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Post-breeding effects of feeding on reproduction in gilts and sows

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ACADEMIC DISSERTATION

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1. ABSTRACT

Early pregnancy losses characterize autumn infertility in pigs. Inadequate pituitary luteinizing hormone (LH) support for corpora lutea (CL) has been hypothesized to induce disruption of pregnancy. However, no consensus exists about the mechanism causing cessation of pregnancy during the less fertile period of the year. High progesterone concentration in early pregnancy is associated with improved embryonic survival and low feeding level. However, there is also evidence that liberal feeding improves conception and farrowing rates in gilts during the same period, despite reported detrimental effects of abundant feeding on embryonic survival in early pregnancy. The underlying mechanism has yet to be determined.

The objectives of this study were threefold. First, we investigated the effect of feeding level on endocrine secretion and reproductive performance during early pregnancy in gilts and multiparous sows. Second, possible differences in progesterone concentrations between peripheral venous blood and blood in the vena cava in early pregnant gilts were evaluated. We then determined whether an episodic pattern of progesterone secretion occurs in pigs. Third, the endocrinological mechanisms of the early disruption of pregnancy was investigated, concentrating on the role of LH. A gonadotrophin-releasing hormone (GnRH) antagonist was used during three phases of the third pregnancy week to suppress LH pulses for a shorter period than that required to cause a direct regression of the CL.

Three feeding regimens, high (HHH), low (LLL) and modified (LHL), were applied to detect the above-mentioned effects of feeding on endocrine secretions. Modified feeding, which is thought to combine the benefits of restricted feeding after ovulation and abundant feeding during implantation, was first tested on three groups of eight gilts in autumn. Blood for progesterone analysis was collected daily during the week of ovulation and then twice a week until the end of the study. For LH assay, blood was collected from five gilts in each group at 15-minute intervals for 10 hours on day 15 of pregnancy. The pregnancy rate was higher in the HHH group (100%) than in the LLL (25%) or LHL (38%) groups, although the HHH group had significantly lower progesterone concentrations on days 9 and 12. The basal and mean LH levels were higher in the HHH group than in the LHL group. The LH amplitude tended to be higher for gilts in the HHH group. The LHL feeding strategy failed to provide the benefits anticipated. Instead, the HHH feeding strategy that provided a distinct advantage in pregnancy rate.

The same regimens were tested during early pregnancy on multiparous sows. In this experiment, dietary treatment did not significantly affect hormonal parameters. However, progesterone concentration tended to be lower in the HHH group than in the LLL group. In the LHL group, progesterone concentration seemed to be associated with the level of feeding. The embryonic recovery rate was 69% in the LLL, 45% in the HHH and 55% in the LHL group. Neither high feeding nor modified feeding provided any benefits for reproductive performance in multiparous sows.

To examine the influence of different feeding regimens on the secretion pattern of progesterone, 19 cross-bred pregnant gilts were offered three different levels of feeding. Ear vein catheters were placed non-surgically and caudal vena cava catheters under general anaesthesia on day 19 of pregnancy. Two consecutive samples taken at 30-minute intervals were collected four times a day for five days. In addition, three gilts were simultaneously sampled from both catheters at 30-minute intervals for 12 hours on day 22. Progesterone concentration was significantly lower in samples from the jugular vein than in those from the caudal vena cava (blood containing progesterone before

its metabolism in the liver) in all three feeding groups. These findings support the theory that the oviduct and uterus are locally supplied with higher concentrations of progesterone than measured in jugular blood during early pregnancy. An episodic pattern of progesterone production was evident in plasma collected from the caudal vena cava, but not in the plasma from the jugular vein. The frequency of progesterone pulses was similar to that of LH pulses. However, feeding rate did not seem to affect progesterone concentrations at this stage of pregnancy.

The mechanism of seasonal early disruption of pregnancy was studied by means of a GnRH antagonist. The duration of LH suppression following a single treatment with a GnRH antagonist was determined. LH pulses were then blocked for only certain days of early pregnancy, and the period of early pregnancy most susceptible to suppression of LH was identified. Fifteen cross-bred gilts were injected with 100 µg/kg of the GnRH antagonist on days 14 (n=6), 16 (n=5) and 19 (n=4) of pregnancy, corresponding to groups LH14, LH16 and LH19, respectively. Blood samples were collected at 20-minute intervals for 12 hours on the day prior to the treatment and on several days thereafter. The dose used suppressed pulsatile LH secretion immediately and was high enough to abolish LH pulsation for a period of more than two days. GnRH antagonist treatment decreased LH pulse amplitude significantly in those animals in which resumed pulses could be identified. Furthermore, there was a decrease in mean LH secretion after antagonist treatment. The suppression in LH secretion by GnRH antagonist seemed to be sufficient to cause a disruption of pregnancy in some pigs. An abnormal development of pregnancy was diagnosed using a real-time ultrasound technique one day before or on the day of abortion, before external signs of abortion manifested.

Impaired LH secretion of less than four days in duration, mimicking a seasonal effect, was adequate to cause early disruption of pregnancy in some pigs. However, this period of LH suppression was not sufficient to cause regression of the CL directly. Lowered LH secretion in autumn is suggested to suppress progesterone secretion either by decreasing frequency and/or amplitude of progesterone pulses or by decreasing mean progesterone concentration, interfering with uterine histotrophes, embryonic viability and oestrogen signals. This might result in a prostaglandin F2alpha (PGF-2α) release back towards the uterine vascular system, which in turn could cause a regression of the CL and cessation of pregnancy.

In conclusion, progesterone was shown to be secreted in an episodic manner from the CL in the pig. A slight association was found between episodic progesterone and LH secretions on day 22 of pregnancy. The findings also provided further indication of the significance of sustained LH suppression in termination of pregnancy. Apparently, only some gilts are susceptible to suppressed LH secretion, and hence, may be seasonally infertile. Gilts benefit from abundant feeding in early pregnancy in contrast to multiparous sows. Based on these findings, two regimens should be used in piggeries to optimize reproductive performance of pigs of all ages.

2. LIST OF ORIGINAL ARTICLES

This thesis is based on the following original publications referred to in the text by their Roman numerals. These articles were reprinted with the kind permission of the journals concerned.

I) Virolainen, J.V., Tast, A., Sorsa, A., Love, R.J., Peltoniemi, O.A.T., 2004. Changes in feeding level during early pregnancy affect fertility in gilts. Anim. Reprod. Sci. 80, 341-352.

II) Virolainen, J.V., Peltoniemi, O.A.T., Munsterhjelm, C., Tast, A., Einarsson, S., 2005. Effect of feeding level on progesterone concentration in multiparous sows. Anim. Reprod. Sci. (in press).

III) Virolainen, J.V., Love, R.J., Tast, A., Peltoniemi, O.A.T., 2005. Plasma progesterone concentration depends on sampling site in pigs. Anim. Reprod. Sci. 86, 305-316.

IV) Virolainen, J.V., Love, R.J., Tast, A., Peltoniemi, O.A.T., 2004 [imprint 2003]. Effect of a gonadotrophin-releasing hormone antagonist on luteinizing hormone secretion and early pregnancy in gilts. Reprod. Fertil. Dev. 15, 451-459.

3. ABBREVIATIONS

ACTH	adrenocorticotropic hormone
CaI	calcium ionophore
CL	corpora lutea
DE	digestible energy
E2	oestradiol-17β
EDTA	ethylenediamine tetra-acetic acid
FFA	free fatty acid
FR	farrowing rate
FSH	follicle-stimulating hormone
G	gravity or gauge
GnRH	gonadotrophin-releasing hormone
HHH	high feeding group
I^{125}	radioactive iodine
IαI	inter-α-trypsin inhibitor
IαIH4	inter-α-trypsin inhibitor heavy chain 4
i.d.	inner diameter
i.m.	intramuscular
i.v.	intravenous
IGF-1	insulin-like growth factor I
kDa	kilodalton
LH	luteinizing hormone
LH14	group of gilts treated with a GnRH antagonist on day 14 of pregnancy
LH16	on day 16 of pregnancy or
LH19	on day 19 of pregnancy
LHL	modified feeding group
LIF	leukaemia inhibitory factor
LLL	low feeding group
LPP	long pseudopregnancy
NRC	National Research Council
o.d.	outer diameter
PGE	prostaglandin E
PGF-2α	prostaglandin F2alpha
PRL	prolactin
RBP	retinol binding protein
RIA	radioimmunoassay
rpm	rounds per minute
RTU	real-time ultrasound
SD	standard deviation
SPP	short pseudopregnancy
WOI	weaning-to-oestrus interval

4. INTRODUCTION AND REVIEW OF LITERATURE

4.1 GENERAL INTRODUCTION

In many wild mammals, including the European wild boar, an ancestor of the domestic pig, a seasonal pattern of reproduction is evident (Mauget, 1982). The same pattern remains in some domestic animals, such as sheep, goats and horses, but the process of domestication has decreased seasonality in other species such as cows and pigs (Lincoln, 1992). However, evidence indicates that the domestic pig retains vestiges of the seasonal breeding pattern of its ancestor. A large number of studies from several countries have shown decreased fertility in pigs during late summer and early autumn, periods of complete anoestrus among European wild boar sows (Delcroix et al., 1990). The European wild boar usually produces only one litter per year, with a breeding season from late autumn to early winter. After farrowing, a wild sow remains anoestrous until late autumn. The pineal response to the annual changes in day length, which modify the pattern of melatonin secretion, is considered to be the main factor synchronizing the secretion of gonadotrophins and the onset of the breeding season.

Nutritional factors also influence reproductive performance in the wild boar (Mauget, 1982). Both day length and nutrition have been reported to also affect reproductive performance in the domestic pig, and by manipulating these factors, fertility can be increased during the seasonal infertility period (Paterson and Pearce, 1990; Prunier et al., 1996). A controlled or artificial lighting regimen imitating the day length in late autumn is already used on some commercial farms to improve fertility. However, in many piggeries, the most practical way to improve fertility during the seasonal infertility period is through dietary control, which plays an important role throughout the year in modern loose-housing piggeries. Dietary control decreases the stress associated with large numbers of animals and restricted feeding rates (Brouns and Edwards, 1994). This thesis focuses on the endocrinological changes and the influence of nutrition during early pregnancy.

Progesterone is produced by the corpora lutea (CL), which are formed after ovulation. Progesterone concentration increases from day 4 or 5 after oestrus, reaching a maximum between days 12 and 14. Regression of the CL, caused by prostaglandin F-2α (PGF-2α) of endometrial origin, begins around day 16 in non-pregnant females, and peripheral progesterone decreases to basal levels ($\leq$ 1 ng/ml) by day 17-18, leading to a return to oestrus. This decrease in progesterone concentration allows the growth and development of follicles, stimulated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) of pituitary origin. The growing follicles start to produce and secrete their own hormones, principally oestrogens. Rising levels of oestrogens in plasma cause oestrus. Behavioural oestrus lasts from 24 to 72 hours. When oestrogen reaches its peak concentration in the vascular circulation, a LH surge occurs followed by ovulation. Ovulation takes place 36-42 hours after onset of oestrus and usually lasts 1-9 (average 2.9) hours after the first follicle has ovulated (Soede et al., 1997). The optimum timing of insemination or mating varies according to whether the female is a gilt or a sow and whether the weaning-to-oestrus interval (WOI) is less than 6 days (Hughes and Pope, 2001). These authors suggested that all females should be checked for oestrus twice daily. Gilts and sows with WOI exceeding 6 days were recommended to be inseminated at first detection of oestrus, whereas for sows with WOI less than 6 days insemination was advised at 12 hours after first detection of oestrus. All pigs were suggested to receive a second insemination 24 hours after the first if still in oestrus. Factors such as restraint stress, undernutrition, age and season, as reviewed by Pope (1988), might cause an increase in embryo mortality or even total failure of pregnancy. Failure of recognition of pregnancy manifests as an extended inter-oestrus interval. The

extended inter-oestrus interval, usually defined as a return to oestrus of over 24 days, is mainly seen in late summer and early autumn in pigs.

To elucidate the role played by early pregnancy loss in seasonal infertility, the main manifestations of seasonal infertility and physiological endocrine changes in early pregnancy are reviewed here. The proposed endocrine mechanisms underlying early disruption of pregnancy are also discussed.

4.2 SEASONAL INFERTILITY

In contrast to its ancestor, a prolonged period of anoestrus is not the only manifestation of seasonality in the domestic pig. According to many studies, seasonal infertility has various manifestations such as delayed puberty in gilts, a prolonged interval from weaning to oestrus, reduced farrowing rate and delayed return to oestrus after mating. Some reports of reduced litter size have also been published, but typically the whole litter is lost, as discussed in following sections.

4.2.1 Delayed puberty

The majority of studies on seasonal infertility indicate that puberty is delayed during the season of low fertility, resulting in an increase in age at first farrowing (Paterson and Pearce, 1990; Paterson et al., 1991). Peltoniemi et al. (1999a) revealed variation in the age of gilts at first farrowing in their herd record data (collected in 1993 from 1081 herds in Finland). The gilts born between December and April and attaining puberty between August and December were older (> 5 days) at farrowing than those born during the rest of the year. The increased number of pigs returning to oestrus after mating at that time could also partly explain the variation in gilts' farrowing ages. However, the same data showed that the culling rate due to anoestrus was significantly higher in August, October and November than in January, particularly in gilts and primiparous sows.
The effect of season on the attainment of puberty under standardized field conditions is well known (Paterson et al., 1991). Gilts expected to reach puberty during long day length (>12 hours) had delayed puberty. This seasonal inhibition of puberty was reduced by boar exposure, but a clear delay remained in the attainment of puberty. The authors concluded that day length is the main factor determining the age of puberty in the domestic pig. There is a general consensus that melatonin is a mediator of the endocrine response to photoperiodic variation. The existence of a circadian rhythm in melatonin secretion has been demonstrated in both the European wild boar and the domestic pig (Tast et al., 2001). In both breeds, the duration of secretion is subject to seasonal alterations. The domestic pig was therefore concluded to be able to recognize and respond to a change in day length, resulting in inhibition of oestrus in summer-autumn. This seasonal delay in puberty could be diminished by an artificial light regimen for breeding gilts.

4.2.2 Prolonged weaning-to-oestrus interval

A normal weaning-to-oestrus interval is generally considered to be less than 10 days. Most pigs return to oestrus after weaning within this interval throughout the year, but some reports indicate that the risk of this interval being prolonged is increased during the autumn. Herd record studies in Finland (Peltoniemi et al., 1999a) and in Sweden (Tummaruk et al., 2000) demonstrated that this risk is more common in primiparous sows than in multiparous sows, which is in accordance with Xue et al. (1994). However, these gilts have an acceptable farrowing rate when mated (Love, 1978).

This manifestation of seasonal infertility is mainly seen among restricted-feed sows with body weight loss in lactation (Zak et al., 1997). A long time is needed for a recovery from the negative energy status, which prolongs the weaning to oestrus interval. In the summer-autumn period, delayed oestrus after weaning might be more common in countries where high ambient temperature is associated with seasonal infertility and where voluntary feed intake is decreased due to the hot environment (Prunier et al., 1997). On the other hand, in Scandinavia, (northern latitude 50-70°), seasonal infertility is evident despite ambient temperature not being high enough in autumn to greatly interfere with appetite (Peltoniemi et al., 1999a). In that study, the photoperiod was instead hypothesized to be responsible for these effects. While both these factors should be optimal to stimulate reproductive activity, one of them alone can probably inhibit this activity. In the European wild boar, the photoperiod is the main regulator of the seasonal variation in reproductive activity, but nutritional factors may influence the start of the breeding season (Mauget, 1982).

4.2.3 Reduced farrowing rate

Reduced farrowing rate (FR) is the most reported manifestation of seasonal infertility, and it is also considered the most important feature economically. However, the severity of this manifestation varies between countries and even between farms. In Britain, a reduced FR of 3-5% (Stork, 1979) to up to 40% (Hancock, 1988) was reported during summer-autumn. In Italy, only 37% of pigs that mated in August farrowed (Enne et al., 1979). The decrease in FR is typically reported to be 5-15% depending on environmental factors and housing conditions (Hurtgen and Leman, 1980; Love, 1981; Elbers et al., 1994; Peltoniemi et al., 1999b). Reduced FR can be alleviated by individual housing systems since confinement after breeding has been shown to improve FR compared with a group-housing system (Hurtgen and Leman, 1980; Love et al., 1995). Today, however, individual housing is not desirable from an animal welfare aspect, with loose housing systems being preferred. The feeding rate in group housing systems may also play an important role. Feeding sows and especially gilts at a high rate during the summer-autumn improves FR and reduces the adverse effect of season (Love et al., 1995; Virolainen et al., 2004). Benefits of roughage feed and bedding in improving fertility in group housing have also been reported (Peltoniemi et al., 1999b).

4.2.4 Prolonged oestrus-to-oestrus interval

Gilts and primiparous sows are most likely to manifest a prolonged oestrus-to-oestrus interval, and pigs mated during the infertility period return to oestrus at intervals greater than 24 days (Love, 1978; Hurtgen and Leman, 1980; Xue et al., 1994). There are two peaks of sows returning to oestrus, the first at 22-37 days and the second at 44-53 days after mating (Love, 1981). Surprisingly, the interval between the peaks is approximately one oestrous cycle, indicating that termination of pregnancy occurs at the same time in both cases, but for some reason certain pigs return to oestrus only after one cycle has elapsed. Some studies have provided evidence of the presence of viable embryos 22-25 days after mating in pigs with delayed return to oestrus (Mattioli et al., 1987; Tast et al., 2002). This delayed return to oestrus after mating is caused by early disruption of pregnancy due to either early embryonic deaths and loss of the entire litter (Xue et al., 1994) or regression of the CL (Wrathall et al., 1986). The precise endocrinological events causing the termination of pregnancy have yet to be clarified. Early disruption of pregnancy and its suggested endocrinological mechanisms will be discussed later.

4.3 PROGESTERONE SECRETION IN PIGS

4.3.1 Role of progesterone in pregnancy

Progesterone is essential for the development of a uterine environment to support embryonic maturation. Progesterone induces secretions of many proteins and vitamins from the uterine endometrium that are required by growing embryos. In all pigs, both pregnant and non-pregnant, peripheral progesterone concentration rises up to day 14 after the onset of oestrus. Progesterone decreases due to luteolysis after day 16 in non-pregnant pigs. However, in pregnant pigs a 30-70% decrease in peripheral plasma progesterone concentration often occurs between days 14 and 30 (Ziecik et al., 1986; King and Rajamahendran, 1988). This decrease is associated either with partial CL regression caused by a constant low secretion of prostaglandin followed by "rescue" of CL by LH support during the time of establishment of pregnancy (Moeljono et al., 1977; Bazer et al., 1982) or with initiation of progesterone metabolism within the uterus (Fischer et al., 1985). The same gradual decrease in plasma progesterone is also observed in pseudopregnant gilts and in hysterectomized gilts (King and Rajamahendran, 1988). The authors compared plasma progesterone profiles in cyclic, pregnant, pseudopregnant and hysterectomized pigs between 8 and 27 days after oestrus. Throughout the study, the mean progesterone concentrations for hysterectomized pigs were consistently higher than for other groups. A lower concentration beyond day 14 for pregnant and pseudopregnant groups was associated with the basal secretion of prostaglandin from the uterus, which prevents the CL from reaching maximum secretory potential. On the other hand, the large dose of oestradiol conjugate used in studies of pseudopregnancy may reduce the production or secretion of progesterone by luteal cells, resulting in a decrease of progesterone. In hysterectomized pigs, the slight decrease beyond 14 days could have been caused by prostaglandin synthesized by luteal tissue (King and Rajamahendran, 1988). Alternatively, the decrease in plasma progesterone may reflect initiation of progesterone metabolism by the pregnant uterine endometrium and/or embryos. Progesterone concentrations in pregnant pigs are consistently lower in the uterine vein than in the uterine artery (Knight et al., 1977). Furthermore, on day 4 of pregnancy, bilaterally ovariectomized gilts treated with various doses of progesterone to maintain pregnancy had plasma progesterone concentrations ranging from 7 to 26.5 ng/ml, whereas non-pregnant gilts had concentrations of 163-428 ng/ml (Bazer et al., 1982). Evidence also suggests that the conceptus and endometrial tissue in early pregnancy and the endometrium in pseudopregnant pigs on day 25 metabolize progesterone in vitro (Fischer et al., 1985). In conclusion, progesterone level in early pregnancy may have an essential role in the development of embryos.

4.3.2 Corpora lutea, the source of progesterone

The CL are the main source of progesterone throughout gestation in pigs. Up to day 12-14 after oestrus, the CL function autonomously without any pituitary support, as demonstrated with hypophyseal blockade (Brinkley et al., 1964) and by using hypophyseal-transectioned pigs (Anderson et al., 1967). Thereafter, the CL regress within four days, and by day 20 only necrotic embryos are detected (Anderson et al., 1967). However, initial pituitary support is necessary for the LH surge in ovulation and formation of functional CL (Brinkley et al., 1964). These results support the theory of autonomous functioning of the CL up to day 12 or even day 16. Moreover, evidence has been shown for CL dependence on LH (Anderson et al., 1967).

The autonomous functioning of the CL seems to disappear at the same time as PGF-2α reaches a peak level in vascular circulation. Whether these two processes are independent of each other is unclear. Bilateral hysterectomy in the early or mid-luteal phase of the oestrous cycle resulted in CL

maintenance throughout the normal pregnancy period (Anderson et al., 1961). This indicates that the uterine endometrium is the source of luteolytic factor (PGF-2α). To avoid these luteolytic effects on the CL, conceptus oestrogens seem to have a biological role in the timing of endometrial secretion and transport of PGF-2α. Exogenous PGF-2α (a total of 80 mg) infused four times intramuscularly at 12-hour intervals on days 4 and 5 after oestrus had no effect on the CL or on progesterone concentration (Hallford et al., 1975). However, luteolysis was initiated and the length of the oestrous cycle was reduced in sows treated with PGF-2α on days 12 and 13. Diehl and Day (1974) treated cycling pigs on day 10 or 12 with 5 mg of PGF-2α but observed no luteolysis. All of these data support the theory that the CL in the pig is refractory to the luteolytic effect of PGF-2α until day 12 of the oestrous cycle and that a large amount of PGF-2α for two days is required to alter luteal function. Increased CL sensitivity to PGF-2α after day 11 is associated with an increase in numbers of PGF-2α receptors on the luteal cells (Gadsby et al., 1990). This sensitivity corresponds to the time when CL function becomes dependent on continued pituitary LH support.

The main mechanism for the maintenance of pregnancy therefore involves prevention of the luteolytic activity of PGF-2α secreted by the uterine endometrium. PGF-2α production in the endometrium is similar for mid-luteal phase and early pregnancy, but utero-ovarian vein plasma PGF-2α concentration is significantly lower in pregnant than in non-pregnant gilts during the time of expected luteolysis (Frank et al., 1977; Shille et al., 1979). During early pregnancy the release of PGF-2α into the vascular system has to be avoided beyond day 12, when the CL are sensitive to its luteolytic action.

4.4 EARLY PREGNANCY IN PIGS

The establishment and maintenance of pregnancy are not controlled by a single mechanism but by a series of complex biochemical and cellular interactions. In mammalian species, an endocrinological signal from embryos is required to allow a pregnancy to continue (Bazer et al., 1986). If there is no signal from the embryos, a normal-length oestrous cycle occurs.

4.4.1 Embryonic signals

After ovulation and successful fertilization, the most critical event of pregnancy seems to be the establishment of pregnancy. During this time it is necessary to have embryos present in the uterus to ensure the maintenance of the CL (Dhindsa and Dziuk, 1968; Moeljono et al., 1977; Meulen et al., 1988). Embryos of many species induce endocrine changes in the mother that ensure continued secretion of progesterone from the CL (Bazer et al., 1986). This requires both signals from the embryos and appropriate responses from the uterus to allow pregnancy to be maintained.
Embryos reach the uterine horn at the four-cell stage about 60-72 hours after the onset of oestrus. Embryos reach the blastocyst stage on day 5 and emerge from the zona pellucida in spherical form between days 6 and 7. Blastocysts grow from a diameter of about 0.5-1 mm to 2-6 mm on day 10. They thereafter grow at a rate of approximately 0.3 mm/hour over the next 30 hours. Once they have reached an ovoid length of 9-10 mm on day 11, they undergo a rapid transition to a tubular form of length 10-60 mm. Finally, on approximately day 12, they elongate to a filamentous form of over 100 mm long, followed by a further elongation to a thread-like organism of up to 700-1000 mm in length and 0.5-1 mm in diameter by days 14-16 of pregnancy. This phenomenal change from a 10-mm sphere to a 100-mm filamentous form may occur within four hours (Geisert et al., 1982a). This estimation is based on the recovery of blastocysts first from one uterine horn and then 2-6 hours later from the other uterine horn on day 11. A great deal of variation is seen in the

development from the four-cell form to the threadlike form. The most profound variation in developmental stages occurs on days 11 and 12 (Pusateri et al., 1990). Several developmental stages of the conceptus are seen on day 12. This variability may be crucial for embryonic survival and maintenance of pregnancy. The more advanced embryos within a litter are hypothesized to elongate first and release oestrogen, which in turn changes the uterine secretory environment such that it becomes incompatible for the least developed embryos, which are then eliminated from the litter (Pope, 1988). However, this detrimental effect of asynchronous oestrogen release is not evident in Meishan pig (Pickard et al., 2003).

The embryonic signals involved in recognition of pregnancy have been described (Gadsby et al., 1980; Fischer et al., 1985). Embryos obviously have to "inform" the maternal host of their presence somehow to avoid luteolysis and the maternal immune response. Embryonic signals in pigs may contain the free oestrogens, oestrone and oestradiol as well as prostaglandin E2, all of which are synthetized and secreted by blastocysts (Fischer et al., 1985). The endometrium itself is known to have a low capacity to produce oestrogens in early pregnancy (Fischer et al., 1985), but oestrogens have been localized in the trophectoderm and endoderm of embryos on day 12. The highest intensity of production appears in yolk sac endoderm on days 14-16 (King and Ackerley, 1985).

The production of oestrogens is thought to occur only when embryos have reached a critical stage of development (Meulen et al., 1988). Embryonic oestrogen content suggests that oestrogen synthesis increases as embryos progress from the spherical to the tubular form, remaining high during the initial period of the elongation and then declining as embryos expand to lengths greater than 100 mm (Pusateri et al., 1990). The embryonic free oestrogens act locally on the uterine endometrium to stimulate vasodilation and angiogenesis, which increase uterine blood flow and water and electrolyte movement. However, these free oestrogens are converted into a biologically inactive form, conjugated oestrogens, mainly oestrone sulphate, before they are transferred to the maternal circulation. Therefore, oestrone sulphate is the primary oestrogen measured in maternal plasma and is detectable by day 16 of pregnancy, achieving a peak value between days 23 and 30 (Robertson and King, 1974). It is suggested that oestrogen, produced by embryos, acts as a luteostatic factor. Embryonic oestrogens or exogenous oestrogens do not increase the resistance of CL to the luteolytic effect of uterine or exogenous PGF-2α (Ford and Christenson, 1991), and embryonic oestrogens are not luteoprotective in response to exogenous prostaglandins (Diehl and Day, 1974). Evidence for embryonic oestrogens as a 'signal for the maternal recognition of pregnancy' is provided by demonstrations that injection of exogenous oestrogen into gilts or sows on or before day 11 of the oestrous cycle results in luteal maintenance (Frank et al., 1977; Geisert et al., 1982c; Pusateri et al., 1996a).

The antiluteolytic signals of blastocysts presumably occur between days 12 and 22 of pregnancy. Dhindsa and Dziuk (1968) concluded that embryos must be present in both uterine horns between days 10 and 12 for continuation of pregnancy, while embryos need to be present in one horn only after day 12. Meulen et al. (1988) observed that flushing the blastocysts from uterine horns on day 10 resulted in a cycle of normal length and a normal progesterone profile, while flushing on day 11, 12 or 13 had no effect or extended the life span of the CL for 3-13 days. Flushing embryos on or after day 18 from one uterine horn will maintain the CL beyond 30 days. This indicates that maintenance of the CL requires a second signal or at least a prolonged signal of oestrogen. Increasing the interval between the first oestrus and flushing of blastocysts also increased the inter-oestrus interval. In gilts with an increased inter-oestrus interval of at least three days, the blastocysts were $\geq$ 8 mm in diameter or were filamentous. Large variation existed in the diameter of blastocysts flushed on the same day, which is consistent with the rapid elongation process during day 11 (Geisert et al., 1982a). The authors concluded that the first signal for maternal recognition of

pregnancy occurs on day 12 and blastocysts are required to be over 8 mm. A second signal is necessary to maintain the CL to the end of normal pregnancy. Further evidence of biphasic conceptus oestrogen production is provided by studies which have measured uterine content of oestrogen in uterine flushings (Geisert et al., 1982b; Stone and Seamark, 1985). The timing of embryonic oestrogen production is concurrent with the rapid elongation, allowing embryos to have a large surface area in contact with the endometrium and to ensure an interaction between embryos and the endometrium.

Administration of exogenous oestrogens to cyclic gilts to mimic the embryonic release of oestrogens has been used to demonstrate the time period in which the maternal recognition of pregnancy occurs. Pusateri et al. (1996a) showed that various dosages and timing of short-acting oestrogen, oestradiol-17β (E2), given daily from day 12 to cycling gilts, will cause an extended inter-oestrus interval. Short pseudopregnancy (SPP) is defined as an extended cycle length from 23 to 35 days. If the oestrus-to-oestrus interval exceeds 50 days, it is defined as a long pseudopregnancy (LPP). The length of treatment corresponded with the length of pseudopregnancy in these gilts. Exogenous E2 given on days 12 and 13 was optimal to cause SPP. If treatment was continued through day 19, it was optimal for inducing LPP. These results indicate that the signal for inducing LPP as well as for the maternal recognition of pregnancy may occur in two phases with continuous exposure to E2. An initial signal should be present between days 11 and 14 and last for at least two days. All gilts that became pseudopregnant received E2 on days 12 and 13. Gilts that received E2 from day 12 to day 19, 22 or 25 remained pseudopregnant over 50 days. If a second administration of E2 was delayed until day 21, the long pseudopregnancy was not induced.
These data may explain one manifestation of seasonal infertility. Many sows return to oestrus after an extended inter-oestrus interval in summer-autumn (Peltoniemi et al., 1999b; Tast et al., 2002), which indicates embryonic loss after the first signal. The length of the inter-oestrus interval after the early disruption of pregnancy is consistent with the length of pseudopregnancy caused by various treatments of exogenous oestrogens.

4.4.2 Maternal response

Peripheral progesterone concentrations begin to rise after ovulation in cyclic and pregnant pigs. The level to which progesterone will rise is highly correlated with the number of CL (Kensinger et al., 1986). Nutrition affects circulating progesterone concentration, possibly through changes in the metabolic clearance rate (Prime and Symonds, 1993). Thus, the interval between ovulation and a detectable rise in plasma progesterone, and the levels reached are affected by feeding rate. Low feeding level (about 25 MJ DE/day), as compared with high feeding level (about 40 MJ DE/day), is associated with a shorter interval from the ovulatory LH surge to the postovulatory progesterone rise and also with a higher progesterone concentration in early pregnancy (Jindal et al., 1997). Elevated hepatic blood flow and enzymatic activity may increase progesterone turnover during high level feeding. Plasma progesterone concentration reflects a balance between luteal synthesis by CL and metabolic clearance by the liver and the kidney. The high progesterone concentration during early pregnancy positively influences oviductal and endometrial development and secretory activity. Post-mating treatment of progesterone supplementation of gilts with high ovulation rates increases the number of viable foetuses (Ashworth, 1991). However, there is also a beneficial effect of high feeding level in early pregnancy, and this will be discussed later.

As discussed earlier, PGF-2α secretion from the uterine endometrium has a luteolytic effect on the CL, and oestrogens have a crucial role in protecting the CL from the luteolytic activity of PGF-2α in pigs. In vitro PGF-2α production from the uterine endometrial tissue is higher in the late luteal

phase than in the mid-luteal phase or during early pregnancy (Watson and Patek, 1979). PGF-2α secretion starts to rise in cycling gilts after day 12, reaching a peak concentration in utero-ovarian vein plasma on days 14 and 15, which is associated with a coincidental decline in plasma progesterone concentration (Moeljono et al., 1977). PGF-2α concentration returns to the basal level on day 18-19 after onset of oestrus. These findings were later supported by Shille et al. (1979), who observed a rise in the prostaglandin metabolite in peripheral vein plasma of pregnant gilts between days 11 and 13. However, base-level secretion was reached again within 48 hours in pregnant gilts, as compared with 3-5 days in non-pregnant gilts. Christenson et al. (1994) characterized and compared the patterns of uterine secretion of prostaglandin E2 (PGE) with those of PGF-2α during the oestrous cycle and the corresponding days of early pregnancy. They observed a similar maximal concentration of PGF-2α and a similar pulse frequency during the late luteal phase of the cycling gilts as in the above-mentioned studies. Consistent with the findings of Shille et al. (1979), pregnant gilts had an earlier (days 11-13) transient rise in prostaglandin secretion, with PGE predominating. Thereafter, both PGE and PGF-2α secretion declined to basal levels, where they remained throughout the sampling period (up to day 16 of pregnancy). The increase in PGE secretion occurred concomitantly with the increase in PGF-2α during days 11-12. In another study, an increased secretion of PGE was observed in the gravid horn compared with the non-gravid horn of pigs. The elevated PGE secretion was associated with an increased progesterone concentration in the CL of the ipsilateral ovary. This increase in PGE secretion was postulated to protect the CL from a luteolytic effect of PGF-2α. It was earlier demonstrated by Ford et al. (1991) that PGE simultaneously administered with PGF-2α at a 4:1 ratio into a single CL was capable of counteracting the luteolytic effect of PGF-2α, whereas oestrogen was unable to prevent luteolysis directly at the level of the CL. Christenson et al. (1994) concluded that conceptus oestrogens may have an initial effect that preferentially increases the secretion of PGE over PGF-2α (maternal recognition of pregnancy) and a second, later effect that prevents both PGE and PGF-2α synthesis/secretion (continued luteal function). Accordingly, one could speculate that PGE may be luteotrophic in the pig and might have a role in the maintenance of the CL in early pregnancy before the secretion of prostaglandins is redirected towards the uterine lumen.

The concentration of PGF-2α in non-pregnant pigs is elevated in peripheral plasma during luteolysis but not between days 14 and 25 of pregnancy or pseudopregnancy. In the study by Pusateri et al. (1996b), oestrogen-induced pseudopregnant gilts had a lower peripheral concentration of PGF-2α than did cyclic gilts between days 10 and 20 after oestrus. However, in all gilts returning to oestrus after treatment, regardless of duration of luteal function, PGF-2α concentrations were elevated and peaked 4-6 days before oestrus. This confirms that luteolysis, whether occurring at the end of pseudopregnancy or at the end of the normal cycle or early pregnancy, is caused by uterine PGF-2α release. In contrast to findings in peripheral blood, PGF-2α concentration measured in the uterine flushings of pregnant and pseudopregnant gilts was significantly higher than that measured in the uterine flushings of cyclic gilts (Zavy et al., 1980). Uterine content of PGF-2α is even higher in pregnant gilts than oestrogen-treated gilts. This difference suggests that conceptuses also have some prostaglandin synthesis. Furthermore, these findings strongly indicate that secretion of PGF-2α is not inhibited during pregnancy or pseudopregnancy. In contrast to pig embryos, embryos of sheep produce and secrete a protein, ovine trophoblast protein-1, between days 10 and 21 of gestation, which is responsible for establishment of pregnancy in sheep. This protein inhibits uterine production of luteolytic amounts of PGF-2α and prevents luteolysis (Fincher et al., 1986). Although porcine embryos secrete two major proteins between days 10 and 16-18, a porcine chorionic gonadotrophin has not been identified (Godkin et al., 1982). Secretory proteins produced by the porcine embryos stimulate rather than suppress uterine production of PGF-2α and PGE. Therefore, it is more likely that

maternal endocrine secretion is changed to the exocrine secretion to maintain pregnancy, as hypothesized by Bazer and Thatcher (1977).

Embryonic oestrogen seems to affect transport of endometrial PGF-2α. Transport of PGF-2α is towards the uterine vasculature (endocrine) in cyclic gilts, causing luteolysis (Moeljono et al., 1977; Shille et al., 1979; Christenson et al., 1994). After oestrogen exposure, uterine transport is directed towards the uterine lumen (exocrine) and PGF-2α is sequestered in the uterine lumen, preventing PGF-2α from reaching the CL (Bazer and Thatcher, 1977). In vitro studies (Fischer et al., 1985; Gross et al., 1988) support this theory since secretion from the endometrium is greater from the myometrial side (endocrine, towards uterine vasculature) in cyclic gilts as well as in gilts on day 10 of pregnancy. However, on day 14 after oestrus, secretion of PGF-2α is from the luminal side (exocrine) in pregnant and in oestrogen-treated pseudopregnant gilts (Gross et al., 1990). The mechanism by which oestrogens alter the direction of PGF-2α transport is not yet completely known. An elevation in oestrogen concentration in the uterine lumen on or after day 12 stimulates release of calcium into the uterus within 12 hours (Geisert et al., 1982b). An elevated calcium concentration is associated with an earlier synchronized release of proteins and specifically with the release of uteroferrin and plasmin inhibitors. Re-uptake of released calcium by endometrial tissue starts about 12 hours after the maximum calcium concentration is reached in uterine secretions. The uterine endometrium appears to respond to oestrogen and to secrete calcium only between days 10 and 14 of the oestrous cycle. The role of calcium ionophore (CaI) (an inducer of calcium cycling across the endometrial epithelium) in changing the orientation of PGF-2α secretion from the myometrium side to the luminal side was elucidated by Gross et al. (1990) in perfusion studies in vitro. When treatment with CaI was terminated, PGF-2α secretion returned to pre-treatment levels and was primarily endocrine-orientated. These results support earlier findings and indicate that calcium may be involved in redirecting endometrial PGF-2α secretion during early pregnancy.
Gross et al. (1990) suggest that an interaction between oestrogen and prolactin (PRL) is required to enhance uterine secretory activity. Hypoprolactinemic pigs are known to have decreased electrolyte release into the uterine lumen (Young et al., 1989). PRL receptors have been detected in pig endometrial membranes in early pregnancy but also six hours after oestradiol treatment in pseudopregnant gilts (Young et al., 1990). Gross et al. (1990) evaluated whether an interaction between oestradiol valerate and PRL affects secretion of PGF-2α in vitro in endometrial tissue samples. PRL treatment did not affect secretion of PGF-2α in perfused endometrial samples obtained before oestradiol valerate treatment, whereas after oestradiol valerate treatment it increased PGF-2α secretion from the luminal surface but decreased secretion from the myometrial surface. However, PRL treatment did not alter PGF-2α secretion in uterine endometrial tissue samples from cyclic, pregnant or oestrogen-treated gilts on day 14 after oestrus. The endometrium was suggested to no longer be capable of responding to PRL once PGF-2α secretion had reoriented from an endocrine to an exocrine direction. The effects of PRL may also be restricted to occurring before day 14 post-oestrus, if involved in the establishment of pregnancy. In conclusion, embryonic oestrogens may act on the uterine endometrium to induce PRL receptors, which then allow PRL to bind to these receptors and stimulate an increase in calcium release across the endometrial epithelium. This calcium flux has been suggested to alter the orientation of PGF-2α secretion from an endocrine to an exocrine direction.

The pig uterine endometrium has also been shown to be a source of oxytocin and relaxin. Oxytocin is present in the intra-uterine environment of pregnant and non-pregnant pigs, but the concentration is higher in pregnant pigs (Vallet et al., 1998). Oxytocin secretion from the uterine endometrium has been suggested to be episodic because of the high variation present among animals. Embryonic oestrogens increase the release of endometrial oxytocin into the uterine lumen and reach peak

values on day 12. Conceptus tissues do not have oxytocin receptors between days 10 and 12, indicating that oxytocin may have an autocrine function in uterine contraction. Higher oxytocin levels during pregnancy may also play a role in regulating the movement of PGF-2α into the uterine lumen. Exogenous oxytocin is also reported to stimulate the release of PGF-2α in cyclic and pregnant gilts, measured as a stable metabolite of PGF-2α in jugular plasma (Carnahan et al., 1996). However, responsiveness to oxytocin was reduced from days 14 to 16 of pregnancy, while it was increased in cyclic pigs. This seems to be consistent with the endocrine-exocrine theory for inhibition of luteolysis in pigs (Bazer and Thatcher, 1977).
The CL are the primary source of circulating relaxin. The initial relaxin expression in the uterus starts around day 12, increases to day 16 and is maintained until day 20 in pregnant pigs. Relaxin has an uterotrophic effect which could stimulate growth of the uterus to accommodate expansion of the allantochorionic membranes from days 18 to 30 of pregnancy (Knox et al., 1994).

4.4.3 Uterine protein secretion in early pregnancy

Uterine proteins (histotrophes) are essential for the development of embryos, especially in species like the pig with epitheliochorial placentation. In this type of placentation, there is no erosion of the uterine epithelium, and the uterine secretions are important for the conceptus throughout the pregnancy. The concentrations of most proteins are increased in both non-pregnant and pregnant gilts between days 10 and 13 (Vallet et al., 1996). Progesterone-treated pigs (both pregnant and non-pregnant) on days 2 and 3 after oestrus have increased total protein, uteroferrin and retinol binding protein (RBP) in uterine flushings (Vallet et al., 1998). However, in pregnant sows, concurrent concentrations of uteroferrin and oxytocin were even higher. In addition, in progesterone-treated gilts, increased uterine protein secretion preceded increased conceptus oestrogen secretion. All of these findings strongly indicate that uterine secretory proteins are mainly progesterone-stimulated and that some of them are released afterwards by embryonic oestrogens from secretory vacuoles on the luminal surface and in glandular epithelial cells of the endometrium. Because these proteins are involved in vitamin and mineral transportation, changes in availability may influence the rate of conceptus development, oestrogen secretion and subsequent embryo survival.
A down-regulation of progesterone receptors and an increase of epithelial oestrogen receptors (Geisert et al., 1993) occur in the surface and glandular epithelium of the endometrium beyond day 10 of the pregnancy or oestrous cycle (Geisert et al., 1994). The disappearance of progesterone receptors is associated with an increase in protein secretion on day 10, followed by an increase in conceptus oestrogen secretion on day 11. Once the proteins are secreted into the lumen, they provide nutrient support for fast-growing conceptuses. Many of these proteins have been identified and are reviewed by Geisert and Yelich (1997). Some of these are discussed below.

Uteroferrin and RBP are the extensively studied uterine proteins in pigs. These glycoproteins have a low molecular weight, and their production is induced from the pig uterine endometrium by progesterone. Uteroferrin is involved in transport of iron, RBP and retinol from the uterine endometrium to the embryos. Uteroferrin is also synthesized and secreted by the surface and glandular epithelium of the endometrium (Chen et al., 1975). In cyclic pigs, uteroferrin is secreted into the lumen of the uterine glands from days 9 to 13 after oestrus. On and beyond day 14, uteroferrin is localized within the uterine endometrial stroma, whereas in pregnant pigs uteroferrin is always localized within the epithelial cells, in the lumen of uterine glands and in the placenta. Upon uteroferrin being secreted into the uterine lumen, it provides a nutrient source to the developing pig embryos.

RPB has been identified in uterine secretions from cyclic gilts in the luteal phase and also in allantoic fluid of pregnant pigs. Concentrations of retinol increase in uterine flushings obtained on days 11-13 of pregnancy, the time when conceptuses reach the filamentous stage of development (Trout et al., 1992). Flushings from non-pregnant gilts or from gilts having only spherical conceptuses on days 11 and 12 contained 7- to 8-fold less retinol than flushings from gilts with filamentous-stage conceptuses. RBP is also synthesized by conceptus tissues as early as at 10 or 11 days of gestation (Harney et al., 1990). After the transfer of retinol to the conceptus, free retinol is converted to retinoic acid, which is associated with activation of trophectoderm remodelling. RBP is induced in ovariectomized sows treated with progesterone or progesterone and oestradiol but not in sows treated with oestradiol or corn oil (Adams et al., 1981). Progesterone-related regulation probably ensures availability of these proteins for most of the pregnancy. However, the presence of the conceptus is associated with increased uteroferrin (Vallet et al., 1998) and RBP (Trout et al., 1992), which suggests that the amount and timing of production of these proteins may also be influenced by the presence of the conceptus.

The pig endometrium contains many other proteins, protease inhibitors and enzymes such as lysozymes, haematopoietic cytokines and several types of cathepsins. Cathepsins are lysosomal cysteine proteases that have been implicated in invasive implantation of rats and cats. Cathepsin L activity increases at the time of conceptus elongation in pigs, reaching peak activity on day 15 of gestation (Geisert and Yelich, 1997). Many kinds of endometrial-origin protease inhibitors regulate the microenvironment during placental attachment. Recently, for example, the inter-trypsin inhibitor (IαI) was detected in the pig endometrium during conceptus elongation and attachment (Geisert et al., 1998). This protease inhibitor is also associated with acute-phase reaction to cardiogenic shock. Uterine secretion of IαI may regulate conceptus attachment and limit proteolysis. Some haematopoietic cytokines have also been detected in the uterine endometrium and secretions during early conceptus development. One of the most notable cytokines is leukaemia inhibitory factor (LIF). The highest concentration of LIF in the uterine secretion is on days 11 and 12 of pregnancy. An essential role of LIF in blastocyst growth and implantation in mice has been shown (Cai et al., 2000). These findings imply that LIF may also have a crucial role in conceptus development and implantation in pigs.

Embryonic growth, development and differentiation are highly dependent on the timing and quantity of growth factors in the uterine secretions. Many growth factors influence the uterine lumen in early pregnancy. Although expression of these growth factors has been demonstrated, their role in development of conceptuses has not been clearly defined. One of the best-characterized factors is insulin-like growth factor I (IGF-I). Uterine secretion of IGF-I may enhance embryonic oestrogen synthesis since secretion reaches a peak concentration on day 12 of gestation and has an impact on P450 aromatase gene expression, which may be important in regulating oestrogen synthesis (Green et al., 1995). IGF-I may also affect uterine growth and development, as the uterine endometrium is reported to contain IGF-I receptors.

Conceptus secretory proteins are reported to change from predominantly acidic proteins to basic proteins. The transition occurs on day 13 (Godkin et al., 1982). Basic uterine proteins dominated one day earlier in highly fed pregnant gilts than in normally fed pregnant and cyclic gilts, suggesting that maternal nutrition status may influence the timing of changes in the conceptus secretory protein profile, which may in turn influence conceptus growth and survival (Soede et al., 1999). Conceptus secretory proteins change from acidic to basic later in Meishan pig (Ashworth et al., 1996), which might be beneficial for embryonic survival.

As described above, numerous different proteins are present in the uterine lumen during early pregnancy, and many proteins are likely to be uncovered in the future. To completely understand the process involved in conceptus elongation and implantation, further investigations are required. In conclusion, progesterone is certainly the primary director of uterine development and secretion since mainly these uterine products are seen in both cyclic and pregnant gilts. However, the marked down-regulation of progesterone receptors in the surface and glandular epithelium from day 10 of the oestrous cycle or pregnancy and the increase in epithelial oestrogen receptors indicate the response of the uterine epithelium to conceptus oestrogens during elongation and implantation. This is confirmed by the secretion of numerous proteins after a release of the first oestrogen signal of embryos on day 12. A perfect synchronization between the endometrium and conceptuses is needed to achieve implantation and continue gestation.

4.4.4 Mechanism of implantation in pigs

The embryos remain free in the uterus for two weeks before implantation. In this period the embryos develop without maternal cues, but during implantation a dialogue is required between the uterine epithelium and the embryos. Oestrogen produced by the conceptus triggers several events that allow implantation to begin. Attachment of the embryo to the uterine epithelial surface is a critical event for early embryonic survival. A large number of uterine and conceptus factors have been identified and suggested to be involved in this interaction. Expansion of the pig embryos delineates the surface area for placental attachment. Embryos form vascular connections after implantation and these are necessary for nutrient transport. In the pig, placentation is non-invasive, diffuse and of epitheliochorial type. Implantation involves complex activities that are sensitive to disruption, as shown by embryonic mortality and disruption of pregnancy.

The mechanism of implantation is well understood in rodents and primates (Cross et al., 1994). In rodents, a secondary surge of oestrogens secreted by ovarian follicles is the factor that induces implantation on day 4 of pregnancy. Abolishing this surge by ovariectomy or lactation prevents the attachment reaction and the embryos remain in diapause. During this delayed implantation the embryos slow their metabolism. Once the surge of oestrogen occurs, it induces implantation. Oestrogen acts on the uterine epithelium, inducing it to secrete cytokines such as different growth factors and leukaemia inhibitory factor (LIF) (Stewart et al., 1992). LIF is a pleiotropic cytokine, a 45- to 56-kDa glycoprotein, secreted by the endometrial glandular epithelium. LIF is involved in many physiological systems, including proliferation, differentiation and cell survival, all of which are also associated with embryo development and implantation. LIF seems to be an essential factor for embryo implantation in the mouse (Stewart et al., 1992; Cai et al., 2000). LIF antibody treatment decreases the number of embryos implanted (Cai et al., 2000), and normal blastocysts can not implant in the uteri of mice with no functional LIF gene (Stewart et al., 1992). It appears that LIF may regulate implantation by acting on the uterine epithelium, preparing the uterus for implantation of the embryo, or alternatively, LIF may have some indirect effect on the embryo. While many other secondary events also affect implantation, oestrogen and LIF are the main factors at least in mice. The same phenomenon has been observed in sheep. A decrease of 33.5% in the pregnancy rate in LIF-immunized sheep was reported by (Vogiagis et al., 1997). LIF was concluded to be facilitatory, but not obligatory, for embryo development and implantation in sheep, although possibly anti-LIF did not completely block the action of uterine LIF.
In pigs, initial expression of LIF receptors is observed in 2-mm spherical conceptuses, with a peak occurring at the 7-mm spherical conceptus stage, which is maintained throughout trophoblast elongation (Yelich et al., 1997). Uterine secretion of LIF at the time of conceptus elongation suggests that LIF may have a vital role in pig conceptus development, as LIF has been reported to

regulate protease activity in the expanding mouse blastocyst (Harvey et al., 1995). Proteases in turn serve as modifiers of the extracellular matrix. However, scarce data are available on the role of LIF in pigs, and further studies are warranted.

Successful implantation in pigs demands close contact between the embryos and the uterine epithelium. During establishment of pregnancy in the uterine endometrium transformations are observed. An increase in endometrial folding and formation of a thick glycocalyx coating on the microvilli of the uterine surface epithelium are associated with oestrogen stimulation and conceptus attachment. Glycoproteins present in the glycocalyx on the uterine luminal epithelium are hypothesized to aid the attachment of the embryos to the uterine surface. The discovery of the inter-α-trypsin inhibitor family (IαI), especially the inter-α-trypsin inhibitor heavy chain 4 (IαIH4) produced by the pig endometrium, has provided a new view on the mechanism of placentation (Geisert and Yelich, 1997). IαIH4 does not have typical protease inhibitory activity, nor does it form complexes with other IαIH chains. The function of IαIH4 has been proposed to be to inhibit embryonic invasion, in addition to offering a target for adhesion molecules. IαIH4 is known to contain a cleavage site for the serine protease kallikrein (Nishimura et al., 1995). Kallikrein acts to cleave the IαIH4 chain into smaller fragments on the uterine epithelial surface. Remaining fragments of IαIH4 on the uterine surface offer a binding target for the conceptus. Release of conceptus oestrogens may stimulate release of kallikrein in pigs, as it was earlier demonstrated that oestrogen plays a role in kallikrein release in rats (Corthorn et al., 1997). Kallikrein protease activity increased in pig uterine lumen during embryo elongation and oestrogen secretion (Vonnahme et al., 1999), and its activity was low in uterine flushings on day 10 of the oestrous cycle and during pregnancy, increasing on day 12 in both pregnant and cycling pigs. Activity in uterine flushing on day 12 of pregnancy was greater than activity in cyclic gilts. Kallikrein activity in uterine flushing also increased progressively with conceptus size. However, on day 15 kallikrein activity was greater in cyclic than in pregnant uterine flushing. The authors concluded that the increase in kallikrein activity in both pregnant and cyclic pigs indicates that this enzyme plays a role in opening sites for conceptus attachment. They further concluded that kallikrein activity is not only induced by embryos but is associated with normal changes in protein secretion during the expected placentation, in contrast to activity in rodents. In addition, kallikrein is involved in the kininogen-kallikrein-kinin system in which kallikrein cleaves kininogens to kinins, particularly to bikunins. Kinins have many bioactive properties, including increased blood flow, decreased membrane permeability, release of calcium and release of prostaglandins, which are also required in embryo elongation and implantation.

It seems therefore that IαIs are crucial in placental attachment to uterine epithelium. Feeding level may affect the timing and secretion of uterine proteins, and this may have a role in the early disruption of pregnancy seen during seasonal infertility. Moreover, at this sensitive time of early pregnancy, luteinizing hormone (LH) helps maintain pregnancy by supporting the CL to produce progesterone.

4.5 LUTEINIZING HORMONE IN EARLY PREGNANCY IN PIGS

Pituitary LH is luteotrophic to day 30 or 50 of gestation (Anderson et al., 1967; Peltoniemi et al., 1995; Tast et al., 2000). According to Peltoniemi et al. (1995), after day 30 of gestation, the CL function is no longer highly dependent on LH secretion. After day 60 of gestation, CL function instead becomes increasingly dependent on prolactin (Szafranska and Tilton, 1993). However, LH secretion was not found to vary significantly throughout pregnancy, although LH pulse frequency declined during the final three weeks (Kraeling et al., 1992). A period of transition has been proposed around day 70 from dependence on LH as the primary luteotropin to dependence on

prolactin, more likely due to a change in biological activity of prolactin than to changes in plasma concentration of these hormones.
LH secretion can be concluded to be necessary for functional CL between days 14 and 29, with the CL thereafter becoming gradually more independent of LH. In addition, LH may affect other organs besides the ovaries, since receptors for gonadotrophins have been detected throughout the reproductive tract, namely in the oviduct, myometrium, endometrium, cervix and the uterine vessel. If this is the case, LH might have an effect on embryonic survival that precedes the CL becoming dependent on pituitary LH.

4.5.1 Luteinizing hormone in the uterus

Recently, attention has been paid to the role of LH receptors in the uterus. LH receptors have been detected in the pig uterus throughout the oestrous cycle (Ziecik et al., 1986; Ziecik et al., 1992). Maximal expression of LH receptors occurs during the luteal phase in the endometrium, myometrium and cervix; this expression is unrelated to the ovulatory LH peak that occurs several days later. There is some evidence that uterine LH receptors might have a physiological function. In the luteal phase, activation of LH receptors is associated with increased induction of endometrial cyclo-oxygenase and its products, PGF-2α production in the endometrium and PGE production in the myometrium (for review see Shemesh, 2001). On the other hand, LH binding to its receptor may affect several systems since LH-dependent messenger systems activate many different enzymes. LH receptors may also have a role in the growth of the uterus and in uterine motility (Ziecik et al., 1992). In addition, LH receptors have been identified in the oviduct, and LH has been revealed to cause relaxation of the oviduct, especially during the periovulatory stage of the oestrous cycle (Gawronska et al., 1999). These authors hypothesized that LH is important in synchronizing the events leading to the fertilization of ova in the ampulla.
The timing of receptor expression in the uterus combined with the association with biochemical changes indicates that LH may have a direct influence on the uterus and play a substantial role in embryo-endometrial interaction during early implantation. This also provides further evidence of another essential role of LH in early pregnancy. Lower LH concentration during seasonal infertility might interfere with uterine production of prostaglandins or change the direction of secretion from endocrine to exocrine. LH receptor concentration in the endometrium and myometrium may also be critical, with insufficient expression resulting in disturbance of uterine secretions.

4.5.2 Factors affecting luteinizing hormone secretion

4.5.2.1 Season

The release of pituitary LH is regulated by gonadotrophin-releasing hormone (GnRH) from the hypothalamus. Both of these hormones are released in an episodic manner, and each pulse of GnRH secretion induces a pulse of LH secretion. Animals with a breeding season have a reduction in LH secretion in the non-breeding season due to the reduced secretion of GnRH. The pineal gland is known to respond to seasonal changes in day length, altering the pattern of melatonin secretion, which in turn is thought to be the mechanism mediating photoperiod information to the hypothalamo-pituitary-ovarian axis (for review see Ebling and Hastings, 1992). In sheep, the seasonal pattern of decreased reproductive efficiency is due to an increased sensitivity of the hypothalamo-hypophyseal axis (GnRH pulse generator) to the negative feedback effects of oestradiol-17β (E2) (Karsch et al., 1984).

LH pulse frequency and amplitude are reported to be significantly higher in pregnant gilts in January and February than in August and September (Smith and Almond, 1991). LH secretion seemed to decrease concurrently with an increase in oestrogen concentration as pregnancy progressed. This decline in LH secretion is proposed to be due to increased serum concentration of E2 (Cox and Britt, 1982). However, the seasonal effect on LH release was apparent only in the natural release of LH, not in the exogenous GnRH-induced release. The neuroendocrine control of pulsatile LH release could be concluded to be inhibited in pregnant gilts during August and September, whereas elevated E2 concentration during mid-gestation suppresses the synthesis and/or release of LH from the anterior pituitary gland. These conclusions were confirmed by Peltoniemi et al. (1997a), who reported significantly greater LH pulse amplitude and intensity in winter than in summer. In winter, LH pulses seemed to have more regular profiles and higher amplitudes than pulses in summer. In summer, the baseline was also irregular, which sometimes made pulse identification difficult. Mean LH concentrations were unaffected by season, in contrast to the report of Smith and Almond (1991). However, the stage of pregnancy was different in these two studies, and E2 negative feedback could have suppressed LH secretion in the former study.
Evidence suggests that a period of 4-9 days of complete abolition of LH pulses is required to induce the CL regression leading to abortion (Peltoniemi et al., 1995; Tast et al., 2000). Thus, the seasonal variation of hypothalamo-pituitary-ovarian axis secretions may not be the only mechanism involved in the aetiology of the seasonal disruption of pregnancy when caused by CL regression, as seasonal changes seen in LH secretion were relatively small.

4.5.2.2 Stress

Prolonged stress is known to impair reproduction in many animals (Dobson and Smith, 2000). The endocrine system appears to be the means by which stressors affect reproductive mechanisms at different levels (i.e. the higher brain, hypothalamus, pituitary and gonads). Hormones of the hypothalamo-pituitary-adrenal axis that are released during stress may interact with hormones of the hypothalamo-pituitary-gonadal axis at any level depending on the stressor. Transport stress is used as a model of acute stress (combined physical and psychological stimuli) in sheep (Dobson et al., 1999) and in pigs (Dalin et al., 1993). In pigs, the rises in cortisol and adrenaline levels occur immediately after the start of transport (Dalin et al., 1993). The maximum level is reached during transport, and after transport the decrease in the cortisol level is rapid. A normal diurnal rhythm is attained four hours later. Group housing of pigs is considered a stressful situation that results in elevated levels of cortisol, particularly among subordinate pigs (Tsuma et al., 1996b). The stress is exacerbated by the restricted feeding used during early pregnancy of group-housed pigs. Group housing is rapidly becoming the most common housing management system in pig production, and therefore, more attention should be paid to group size, feeding level and feeding system to avoid problems in early pregnancy.
Repeated acute and sustained elevations of cortisol were induced to simulate acute and chronic stress in ovariectomized gilts (Turner et al., 1999a). The authors hypothesized that the effect of cortisol would be enhanced in the presence of oestradiol negative feedback. However, when the elevation of cortisol was sustained, the mean plasma LH concentrations and the pre-LH pulse nadir were significantly ($P<0.05$) lower on the 8^{th} day of the treatment compared with the day before treatment or the 4^{th} day of treatment. This reduction in mean LH was not associated with a decrease in the frequency or amplitude of LH pulses. This indicated that the basal secretion of LH was impaired. The authors concluded that cortisol needed to be elevated for more than four days to impair the secretion of LH. Oestradiol did not enhance the effect of cortisol on LH secretion in ovariectomized pigs. The mechanism by which cortisol acted to produce these results remains obscure. Cortisol did not affect the response of the pituitary to a large dose of exogenous GnRH in

these ovariectomized gilts. The same protocol was later used with cycling gilts, and again, sustained elevation of cortisol affected LH secretion (Turner et al., 1999b). LH surge, oestrus and ovulation were inhibited in some cyclic gilts. The same detrimental effect of cortisol on LH secretion may occur during early pregnancy in low-ranking pigs. This effect combined with the seasonal suppression of LH might be sufficient to interfere with development of conceptuses, resulting in an early disruption of pregnancy.

4.5.2.3 Feeding

Evidence indicates that a reduced feeding rate affects the hypothalamo-hypophyseal-gonadal axis, reducing LH secretion (Armstrong and Britt, 1987; Booth, 1990; Prunier et al., 1993). Many of these studies have focused on gonadotrophin secretion related to the follicular phase and ovulation. The animals involved in these kinds of studies are often prepubertal gilts.
A severe level of feed restriction (0.23 kg/day) in cycling gilts caused a cessation of the oestrous cycle after 46 days (Armstrong and Britt, 1987). The catabolic state of these animals was associated with lower basal plasma insulin concentrations and a tendency for higher preprandial free fatty acids (FFA) in plasma compared with gilts receiving the control diet. This nutritionally induced (for 80 days) anoestrus was characterized by suppression of episodic release of LH. Two weeks after realimentation, no differences were evident in metabolic variables between control and restricted gilts. A resumption of oestrus did not occur until 36 days after realimentation, accompanied by a linear increase in concentration and frequency of LH release. However, there is an indication that feed restriction inhibits LH release more than LH synthesis. Exogenous administration of GnRH in nutritionally anoestrous gilts induces an elevated basal concentration of LH (Prunier et al., 1993) and even ovarian follicular growth (Armstrong and Britt, 1987). Therefore, the low secretion of LH in feed-restricted gilts is more likely to be due to an inhibition of the GnRH pulse generator than to inadequate pituitary synthesis or storage of LH. However, the observations of Armstrong and Britt (1987) indicated no direct relationship between the metabolic status and the GnRH pulse generator; the response to the metabolic changes was very slow. On the other hand, these animals were in a deep catabolic state since they had lost on average 30 kg in weight and 8 mm backfat in depth during the 80-day diet. A reduced protein intake and/or a huge protein loss (> 16%) in lactating sows is associated with reduced ovarian function and prolonged weaning-to-oestrus interval (Jones and Stahly, 1999; Clowes et al., 2003). Therefore, changes in protein levels might also modulate reproductive function in the pig.

The mechanism mediating nutritional effects on reproduction via the hypothalamo-pituitary-ovarian axis is hypothesized to involve factors regulating the metabolic status of the animal. Insulin and glucose are considered to be the main nutritional mediators the hypothalamo-pituitary-gonadal axis. Nutritional modulation of the reproductive axis can occur even without any change in body weight or composition (Booth, 1990). Gilts with restricted feeding had an increase in LH secretion within six hours of ad libitum feeding. The observed advantage in LH secretion in gilts fed to appetite was concluded to be mediated by the effects of elevated plasma concentrations of insulin and IGF-1 present in the realimented condition. The same positive effect on LH secretion in restricted fed gilts was achieved with a series of glucose injections. These results revealed that glucose administration to restricted-feed gilts and the associated plasma insulin rise induce a rapid increase in episodic LH secretion similar to that observed in response to realimentation. However, contradictory studies also exist, showing either no effect (Tokach et al., 1992) or a detrimental effect (Barb et al., 1991) of glucose on LH response to GnRH. One explanation could be that when variation in glycaemia is within a physiological range glucose administration will not affect reproduction.

A high rate of feeding during the seasonal infertility period significantly improves the farrowing rate, reducing the adverse effect of the season (Love et al., 1995). This improvement was hypothesized to be mediated by LH secretion. Therefore, a study was conducted to determine the effect of season and feed restriction on LH and metabolic hormones in early pregnant gilts (Peltoniemi et al., 1997a). The findings were consistent with earlier studies showing that feed restriction can decrease LH frequency. These changes were closely related to the metabolic state of the gilts, as indicated by insulin and FFA concentrations. LH pulses were more frequent in gilts showing less variation than in those with large variation in insulin and FFA concentrations in relation to feeding. However, the difference was not significant between feeding groups in summer. The authors concluded that energy metabolism may not be the only mechanism controlling the hypothalamo-pituitary-ovarian axis in seasonal disruption of pregnancy since changes in LH secretion induced by the different feeding levels were relatively small. LH secretion (amplitude and frequency) showed a tendency towards higher values when the gilts were fed a higher energy diet (Peltoniemi et al., 1997b). However, these gilts were individually housed in stalls, in environmental conditions not conducive to causing seasonal infertility. In addition, the energy supplement was in the form of fat (soybean oil), but the carbohydrate supplement could have had stronger influence through the insulin-glucose mechanism. The exact mechanism, by which abundant feeding influences reproductive success, is yet to be defined. The findings that in multiparous sows different levels of food intake during lactation or during the interval between weaning and mating have no profound or significant effects on LH concentration either before oestrus or during the LH surge (Varley et al., 1996) add to the confusion. This may indicate that a difference exists in the response of multiparous sows and that of gilts or primiparous sows. Seasonal infertility is mainly seen in gilts or primiparous sows.

5. AIMS OF THE STUDY

This study was carried out to investigate effects of feeding level during early pregnancy on endocrine secretion and reproductive performance in gilts and multiparous sows. In addition, the endocrinological mechanism of the early disruption of pregnancy was investigated. Specific aims of the work were as follows:

1) To investigate the relationship between feeding level and endocrinological secretions in early pregnant pigs (I, II, III).

2) To investigate whether a low feeding level for the first 10 days post-insemination followed by a period of liberal feeding would combine the benefits of an early rise in progesterone concentration related to restricted feeding after ovulation and the positive effect of a high feeding level around implantation in gilts (I) and multiparous sows (II).

3) To determine how different levels of feeding affect the synthesis and the clearance of progesterone in early pregnancy (III).

4) To investigate the effect of sampling site on progesterone concentration (III).

5) To further investigate the endocrinological mechanism of the early disruption of pregnancy, concentrating on the role of LH, and to determine whether any stage of early pregnancy is more susceptible to suppressed LH secretion (IV).

6. MATERIALS AND METHODS

An overview of materials and methods is presented in this section. More detailed descriptions are available in original publications (I-IV). The feeding trial with gilts (I) was carried out in a commercial environment with standardized housing and management practices. The other experiments (II, III), including the study investigating the mechanism of early disruption of pregnancy (IV), were carried out in a strictly controlled environment.

6.1 EXPERIMENTAL DESIGN

The effect of three feeding regimens on hormonal patterns, embryonic mortality and pregnancy rate was tested in early pregnant gilts (I) and multiparous sows (II). Two groups received a constant high-energy diet (HHH) or low-energy diet (LLL) after insemination, and one group was fed a high-energy diet during implantation and a low-energy diet otherwise (LHL).
The same feeding regimens were used when investigating effects of feeding on progesterone concentration before and after its metabolism in the liver (III). Two groups were provided with constant high or low feeding commencing around day 12 of pregnancy. Feeding level was increased from low to high in the third group on day 21 of pregnancy at the beginning of sampling period (days 20-24).
A GnRH antagonist was used to suppress LH pulses during the third week of pregnancy (IV). Gilts in three groups were treated with a single injection of a GnRH antagonist on day 14, 16 or 19 of pregnancy (groups LH14, LH16 and LH19, respectively) and intensive sampling for LH was performed thereafter.

6.2 ANIMALS AND MANAGEMENT

Cross-bred (Finnish Yorkshire X Landrace) gilts (7-8 months old) from a Finnish commercial farm and sows in mean of their eighth parity (range,2-11) from a Finnish sow pool were used in Studies I and II, respectively. The three groups of eight gilts each were housed in pens with individual feeding stalls, whereas the sows were housed in paired pens. The general health status of the animals was carefully followed and recorded before and during the study. Pigs were fed according to National Research Council (NRC) recommendations before artificial insemination. After ovulation, they were randomly allocated to one of the three feeding groups.

The cross-bred (Landrace x Large White) gilts used in Studies III and IV were selected from an Australian commercial piggery based on naturally occurring oestrus and were naturally mated. The gilts were housed in individual stalls and fed 2.5 kg of a commercial ration once a day. All pigs in each experiment had free access to water.

Table 1. Data on Studies I-IV.

Study No.	No. of animals	Treatment / group size	Parity	Days of pregnancy monitored	Type of housing
I	24	8	gilt	0 – 34	Group housing
II	18	6	2-11	0 – 35	Pair pens
III	16	5 - 6	gilt	5 – 24	Individual
IV	15	4 - 6	gilt	5 – 28	Individual

At the end of Studies I and II, animals were slaughtered at a local abattoir and the reproductive tract of each animal was recovered to determine the embryo survival rate. The ovaries and uterus were dissected to determine the number of CL and embryos. Individual embryo weights and crown-rump lengths were determined after dissection, and the mean of the litter was calculated for each pig. Each embryo was classified as viable or non-viable based on appearance within the amnion and crown-rump length. A crown-rump length of 2 SD below the mean of the same litter was used as an objective measure of abnormal development. Embryo survival was determined both as viable embryo survival, expressed as a percentage of normally developed embryos from the number of CL present, and total embryo survival, expressed as a percentage of all embryos from the number of CL present. Gilts in Studies III and IV were not sacrificed but were returned to the piggery. Information about their next farrowing was collected.

6.3 FEEDING REGIMENS (I, II, III)

Prior to insemination or mating, Australian gilts (III, IV) received ad libitum feeding, whereas all Finnish gilts (I) and sows (II) were fed twice a day. Gilts received a total of 3.1 kg (40 MJ/day) and sows 4 kg (52 MJ/day) of a commercial ration, containing 13 MJ DE, 7.4 g lysine/kg and 14.5% crude protein. Pigs had free access to water. Following insemination, the animals were randomly allocated to one of three feeding regimens. They were fed twice a day a total of 2 kg (27 MJ/day) (LLL) or 4 kg (54 MJ/day) (HHH) until the end of the study or 2 kg (27 MJ/day) for 10 days, 4 kg (54 MJ/day) for 7 days and 2 kg (27 MJ/day) until the end of study (LHL). In the modified group, the feeding level was increased within one day but decreased in 0.5 kg/day increments until the low level was reached. A sudden decrease in feeding level was assumed to be more stressful for early pregnant pigs than a sudden increase and therefore the procedure described above was used when changing feeding levels. In Study III, the same regimens were used with modified timing.

6.4 OESTRUS DETECTION, OVULATION AND PREGNANCY TESTING

Oestrus was stimulated and status was determined twice a day by fence-line contact with a mature boar in Studies I and II. At the first detection of standing oestrus, the pigs were inseminated in the presence of a boar, and the insemination was repeated at 12-hour intervals until standing oestrus subsided. Pigs were examined twice daily by a transcutaneous or transrectal real-time ultrasound (RTU) instrument (Pie Medical) equipped with a 5 MHz sector probe until detection of ovulation. The first day of ovulation was considered day 0. Gilts were pregnancy-tested by ultrasound on days 19 and 23. All gilts assessed as pregnant had embryonic sacs in the uterus.
Gilts in Studies III and IV were naturally mated and transferred to a controlled environment around day 5 of pregnancy. Pregnancy was checked with a RTU (Hondex®, Honda Electronics CO, Japan, equipped with a 5 MHz linear probe) transrectally on days 14 and 15 (Knox and Althouse, 1999) and transcutaneously daily thereafter.

6.5 BLOOD COLLECTION

6.5.1 Single blood samples (I, II, III, IV)

The pigs were bled once a day for progesterone and/or cortisol analyses via the medial vena saphena, the coccygeal vein or the jugular vein, if they were not fitted with an intravenous indwelling jugular catheter.

6.5.2 Jugular vein catheters (I, II, III, IV)

The day before intensive sampling, gilts were fitted non-surgically with an indwelling jugular vein cannula via an ear vein. Gilts were fastened around the upper jaw with a rope snare and tied to a fence. The dorsal surface of an ear was disinfected with iodine and dried. The veins were distended by pressure at the ear base and an intravenous (i.v.) catheter placement unit (Optiva^{TM} 14 G, 57 mm) was inserted into an ear vein. A vinyl tube (o.d. 1.5 x i.d. 1.0 mm, Biocorp Australia Pty Ltd, Australia) was passed through the catheter for approximately 50 cm, with another 50 cm remaining free. The catheter placement unit was removed and the free tube secured to the ear with adhesive tape (Elastoplast^{TM}, Smith+Nephew, Australia). The ear was fixed to the neck by a collar of adhesive tape. A blunted needle (BD® 18 G, 1.2 x 38 mm) was inserted into the end of tube. The catheter was flushed with 4 ml of EDTA saline solution (0.9% K-EDTA, 0.9% NaCl), and a stopper was inserted to prevent backflow. The free end of the tube was stored in a Velcro pouch attached to the neck for easy sampling. The time needed for catheterization was approximately 5-10 minutes. All samples (10 ml) were collected in glass tubes containing 100 µl of EDTA (250 mg/ml) and then centrifuged (Eppendorf, Hamburg, Germany) at 3000 x g for 10 min at 10ºC within 2 hours. Plasma was stored at -20ºC until radioimmunoassay for progesterone and/or LH.

6.5.3 Caudal vena cava catheters (III)

Ear vein catheters were placed non-surgically on the day before intensive sampling as described above followed by catheterization of the caudal vena cava via the lateral saphenous vein (Benoit and Dailey, 1991). The sampling site of the caudal vena cava is depicted in Figure 1. This method was earlier validated in gilts by Benoit and Dailey (1991) and our own pilot study. A great majority of catheters seemed to be placed correctly when secured 52 cm in gilts. In preparation for catheterization of the caudal vena cava, anaesthesia was induced with an i.v. bolus injection of 1.25 g of thiopentone sodium (Pentothal®, Merial Limited, Australia) diluted in 25 ml of sterile water. Anaesthesia was maintained with a halothane/oxygen mixture delivered by a face mask (Rhodia Halothane, Merial, Australia). Gilts were laid on the left side, the lateral aspect of the right rear leg was shaved approximately 20 cm dorsal to the hock and the area was disinfected with 70% ethanol. An incision was made through the skin approximately 3 cm dorsal to the hock and 2 cm lateral to the Achilles tendon. The subcutaneous fat (1-2 cm) was separated and the lateral saphenous vein was isolated by blunt dissection. Tweezers were placed under the vessel and two loose ligatures were inserted approximately 1 cm apart. Slight traction was maintained on the distal ligature, a small transversal incision was made in the vein and a catheter (1.0 mm i.d., 1.5 mm o.d., Biocorp Limited, Australia) was inserted. Each catheter had previously been marked 48, 52 and 56 cm from the tip. Plasma samples were collected at each mark as the catheter was pushed forward to ensure its correct position. Approximately 2 ml of blood was withdrawn and discarded before collection of a 4-ml sample. A jugular sample was collected after the last sample from the vena cava. Sampling from all sites took approximately 3 minutes. Catheters were flushed after each sample with 3 ml of EDTA saline solution (0.9% K-EDTA, 0.9% NaCl). After samples had been collected, catheters were pulled back to the 52 cm mark and secured with two ligatures around the vein. Before the catheter was secured, the distal ligature was used to tie off the vein. The wound was dressed with antibiotic powder (Terramycin Pinkeye Powder, Pfizer, Australia) and sutured (Coated Vicryl 0 (3.5 m), Ethicon, Australia). The free end of the catheter (1 m) ran dorsally along the caudal surface of the rear leg lateral to the tail and ended on the back of the pig. Catheters were sutured to the skin and Velcro pouches were used to protect the free end. The pouches were fixed to the animal with adhesive tape wrapped around the abdomen. The legs were bandaged and catheters covered with adhesive tape (Elastoplast, Smith+Nephew, Australia). Catheterization took approximately 40 – 60

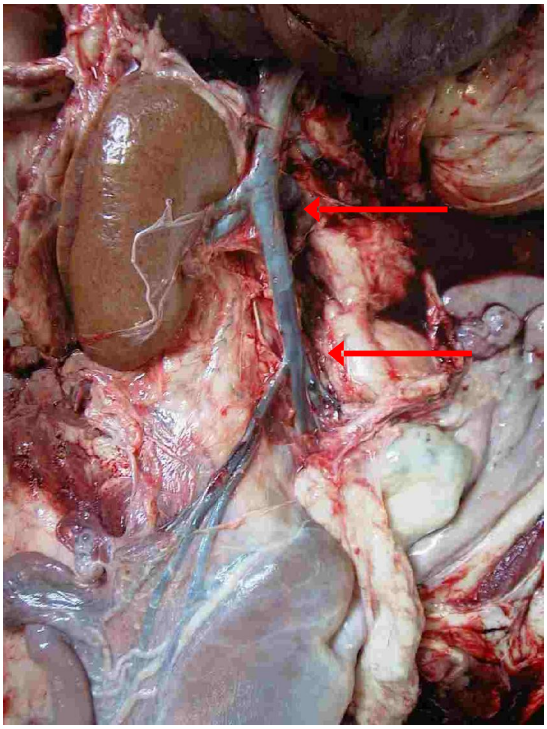

Figure 1. The sampling site used (the end of the tube) in the vena cava is situated between the two arrows. The margin of the correct placement for a catheter was approximately 10 cm. This site was typically achieved with a 52 cm (intravenous length) catheter via the lateral saphenous vein.

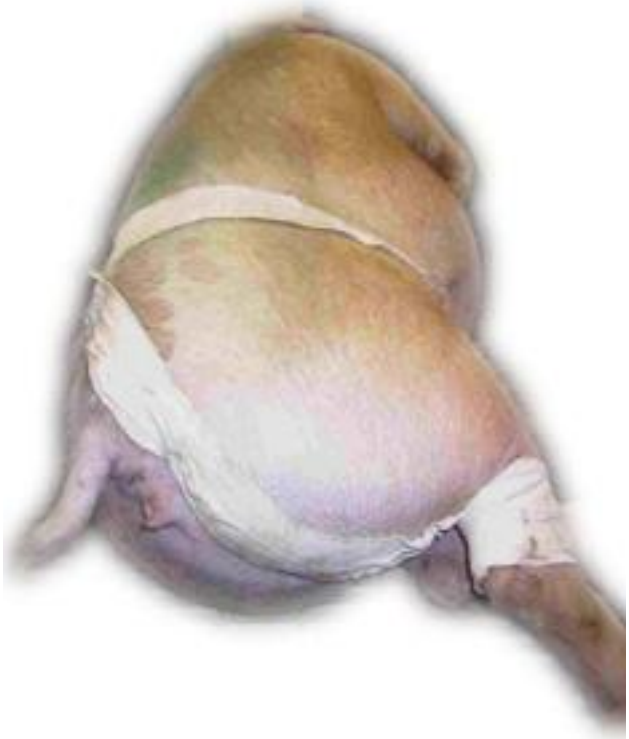

Figure 2. A gilt cannulated via the lateral saphenous vein.

minutes. A gilt with vena cava catheter is shown in Figure 2.

6.6 GONADOTROPHIN-RELEASING HORMONE ANTAGONIST

A single intramuscular (i.m.) injection of a GnRH antagonist was used to suppress LH pulses on days 14, 16 and 19 after mating in groups LH14, LH16 and LH19, respectively (IV). All gilts received a dose of 100 μg/kg of GnRH antagonist D-Phe^{2}, D-Trp^{3}, D-Phe^{6}, D-Ala^{10} LHRH (BIM-21009, Biomeasure Inc., USA) as a single injection (Patterson-Bay et al., 1997) (D.H. Coy, pers. comm.). For the first group, the GnRH antagonist was prepared in sterile saline (1.5 mg/ml) less than 1 hour before treatment. Due to difficulties with solubility, for the other treatment groups, GnRH antagonist was first dissolved in 10 ml of propylene glycol and then diluted in 20 ml of sterile saline (3 mg/ml) (D.H. Coy, pers. comm.). The gilts were immobilized using a nose snare to ensure a satisfactory i.m. injection.

6.7 HORMONE ASSAYS

6.7.1 Progesterone (I, II, III, IV)

Blood samples were analysed for progesterone using a commercial radioimmunoassay (RIA) (Spectria, Orion Diagnostica, Finland), validated to measure progesterone in pig plasma (Peltoniemi, 1994). Fifty microlitres of plasma sample and 500 μl of buffered I^{125} label were added into antibody-coated tubes. After vortex mixing, tubes were incubated at room temperature for 2 hours. The supernatant was decanted and the tubes were left standing upside down for 5 minutes. Each tube was counted for 1 minute in a γ-counter (Wallac®, LKB-Wallac, Turku, Finland). The sensitivity of the assay was ≤ 0.09 ng/ml.

6.7.2 Luteinizing hormone (I, II, III, IV)

Plasma LH concentrations were determined using a previously validated direct homologous double antibody RIA (Peacock, 1991). Purified porcine LH (LER-786-3) supplied by Professor L.E. Reichert Jr. was used and labelled with I^{125} (Amersham Australia Pty Ltd., Baulkham Hills, NSW, Australia). The labelled LH was stored at -20°C and used within 5 weeks. The antibody used was anti-porcine NIESWENDER No. 566, donated by Professor G. Nieswender. It was frozen in a final dilution of 1:17 000 in buffer. A solid-phased second antibody-coated cellulose suspension raised in donkeys against rabbit serum (Sac-Cel®, A-SAC1, Abacus Diagnostics, Brisbane, Australia) was used to separate bound and unbound label. The assay buffer supplemented with bovine serum albumin (200 mg in 20 ml of buffer) was used to prepare the standards. An aliquot of 100 μl of the purified LH was added to 3.9 ml of the buffer and 2 ml of the previous standard serially diluted to 2 ml of the buffer to obtain standards from 16 to 0.125 ng/ml. Aliquots of 200 μl of plasma sample (duplicates) or standard (triplicates) were added to 600 μl of the buffer; 100 μl of antiserum was added and tubes were incubated at 4°C for 24 hours. Following the incubation, 100 μl of labelled LH was added and further incubated at 4°C for 48 hours. The second antibody (100 μl) was then added, and the tubes were incubated for 30 minutes at room temperature and centrifuged (3000 rpm for 10 minutes) at 4°C. The supernatant was then poured out and the tubes were counted for 1 minute in a γ-counter (Wallac®, LKB-Wallac, Turku, Finland). The assay sensitivity was 0.25 ng/ml.

6.7.3 Cortisol (II)

Serum cortisol concentrations were analysed by using a solid-phase RIA (Coat-A-count Cortisol, Diagnostic Products Corporation, Los Angeles, CA, USA), according to the manufacturer's instructions. The method has recently been used in many studies and it was earlier validated for use in pigs (Griffith and Minton, 1992). Aliquots of 25 µl of serum sample and 1.0 ml of I^{125} cortisol were added into cortisol antibody-coated tubes. After vortex mixing, the tubes were incubated for 45 minutes at 37°C in a waterbath. The supernatant was decanted thoroughly and tubes were left draining on absorbent paper for 3 minutes. Each tube was counted for 1 minute in a γ-counter (Wallac®, LKB-Wallac, Turku, Finland). The sensitivity of the assay was 2.0 ng/ml.

6.8 STATISTICAL ANALYSIS

Statistical analyses were carried out using a Stata Intercooler, 7.0 statistical package (Stata Corporation, College Station, TX, USA), SPSS for Windows, 11.0.1 (SPSS Inc., Chicago, IL, USA) and a Minitab statistical package (Minitab, Boston, MA, USA).

6.8.1 Identification of luteinizing hormone pulse

The method for identification of LH pulses was modified from a procedure described earlier (Shaw and Foxcroft, 1985). For LH pulse analyses, the basal LH level was defined as the mean of six consecutive samples preceding a potential LH pulse. The potential LH pulses were found from the profile after plotting an individual's samples against time. If there were more than two possible pulses, the basal level was calculated using the value that gave the lowest LH concentration. A pulse was defined as any increase in the concentration of LH that exceeded by at least 3 SD the basal level for at least two consecutive samples, with the peak concentration being reached within two subsequent sampling intervals. The rise must also have been followed by a decline, the duration of which was at least two sampling intervals and which went down to the basal level or showed a steep decline close to the basal level. In addition, the decrease could not have lasted longer than the known half-life of the LH. If no pulses were found, the mean concentration was used as a basal level. The amplitude of a LH pulse was calculated as the difference between the peak and the basal LH level. If there was more than one pulse, the amplitude was calculated as the average of these amplitudes. The mean plasma concentration of LH was calculated as the mean of all points in the sampling.

6.8.2 Data analyses

The LH pulse characteristics (I, II, IV) and total and viable embryo survival (%) (I, II) were analysed using one-way analysis of variance. If a significant variance was found, the data were further analysed using Tukey's pair-wise comparison, where the critical value was 3.77. Due to the non-normality of distribution for some data, logarithmic transformations were performed. Progesterone (I, II, III, IV) and cortisol (II) profiles were analysed using analysis of variance with repeated measurements. Where appropriate, Student's t-test was used for further analyses. The effect of dietary treatment (level of feeding) on body weight gain was analysed using the general linear model. Pregnancy rate (%) was analysed using Fischer's exact probability test (I).

7. RESULTS

This section provides an overview of the main results. All results are described in detail in original publications I-IV.

7.1 EFFECT OF FEEDING ON MAINTENANCE OF PREGNANCY AND EMBRYONIC SURVIVAL (I, II, III)

The percentage of pregnant group-housed gilts (I) five weeks after insemination was significantly higher ($P<0.005$) in the high-fed group (HHH, 100%) than in the other groups (LLL and LHL), which did not differ significantly from each other, 25% and 38%, respectively. According to a transcutaneous ultrasound examination on days 19 and 23 of pregnancy, one LLL gilt and three LHL gilts lost their pregnancy during the fourth and fifth week of pregnancy. The mean daily gains during pregnancy for groups HHH, LLL and LHL were 1201, 287 and 438 g/day ($P<0.05$), respectively, whereas before insemination with equal feeding they were 523, 424 and 513 g/day. The mean daily gains for pregnant and non-pregnant gilts in groups LLL and LHL were 453 and 521 g/day and 104 and 388 g/day, respectively. No significant difference was detected in total embryo survival or viable embryo survival for those gilts that remained pregnant; these were 70±18% and 67±18%, 91±13% and 75±2%, and 69±14% and 69±14% for HHH, LLL and LHL groups, respectively. The feeding regimen in individually housed gilts (III) did not significantly affect total litter size or number of piglets born alive, 11.5±1 and 11.5±1, 12.7±3.3 and 11.8±2.5, and 11.0±2.6 and 11.0±2.6 for groups HHH, LLL and LHL, respectively. In the three groups of multiparous sows (II), pregnancy rates did not differ from each other. Embryonic recovery was high in the LLL group (69%), low in the HHH group (45%) and moderate in the LHL group (55%). The length of viable embryos at the time of sacrificing in group HHH was significantly shorter than that in the other groups. Data concerning recovery of the genital tract and embryos are depicted in Table 2 (gilts) and Table 3 (sows).

7.2 EFFECT OF FEEDING ON PROGESTERONE AND LUTEINIZING HORMONE PATTERNS IN PERIPHERAL BLOOD (I, II)

7.2.1 Progesterone

The mean progesterone level in peripheral blood was not affected by dietary treatment in gilts until day 9 of pregnancy (I). Gilts in the LLL group had higher ($P<0.05$) progesterone concentrations on days 9 and 12 (19.3±4.8 and 17.2±5 ng/ml) than gilts in the HHH group (14±2.2 and 11.5±2 ng/ml), gilts in the LHL group had intermediate progesterone concentrations (15.4±4.6 and 13.8±4.2 ng/ml) (Figure 3).

Progesterone concentrations did not differ significantly between treatment groups in multiparous sows, although progesterone concentrations tended ($P=0.08$) to be lower in HHH sows (II). Furthermore, an increase in feeding level in LHL sows tended to decrease progesterone concentration to the level measured in HHH sows (Figure 4). Progesterone concentration in allantoic fluid was unaffected by the feeding level on day 35 of pregnancy.

At three weeks of pregnancy (III), feeding level significantly ($P<0.05$) affected the concentration of progesterone in the jugular vein but not in the caudal vena cava. Progesterone concentrations in the jugular vein in the LLL group were significantly ($P<0.05$) lower than those in the HHH group, but only tended ($P=0.054$) to be lower than those in the LHL group. Progesterone concentration in the

Tables 2 and 3. Day of slaughter, crown-to-rump length, number of corpora lutea (CL), progesterone concentration in allantoic fluid (al) and in plasma of the jugular vein (jug), number of foetuses and embryo survival rate (means ± SD) in the high (HHH), low (LLL) and modified (LHL) feeding groups on day of slaughter. Table 2 presents information on gilts (I) and Table 3 information on sows (II).

Table 2.

GILTS	**HHH** mean	**± SD**	**LLL** mean	**± SD**	**LHL** mean	**± SD**
Day of slaughter	33.9	0.4	34.3	0.5	33.9	0.4
Crown-to-rump length (cm)	3.8	0.2	3.8	0.2	3.9	0.1
No. of CL	16.8	2.9	26.5	0.7	19.3	9.5
Progesterone (ng/ml), jug	8.2	1.7	12.7	3.1	10.4	1.6
No. of viable foetuses	11.3	4.1	20.0	0	13.3	5.0
Total no. of foetuses	11.8	4.3	24.0	2.8	13.3	5.0
Embryo survival rate (%)	67	18	75	2	69	14
Pregnancy rate (%)	100		25		37.5	

Table 3.

MULTIPAROUS SOWS	**HHH** mean	**± SD**	**LLL** mean	**± SD**	**LHL** mean	**± SD**
Day of slaughter	35.0	0	35.2	1.0	34.8	1.5
Crown-to-rump length (cm)	3.6	0.3	3.8	0.2	3.9	0.4
No. of CL	26.2	5.3	22.6	3.4	18.7	2.5
Progesterone (ng/ml), al	2.2	0.7	1.8	1.0	1.4	1.0
Progesterone (ng/ml), jug	10.1	3.2	13.5	2.9	13.5	3.3
No. of viable foetuses	11	5.1	15.6	3.1	10.3	2.9
Total no. of foetuses	13	7	16.8	2.9	13	1
Embryo survival rate (%)	45	25	69	8	55	10
Pregnancy rate (%)	83		83		60	

jugular plasma of gilts in all feeding groups decreased steadily throughout the day after ingestion of food. An episodic pattern of progesterone production was not evident in plasma samples collected from the jugular vein, unlike in plasma samples from the caudal vena cava.

7.2.2 Luteinizing hormone

LH characteristics in Studies I and II on day 14 of pregnancy are shown in Table 4. Mean and basal LH concentrations were affected by dietary treatment in group-housed gilts (I). Gilts in the HHH group had higher mean and basal LH concentrations than those in the LHL group ($P<0.05$). A tendency for a higher mean LH concentration was seen in the HHH group as compared with the LLL group ($P=0.09$), but no difference was detected in the basal LH level. The amplitude of LH pulses tended to be higher ($P=0.058$) for gilts in the HHH group than in either of the other two groups.

LH characteristics were not significantly affected by feeding level in multiparous sows (II). However, when LH characteristics on days 14 and 21 of pregnancy were compared, a significant interaction was present between the level of feeding and the basal LH, as well as between the level of feeding and the mean LH. While basal and mean LH concentrations remained constant in the HHH group, they decreased in the LLL group and increased in the LHL group. The amplitude decreased significantly in all groups with progression of pregnancy.

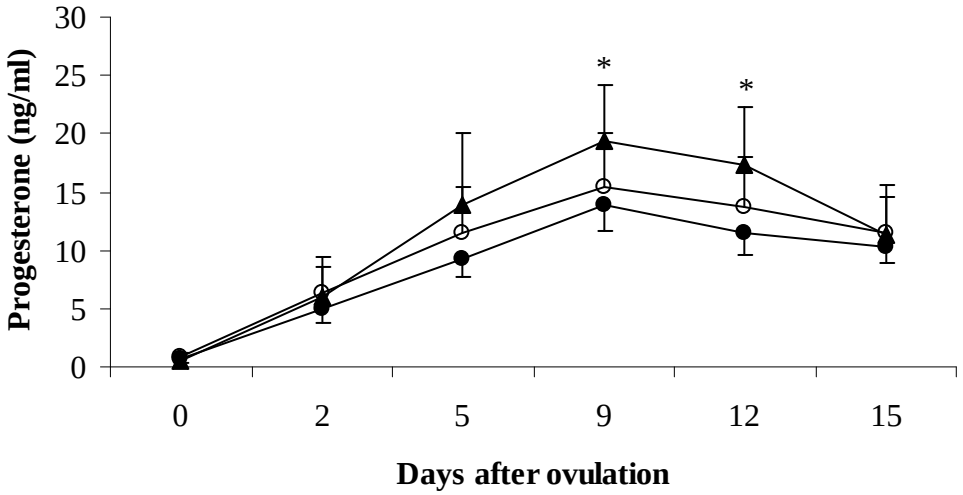

Figure 3. Progesterone concentrations in peripheral blood (mean ± SD) high (●), low (▲) and modified (○) feeding groups in gilts for 15 days after artificial insemination. * $P<0.05$.

Table 4. Luteinizing hormone characteristics for high (HHH), low (LLL) and modified (LHL) feeding groups (mean ± SD) determined from plasma samples collected on day 14 of pregnancy. A sampling window of 10 hours was used in gilts and 12 hours in multiparous sows.

Feeding	Parity	n	Frequency	Amplitude (ng/ml)	Basal level (ng/ml)	Mean level (ng/ml)
HHH	Gilts	5	1.80±0.45	1.24±0.36	0.98±0.22	1.18±0.24
	Sows	6	2.17±0.75	1.19±0.81	0.73±0.21	0.90±0.19
LLL	Gilts	5	2.10±0.74	0.75±0.32	0.79±0.12	0.93±0.15
	Sows	5	2.40±0.89	1.08±0.16	0.94±0.39	1.13±0.38
LHL	Gilts	5	1.50±0.50	0.79±0.28	0.60±0.08	0.70±0.07
	Sows	4	2.50±0.58	1.18±0.12	0.69±0.21	0.91±0.18

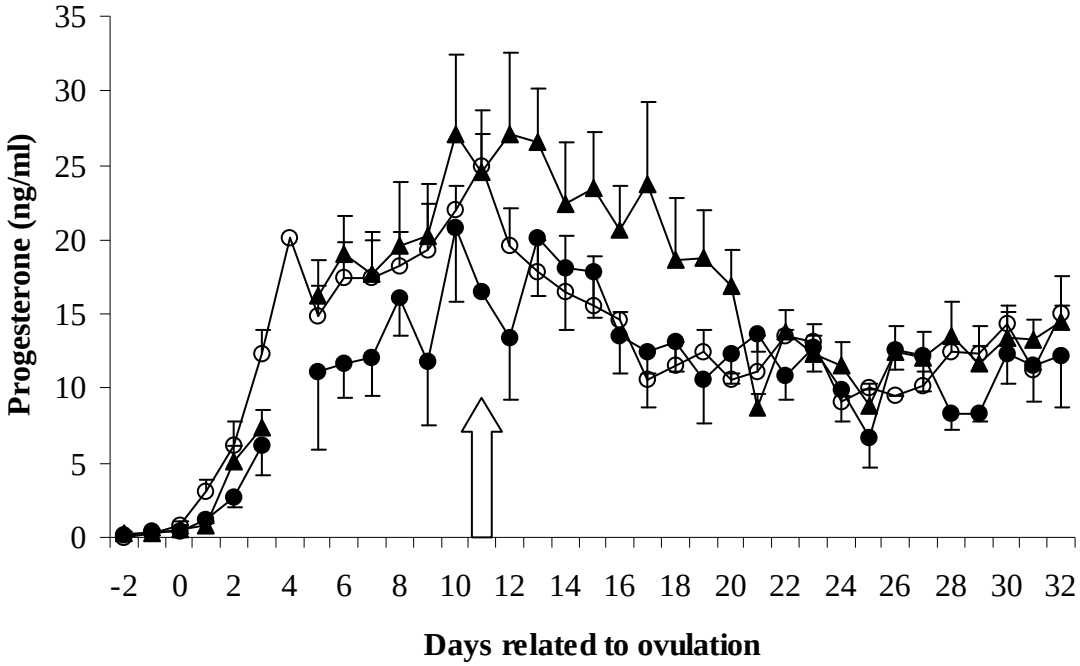

Figure 4. Progesterone concentrations in peripheral blood (mean ± SD) of high (●), low (▲) and modified (○) feeding groups of sows throughout the trial. On day 11 (arrow), feed intake increased in the modified group from 2 to 4 kg per day.

7.3 EFFECT OF SAMPLING SITE ON PROGESTERONE CONCENTRATION (III)

The progesterone concentrations were higher ($P<0.001$) in plasma from the caudal vena cava than in plasma from the jugular vein throughout the sampling period (Figure 5), being 19±11.5 ng/ml and 8±2.6 ng/ml (mean ± SD), respectively. The variation (mean ± SD) between two consecutive 30-minute progesterone samples was greater in plasma from the caudal vena cava than in plasma from the jugular vein, being 8.7±9.4 ng/ml and 1.8±0.9 ng/ml, respectively. After feeding, the progesterone concentration in jugular plasma from gilts decreased steadily in all groups throughout the day (Figure 5). In vena cava plasma, by contrast, marked variation in progesterone concentrations was observed throughout the day. Based on intensive sampling, an episodic pattern of progesterone production was evident in plasma samples collected from the vena cava but not from those collected from the jugular vein (Figure 6). Some progesterone pulses were associated with preceding LH pulses. A LH pulse preceded every episodic release of progesterone in the vena cava in one of the gilts. Another gilt did not have any pulses that fulfilled the criteria set for a progesterone pulse. However, progesterone concentration rose whenever a LH pulse occurred.

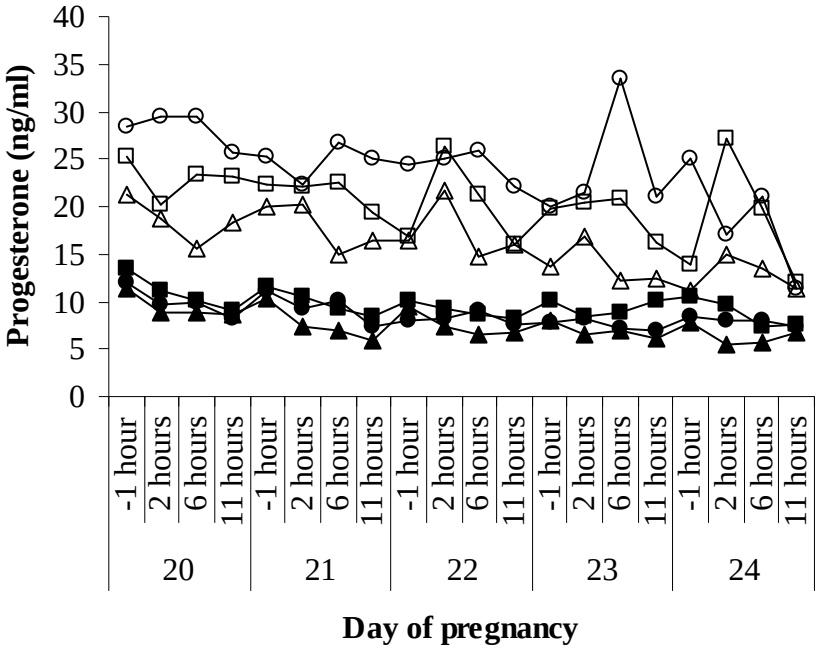

Figure 5. Means of progesterone concentrations in caudal vena cava (VC) and jugular vein (VJ) plasma of high (VC □-□; VJ ■-■), low (VC ∆-∆; VJ ▲-▲) and modified (VC ○-○; VJ ●-●) feeding groups on sampling days. Two consecutive samples were taken 30 minutes apart four times a day, at 1 hour before feeding (9.00 am), and at 2, 6 and 11 hours after feeding.

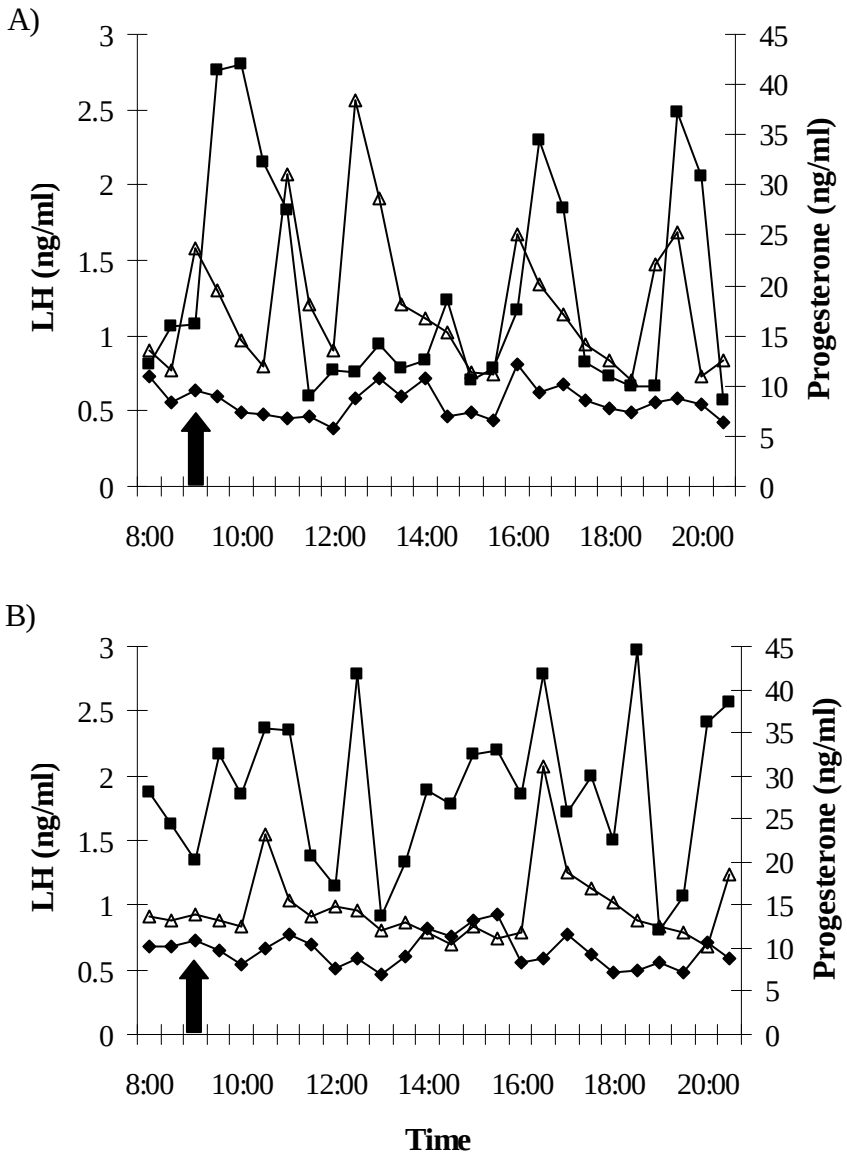

Figure 6. Plasma progesterone profiles measured from the caudal vena cava (■-■) and the jugular vein (♦-♦), and luteinizing hormone (LH) profiles (Δ-Δ) during a 12-hour sampling window at 30-minute intervals on day 22 of pregnancy for two gilts. The gilt with profile A had a low feeding level since day 11 of pregnancy and the gilt with profile B had a high feeding level since day 20 of pregnancy. Animals were fed once a day at 9:00 am after bleeding (shown with arrow). All feed offered was consumed within 10 minutes by gilt A but not until late afternoon by gilt B.

7.4 GONADOTROPHIN-RELEASING HORMONE ANTAGONIST, LUTEINIZING HORMONE SECRETION AND MAINTENANCE OF PREGNANCY (IV)

The GnRH antagonist treatment suppressed pulsatile LH secretion immediately and was sufficient to abolish LH pulses for a period of more than two days (Figure 7). Seven out of 15 gilts (2/6, 1/5 and 4/4 gilts for groups LH14, LH16 and LH19, respectively) resumed episodic LH secretion within the sampling period, with the mean (±SD) suppression lasting 2.7±1.8 days. All gilts in the LH19 group showed recognizible pulses during day 3 or 4 after treatment. GnRH antagonist treatment influenced LH pulse amplitude significantly (on days 0-3 $P<0.01$, and on day 4 $P<0.05$) in those animals in which pulses were identified. The treatment had only a slight effect on basal LH concentration, but mean LH secretion was sustained until the end of the trial. The antagonist treatment did not affect progesterone concentrations differently between the groups.

One gilt in each group aborted. The mean treatment-to-abortion period was 4.7 days (6, 5 and 3 days in animals treated on days 14, 16 and 19, respectively). In animals that aborted, progesterone decreased below 1 ng/ml on average 4.3±1.2 days after treatment. Abnormal development of pregnancy was indicated by RTU assessment on the same day or the day preceding abortion, before any external signs of abortion were apparent. The volume of fluid in embryonic sacs decreased or remained static in gilts that aborted, while in gilts with normal pregnancies the volume of allantoic fluid increased. Visible signs following abortions included swelling of the vulva and clear sticky discharge. Two of the three gilts formed small (ø1.2-2 cm) cystic follicles, and one seemed to stay anoestrous.

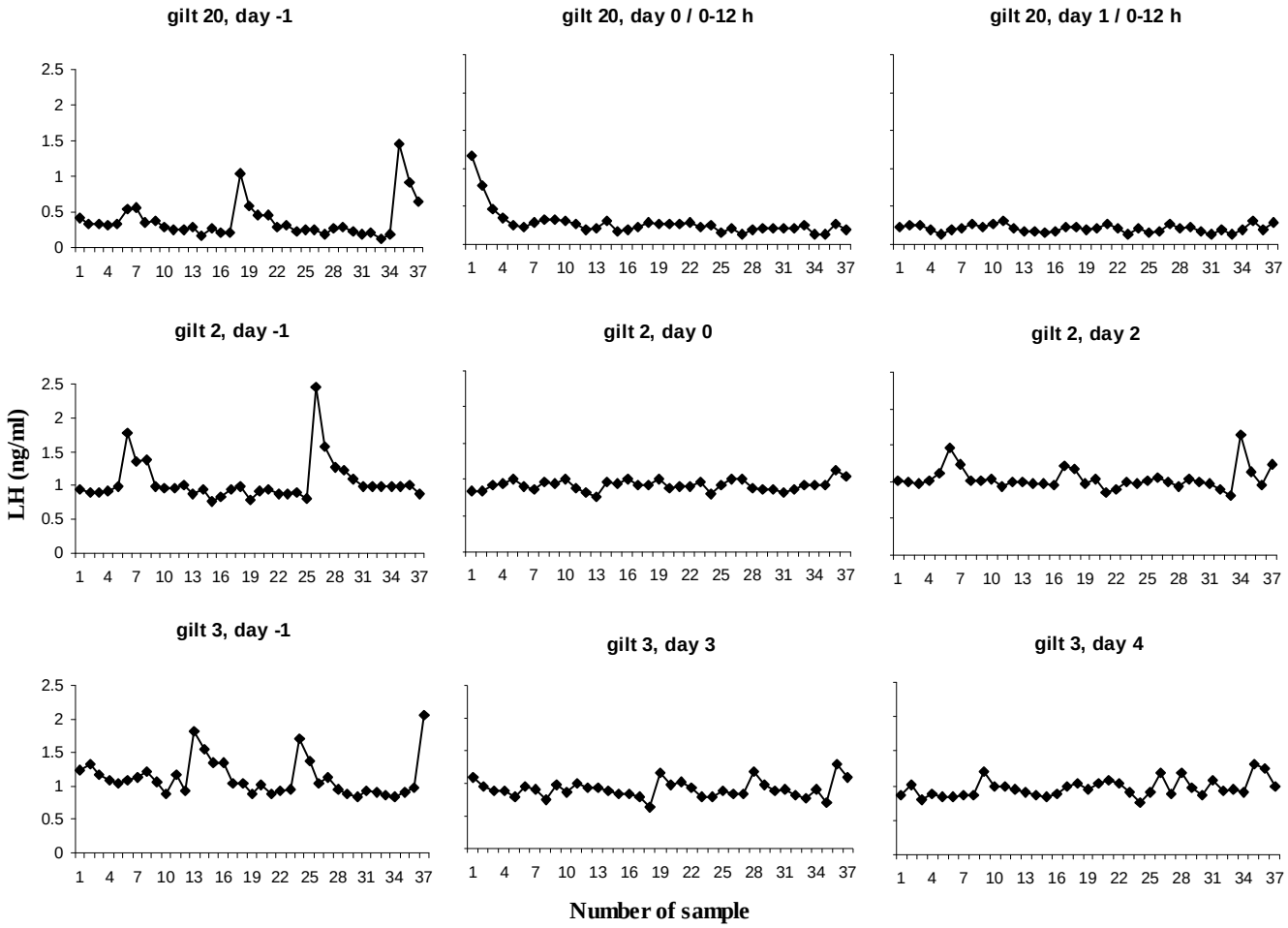

Figure 7. Individual 12-hour luteinizing hormone (LH) profiles one day before the GnRH antagonist treatment (day -1) and two sampling windows after treatment for three gilts. Gilts 2, 20 and 3 were treated on days 14, 16 and 19 of pregnancy. Samples were taken at 20-minute intervals. Day 0 was the day of the treatment. Note the variation on the post-treatment sampling day. None of these gilts had aborted.

8. DISCUSSION

8.1 GILTS BENEFIT FROM HIGH FEEDING

Study I demonstrated a favourable effect of a continued abundant feeding strategy on pregnancy rate of early pregnant gilts during reduced fertility in autumn as reported earlier in a field study by Love et al. (1995). The modified feeding strategy did not provide the benefits anticipated for pregnancy rate or embryonic survival in gilts or in multiparous sows. However, LHL gilts tended to benefit from the higher feeding level during implantation from day 11 to day 17, as evidenced by the pregnancy rate on ultrasound examinations on days 19 and 23. A risk for failure in maternal recognition of pregnancy appeared to be present in the LLL and LHL groups. The feeding level should probably be increased before day 10, and for the LHL group to derive the same benefit as the HHH group the feeding should be continued at a higher level for longer. In the LLL group, with strongly restricted feeding, one gilt lost 15.5 kg during the experiment and two gilts gained only 125 g/day. Both pregnant animals in this group gained more than 400 g/day and had the highest growth rate in the LLL group. The traditionally recommended feeding level for gilts prior to puberty combined with the recommended decrease in feed intake after insemination may therefore have detrimental effects on metabolic status, ovarian activity and reproductive function. Maternal recognition of pregnancy is based on a two-phase oestrogen signal by embryos between days 11 and 18 of pregnancy (Pusateri et al., 1996b). A failure in both oestrogen signals will result in a regular oestrus-to-oestrus interval, but pigs experiencing a failure in the second signal will have a delayed return to oestrus (Pusateri et al., 1996a). In Study I, the second signal was either inadequate in gilts that aborted or these gilts failed to respond to this signal. In LHL gilts detected as pregnant by ultrasound (on days 19 and 23) but eventually interrupting their pregnancy, progesterone level had reached a nadir on day 26 and remained low until day 30. These findings are consistent with those of Tast et al. (2002), who showed that the seasonally decreased farrowing rate is mainly caused by early disruption of pregnancy. Love et al. (1995) also reported that a low level of feeding in summer-autumn was associated with increased numbers of delayed returns to oestrus and low farrowing rates.

8.2 ENDROCRINOLOGICAL BACKGROUND

Complete food deprivation for two days during early pregnancy affects endocrine secretion (Tsuma et al., 1996a), and evidence also indicates that longer term feed restriction affects endocrine secretion, increasing cortisol concentrations and reducing LH secretion (Prunier et al., 1993). Food deprivation during early pregnancy is stressful and may cause changes in hormonal concentrations, which may in turn affect reproductive performance. The manifestation of stress is associated with elevated circulating cortisol levels (Einarsson et al., 1996; Dobson and Smith, 2000). Chronically elevated circulating cortisol levels inhibit LH secretion (mean LH concentration and pulse frequency) in pigs (Estienne et al., 1991; Turner et al., 1999b) and can even block the LH surge and ovulation in gilts (Barb et al., 1982). However, a complete blockage of LH pulses for 4 days (Anderson et al., 1967) or 4-9 days (Peltoniemi et al., 1995; Tast et al., 2000) is needed to initiate regression of CL in early pregnant pigs. In Study II, neither cortisol concentrations nor the response to adrenocorticotropic hormone (ACTH) challenge were affected by feeding level in multiparous sows. This might be explained by the straw bedding that the sows used to supplement their diet, decreasing stress produced by the low feeding rate. In addition, these sows were not a socially stressed group and management was optimal. Daily sampling was also performed with minimal stress during feeding via vena saphena or coccygea. Moreover, our study was not performed during

a known period of infertility. Differences in cortisol concentrations might, however, have been lost because of the diurnal rhythm of cortisol (Janssens et al., 1995). An increase in cortisol concentration in chronically stressed pigs has been found to be significant only when measured from evening plasma and not from morning plasma. Further studies are needed to clarify the role of cortisol as a marker of chronic stress caused by group housing and reduced feeding in early pregnant pigs, especially in gilts.

8.3 PROGESTERONE AND FEEDING LEVELS

Abundant feeding improves pregnancy rate during the seasonal infertility period (Love et al., 1995; Study I). The underlying mechanism is not clear. A high rate of feeding during the immediate period after ovulation has a detrimental effect on embryonic survival (Jindal et al., 1996; Ashworth et al., 1999). This detrimental effect is mainly associated with lowered circulating progesterone concentrations in early pregnancy (Pharazyn et al., 1991; Jindal et al., 1996; Jindal et al., 1997) and with a longer period from ovulation to peak progesterone (Jindal et al., 1997). Lower progesterone concentrations detected in peripheral blood in liberally fed gilts and multiparous sows are probably due to increased blood flow in the portal vein and increased metabolic clearance of progesterone (Prime and Symonds, 1993). Contrary, in Meishan gilts pre-mating diet seems to affect embryonic survival more than post-mating diet (Ashworth et al., 1999). However, in Study I and II, a decreased survival rate of embryos was clearly seen in only multiparous sows, not in gilts. This difference between gilts and sows might be explained by their different immune responses and metabolic status in general. In gilts, LH secretion increased while circulating progesterone concentration decreased with liberal feeding. However, in LHL group an effect of feeding on progesterone level was not so clear, which might indicate a presence of other factors affecting progesterone level. Booth (1990) suggested that increased insulin (due to abundant feeding) might increase progesterone production and concentration at the uterine level directly or be mediated by enhanced LH secretion. Furthermore, some earlier studies have provided evidence of a countercurrent system in pigs, which results in locally elevated progesterone levels in the uterus (Pharazyn et al., 1991; Stefanczyk-Krzymowska et al., 1998). This results in the countercurrent transfer of steroid hormones from venous blood flowing from ovaries to arterial blood supplying the oviduct and uterus. Progesterone production and its concentration in the uterus are hypothesized to be enhanced during the seasonal infertility period due to liberal feeding despite lowered peripheral levels of progesterone.

8.4 PROGESTERONE AND EMBRYONIC SURVIVAL

Study III demonstrated a great difference in progesterone concentrations before and after metabolism, i.e. in the caudal vena cava versus the jugular vein, representing the dilution effect and the metabolic effect of the liver. This may imply a higher progesterone concentration locally in the oviduct and/or in the uterus, supporting the secretion of proteins from the endometrium into the uterine lumen and the involvement of the conceptus. Metabolism of progesterone into oestrone, oestradiol and oestriol by the tubular and filamentous conceptus and by endometrial tissue is evident in early pregnancy (Fischer et al., 1985), and higher progesterone concentrations in the uterus may increase embryonic oestrogen secretion. Exogenous oestrogens given on days 12 through 19 or 25 after oestrus seem to be optimal for maintaining the CL for more than 50 days in cyclic gilts (Pusateri et al., 1996a). This probably mimics secretion of embryonic oestrogens after day 12 of pregnancy to maintain the CL. Although benefits of liberal feeding during a period of low fertility are apparent (Love et al., 1995) and suggested to be mediated by progesterone, Study III

failed to provide evidence of a relationship between progesterone production and feeding level during days 20-24 of pregnancy. A different outcome also in pregnancy rate between these two studies (Studies I and II) might be due to differences in feeding regime and housing system. Liberal feeding should probably commence immediately after mating to provide any benefit to fertility or to LH/ progesterone production. Study III was not carried out under conditions known to predispose to seasonal infertility.

Study III provided evidence of pulsatile progesterone secretion in early pregnant gilts, a finding also made in late pregnant miniature pigs (Parvizi et al., 1976). The role of these progesterone pulses in endometrial activity remains unclear. Samples for progesterone concentrations should be obtained at an earlier stage of pregnancy than done here; samples were obtained when the CL had already become dependent on LH. Decreased progesterone secretion (decreased episodic pattern, decreased basal concentration) might result in decreased countercurrent progesterone transfer, which in turn may interfere with secretions of vital histotrophes from the endometrium (Figure 8). This may disturb development of embryos to the extent that the embryonic oestrogen signals are insufficient to maintain pregnancy. Alternatively, LH and progesterone may be involved directly in PGF-2α secretion and reorientation. According to the result of Study III, a single blood sample from a conventional peripheral sampling site is not adequate to give information of progesterone production and feeding interaction. In future studies, the effect of feeding on progesterone production should be examined using intensive sampling from the caudal vena cava or from the utero-ovarian veins.

8.5 DISRUPTION OF PREGNANCY

The mechanism causing disruption of pregnancy during late summer and autumn is thought to be luteolysis due to reduced LH secretion, followed by a decline in progesterone concentration, which results in embryonic death and abortion (Wrathall et al., 1986). A number of earlier studies have shown that prolonged complete suppression of LH results in cessation of pregnancy within 4-9 days (Anderson et al., 1967; Peltoniemi et al., 1995; Tast et al., 2000). According to these studies, decreased progesterone secretions due to regression of the CL preceded an abortion. However, reduced LH secretion may be insufficient to cause regression of the CL directly. Total abolition of LH pulses has not been associated with the seasonal infertility period. Tast et al. (2000) suggested that impaired LH secretion might first interfere with development of embryos in the early stage of pregnancy, which then leads to regression of the CL through a change in the direction of PGF-2α transport in the uterus. If the CL work autonomously for 12 days, with a peak progesterone secretion between days 10 and 14, the first oestrogen signal by embryos should be entirely independent of LH secretion. LH may, however, have a direct effect on the uterus. Progesterone concentrations are dependent on the number of newly formed CL as well as on the rate of feeding. Any factor decreasing the viability of embryos and their ability to produce the essential oestrogen signals or any factor interfering with the maternal response to these signals could be involved in the mechanism of the early disruption of pregnancy. The following discussion provides a hypothesis for the mechanism underlying the early disruption of pregnancy and how feeding could diminish detrimental seasonal effects.

The results of Study IV provide further evidence of the role played by LH in early disruption of pregnancy. A temporary suppression of LH was achieved by a GnRH antagonist; LH pulses were abolished for less than three days after treatment. Thereafter, LH pulses were of lower amplitude for a prolonged period than pulses prior to treatment. LH secretion after the GnRH antagonist treatment seemed to mimic the seasonal suppression of LH secretion, with an irregular basal level and low

amplitude of pulses detected (Paterson et al., 1992; Peltoniemi et al., 1997b). While the GnRH treatment followed by a decreased LH secretion did not appear to affect progesterone concentrations in peripheral blood, a difference in progesterone concentration at the uterine level may exist that is not evident in the peripheral blood. Progesterone can be considered the main hormonal mediator involved in pregnancy failure since progesterone orchestrates the secretions of histotrophes from the endometrium. Progesterone concentrations are higher at the uterine level than peripherally indicating a local and vital role for progesterone in the uterus. Results in peripheral blood are similar to those of Tast et al. (2002), who found no difference in progesterone levels between spring and autumn in early pregnant pigs. They did, however, report a tendency for lower progesterone concentrations beyond day 13 in animals loosing their pregnancy than in those remaining pregnant. Nevertheless, the difference was not significant until day 20 of pregnancy. This may indicate individual variation in the time of abortion, seen also in the gilts that aborted after GnRH antagonist treatment.

8.6 CONCLUDING REMARKS

In conclusion, high feeding level during early pregnancy improves weight gain and pregnancy rate in gilts, at least during the decreased fertility period. Findings in early pregnant multiparous sows suggest that to achieve optimal reproductive performance multiparous sows should be fed differently from gilts. Our results support the theory that the oviduct and uterus are supplied with higher concentrations of progesterone than in the peripheral circulation during early pregnancy. Progesterone is secreted in an episodic pattern, which is not detectable in peripheral blood. The frequency of progesterone pulses is similar to the frequency of LH pulses. Impaired LH secretion (induced by a GnRH antagonist) of less than four days' duration, mimicking a seasonal effect, is adequate to cause early disruption of pregnancy in some pigs. However, this time period is insufficient to cause regression of the CL directly. Lowered LH secretion in autumn may suppress progesterone secretion at the uterine level by decreasing either progesterone pulses or mean progesterone concentration. Decreased local progesterone levels interfere with uterine histotrophes and viability of embryos and oestrogen signals. This may cause PGF-2α secretion to be redirected towards the uterine vascular system, which can eventually result in regression of the CL and loss of the whole litter. After these endocrinological changes, the sow or gilt returns to oestrus between days 24 and 30 after mating, as seen in many piggeries. A schema of proposed season and feeding level interactions affecting early pregnancy in pigs is presented in Figure 8. Further studies are needed to clarify association between episodic progesterone and LH secretion during the period of pregnancy recognition and to examine effects of feeding levels on these hormones.

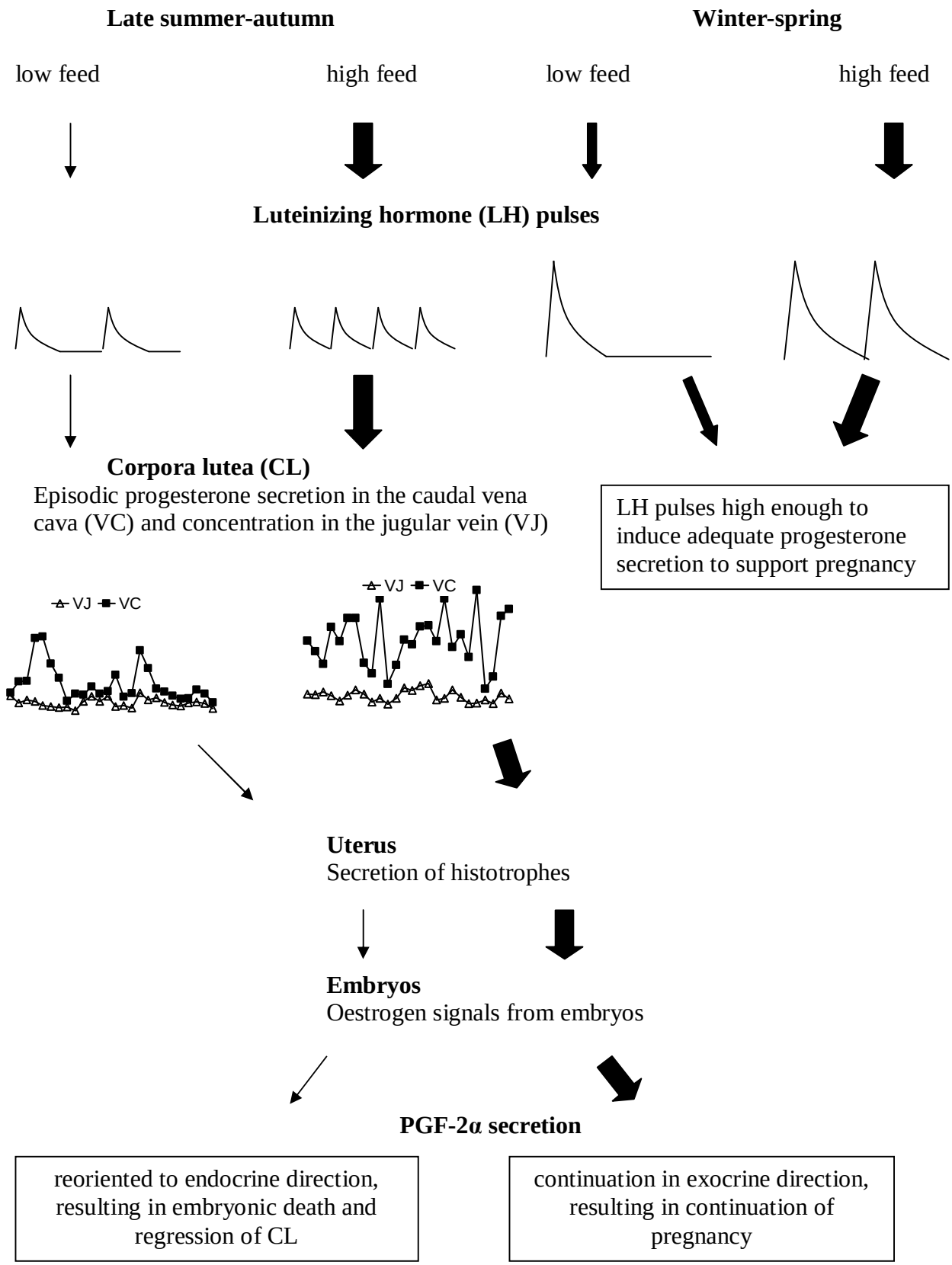

Figure 8. Schematic presentation of the proposed mechanism mediating effects of different feeding levels on the establishment of pregnancy. The width of arrows describes support from the previous stage to the following stage. There is evidence during late summer and autumn of benefits of high feeding level on fertility.

9. CONCLUSIONS

1. Abundant feeding during early pregnancy improves weight gain and pregnancy rate in gilts, at least during the decreased fertility period.

2. Findings in early pregnant multiparous sows indicate that a reduced feeding strategy might be beneficial for reproductive performance in older sows but not in gilts. This is particularly the case when pigs are given an optimal environment and handling.

3. Some evidence was provided of an episodic pattern of progesterone secretion, which is not detectable in jugular blood in early pregnant pigs. Further studies are needed to verify the episodic nature of progesterone secretion and to clarify the relationship between LH pulses and progesterone secretion.

4. The role of progesterone as a mediator of the effects of post-mating feeding levels on the uterine environment and embryonic viability has previously been examined by progesterone concentrations in jugular plasma, which may not be informative enough. Therefore, intensive sampling from the caudal vena cava or uterine veins should be preferred.

5. Impaired LH secretion induced by a GnRH antagonist mimicking a seasonal effect was able to cause early disruption of pregnancy in some pigs. However, the period of LH suppression was not sufficiently long to cause regression of the CL directly.

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