987; Weiler et al., 1996). Since the seasonal infertility problems of modern pig production coincide with the anoestrus period of the European wild boar, the assumption, that vestiges of a seasonal breeding pattern may influence the reproductive performances of modern domestic pigs, appears reasonable.

Seasonal infertility can be displayed as either reduced farrowing rate due to disrupted pregnancy, delayed female puberty (which to some degree can be overcome by the presence of a boar), a prolonged weaning-to-oestrus interval, reduced litter size (see Claus and Weiler, 1985; and Love et al., 1993), or reduced sperm quality and libido in the boar (Claus et al., 1985). Several environmental factors have been suggested to be responsible for these seasonal variations in fertility. Increased temperatures can cause heat stress and have a negative effects on reproduction in pigs (Omtvedt et al., 1971; Wettemann and Bazer, 1985), including boars (Malmgren and Larsson, 1984). Higher sensitivity to stress has been suggested as an explanation for why gilts are more susceptible to seasonal effects than are sows (Wan et al., 1994). Love et al. (1995) have shown that nutrition and management systems can mask seasonal effects on farms, as both higher feeding levels and individual housing of sows decrease problems of seasonal infertility. All these factors influence pig reproduction, and may be involved in modifying the photoperiodic effects on reproduction during late summer and early autumn.

Boar taint

In many countries, the release of an unpleasant odour ('boar taint') from meat during cooking has prevented the use of intact males in pork production. Although only a small proportion of the consumers are highly sensitive to boar taint and only a small percentage of the male finishing pigs have high levels of boar taint, male piglets are routinely castrated at a young age, which causes concern in terms of animal welfare. The lack of anabolic steroids in castrates results in decreased production efficiency and reduced feed efficiency (Newell and Bowland, 1972). Entire males also have less total fat than do castrates and the lipid fraction of boars contains a higher proportion of unsaturated fatty acids (Malmfors et al., 1978). Castrates tend to have less muscle tissue than do entire males. Therefore, more of the ingested energy goes to fat tissue than to muscle tissue.

Boar taint can mainly be attributed to the accumulation of 3-methyl-indole (skatole) and the testicular steroid androstenone (5α-androstenone) in adipose tissue (Lundström et al., 1988). Skatole has a faecal odour and in monogastric animals, is produced from L-tryptophan by microbes in the large intestine (Yokoyama and Carlson, 1979). Entire males have higher fat skatole levels than do castrates and gilts, possibly because testicular steroids cause increased gut formation (Claus and Raab, 1999) or reduced liver degradation of skatole (Babol

et al., 1998). The biosynthesis and secretion of androstenone are parallel with those of testicular steroid hormones (Gower, 1972; Claus et al., 1983). Androstenone is then transported to the saliva glands where it binds to a specific binding protein, pheromaxein (Booth and White, 1988). After its release into the saliva, androstenone acts as a pheromone, stimulating both attraction to the male and receptive mating behaviour in oestrous females (see Booth and Signoret, 1992). Owing to its hydrophobic character, androstenone is accumulated in fat (Brooks and Pearson, 1986) and after slaughter, when meat from entire males is heated, fat androstenone will cause an odour of 'boar' or 'urine'.

Many strategies for dealing with the boar taint problem have been suggested. Since skatole levels are strongly influenced by environmental factors, such as water levels in feed, cleanness, ventilation, feed composition and feed antibiotics (Lundström et al., 1988; Hansen et al., 1994; Claus et al., 1994; Andersson et al., 1997), management systems can attribute to reducing its levels in fat. Fat androstenone levels are, however, less affected by management, although in same-sex groups, the presence of a strongly tainted boar has been seen to increase the taint levels of the other males (Giersing et al., 2000). Other approaches to reduce fat androstenone, such as immunocastration (immunization against gonadotropins, Bonneau et al., 1993), treatment with GnRH depot formulation (Schneider et al., 1998) and treatment with a GnRH agonist (Xue et al., 1994), have been investigated. There are breed differences in tissue levels of boar taint compounds (Xue et al., 1996) and Sellier et al. (2000) have shown that fat androstenone levels can be reduced by selection.

Aims

The routine castration of male piglets is performed in many countries to avoid boar taint in meat. In addition to causing great concern in terms of animal welfare, castrates have reduced feed efficiency and more fat than do entire males. Since boar taint is closely associated with male sexual maturation, and there are indications that domestic pigs remain responsive to photoperiod, the use of artificial light regimens to delay puberty could be a non-invasive method to reduce boar taint. The primary objective of this study was therefore to investigate whether photoperiod influences reproductive development and thus, boar taint in entire males.

In mammals, pineal melatonin mediates photoperiodic information and synchronizes circannual rhythms. However, in pigs, there have been considerable difficulties in demonstrating a typical melatonin profile which reflects the period of darkness. An additional objective was therefore to find and evaluate a reliable assay for porcine melatonin, and to investigate whether pigs are capable of transforming photoperiodic information into an endocrine signal (the 'circadian melatonin profile'). To be able to evaluate lighting requirements on farms we wanted to measure melatonin secretion in relation to the light-dark cycle in a conventional stable environment. Further, as the European wild boar is a seasonal

short-day breeder, we wanted to compare melatonin profiles between wild and domestic pigs in order to establish the level at which artificial selection has altered the seasonal breeding pattern in pigs.

Methodological Considerations

The thesis is based on five studies (papers I-V) referred to by their Roman numerals. Detailed descriptions and references of materials and methods used are given separately in each paper.

Timing of male puberty

In papers I and II, intact crossbred males born during winter (i.e. the short-day photoperiod) were divided into three groups after weaning. A control group (the 'natural spring' group) were maintained in a room with windows, and exposed to approximately 150 lx of supplementary light during working hours. The other two groups were maintained under artificial photoperiods in light-sealed rooms. The artificial light sources, fluorescent tubes with a spectrum closer to sunlight than standard fluorescent lamps, created a light intensity of approximately 1400 lx at the level of the pigs' eyes. The photoperiod was adjusted weekly. Both experiments began in January/February (in January/February at 60°N there are 6-7 h of daylight/day), when the pigs were between 5 and 9 weeks old, and continued until mid-June.

In other short-day breeding ungulates, exposure to a decreasing photoperiod (i.e. long days followed by short days) is necessary to correctly time puberty (e.g. female sheep, Yellon and Foster, 1985). Therefore, in Paper I, the 'artificial autumn' group was started off with 17 hours of light and 7 hours of darkness (equivalent to July at 60°N); thereafter, the photoperiod was gradually decreased. The 'artificial spring' group were exposed to an increasing photoperiod that was equivalent to the natural changes in photoperiod experienced by the 'natural spring' group (Figure 2).

After having observed the outcome of Paper I, different light regimens were used in the following study (Paper II). Conditions for the 'natural spring' group were as in the first study, but the 'artificial summer' group were exposed to a photoperiod that simulated natural changes in day length from the vernal equinox (mid-March) to August at 60°N. The 'artificial winter' group were exposed to a photoperiod that simulated natural changes in photoperiod from the autumnal equinox (mid-September) to February at 60°N. By this approach, both the artificial light regimen groups were exposed to the same shift in photoperiod at the start of the experiment (Figure 2).

In both papers I and II, the animals were weighed and blood was collected biweekly until slaughter at 115 kg. The plasma samples were analysed for content of total testosterone using a radioimmunoassay (Diagnostic Product Corporation, Los Angeles, CA, USA, 1982), oestrone-sulphate using double antibody

enhanced luminescence immunoassay, total thyroxin using enhanced luminescence immunoassay (Amerlite, Johnson and Johnson Clinical Diagnostics Ltd, Amersham, UK, 1994) and prolactin using an end-point enzyme immunometric assay for canine prolactin (Diagnostic Product Corporation, Los Angeles, CA, USA, 1994) and a porcine standard. In Paper I, plasma 5α-androstenone was analysed by double antibody-enhanced luminescence immunoassay.

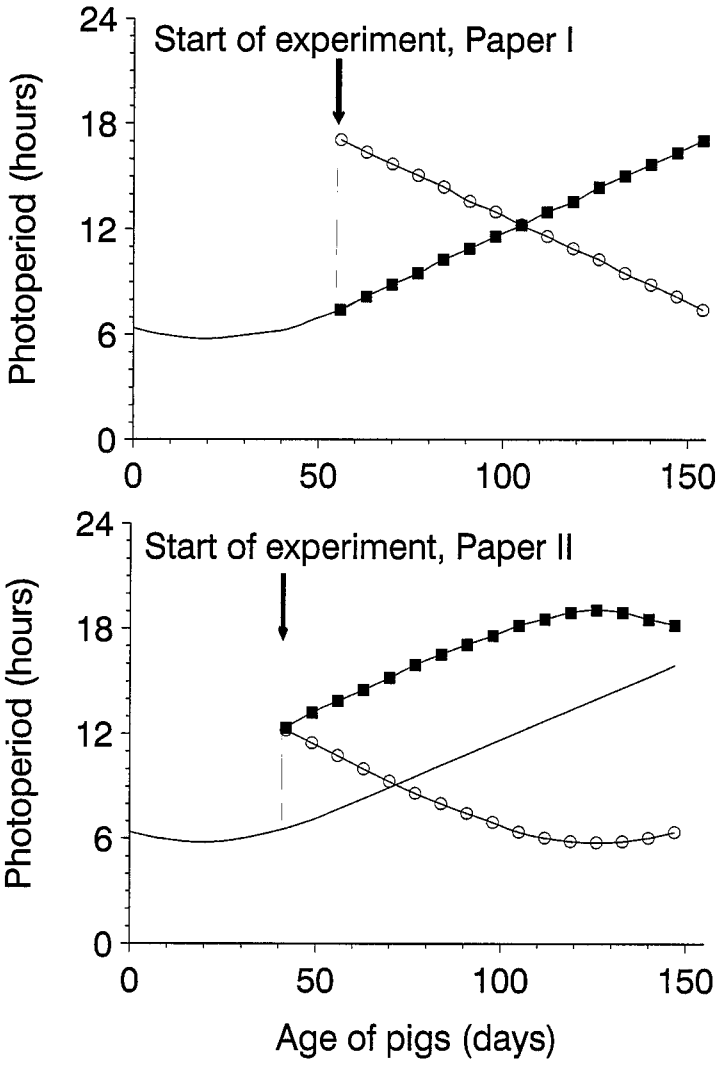

Figure 2. Light regimens in papers I and II. Unmarked lines represent natural photoperiod at 60°N. Filled squares show 'artificial spring' in Paper I and 'artificial summer' in Paper II. Open circles show 'artificial autumn' in Paper I and 'artificial winter' in Paper II. Control groups were exposed to the natural photoperiod in both studies.

To estimate the activity of the anterior pituitary gland and the testicular Leydig cells, LH and testosterone release after GnRH challenge was studied in Paper II. Using information about the temporal pattern of testicular steroids from Paper I, nine animals were allocated to individual pens in March and plasma samples were collected every 15 minutes for 6.5 h. At this time, the 'natural spring' group were exposed to 12 h of light, the 'artificial summer' group to 18 h of light and the 'artificial winter' group to 6 h of light. After 1.5 h of sampling a GnRH-analogue, Buserelin (Receptal® vet.) was injected intravenously. Since we hypothesized that at this age (100-110 days), some animals had started the pubertal increase in Leydig cell numbers, whereas other animals still had pre-pubertal capacity steroid synthesis, a fairly high dosage of GnRH (50 μg) was used to distinguish between pubertal and pre-pubertal animals. After the sampling period the catheter was removed and the animals were immediately returned to their group. Testosterone was analysed as above and LH was analysed using a heterologous radioimmunoassay.

The testes, epididymides and bulbourethral glands were weighed after slaughter. Lean meat percentage was estimated (papers I and II). In Paper I, reproductive organ development relative to carcass weight was estimated.

Skatole concentration in fat was measured with spectrophotometry and the concentration of 5α-androstenone in fat was determined by gas chromatography (papers I and II). A trained sensory-evaluation panel of five (Paper II) to seven (Paper I) assessors scored fat samples heated to melting point for boar taint on a scale of 1 (no taint) to 5 (very strong taint).

To gain a better estimate of pubertal maturation, tissue specimens from two parts of the testes (proximal and distal) and semen from the cauda epididymis were collected for light microscopy in Paper II. Cross-sections of testicular tubules were examined for maturation of the seminiferous epithelium and in the semen samples, special attention was given to abnormalities related to sexual maturation of the spermatozoa. The maturity of spermatogenesis was classified into four classes: not in puberty, immature, almost mature or mature.

All statistical analyses were made by analysis of variance (ANOVA) using the MIXED procedure of SAS/STAT (SAS Institute Inc., 1989 and 1997) and the least squares means option was used to compare different means. Treatment and group within treatment were tested for difference in all models. For repeated measurements, effect of time of measurement, individual animal within treatment, and the interaction between treatment and time of measurement were considered. Effects of litter (mother) and age, where appropriate, were included in the model for measurements obtained at slaughter. Spermatogenesis maturity scores were ranked before analysis, which equals a Kruskal-Wallis test. The taint scores were tested for differences of treatment, group within treatment, and assessor.

Melatonin

To establish whether domestic pigs show a circadian melatonin profile in agreement with the light-dark cycle, three male siblings from each of six litters in papers I and II, were sampled hourly for 24 h (Paper III). In addition, three

younger, unrelated males were sampled for 48 h, to study repeatability of the circadian rhythms. The animals were kept in their groups until the day before blood sampling, when they were allocated to an individual pen in the same room. During the last day of February and the first days of March, when the three boars used for a 48-h study were 12 weeks old blood samples were collected at 2-h intervals for 48 h. Blood was sampled with a jugular catheter and low-intensity red light (<5 lx) was used in order to facilitate night sampling. In May, when the remaining 18 animals were 5 months old, blood samples were collected hourly for 24 h. At this point, the 'natural long-day' group were receiving 16 to17 h of light, the 'artificial long-day' group were exposed to 17 h of light, and the 'artificial short-day' group were given 8 h of light

A comparison of melatonin profiles between European wild boars (both males and females) and crossbred domestic gilts was made in southern Finland during four seasons (Paper IV). The blood samples were collected at the vernal and autumnal equinoxes and the summer and winter solstices. The wild boars were purebred European wild boars living in a semi-natural environment. During the experiment the wild boars were housed under natural lighting conditions in small individual stalls located outdoors. The domestic gilts were housed indoors in individual pens. Lights were switched on between 06:00 and 18:00, except during the winter sampling when lights were on between 09:00 and 21:00, because of technical difficulties with the catheters. The indoor supplementary light at the level of the pigs' eyes was approximately 120 lx. The light intensity was measured at each blood sampling using a digital light intensity meter. Blood samples from the wild boars were obtained via medial saphenous arterial catheters and from the domestic gilts, via ear vein catheters. Blood samples were collected at 2-h intervals for 48 hours.

To further study melatonin levels in a conventional piggery environment, 31 female Yorkshire pigs, and three Hampshire boars were bled during November-February at 60°N (Paper V). In August, 48 crossbred piglets, 24 females and 24 males (10-14 weeks of age), offspring of four gilts, four sows and two boars from the winter bleeding, were bled. The animals were kept under standard stable management, with windows and additional light (light bulbs) during working hours. Daytime light intensity varied, depending on weather conditions, between 150 lx and 300 lx, with occasional higher intensities. Overall night-time light conditions for the gilts and piglets were very low (< 5 lx). The sows and boars had low-intensity night illumination (light bulbs), creating a night-time light intensity between 5-10 lx. Three daytime samples and three night-time samples from each animal were collected by jugular venipuncture between 10:00 and 15:00 and 22:00 and 03:00, respectively, with approximately 1-h intervals. To facilitate sampling during the night, dim red light and a small flashlight were used.

Plasma melatonin samples in Paper III were first analysed by radioimmunoassay (Nichols Institute Diagnostics BV, Wijchen, The Netherlands), using a rabbit anti-melatonin antiserum, according to the manufactures' instructions. Before assay, controls and samples were extracted

twice in diethyl ether. Serial dilutions of pig plasma containing high concentrations of melatonin produced displacement curves parallel to the standard curve. The intra-assay and inter-assay coefficients of variation for 16 assays were 6.3 % and 14.7 % (42.4 pg/ml), respectively and the sensitivity of the assay was 1.3 pg/ml. The specificity of the assay has been evaluated by Nichols Institute Diagnostics and shows 1 % cross-reactivity with 6-hydroxy-melatonin, whereas all other measured compounds show less than 0.05 % cross-reactivity. Selected samples were re-analysed using the same assay on a later occasion, in order to confirm previous findings.

Although conventional assay evaluation indicates that the Nichols assay performs well with pigs, we were not content with the results. Therefore, all melatonin samples in papers III-V were reanalysed by a radioimmunoassay for human melatonin (Bühlmann Laboratories AG, Switzerland), with a caprine against melatonin conjugated to bovine thyroglobulin anti-melatonin antiserum, Kennaway G280 (Vaughan, 1993), according to the manufactures' instructions. Before assay, controls and samples were extracted twice in diethyl ether. Serial dilutions of pig plasma containing high concentrations of melatonin produced displacement curves parallel to the standard curve. The intra-assay and inter-assay coefficients of variation for 20 assays were 13.1 % and 8.2 % (2.4 pg/ml), and 8.4 % and 8.0 % (19.5 pg/ml), respectively. The sensitivity of the assay was 0.3 pg/ml. The specificity of the assay has been evaluated by Bühlmann Laboratories AG and all measured compounds show less than 0.05% cross-reactivity.

For a circadian 'reference', plasma cortisol, which in pigs shows a morning peak and an evening nadir (Barnett et al., 1981; Janssens et al., 1995), was analysed by a luminescence immunoassay (Amerlite, Kodak Clinical Diagnostics Ltd., UK) in Paper III. Standards and controls were provided in human plasma. Before assay, standards, controls and samples were extracted twice in diethyl ether.

In study III and V, the statistical analyses were made by ANOVA using the MIXED procedure of SAS/STAT (SAS Institute Inc., 1997) and the least squares means option was used to compare different means. Melatonin concentrations among the matched siblings (Paper III) were tested for differences between phase, treatment, father, litter nested with father, the interaction between phase and treatment, and the interaction between phase and father. Plasma cortisol concentrations were tested for differences between phase, treatment, father, litter nested with father, and the interaction between phase and treatment.

In Paper V, melatonin from the winter bleeding was tested for effects of time of day, sampling order within time of day, sex and age within sex. Melatonin of the gilts was also tested for the effect of mother (litter). Melatonin levels from the summer bleeding were analysed for effects of time-of-day, sampling order within time of day, sex, father, mother (litter) within father and the interaction of mother litter) and time of day.

In the wild boar study (Paper IV), the statistical analyses were carried out using the Stata Intercooler, version 5.0 statistical package (Stata Corporation, Texas, U.S.A.). In the model, effects of season, breed, individual animal and night were

checked for source of variation. An ANOVA with repeated measures (Gill and Hafs, 1971) was used to study variation in night-time melatonin levels. Differences in duration of nocturnal melatonin secretion between summer and winter in both animal groups were analysed using Student's *t*-test.

Results and Discussion

Timing of male puberty

As the wild ancestor of modern pig breeds is a seasonal short-day breeder, we hypothesized that a decreasing the photoperiod would stimulate, and a increasing the photoperiod would inhibit, male reproductive development. After weaning, when winter-born male piglets were exposed to either an 'artificial autumn' or an 'artificial spring' treatment, or kept under a 'natural spring' conditions, the longitudinal measurements of hormones showed an accelerated increase of testosterone in the 'artificial autumn' group. A parallel increase in oestrone-sulphate was not observed in this group. In fact, at the time of the peripubertal increase in testicular steroid production, oestrone-sulphate was lower in the 'artificial autumn' group. In the 'artificial spring' and 'natural spring' groups, secretion of testicular steroids was parallel, as previously observed (Schwarzenberger, 1993). Plasma prolactin was generally low, as reported in other studies (Ravault et al., 1982; Meijer et al., 1988). However, at the start of the experiment, when the 'artificial autumn' group were exposed to long days and the other groups were exposed to short days, prolactin levels were higher in the 'artificial autumn' group. Some months later, when the 'artificial spring group' and the 'natural spring' group were exposed to long days and the 'artificial autumn' group, to short days, the situation was the reversed.

At slaughter, the weight of the epididymis was lower in the 'artificial autumn' and 'artificial spring' groups than in the 'natural spring' group, and testis weight tended to be lower in the 'artificial autumn' group than in the 'natural spring' group. Correspondingly, the percentage of proximal droplets was higher in the 'artificial autumn' group than in the 'natural spring' group, indicating that animals in the 'artificial autumn' group were less mature. The 'artificial autumn' and the 'artificial spring groups had lower meat percentage than did the 'natural spring' group. Skatole concentrations in fat were higher in the 'natural spring' group than in the 'artificial autumn' and 'artificial spring' groups, and fat androstenone tended to be higher in the 'natural spring' group than in the 'artificial autumn' group.

Together, the results from Paper I showed, that although testosterone increased at an earlier age in the 'artificial autumn' group, the animals in the 'natural spring' group were more sexually mature at slaughter. The 'artificial autumn' treatment therefore appears to have delayed puberty instead of having stimulated it. These results are the opposite of those reported in a previous study comparing entire males raised under natural spring or autumn conditions (Claus and Weiler, 1985). Our 'artificial autumn' group had been switched from 7 h of daylight to 17 h of

artificial light, and this abrupt increase in photoperiod may have negatively affected pubertal development, leading to a disruption of the parallel secretion of testicular steroids and the lower weight of reproductive organs at slaughter. This indicates that by the time of weaning, male piglets have received photoperiodic information which can influence the response to the subsequent photoperiod. Photoperiodic information may already be mediated to the foetus by maternal melatonin and may influence the timing of puberty (Siberian hamster, Stetson et al., 1989; red deer, Adams et al., 1994).

In Paper II, both the 'artificial summer' group and the 'artificial winter' group were switched, at the start of the experiment, from 6.5 h of daylight to 12 h of artificial light, while the controls were again a 'natural spring' group. When the animals were between 9 and 11 weeks old, there was a transient peak in testosterone, in both the 'artificial summer' and the 'artificial winter' groups but not in the 'natural spring' group. There was no increase in oestrone-sulphate at this time. After the peak, testosterone remained at pre-pubertal levels until the pubertal increase. Just before slaughter, testosterone concentrations were higher in the 'artificial winter' group than in the 'artificial summer' and 'natural spring' groups. Thyroxin decreased with age, as has been observed in other species (e.g. Irvine, 1984; Reimers et al., 1990). There were no significant group differences in plasma prolactin, but some high values were measured in both the 'artificial summer' and the 'natural spring' groups when they were exposed to long days. All the nine matched siblings (three from each treatment), which were injected using a GnRH analogue, showed a strong response in LH and testosterone, and there was no difference between the treatments. Live-weight gain was lower in the 'natural spring' group than in either the 'artificial summer' group or the 'artificial winter' group.

At slaughter, the bulbourethral gland weight was lower in the 'artificial summer' group than in the 'artificial winter' group, and tended to be lower than in the 'natural spring' group. The fixed percentage of proximal droplets was higher in the 'artificial summer' than in both the other treatment groups. Estimates of the maturation of the seminiferous epithelium and sperm morphology showed that the 'artificial winter' group had a more mature spermatogenesis than did the 'artificial summer' and 'natural spring' groups Also, the 'artificial summer' group tended to have less developed spermatogenesis than did the 'natural spring' group. Animals in the 'artificial winter' group were therefore more sexually mature than were the males in the 'artificial summer' group; the animals under 'natural spring' conditions were somewhere in between. Fat concentrations of skatole and androstenone were somewhat higher in the 'natural spring' group. The sensory evaluation showed a significantly lower taint score for animals in the 'artificial summer' group than in the 'artificial winter' group (Table 1).

There was no single simple measurement of male pubertal development that consistently reflected the influence of photoperiod. Longitudinal measurements of testosterone (papers I and II) and testicular histology (Paper II), in combination with sperm morphology (papers I and II), appeared best to reveal differences between treatments. The weight of accessory sex organs also showed differences

between treatments (papers I and II). In many seasonal breeders, weight or spatial measurements of the testes show prominent seasonal changes and have been useful in research (see e.g. red deer, Lincoln, 1971; rock hyrax, Millar and Glover, 1973; ferret, Neal et al., 1977; rhesus monkey, Chik et al., 1992). However, weight, length or width of the testes did not reveal any significant differences between treatments in our studies. The endocrine response to GnRH challenge did not reveal any difference between treatments, but since only nine animals were studied, photoperiodic effects on the maturation of the pituitary-testicular axis cannot be dismissed (Paper II).

Table 1. Maturity of spermatogensis, bulbourethral gland weight and boar taint in pigs exposed to an 'artificial summer' or an 'artificial winter' (Paper II).

	Summer	Winter	
Frequency of mature spermatogenesis	13 %	57 %	***
Bulbourethral gland (g) (mean ± SD)	46 ± 10	63 ± 24	**
Taint evaluation (1-5) (mean ± SD)	1.7 ± 0.6	2.5 ± 1.0	*

SD = standard deviation.
* = P<0.05, ** = P<0.01 and *** = P<0.001.

All three groups that were subjected to an abrupt increase in photoperiod when allocated to the experimental rooms showed a transient increase in plasma testosterone, but this was not accompanied by a parallel increase in oestrone-sulphate (papers I and II). A similar phenomenon has previously been observed in adult boars exposed to an abrupt shift in photoperiod (Claus et al., 1985). Gradual (natural) versus abrupt changes in photoperiod have shown differential effects on reentrainment of circadian rhythms, as well as on reproductive responses in Siberian hamsters (Gorman et al., 1997). Oestrogens are synthesized in porcine testicular Leydig cells from 19-norandrogens by aromatase (Raeside et al., 1989). The discrepancy between testicular androgen and oestrogen secretion may be due to a time delay in the reaction to the photoperiodic shift between the hypothalamus-pituitary-testicular axis and some other (possibly endocrine) factor which plays a role in the aromatization in testicular tissue. Normally, aromatization takes place in the Leydig cells but there are indications that the Sertoli cells have the capacity to aromatize androgens during sexual development (Dorrington et al., 1978). The animals which experienced a large photoperiodic shift (i.e. of 10 h) showed a delay in sexual development. When two groups were exposed to a smaller, abrupt increase in photoperiod (5 h), effects on the endocrine pattern were noted but no effects of reproductive development could be

ascribed to the shift in photoperiod. Some authors have suggested that oestrogens are important for maturation of accessory sex glands during sexual development (Booth, 1983) and that oestrogens in boar semen stimulate the reproductive tract of the female pig at mating (see Claus, 1990).

The difference in growth rate between animals in a standard stable environment and animals under artificial light regimens (Paper II) is difficult to explain. In papers I and II, all three rooms were in one wing of the stable, with the windows sealed in two of the rooms. Therefore, except for the light environment, very few factors could be different between the groups. Since animals in a standard stable environment compared with animals under artificial light regimens had a higher meat percentage in one study (Paper I) and a lower growth rate in the other (Paper II), no general conclusions could be drawn on the metabolic effects of light environment. Plasma thyroxin showed little variation between groups and was difficult to correlate with the differences in growth rate. Fat concentrations of skatole and androstenone were higher among animals kept in a standard stable environment and the sensory evaluation correlated better with reproductive parameters than with the fat concentrations of the compounds considered to be the main contributors to boar taint (Paper II).

Together, these studies show a stimulating effect of short days and a corresponding inhibiting effect of long days on boar puberty, which is well in agreement with the seasonal breeding pattern of the European wild boar (Mauget, 1985). A clear photoperiodic effect on spermatogenesis and on sensory score (Paper II) indicates that long artificial daylight can delay sexual development and thus reduce the expression of boar taint without any major negative effects on production traits. These studies also show that pre-weaning photoperiodic conditions influence the response to the light regimens (Paper I). Therefore, lighting programmes in pig stables must be viewed in their entirety.

Melatonin

Measuring porcine melatonin has proven to be difficult. Assay systems can appear to be working well when results do not show a typical melatonin profile (McConnell and Ellendorff, 1987; Reiter et al., 1987; Minton et al., 1989; Minton and Cash, 1990; Peacock et al., 1991, and 1995; Diekman et al., 1992; Griffith and Minton, 1992; Green et al., 1996, and 1999; Bassett, 1996; Bollinger et al., 1997; Diekman and Green, 1997; Lewczuk and Przybylska-Gornowicz, 1997; Klupiec at al., 1997; Bubenik et al., 2000). We experienced the very same thing when we measured melatonin by two commercial melatonin radioimmunoassays in plasma from peripubertal boars kept under conditions of long or short artificial photoperiod or natural long days. According to standard assay evaluations, both assays performed well, but only the Bühlmann assay measured low to undetectable concentrations during the light phase and elevated melatonin levels during the dark phase, for all individuals. This emphasizes that careful and critical evaluations must be performed when biochemical methods are applied to a previously untested species. Low peripheral levels (papers III and V) and unspecific disturbance in porcine blood (Klupiec et al., 1997) are probably the

main factors causing the problems of measuring porcine melatonin with radioimmunoassays. Unfortunately, these disturbances are not always revealed by conventional technical evaluation steps such as parallelism and sensitivity. Thus, to confirm these findings, very sensitive non-immunologically based methods therefore need to be applied.

Using the Bühlmann assay, we could demonstrate a typical circadian melatonin profile, with low levels during the day and increased secretion during darkness, as described by Paterson et al. (1992b). Since the Bühlmann assay is very sensitive we could measure dark-phase melatonin concentrations even in animals with a very low nocturnal secretion, which has been difficult in other studies (Paterson et al., 1992b; Klupeic et al., 1997). Dark-phase melatonin concentrations were higher in the 'natural long-day' group than in the 'artificial long-day' and 'artificial short-day' groups (Figure 3). The six sons of Father I had higher dark-phase melatonin concentrations than did the nine sons of Father II and the two sons of Father III. An evaluation of individual melatonin profiles showed that the duration of the elevated melatonin phase was synchronized to the period of darkness in the artificial light regimens. The recorded timing of the evening increase and of the morning decline in melatonin among the animals in the 'natural long-day' varied between individuals, but for all animals in this group, both the evening increases and the morning decreases in melatonin took place when the supplementary light was off, and before sunset and after sunrise. The duration of the elevated melatonin phase was 15-17 h in the 'artificial short-day' group, 7-12 h in the 'artificial long-day' group and 12-14 h in the 'natural long-day' group (Figure 3).

In all three groups, cortisol showed a typical diurnal pattern of circadian rhythmicity, with a morning peak and an evening nadir (Barnett et al., 1981; Janssens et al., 1995). The 'natural long-day' group had higher light-phase peak concentrations in cortisol than did the 'artificial long-day' and 'artificial short-day' groups, but there was no difference in cortisol levels between treatments during the dark phase (Figure 3).

After having observed the abstruse results from the first assay (Paper III), we hoped that synchronous measurement of melatonin in both wild and domestic pigs would show whether the Bühlmann assay is practicable for measuring porcine melatonin. It would be highly unlikely that the temperate, seasonally breeding European wild boar would not express a typical melatonin profile entrainable by photoperiod. The study was to reveal whether domestication has altered melatonin secretion in pigs.

The study showed a circadian melatonin profile entrained by the photoperiod for both the European wild boars and the domestic gilts. There was no apparent difference between wild and domestic pigs, although large differences between individual animals in the increase of nocturnal melatonin secretion were again observed (Figure 4).

When plasma melatonin was compared among a larger number of domestic pigs in a conventional piggery, night-time melatonin concentrations were clearly higher than daytime melatonin levels. An unexpected finding was that the three

adult boars had higher daytime melatonin concentrations than did the 31 sows and gilts. Despite of the low numbers of animals per litter, the gilts from one of the litters had significantly higher plasma melatonin concentrations than did the gilts in three other litters.

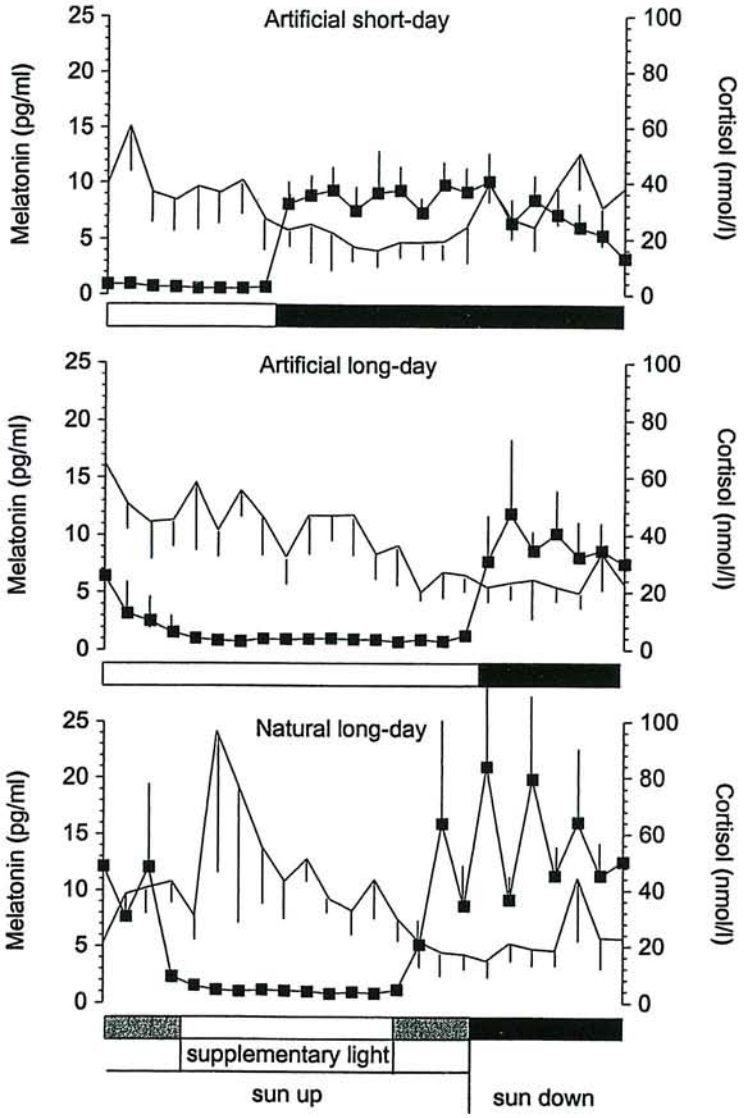

Figure 3. Circadian profiles of melatonin (filled squares) and cortisol (no markers) in plasma (mean ± standard error of the mean [SEM]), in the 'artificial short-day' group, the 'artificial long-day' group and the 'natural long-day' group. Black horizontal bars indicate dark phase, white horizontal bars indicate light phase, and grey bars indicate dawn and dusk in the 'natural long-day' group. (Paper III)

Among the 48 piglets tested, there was an interaction between light phase and litter, as night-time but not daytime melatonin concentrations differed between litters. However, in contrast to Paper III, the effect of father in Paper V was not quite significant (P=0.12). There was no difference in melatonin concentrations between the male and female piglets.

A relatively low amplitude of dark-phase melatonin secretion seems characteristic to pigs (papers III-V), and has probably been one factor which has contributed to the difficulties of measuring porcine melatonin in blood. A genetically determined capacity for pineal melatonin synthesis is responsible for the individual levels of dark-phase melatonin in sheep (Zarazaga et al., 1998a; Zarazaga et al., 1998b). Differences in nocturnal melatonin levels between sibling groups in papers III and V, suggest that the genetic effect is important also in pigs.

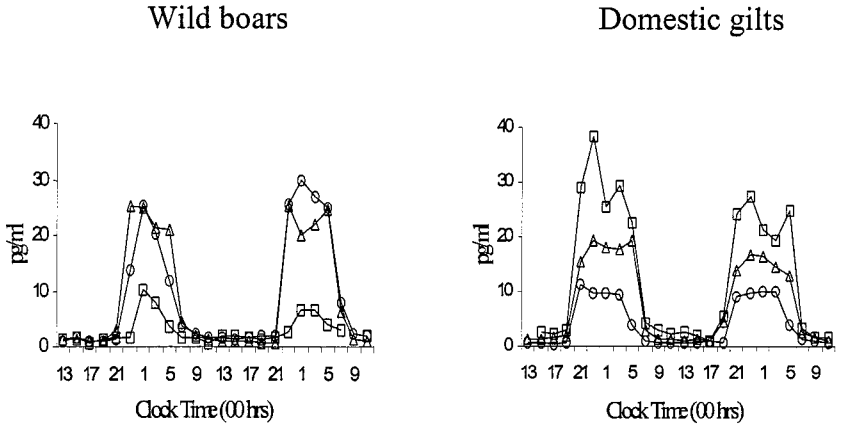

Figure 4. Individual melatonin patterns in summer in three wild boars and three domestic gilts (Paper IV).

In the rat, the rate of melatonin synthesis is regulated mainly by NAT (Klein et al., 1997) but in the Siberian hamster, which has higher levels of nocturnal melatonin during short days than during long days, there are indications that it is the pineal enzyme hyroxyindole-*O*-methyltranseferas (turning *N*-acetyl-serotonin into melatonin) which drives the photoperiodic changes in nocturnal melatonin (Ribelayga et al., 2000). The pineal enzyme(s) responsible for the low levels of nocturnal melatonin secretion in pigs is not known and, in contrast to findings reported for the Siberian hamster, we did not find any seasonal differences in levels of nocturnal secretion (Paper IV). The lower levels of nocturnal melatonin in animals kept under high-intensity light conditions (Paper III) may point to quality effects, such as light intensity and the gradual changes of dawn and dusk, rather than to a duration effect of the light environment on porcine melatonin

synthesis. An effect of light environment was also observed for peak concentrations of the circadian cortisol rhythm.

The results in this thesis suggest that the melatonin rhythm of the pig does not differ greatly from that of other species (see Reiter, 1993). Possible exceptions are the higher diurnal plasma concentrations in three boars sampled by jugular venipuncture (Paper V), but these may have been caused by disturbances due to the sampling method or, more likely, a cross-reaction with some unknown compound(s). Among the animals kept in a standard stable environment during long days, both the natural photoperiod and the supplementary lighting shaped the melatonin profile. The duration of the nocturnal melatonin secretion therefore did not directly correspond with either the longer natural photoperiod or the duration of the light regimen (Paper III). As the seasonal changes in the duration of melatonin secretion are mainly dictated by the inhibitory effects of light (Picazo and Lincoln, 1995), this indicates that the size and placing of windows and the light intensity of the supplementary lighting will influence the melatonin profile and therefore, the response to seasonal changes. Interestingly, when wild boars kept outside and domestic gilts kept indoors were compared at different times of the year, no major differences were observed (Paper IV). However, the 2-h sampling interval in this study prevented any detailed comparison of nocturnal duration of the elevated phase.

These studies show that pigs, both wild and domestic, express a typical circadian melatonin profile with a clear nocturnal increase of pineal secretion (papers III and IV), which is a prerequisite to mediating photoperiodic effects on reproduction as well as other seasonal rhythms in mammals. The nocturnal increase in melatonin secretion is relatively low (papers III-V), but as there is no apparent difference in melatonin secretion between wild and domestic pigs (Paper IV), it is unlikely that impaired pineal synthesis is the cause of reduced seasonality in the breeding pattern of domestic pigs.

Practical implications

This thesis, together with studies on photoperiodic effects on female puberty (Paterson and Pearce, 1990) and boar fertility (Claus et al., 1985), shows the stimulative effect of short days and a corresponding inhibiting effect of long days on reproductive parameters in pigs.

Although these studies are insufficient for making detailed recommendations for light regimens on commercial pig farms, some general points may be outlined. Artificial long days can delay pubertal development and thus reduce boar taint among male finishing pigs in same-sex groups (Paper II). Since the photoperiod of the light regimens would be as long as, or longer than, the natural photoperiod, stable windows would probably not be a problem as long as the light intensity is sufficiently high. As long as large shifts in photoperiod are avoided at the introduction to the new stable (Paper I), artificial long days would either delay male puberty or have a neutral effect, depending on whether the male piglets have previously been exposed to short days or long days, respectively. Effects of

exposure to long days over a prolonged period of time have not been studied in pigs, but would probably only result in photorefractoriness.

Stable windows make the use of artificial light regimens to stimulate pig reproduction more complicated. Only during the darker part of the year can artificial light regimens be used to create long-day exposure followed by short days and thus stimulate reproduction. Such a programme could be used to stimulate puberty in gilts (Paterson and Pearce, 1990), but since many other stimulative options, such as contact with a boar, or relocation, are available, short daylight regimens would probably be of limited additional benefit. Unless the period of natural light can be regulated, it is difficult to see how artificial short days could be used to decrease the problems of seasonal infertility during summer and early autumn, as the long natural photoperiod probably would undermine the influence of the lighting regimens. If, however, the access to natural light could be restricted to 6-8 h per day (short days), a gradual shift from artificial long days to natural short days 1-2 months before mating or artificial insemination (AI) may possibly have a positive effect on the pregnancy rate. Mimicking short days by exogenous administration of melatonin (Paterson et al., 1992a) could have a positive effect on seasonal infertility problems, if the melatonin is administered well in advance of the time of desired effect. Examples of other management tools to reduce problems with seasonal infertility are cooling systems (Wettemann and Bazer, 1985), feeding (Love et al., 1995) and exogenous oxytocin (Pena et al., 1998).

Seasonality and domestication

Many mammals, both seasonal and non-seasonal breeders, show a photoperiod-regulated seasonal prolactin secretion, which is high during long days and shows a nadir during short days, a pattern also observed in European wild boars (Ravault et al., 1982). We measured low concentrations of prolactin (papers I and II) but somewhat higher levels were observed among animals kept under long-day conditions in both studies. As neither a typical circadian melatonin rhythm nor a circannual prolactin rhythm had previously been described for modern pig breeds, the hypothesis that domestication has disrupted all seasonal patterns in domestic breeds has been proposed (see, e.g., Green et al., 1996). Since both wild and domestic pigs express a typical circadian melatonin rhythm entrainable by photoperiod (papers III and IV) and functional melatonin binding sites in the pars tuberalis have been identified in neonatal pigs (Williams et al., 1999), it is unlikely that impaired pineal synthesis is the cause of reduced seasonality in the breeding pattern or prolactin secretion of domestic pigs. The reduction in seasonality caused by artificial selection of pigs therefore probably occurs post-pineally, at the level of the hypothalamus or the pituitary, respectively. In contrast to the seasonal regulation of GnRH release, photoperiod, through melatonin, acts directly on the pituitary gland, and entrains the circannual prolactin rhythm (Lincoln, 1999). During the domestication of pigs, alterations of the circannual prolactin rhythm may therefore have occurred separately from effects on the reproductive system.

The term 'seasonal reproduction' usually refers to a restriction of breeding activities to a limited period of the year. Domestic pigs can breed all year around, yet they remain responsive to photoperiod. Specific photoperiod cues are not obligate prerequisites to reproductive success in pigs; instead, photoperiod appears to act like a modifier of fertility, effects of which may be more, or less, discernible, depending on management factors (Love et al., 1995). Therefore, photoperiodism probably evolved in species with restricted mating and birth periods, but the retinal-pineal-hypothalamic pathway can be functional also in species which breed opportunistically, such as the domestic pig.

Conclusions

- Photoperiod influences the timing of puberty in boars. Short days stimulate pubertal maturation of spermatogenesis compared with long days.
- An artificial long-day light regimen can, compared with short days, reduce the sensory score of boar taint at slaughter in entire male pigs. However, by the time of weaning, male piglets have acquired photoperiodic information which may affect their response to the subsequent photoperiod.
- An abrupt increase in photoperiod can cause a transient dissociation in the normally parallel testicular secretion of androgens and oestrogens in peripubertal boars.
- Pigs express a typical circadian melatonin profile with clearly elevated secretion during darkness and low to undetectable levels during the light phase. There are no apparent differences between European wild boars and domestic breeds in melatonin secretion.
- The circadian melatonin rhythm is entrained by photoperiod in pigs, and in the stable, natural photoperiod and supplementary lighting interact in shaping the melatonin profile.
- Characteristic for pigs is a low increase of nocturnal melatonin secretion. The variation between individuals in melatonin levels during the night appears to be genetically based.

References

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

Adam CL, Kyle CE, Young P and Hirst DJ (1995) Influence of prenatal photoperiod on postnatal plasma concentrations of progesterone and prolactin in female red deer (*Cervus elaphus*) reared in constant equatorial photoperiod. J Pineal Res. 18: 77-83.

Allrich RD, Christenson RK, Ford JJ and Zimmerman DR (1983) Pubertal development of the boar: Age-related changes in testicular morphology and in vitro production of testosterone and estradiol-17 beta. Biol Reprod. 28: 902-909.

Almeida OF and Lincoln GA (1984) Reproductive photorefractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion. Biol Reprod. 30: 143-158.

Andersson K, Schaub A, Andersson K, Lundström K, Thomke S and Hansson I (1997) The effects of feeding system, lysine level and gilt contact on performance, skatole levels and economy of entire male pigs. Livest Prod Sci. 51: 131-140.

Aurich C, Sieme H, Hoppe H and Schlote S (1994a) Involvement of endogenous opioids in the regulation of LH and testosterone release in the male horse. J Reprod Fertil. 102: 327-336.

Aurich C, Schlote S, Hoppen HO, Klug E, Hoppe H and Aurich JE (1994b) Effects of the opioid antagonist naloxone on release of luteinizing hormone in mares during the anovulatory season. J Endocrinol. 142: 139-144.

Babol J, Squires EJ and Lundström K (1998) Relationship between oxidation and conjugation metabolism of skatole in pig liver and concentrations of skatole in fat. J Anim Sci 76: 829-838

Barnett JL, Winfield CG, Cronin GM and Makin AW (1981) Effects of photoperiod and feeding on plasma corticosteroid concentrations and maximum corticosteroid-binding capacity in pigs. Aust J Biol Sci. 34: 577-585.

Barrell GK, Thrun LA, Brown ME, Viguié C and Karsch FJ (2000) Importance of photoperiodic signal quality to entrainment of the circannual reproductive rhythm of the ewe. Biol Reprod. 63: 769-774.

Bartness TJ, Powers JB, Hastings MH, Bittman EL and Goldman BD (1993) The timed infusion paradigm for melatonin delivery: What has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? J Pineal Res. 15: 161-190.

Bassett JM, Bray CJ and Sharpe CE (1996) Summer infertility in outdoor sows: Lessons from studies on 'seasonally barren' sows. Pig J. 36: 65-85.

Berger T, Mahone JP, Svoboda GS, Metz KW and Clegg ED (1980) Sexual maturation of boars and growth of swine exposed to extended photoperiod during decreasing natural photoperiod. J Anim Sci. 51: 672-678.

Berria M, Joseph MM and Mead RA (1989) Role of prolactin and luteinizing hormone in regulating timing of implantation in the spotted skunk. Biol Reprod. 40: 232-238.

Bittman EL and Karsch FJ (1984) Nightly duration of pineal melatonin secretion determines the reproductive response to inhibitory day length in the ewe. Biol Reprod. 30: 585-593.

Bittman EL, Dempsey RJ and Karsch FJ (1983) Pineal melatonin secretion drives the reproductive response to daylength in the ewe. Endocrinol. 113: 2276-2283.

Boissin-Agasse L, Barbelis C, Audigier S, Roch G and Boissin J (1992) Localization of melatonin binding sites in the pars tuberalis of the mink at three times of the seasonal cycle. Neurosci Lett. 144: 147- 151.

Bollinger AL, Wilson ME, Pusateri AE, Green ML, Martin TG and Diekman MA (1997) Lack of a nocturnal rise in serum concentrations of melatonin as gilts attain puberty. J Anim Sci. 75: 1885-1892.

Bonneau M, Dufour R, Chouvet C, Roulet C, Meadus W and Squires EJ (1993) The effects of immunization against luteinizing hormone-releasing hormone on performance, sexual development, and levels of boar taint-related compounds in intact male pigs. J Anim Sci. 72: 14-20.

Bonnefond C, Monnerie R, Richard J-P and Martinet L (1993) Melatonin and the circadian clock in mink: Effects of daily injections of melatonin on circadian rhythm of locomotor activity and autoradiographic localization of melatonin binding sites. J Neuroendorinol. 5: 241- 246.

Booth WD (1980) A study of some major testicular steroids in the pig in relation to their effect on the development of male characteristics in the prepubertally castrated boar. J Reprod Fertil. 59: 155-162.

Booth WD (1983) Development of some male characteristics supported by oestrone but not dehydroepiandrosterone in the boar. J Reprod Fertil. 68: 9-16.

Booth WD and White CA (1988) The isolation, purification and some properties of pheromaxein, the pheromonal steroid-binding protein, in porcine submaxillary glands. J Endocrinol. 128: 205-212.

Booth WD and Signoret JP (1992) Oflaction and reproduction in ungulates. Oxford Rev. Reprod Biol. 14: 264-301.

Brooks RI and Pearson AM (1986) Steroid hormone pathways in the pig, with special emphasis on boar odour. J Reprod Fertil. 59: 155-162.

Bubenik GA, Hacker RR, Brown GM and Bartos L (1999) Melatonin concentrations in the luminal fluid, mucosa, and muscularis of the bovine and porcine gastrointestinal tract. J Pineal Res. 26: 56-63.

Bubenik GA, Pang SF, Cockshut JR, Smith PS, Grovum LW, Friendship RM and Hacker RR (2000) Circadian variation of portal, arterial and venous blood levels of melatonin in pigs and its relationship to food intake and sleep. J Pineal Res. 28: 9-15.

Chik CL, Almeida OF, Libre EA, Booth JD, Renquist D and Merriam GR (1992) Photoperiod-driven changes in reproductive function in male rhesus monkeys. J Clin Endocrinol Metab. 74: 1068-1074.

Claus R (1990) Physiological role of seminal components in the reproductive tract of the female pig. J Reprod Fertil Suppl. 40: 117-131.

Claus R and Raab S (1999) Influences on skatole formation from tryptophan in the pig colon. Adv Exp Med Biol. 467: 679-684

Claus R and Weiler U (1985) Influence of light and photoperiodicity on pig prolificacy. J Reprod Fertil Suppl. 33: 185-197.

Claus R, Schopper D and Wagner H-G (1983) Seasonal effect on steroids in blood plasma and seminal plasma of boars. J Steroid Biochem. 19: 725-729.

Claus R, Weiler U and Herzog A (1994) Physiological aspects of androstenone and skatole formation in the boar - A review with experimental data. Meat Sci. 38: 289-305.

Claus R, Weiler U and Wagner H-G (1985) Photoperiodic influences on reproduction of domestic boars. II. Light influences on semen characteristics and libido. Zbl Vet Med A. 32: 99-109.

Concannon PW, Castracane D, Rawson, RE and Tennant BC (1999) Circannual changes in free thyroxine, prolactin, testes and relative food intake in woodchucks, *Marmota monax*. Am J Physiol. 277: R1401-1409.

Concannon PW, Roberts P, Graham L and Tennant BC (1998) Annual cycle in LH and testosterone release in response to GnRH challenge in male woodchucks (*Marmota monax*). J Reprod Fertil. 114: 299-305.

Curlewis JD (1992) Seasonal prolactin secretion and its role in seasonal reproduction: A review. Reprod Fertil Dev. 4: 1-23.

Curlewis JD, Loudon ASI, Milne JA and McNeilly AS (1988) Effects of chronic long-acting bromocryptine treatment on liveweight, voluntary food intake, coat growth and breeding season in non-pregnant red deer hinds. Neuroendocrinol. 119: 413-420.

Dahl GE, Evans NP, Thrun LA and Karsch FJ (1995) Thyroxine is permissive to seasonal transitions in reproductive neuroendocrine activity in the ewe. Biol Reprod. 52: 690-696.

Darrow JM, Davis FC, Elliot JA, Stetson MN, Turek FW and Menaker M (1980) Influence of photoperiod on reproductive development in the golden hamster. Biol Reprod. 22: 443-450.

Davis FC and Mannion J (1988) Entrainment of hamster pup circadian rhythms by prenatal melatonin injections to the mother. Am J Physiol. 255: R439-448.

Diekman MA and Green ML (1997) Serum concentrations of melatonin in prepubertal gilts exposed to artificial lighting and sunlight. Theriogenol. 47: 923-928.
Diekman MA, Arthington JA, Clapper JA and Green ML (1997) Failure of melatonin implants to alter onset of puberty in gilts. Anim Reprod Sci. 46: 283-288.
Diekman MA, Clapper JA, Green ML and Stouffer DK (1991) Reduction in age of puberty in gilts consuming melatonin during decreasing or increasing daylength. J Anim Sci. 69: 2524-2531.
Diekman MA, Brandt KE, Green ML, Clapper JA and Mayaler JR (1992) Lack of a nocturnal rise of serum melatonin in prepubertal gilts. Domest Anim Endocrinol. 9: 161-167.
Dombrowicz D, Sente B, Closset J and Hennen G (1992) Dose-dependent effects of human prolactin on the immature hypophysectomized rat testis. Endocrinol. 130: 695-700.
Donham RS, Palacio E and Stetson MH (1994) Dissociation of the reproductive and prolactin photoperiodic responses in male golden hamsters. Biol Reprod. 51: 366-372.
Dorrington JH, Fritz IB and Armstrong DT (1978) Control of testicular estrogen synthesis. Biol Reprod. 18: 55-65.
Ducker MJ, Bowman JC and Temple A (1973) The effect of constant photoperiod on the expression of oestrus in the ewe. J Reprod Fertil Suppl. 19: 143-150.
Duncan MJ and Mead RA (1992) Autoradiographic localization of binding sites for 2-(^{125}I)iodomelatonin in the pars tuberalis of the Western Spotted Skunk (*Spilogaleputorius latifrons*). Brain Res. 569: 152-155.
Ebling FJ and Lincoln GA (1985) Endogenous opioids and the control of seasonal LH secretion in Soay rams. J Endocrinol. 107: 341-353.
Foster DL, Yellon SM and Olster DH (1985) Internal and external determinants of the timing of puberty in the female. J Reprod Fertil. 75: 327-344.
Gaston S and Menaker M (1967) Photoperiodic control of hamster testis. Science 158: 925-928.
Gerlach T and Aurich JE (2000) Regulation of seasonal reproductive activity in the stallion, ram and hamster. Anim Reprod Sci. 58: 197-213.
Giersing HM, Lundström K and Andersson A (2000) Social effects and boar taint: Significance for production of slaughter boars (*Sus scrofa*). J Anim Sci. 78: 296-305.
Gill JL and Hafs HD (1971) Analysis of repeated measurements of animals. J Anim Sci. 33: 331-336.
Gorman MR and Zucker I (1997a) Environmental induction of photononresponsiveness in the Siberian hamster, *Phodopus sungorus*. Am J Physiol. 272: R887-895.
Gorman MR and Zucker I (1997b) Pattern of change in melatonin duration determines testicular responses in Siberian hamsters, *Phodopus sungorus*. Biol Reprod. 56: 668-673.
Gorman MR and Zucker I (1998) Mammalian seasonal rhythms: New perspectives gained from the use of simulated natural photoperiods. In Biological Clocks: Mechanisms and Applications, Ed Y Touitou, pp 195-204. Elsevier, Amsterdam.
Gorman MR, Freeman DA and Zucker I (1997) Photoperiodism in hamsters: Abrupt versus gradual changes in day length differentially entrain morning and evening circadian oscillators. J Biol Rhythms 12: 122-135.
Goss RJ (1984) Photoperiodic control of antler cycles in deer. VI. Circannual rhythms of altered lengths. J Exp Zool. 230: 265-271.
Gower DB (1972) 16-unsaturated C19 steroids, a review of their chemistry, biochemistry and possible physiological role. J Steroid Biochem. 3: 44-103.
Granholm A-Ch (1985) Effects of thyroid hormone deficiency on glia constituents in developing cerebellum of the rat. Exp Brain Res. 59: 451-456.

Green ML, Clapper JA and Diekman MA (1999) Serum concentrations of melatonin during scotophase and photophase in 3, 4, 5 and 6 months old gilts and barrows. Anim Reprod Sci. 57: 99-110.

Green ML, Clapper JA, Andres CJ and Diekman MA (1996) Serum melatonin in prepubertal gilts exposed to either constant or stepwise biweekly alteration in scotophase. Domest Anim Endocrinol. 13: 307-323.

Griffith MK and Minton JE (1992) Effect of light intensity on circadian profiles of melatonin, prolactin, ACTH, and cortisol in pigs. J Anim Sci. 70: 492-498.

Gwinner E (1986) Circannual Rhythms: Endogenous annual clocks in the organization of seasonal processes. Springer-Verlag, Berlin, Heidelberg.

Gwinner E (1995) Circannual clocks in avian reproduction and migration. Ibis 138: 47-63.

Hansen LL, Ekelundh Larsen A, Boso Jensen B, Hansen-Möller J and Barton-Gade P (1994) Influence of stocking rate and faeces deposition in the pen at different temperatures on skatole concentration (boar taint) in subcutaneous fat. Anim Prod. 59: 99-110.

Hastings MH, Walker AP, Powers JB, Hutchison J, Steel EA and Herbert J (1989) Differential effects of photoperiodic history on the responses of gonadotrophins and prolactin to intermediate daylengths in the male Syrian hamster. J Biol Rhythms 4: 335-350.

Herbison AE and Theodosis DT (1992) Localization of oestrogen receptors in preoptic neurones containing neurotensin but not tyrosine hydroxylase, cholecystokinin or luteinizing hormone-releasing hormone in the male and female rat. Neurosci. 50: 283-298.

Heuther G (1993) The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. Experientia 49: 665-670.

Hiebert SM, Thomas EM, Lee TM, Pelz KM, Yellon SM and Zucker I (2000) Photic entrainment of circannual rhythms in golden-mantled ground squirrels: Role of the pineal gland. J Biol Rhythms. 15: 126-134.

Hileman SM and Jackson GL (1999) Regulation of gonadotrophin-releasing hormone secretion by testosterone in male sheep. J Reprod Fertil Suppl. 54: 231-242.

Hoffman K, Illnerova H and Vanecek J (1986) Changes in the duration of the nighttime melatonin peak may be a signal driving photoperiodic response in the Djungarian hamster (*Phodopus sungorus*). Neurosci Lett. 67: 68-72.

Hoffman RA and Reiter RJ (1965) Pineal gland: Influence on gonads of male hamsters. Science 148: 1609-1611.

Howles CM, Craigon J and Haynes NB (1982) Long-term rhythms of testicular volume and plasma prolactin concentrations in rams reared for 3 years in constant photoperiod. J Reprod Fertil. 35: 439-446.

Irvine CH (1984) Hypothyroidism in the foal. Equine Vet J. 16: 302-306.

Irvine CH and Alexander S (1982) Importance of testicular hormones in maintaining the annual pattern of LH secretion in the male horse. J Reprod Fertil Suppl. 32: 97-102.

Jackson GL and Jansen HT (1991) Persistence of a circannual rhythm of plasma prolactin concentrations in ewes exposed to a constant equatorial photoperiod. Biol Reprod. 44: 469-475.

Jansen HT and Jackson GL (1993) Circannual rhythms in the ewe: Pattern of ovarian cycles and prolactin secretion under two different constant photoperiods. Biol Reprod. 49: 627-634.

Janssens CJ, Helmond FA and Wiegant VM (1995) The effect of chronic stress on plasma cortisol concentrations in cyclic female pigs depends on the time of day. Domest Anim Endocrinol. 12: 167-177.

Jarrige J-F and Boucher D (1992) Androgenic function in adult rats: Influence of the pineal gland of the mother and the offspring. J Pineal Res. 13: 66-71.

Jarrige J-F, Laurichesse H and Boucher D (1992) Androgenic activity in 15-day-old male rats: Role of the maternal pineal gland. Biol Reprod. 46: 386-391.

Karsch FJ, Dierschke DJ, Robinson JE, Yellon SM, Wayne NL, Olster DH and Kaynard AH (1986) Melatonin and photorefractoriness: Loss of response to the melatonin signal leads to seasonal reproductive transitions in the ewe. Biol Reprod. 34: 265-274.

Karsch FJ, Robinson JE, Woodfill CJ and Brown MB (1989) Circannual cycles of luteinizing hormone and prolactin secretion in ewes during prolonged exposure to a fixed photoperiod: Evidence for an endogenous reproductive rhythm. Biol Reprod. 41: 1034-1046.

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

Kennaway DJ and Rowe SA (1997) Controlled-release melatonin implants delay puberty in rats without altering melatonin rhythmicity. J Pineal Res. 22: 107-116.

Kennedy BW and Wilkins JN (1984) Boar, breed and environmental factors influencing semen characteristics of boar used in artificial insemination. Can J Anim Sci. 64: 833-843.

Klein DC (1972) Evidence for the placental transfer of ^{3}H-acetyl-melatonin. Nature 237: 118-119.

Klein DC, Coon SL, Roseboom PH, Weller JL, Bernard M, Gastel JA, Zatz M, Iuvone PM, Rodriguez IR, Begay V, Falcon J, Cahill GM, Cassone VM and Baler R (1997) The melatonin rhythm-generating enzyme: Molecular regulation of serotonin *N*-acetyltransferase in the pineal gland. Recent Prog Horm Res. 52: 307-357.

Klupiec CEG, Love RJ and Kennaway DJ (1997) Clarifying plasma melatonin profiles in domestic pigs: A critical and comparative evaluation of two radioimmunoassay systems. J Pineal Res. 22: 65-74.

Lewczuk B and Przybylska-Gornowicz B (1997) Effects of sympathicolytic and sympathicomimetic drugs on plasma immunoreactive melatonin in the domestic pig. J Pineal Res. 23: 209-220.

Lieberman S, Greenfield N and Wolfson A (1984) A heuristic proposal for understanding steroidogenic processes. Endocr Reviews 5: 128-148.

Lincoln GA (1971) Puberty in a seasonally breeding male, the red deer stag (*Cervus elaphus L.*). J Reprod Fertil. 25: 41-54.

Lincoln GA (1981) Seasonal aspects of testicular function. In: The Testis, editors H Burger and D de Kretser, pp. 255- 302, Raven Press, New York.

Lincoln GA (1984) Central effects of photoperiod on reproduction in the ram revealed by the use of a testosterone clamp. J Endocrinol. 103: 233-241.

Lincoln GA (1999) Melatonin modulation of prolactin and gonadotrophin secretion. Systems ancient and modern. Adv Exp Med Biol. 460: 137-153.

Lincoln GA and 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. J Neuroendocrinol. 6: 251-260.

Lincoln GA and Clarke IJ (2000) Role of the pituitary gland in the development of photorefractoriness and generation of long-term changes in prolactin secretion in rams. Biol Reprod. 62: 432-438.

Lincoln GA and Ebling FJ (1985) Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams. J Reprod Fertil. 73: 241-253.

Lincoln GA and Maeda KI (1992) Reproductive effects of placing micro-implants of melatonin in the mediobasal hypothalamus and preoptic area in rams. J Endocrinol. 132: 201-215.

Lincoln GA, Ebling FJ and Martin GB (1987) Endogenous opioid control of pulsatile LH secretion in rams: Modulation by photoperiod and gonadal steroids. J Endocrinol. 115: 425-438.

Lincoln GA, Lincoln CE and McNeilly AS (1990) Seasonal cycles in the blood plasma concentrations of FSH, inhibin and testosterone, and testicular size in rams of wild, feral and domestic breeds of sheep. J Reprod Fertil. 88: 623-633.

Love RJ, Evans G and Klupiec C (1993) Seasonal effects on fertility in gilts and sows. J Reprod Fertil Suppl. 48: 191-206.

Love RJ, Klupiec C, Thornton EJ and Evans G (1995) An interaction between feeding level and season affects fertility in sows. Anim Reprod Sci. 39: 275-284.

Lubbers LS and Jackson GL (1993) Neuroendocrine mechanisms that control seasonal changes of luteinizing hormone secretion in sheep are sexually differentiated. Biol Reprod. 49: 1369-1376.

Lundström K, Malmfors B, Malmfors G, Stern S, Petersson H, Mortensen AB and Sørensen SE (1988) Skatole, androstenone and taint in boars fed two different diets. Livest Prod Sci. 18: 55-67.

Lunstra DD, Ford JJ, Christenson RK and Allrich RD (1986) Changes in Leydig cell ultrastructure and function during pubertal development in the boar. Biol Reprod. 34: 145-158.

Lunstra DD, Ford JJ, Klindt J and Wise TH (1997) Physiology of the Meishan boar. J Reprod Fertil Suppl. 52: 181-193.

Malmfors B, Lundström K and Hansson I (1978) Fatty acid composition of porcine back fat and muscle lipids as affected by sex, age and anatomical location. Swedish J Agric Res. 8: 25-38.

Malmgren L and Larsson K (1984) Seamen quality and fertility after heat stress in boars. Acta Vet Scand. 25: 425-435.

Malmgren L, Rodriguez-Martinez H and Einarsson S (1996) Attainment of spermatogenesis in Swedish cross-bred boars. J Vet Med A. 43: 169-179.

Malpaux B, Robinson JE, Brown MB and Karsch FJ (1987) Reproductive refractoriness of the ewe to inductive photoperiod is not caused by inappropriate secretion of melatonin. Biol Reprod. 36: 1333-1341.

Malpaux B, Robinson JE, Wayne NL and Karsch FJ (1989) Regulation of the onset of the breeding season of the ewe: Importance of long days and of an endogenous reproductive rhythm. J Endocrinol. 122: 269-278.

Malpaux B, Daveau A, Maurice F, Gayrard V and Thiery JC (1993) Short-day effects of melatonin on luteinizing hormone secretion in the ewe: Evidence for central sites of action in the mediobasal hypothalamus. Biol Reprod. 48: 752-760.

Martinet L, Allais C and Allain D (1981) The role of prolactin and LH in luteal function and blastocyst growth in mink (*Mustela vison*). J Reprod Fertil Suppl. 29: 119-130.

Mauget R (1985) Seasonality of reproduction in the wild boar. In Control of Pig Reproduction. DJA Cole and GR Foxcroft eds. pp 509-526. Butterworths, London.

Mauget R and Boissin J (1987) Seasonal changes in testis weight and testosterone concentration in the European wild boar (*Sus scrofa L.*). Anim Reprod Sci. 13: 67-74.

Maywood ES and Hastings MH (1995) Lesions of the iodomelatonin-binding sites of the mediobasal hypothalamus spare the lactotropic, but block the gonadotropic response of male Syrian hamsters to short photoperiod and to melatonin. Endocrinol. 136: 144-153.

Maywood ES, Bittman EL, Ebling FJ, Barrett P, Morgan P and Hastings MH (1995) Regional distribution of iodomelatonin binding sites within the suprachiasmatic nucleus of the Syrian hamster and the Siberian hamster. J Neuroendocrinol. 7: 215-223.

McConnell SJ and Ellendorff F (1987) Absence of nocturnal plasma melatonin surge under long and short artificial photoperiods in the domestic sow. J Pineal Res. 4: 201-210.

Meijer JC, Trudeau VL, Colenbrander B, Poot P, Erkens JH and van de Wiel DF (1988) Prolactin in the developing pig. Biol Reprod. 39: 264-269.
Meyer SL and Goodman RL (1985) Neurotransmitters involved in mediating the steroid-dependent suppression of pulsatile luteinizing hormone secretion in anestrous ewes: Effects of receptor antagonists. Endocrinol. 116: 2054-2061.
Michael RP and Bonsall RW (1977) A 3-year study of an annual rhythm in plasma androgen levels in male rhesus monkeys (*Macaca mulatta*) in a constant laboratory environment. J Reprod Fertil. 49: 129-131.
Millar RP and Glover TD (1973) Regulation of seasonal sexual activity in an ascrotal mammal, the rock hyrax, *Procavia capensis*. J Reprod Fertil Suppl. 19: 203-220.
Minton JE and Cash WC (1990) Effect of cranial sympathectomy on circadian rhythms of cortisol, adrenocorticotropic hormone and melatonin in boars. J Anim Sci. 68: 4277-4284.
Minton JE, Davies DL and Stevenson SJ (1989) Contribution of the photoperiod to circadian variations in serum cortisol and melatonin in boars. Domest Anim Endocrinol. 6: 177-181.
Morgan PJ, Barrett P, Howell HE and Helliwell R (1994) Melatonin receptors: Localization, molecular pharmacology and physiological significance. Neurochem Int. 24: 101-146.
Morgan PJ and Williams LM (1996) The pars tuberalis of the pituitary: A gateway for neuroendocrine output. Reviews of Reprod. 1: 153-161.
Moore RY (1996) Neural control of the pineal gland. Behav Brain Res. 73: 125-130.
Murphy BD, Concannon PW, Travis HF and Hansel W (1981) Prolactin: The hypophyseal factor that terminates embryonic diapause in mink. Biol Reprod. 25: 487-491.
Neal J, Murphy BD, Moger WH and Oliphant LW (1977) Reproduction in the male ferret: Gonadal activity during the annual cycle; recrudescence and maturation. Biol Reprod. 17: 380-385.
Newell JA and Bowland JP (1972) Performance, carcass composition, and fat composition of boars, gilts and barrows fed two levels of protein. Can J Anim Sci. 52: 543-551.
Nicholls TJ, Follett BK, Goldsmith AR and Pearson H (1988) Possible homologies between photorefractoriness in sheep and birds: The effect of thyroidectomy on the length of the ewe's breeding season. Reprod Nutr Dev. 28: 375-385.
Niklowitz P, Lerchl A and Nieschlag E (1994) Photoperiodic responses in Djungarian hamsters (*Phodopus sungorus*): Importance of light history for pineal and serum melatonin profiles. Biol Reprod. 51: 714-724.
O'Callaghan D, Karsch FJ, Boland MP and Roche JF (1991) What photoperiodic signal is provided by a continuous-release melatonin implant? Biol Reprod. 45: 927-933.
Omtvedt IT, Nelson RE, Edwards RL, Stephens DF and Turman EJ (1971) Influence of heat stress during early, mid and late pregnancy of gilts. J Anim Sci. 32: 312-317
Pang SF, Lee PPN, Chan YS and Ayre EA (1993). Melatonin secretion and its rhythms in biological fluids. In: Melatonin. Editors H-S Yu and RJ Reiter. pp 129-153. CRC Press Inc.
Parkinson TJ, Douthwaite JA and Follett BK (1995) Responses of prepubertal and mature rams to thyroidectomy. J Reprod Fertil. 104: 51-56.
Paterson AM and Pearce GP (1990) Attainment of puberty in domestic gilts reared under long-day or short-day artificial light regimens. Anim Reprod Sci. 23: 135-144.
Paterson AM, Maxwell CA and Foldes A (1992a). Seasonal inhibition of puberty in domestic gilts is overcome by melatonin orally, but not by implant. J Reprod Fertil. 97: 97-105.

Paterson AM, Martin GB, Foldes A, Maxwell CA and Pearce GP (1992b) Concentrations of plasma melatonin and luteinizing hormone in domestic gilts reared under artificial long and short days. J Reprod Fertil. 94: 85-95.

Peacock AJ, Evans G and Love RJ (1991) The role of melatonin in seasonal infertility of pigs. In: Advances in Pineal Research, Volume 6. Editors. A Foldes and RJ Reiter, pp. 189-198, John Libbey and Co. Ltd, London.

Peacock AJ, Thacker PA, Kirkwood RN and Tadesco S (1995) The effect of feed restriction and of ß-adrenergic agonist on plasma melatonin concentrations in domestic pigs. Can J Anim Sci. 75: 203-207.

Pellengey ET and Fischer KC (1957) Onset and cessation of hibernation under constant temperature and light in the golden-mantled ground squirrel, *Citellus lateralis*. Nature 180: 1371-1372.

Pelletier J, Bodin L, Hanocq E, Malpaux B, Teyssier J, Thimonier J and Chemineau P (2000) Association between expression of reproductive seasonality and alleles of the gene for Mel(1a) receptor in the ewe. Biol Reprod. 62: 1096-1101.

Pena FJ, Dominguez JC, Carbajo M, Anel L and Alegre B (1998) Treatment of swine summer infertility syndrome by means of oxytocin under field conditions. Theriogenol. 49:829-836.

Picazo RA and Lincoln GA (1995) Light control of the duration of the daily melatonin signal under long and short days in the Soay ram. J Pineal Res. 10: 55-63.

Raeside JI, Renaud RL and Friendship RM (1989) Aromatization of 19-norandrogens by porcine Leydig cells. J Steroid Biochem. 32: 729-735.

Ravault JP, Martinat-Botte F, Mauget R, Martinat N, Locatelli A and 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. Biol Reprod. 27: 1084-1089.

Reimers TJ, Lawler DF, Sutaria PM, Correa MT and Erb HN (1990). Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. Am J Vet Res. 51: 454-457.

Reiter RJ (1993) The melatonin rhythm: Both a clock and a calendar. Experientia 49: 654-664.

Reiter RJ, Britt JH and Armstrong JD (1987) Absence of a nocturnal rise in either norepinephrine, *N*-acetyltransferase, hydroxyindole-*O*-methyltransferase or melatonin in the pineal gland of the domestic pig kept under natural environment photoperiods. Neurosci Lett. 81: 171-176.

Reppert SM (1997) Melatonin receptors: Molecular biology of a new family of G protein-coupled receptors. J Biol Rhythms. 12: 528-531.

Reppert SM, Chez RA, Anderson A and Klein DC (1979) Maternal-fetal transfer of melatonin in the non-human primate. Pediat Res. 13: 788-791.

Ribelayga C, Pevet P and Simonneaux V (2000) HIOMT drives the photoperiodic changes in the amplitude of the melatonin peak of the Siberian hamster. Am J Physiol Regul Integr Comp Physiol. 278: R1339-1345.

Robinson JE and Karsch FJ (1984) Refractoriness to inductive day lengths terminates the breeding season of the Suffolk ewe. Biol Reprod. 31: 656-663.

Robinson JE and Karsch FJ (1987) Photoperiodic history and changing melatonin pattern can determine the neroendocrine response of the ewe to daylength. J Reprod Fertil. 80: 159-165.

Robinson JE, Wayne NL and Karsch FJ (1985) Refractoriness to inhibitory day lengths initiates the breeding season of the Suffolk ewe. Biol Reprod. 32: 1024-1030.

SAS Institute Inc. (1989 and 1997) The SAS system for Windows. Cary NC USA.

Schneider F, Falkenberg H, Kuhn G, Nurnberg K, Rehfeldt C and Kanitz W (1998) Effects of treating young boars with a GnRH depot formulation on endocrine functions,

testis size, boar taint, carcass composition and muscular structure. Anim Reprod Sci. 50: 69-80.
Schwarzenberger F, Toole GS, Christie HL and Reaside JI (1993) Plasma levels of several androgens and estrogens from birth to puberty in male domestic pigs. Acta Endocrinol. 128: 173-177.
Scott CJ and Clarke IJ (1993) Evidence that changes in the function of the subtypes of the receptors for gamma-amino butyric acid may be involved in the seasonal changes in the negative-feedback effects of estrogen on gonadotropin-releasing hormone secretion and plasma luteinizing hormone levels in the ewe. Endocrinol. 133: 2904-2912.
Sellier P, Le Roy P, Fouilloux MN, Gruand J and Bonneau M (2000) Responses to restricted index selection and genetic parameters for androstenone level and sexual maturity status of young boars. Livst Prod Sci. 63: 265-274.
Shaw D and Goldman BD (1995a) Influence of prenatal and postnatal photoperiods on postnatal testis development in the Siberian hamster (*Phodopus sungorus*). Biol Reprod. 52: 833-838.
Shaw D and Goldman BD (1995b) 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*). Endocrinol. 136: 4237-4246.
Skinner TM, Lopez-Corrales NL, Anderson SI, Yerle M and Archilbald AL (1999) Physical mapping of porcine seasonality genes. Anim Biotechnol. 10: 143-146.
Smith AJ, Mondain Monval M, Simon P, Andersen-Berg K, Clausen OPE, Hofmo PO and Scholler R (1987) Preliminary studies of the effects of bromocryptine on testicular regression and the spring moult in a seasonal breeder, the male blue fox (*Alopex lagopus*). J Reprod Fertil. 81: 517-524.
Stetson MH, Ray SL, Creyaufmiller N and Horton TH (1989) Maternal transfer of photoperiodic information in Siberian hamsters. II. The nature of the maternal signal, time of signal transfer, and the effect of the maternal signal on peripubertal reproductive development in the absence of photoperiodic input. Biol Reprod. 40: 458-465.
Thrun LA, Dahl GE, Evans NP and Karsch FJ (1997) A critical period for thyroid hormone action on seasonal changes in reproductive neuroendocrine function in the ewe. Endocrinol. 138: 3402-3409.
Thrun LA, Moenter SM, O'Callaghan DO, Woodfill, CJI and Karsch FJ (1995) Circannual alterations in the circadian rhythm of melatonin secretion. J Biol Rhythms 10: 45-54.
Tortonese DJ and Lincoln GA (1994) Photoperiodic modulation of the dopaminergic control of pulsatile LH secretion in sheep. J Endocrinol. 143: 25-32.
Turek FW, Elliot JA, Alvis JD and Menaker M (1975) Effect of prolonged exposure to non-stimulatory photoperiods on the activity of the neuroendocrine-testicular axis of golden hamsters. Biol Reprod. 13: 475-481.
Turek FW and Van Cuter E (1994) Rhythms in reproduction. In: The physiology of reproduction. Editors E Knobil and JD Neill, pp 487-540. Raven Press, New York.
Vaughan GM (1993) New sensitive serum melatonin radio immunoassay employing the Kennaway G280 antibody: Syrian hamster morning adrenergic response. J Pineal Res. 15: 88-103.
Viguié C, Thibault J, Thiery JC, Tillet Y and Malpaux B (1997) Characterization of the short day-induced decrease in median eminence tyrosine hydroxylase activity in the ewe: Temporal relationship to the changes in luteinizing hormone and prolactin secretion and short day-like effect of melatonin. Endocrinol. 138: 499-506.
Weaver DR and Reppert SM (1990) Melatonin receptors are present in the ferret pars tuberalis and pars distalis but not in the brain. Endocrinol. 127: 2607-2609.
Weaver DR, Keohan JT and Reppert SM (1987) Definition of a prenatal sensitive period for maternal-fetal communication of day length. Am J Physiol. 253: E701-704.

Weaver DR, Liu C and Reppert SM (1996) Nature's knockout: The Mel_{1b} receptor is not necessary for reproductive and circadian responses to melatonin in Siberian hamsters. Mol Endocrinol. 10: 1478-1487.

Wan SS, Hennessy DP and Cranwell PD (1994) Seasonal infertility, stress and adrenocritical responsiveness in pigs. Anim Reprod Sci. 34: 265-279.

Weiler U, Claus R, Denhard M and Hofäcker S (1996) Influence of the photoperiod and a light reverse program on metabolically active hormones and food intake in domestic pigs compared with a wild boar. Can J Anim Sci. 76: 531-539.

Wettemann RP and Bazer FW (1985) Influence of environmental temperature on prolificacy of pigs. J Reprod Fertil Suppl. 33:199-208.

Williams L-M, Hannah LT and Bassett JM (1999) Melatonin receptors in neonatal pig brain and pituitary gland. J Pineal Res. 26: 43-49.

Wilson ER, Johnson RK and Wettermann RP (1977) Reproductive and testicular characteristics of purebred and crossbred boars. J Anim Sci. 44: 939-947.

Wood RI, Ebling FJP, I'anson H and Foster DL (1991) The timing of neuroendocrine sexual maturity in the male lamb by photoperiod. Biol Reprod. 445: 82-88.

Woodfill CJI, Wayne NL, Moenter SM and Karsch FJ (1994) Photoperiodic synchronization of a circannual reproductive rhythm in sheep: Identification of season-specific time cues. Biol Reprod. 50: 965-976.

Xiong J-J, Karsch FJ and Lehman MN (1997) Evidence for seasonal plasticity in the gonadotropin-releasing hormone (GnRH) system of the ewe: Changes in synaptic inputs onto GnRH neurones. Endocrinol. 138: 1240-1250.

Xue JL, Dial CD, Bartsh S, Kertaert B, Squires EJ, Marsh WE and Ferre G (1994) Influence of a gonadotropin-releasing hormone agonist on circulating concentrations of luteinizing hormone and testosterone and tissue concentrations of compounds associated with boar taint. J Anim Sci. 72: 1290-1298.

Xue J, Dial GD, Holton EE, Vickers Z, Squires EJ, Lou Y, Godbout D and Morel N (1996) Breed differences in boar taint: Relationship between tissue levels boar taint compounds and sensory analysis of taint. J Anim Sci. 74: 2170-2177.

Yellon SM and Foster DL (1985) Alternate photoperiods time puberty in the female lamb. Endocrinol. 116: 2090-2097.

Yellon SM and Foster DL (1986) Melatonin rhythms time photoperiod-induced puberty in the female lamb. Endocrinol. 119: 44-49.

Yellon SM, Bittman EL, Lehman MN, Olster DH, Robinson JE and Karsch FJ (1985) Importance of duration of nocturnal melatonin secretion in determining the reproductive response to inductive photoperiod in the ewe. Biol Reprod. 32: 523-529.

Yokoyama MT and Carlson JR (1979) Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. Am J Clin Nutr. 32: 173-178.

Zarazaga L, Malpaux B, Bodin L and Chemineau P (1998a) The large variability in melatonin blood levels in ewes is under strong genetic influence. Am J Physiol. 274: E607-610.

Zarazaga L, Malpaux B, Guillaume D, Bodin L and Chemineau P (1998b) Genetic variability in melatonin concentrations in ewes originates in its synthesis, not in its catabolism. Am J Physiol. 274: E1086-1090.

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