How does nutrition influence luteal function and early embryo survival

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The pre-ovulatory LH surge triggers luteinisation of follicle tissue, but subsequent development of corpora lutea to full size is independent of LH up to around day 12 of pregnancy. Thereafter, severe (pharmacological) inhibition of LH secretion for 3 to 5 days will result in luteal failure and loss of pregnancy. It is unlikely that nutritional circumstances will have a similar effect, although scenarios with severe undernutrition have hardly been studied during early pregnancy. Milder levels of pre- and postmating undernutrition (around maintenance requirements), do affect luteal tissue development, but whether this is related to variation in LH is not clear as studies are equivocal, and there are indications that other nutrition related factors, like IGF-1 and insulin, may mediate these effects. A high plane of nutrition seems to increase progesterone secretion by the ovaries, even though systemic progesterone is reduced at the same time. Since there is direct transfer of progesterone from ovarian veins to the uterus, this may explain why a high plane of nutrition may actually benefit embryo survival and pregnancy, although very early during luteal formation (first days after mating), secretion by the ovaries may be overridden by systemic clearance of progesterone on a high feed level. Direct measurement of progesterone secretion by the ovaries is poorly understood as is the transfer of progesterone to the uterus and effects of specific nutrition related mediators such as IGF-1 and insulin on these processes.

Introduction

After rupture of the pre-ovulatory follicles at ovulation, reformation and reorganization of tissue take place at a remarkable speed, ultimately resulting in the formation of fully functioning corpora lutea by 7-10 d after ovulation, and in sheep, the proliferation rate of different cells involved in luteal tissue formation (luteal cells, endothelial cells, fibroblasts) has been likened to that of rapidly growing tumors (Niswender *et al*., 2000). The pre-ovulatory LH surge triggers the cascade of luteinising processes but as will be discussed in this paper, other factors control the development and later on, the function of established corpora lutea. Angiogenic factors such as VEGF influence corpus luteum formation and function early on (Schams and Berisha, 2004). Factors other than angiogenic factors that have been described are cytokines in cattle (Webb *et al*. 2002), luteotrophic factors like LH (see below), and IGF-1 (Ptak *et al*., 2003, 2004; Miller *et al*., 2003; Schams *et al*., 1999). The importance of some factors in the development

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and function of established luteal tissue in the pig will be discussed, only where there is an identified relation to nutrition, based on the available literature.

Figure 1 shows the development of luteal tissue and a typical profile of systemic progesterone during the embryonic phase. From figure 1 it can be deducted that porcine corpora lutea reach their full size between day 10 and 12 after ovulation, with total luteal mass at this stage ranging between 6 to 8 g in gilts and 10 to 15 g in multiparous sows (Langendijk 2012, unpublished results). A major factor determining total luteal mass is ovulation rate, with the correlation between ovulation rate and luteal mass ranging between 0.45 and 0.62 (Almeida *et al*., 2001; Willis *et al*., 2003; Athorn *et al*., 2012). This explains why multiparous sows have more luteal tissue than gilts and first parity sows. Systemic concentration of progesterone is related to total luteal mass (r = 0.26–0.45; Athorn *et al*, 2012), although this relationship is probably underestimated because it is generally based on blood samples obtained at a different time point than the assessment of luteal mass. Reflecting this relationship, progesterone in the circulation roughly follows the development in luteal tissue mass. After day 12-13 the luteal tissue mass decreases again but the reason for this is unknown.

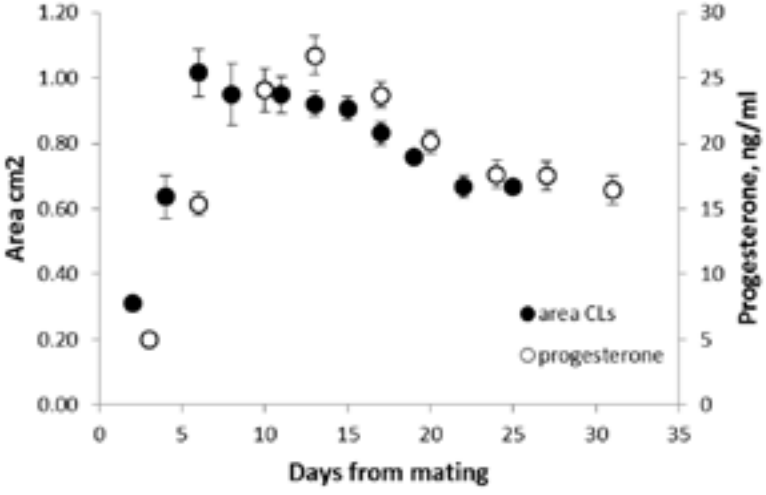

Fig. 1 Development of luteal tissue (average area of CLs measured using ultrasound) in 6 sows and gilts during early gestation (closed circles), and systemic progesterone measured in a different group of sows (open circles). Data from Tast *et al*. (2002) and from Bouwman *et al*. (2012, unpublished)

Role of LH in establishing and maintaining luteal tissue

In pigs, development of the corpora lutea after ovulation and the secretion of progesterone occur independent of LH input from the pituitary, at least until 10–12 days after ovulation (Peltoniemi *et al*., 1995). Hypophysectomy on the day after oestrus or mating does not prevent the development of normal-sized, progesterone-secreting corpora lutea by day 12 after oestrus (Anderson *et al*., 1967), but corpora lutea do regress between days 16 and 20 in pregnant, hypophysectomised sows. Meduri *et al*. (1996) showed that at 48 h after follicle rupture, there is a marked decrease in the density of LH receptors in luteal cells, and 6 days after ovulation the receptor density seems to increase again. These findings indicate an LH-independent and LH-insensitive window during early development of the corpus luteum.

More recent studies by the group of Peltoniemi *et al*. have approached the role of LH in the maintenance of luteal tissue using three different models. First, pregnant gilts received GnRH agonist implants to down regulate GnRH receptors and suppress LH pulsatility (Peltoniemi *et al*., 1995). Second, active and passive immunisation against GnRH was used to reduce LH pulsatility in the early pregnant gilt (Tast *et al*., 2000). Third, a GnRH antagonist was used to directly down regulate LH pulses (Virolainen, 2003).

Based on these models, beyond days 10 and 12 of pregnancy, support of the corpora lutea by LH does become important, although in some studies it seems that reduction in gonadotrophic support has to be severe and chronic to result in luteal regression and pregnancy failure. LH secretion during the luteal phase of the oestrous cycle and during early pregnancy is characterised by a lesser frequency of greater amplitude LH pulses (Langendijk *et al*., 2007 and figure 2). Chronic treatment with a GnRH agonist from days 14 to 21 of pregnancy abolished LH secretion and resulted in a decrease in progesterone secretion and loss of pregnancy in all sows at around 15 days after the start of treatment (Peltoniemi *et al*., 1995). Similarly, Easton *et al*. (1993) observed a decline in progesterone between 13 and 21 days after implantation with a slow-release agonist of GnRH (at oestrus), which is 3 d to 11 d after corpora lutea have started to become sensitive to LH. The use of a GnRH agonist by its nature initially increases LH release before down-regulating LH pulses, and may also cause some extra luteal tissue to be formed, delaying the suppression of LH and effects on luteal function. In contrast, single injection with a GnRH antagonist between days 14 and 19 after ovulation resulted in a more immediate disruption of LH secretion for a period of 2.7 days, on average, and loss of pregnancy in three of 15 sows (Virolainen *et al*., 2003). Active or passive immunisation against GnRH (Tast *et al*., 2000) also had a more immediate effect with a reduction in progesterone within 2 to 4 days, and luteal failure evident within 7-10 days from immunization. In the immunisation model none of the sows maintained pregnancy. Interestingly, immunization at day 10 of pregnancy seemed to cause a reduction in progesterone and failure to establish pregnancy before total luteal failure occurred, whereas immunization at day 20 of pregnancy resulted in total luteal failure before abortion occurred. The first observation may provide some explanation for seasonally related pregnancy failure.

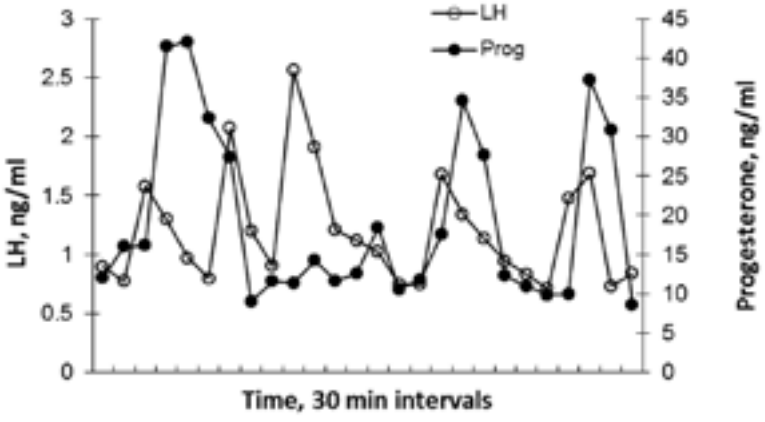

Fig. 2 Pulsatile profile of progesterone in the caudal vena cava (closed circles), and LH (open circles) for an individual gilt during a 12 h sampling period at day 22 of pregnancy. Data from Virolainen *et al*. (2005).

The models described above indicate that a strong suppression of LH that lasts 3 to 5 days will result in luteal failure and as a consequence result in no pregnancy being established or

abortion, depending on the stage of pregnancy. This is supported by recent data from O' Leary *et al*. (2012, personal communications), who treated gilts with 40 mg of altrenogest daily from day 14 through day 24 of pregnancy, and reported an immediate regression of luteal tissue (determined by ultrasound) and a drop in progesterone to basal levels by day 18, in 4 out of 5 gilts. The gilts aborted when altrenogest treatment ceased. As a conclusion, there is enough evidence to state that LH secretion or a minimum of LH pulses is important to support CL and maintenance of early pregnancy in the pig, beyond day 12 after fertilisation.

It is less clear how physiological variation in LH secretion directly affects progesterone secretion. Progesterone is secreted by the ovaries in pulses, which only becomes clear when blood is sampled frequently from the caudal vena cava. However, only some of these pulses are temporally associated with LH pulses (Virolainen *et al*. 2005; Brüssow *et al*., 2011). One could speculate that a low level of LH secretion is sufficient for the function of corpora lutea in terms of progesterone secretion. In fact, Easton *et al*. (1993) suggested that a few pulses of LH, rather than a certain basal level of LH, are important for luteal function. If so, variation in LH secretion within a physiological range, as observed in some reports (Prunier *et al*., 1993; Peltoniemi *et al*., 1997a; Peltoniemi *et al*., 1997b), would not have much influence on luteal maintenance. Nevertheless, progesterone concentration is transiently elevated once LH secretion is stimulated pharmacologically by a GnRH agonist (Peltoniemi *et al*., 1995), and treatment with eCG at day 10 of gestation had an immediate effect on progesterone (O'Leary *et al*., 2011). Even though normal variation in LH secretion during the luteal phase may not have a direct effect on progesterone concentration, LH secretion may be important for luteal function in the long term. It has been shown that LH affects the number of luteal cells and treatment of sheep with hCG in the early luteal phase causes a shift in the size distribution of luteal cells, from small to large luteal cells (Fitz *et al*. 1982). Therefore, differences in LH secretion may affect progesterone secretion in the long term due to differences in steroidogenic properties of small and large luteal cells (Fitz *et al*. 1982; Niswender *et al*. 1985). These differences may be reflected by changes in total luteal mass, as shown by Quesnel *et al*. (2000) in cyclic gilts treated with an LH antagonist that diminished LH pulses in the luteal phase.

Pre mating nutrition

The effects of pre-mating nutrition on luteal function after ovulation have not been studied as such. However, there is an abundance of studies designed to explore the effects on follicle development and ovulation rate. Some of these studies report on carry-over effects on postmating luteal function. These studies have mainly focused on the effects of feed level during the follicular phase, or earlier, during the luteal phase of the preceding cycle in gilts, and lactation in primiparous sows. Feed restriction (maintenance vs 2.5-3.5 kg) during the second week of the luteal phase in gilts for example, resulted in a slower rise in progesterone after subsequent ovulation (Almeida *et al*., 2001; Chen *et al*., 2012a). Administration of insulin counteracted this effect in vivo (Almeida *et al*., 2001) and resulted in increased progesterone secretion in vitro (Mao *et al*., 2001). Ashworth *et al*. (1999) also reported increased progesterone in gilts fed a high feed level (3.5 kg vs 1.15 kg) pre-mating, and reported an increase in the weight of corpora lutea. In primiparous sow models, feed restriction during late lactation had similar carry-over effects on post-ovulatory luteal function with progesterone being lower in feed restricted sows (Mao *et al*., 1999). Van den Brand *et al*. (2001) compared a fat rich and starch rich diet post weaning and reported higher insulin levels but a lower luteal tissue mass (at d35 of gestation) in sows on the starch diet (6.2 g vs 7.0 g), however, progesterone was not measured. Chen *et al*. (2012b), on the other hand, fed a starch/sugar rich supplement during late lactation and reported

higher progesterone at d4 after ovulation compared to control sows and sows fed a fat-rich supplement. In multiparous sows post-weaning insulin characteristics were positively related to post-ovulatory progesterone. This was even though sows on a diet designed to increase insulin did not have a higher progesterone, and even had less luteal mass (9.7 g vs 11.2 g) at d10 after ovulation (Wientjes *et al*., 2011). It seems that pre-mating nutritional treatments (specifically feed level), that improve follicle function and development in general, have positive carry-over effects on post-ovulatory secretion of progesterone. This may be a remnant effect on number or quality of follicle cells, that may influence their secretory capacity after luteinisation, and the effect may be mediated by factors related to metabolic condition such as IGF-1 and insulin. However, the mechanism in terms of development of luteal tissue mass or secretory capacity of that tissue, is not yet clear.

Post mating nutrition

Post mating effects of nutrition on luteal function have to be separated in those that influence formation and establishment of luteal tissue mass and secretory capacity, and direct effects on secretory function. A number of studies report an increase in luteal tissue mass in gilts fed a high feed level compared to those fed around maintenance during the first 2-3 weeks after ovulation. In gilts fed 2.4 M vs 1.2 M during the first 25 d of pregnancy (Athorn *et al*., 2012), luteal weight at d35 of gestation was increased (7.2 vs 6.7 g). In multiparous sows that ovulated during lactation on a reduced suckling regime a 2.5 kg feed reduction during the first week of pregnancy resulted in reduced luteal tissue mass (7.7 vs 9.5 g) at d30 of gestation (Gerritsen *et al*., 2008). These reports assessed luteal tissue mass late after treatments, and short term effects have not been reported much although Athorn *et al*. (2012b) found a lower luteal tissue mass in feed restricted gilts (7.9 vs 8.2 g) at d10 of gestation (n.s.). Partial replacement of cereals in the early pregnancy diet by a fat source did not alter total luteal tissue mass (Athorn *et al*., 2012).

The *indirect* effects of feed level on luteal tissue formation may be mediated by LH, since complete blocking of LH pulses (but basal LH unaffected) with a GnRH antagonist (Antarelix) in the luteal phase of cyclic gilts reduced the weight of corpora lutea and systemic progesterone (Quesnel *et al*., 2000). However, effects of feed level on LH are equivocal. The effect of undernutrition on secretion of gonadotrophins has mostly been studied in prepubertal gilts, mature cyclic gilts, or lactating sows. Moderate feed restriction (fed to maintenance) for at least a week will reduce LH secretion in prepubertal and cyclic gilt models (Booth *et al*., 1990; Booth *et al*., 1996), and severe feed restriction (under maintenance) has been reported to have the same effect on the long term (Armstrong and Britt, 1987). Acute feed deprivation for a short period (one or two days), however, does not necessarily affect LH secretion within 24 h in mature and prepubertal gilts (Barb *et al*., 1997; Barb *et al*., 2001). During the luteal phase in cyclic gilts or during early pregnancy, when LH secretion is limited by negative feedback from progesterone anyway, effects of feed restriction (close to maintenance requirement) have not been studied as much and the available results are equivocal. Some studies report no effect of moderate feed restriction on LH during the luteal phase or early pregnancy (Quesnel *et al*., 2000, Peltoniemi *et al*., 1997a, only amplitude of LH pulses). In contrast, some studies have reported a reduction in LH secretion in feed restricted pregnant gilts (Ferguson *et al*., 2003; Peltoniemi *et al*., 1997b). These studies did not report on luteal tissue mass, except for Quesnel *et al* (2000), who observed no effect of feed restriction on LH or luteal tissue mass. Therefore it is hard to establish whether the reported effects of feed level on LH, which are mild compared to the studies presented earlier with exogenous manipulation of LH, do have a biologically significant impact on luteal tissue formation. This paradigm is poorly established

and requires more research on the effect of moderate and severe feed restriction on LH, IG-1, and insulin, luteal tissue mass, and progesterone secretion, during the windows when luteal tissue is "independent" of LH and thereafter.

Direct effects of nutrition on secretory function of luteal tissue may be mediated by IGF-1 and insulin, since these have been reported to be higher in gilts fed a high feed level during early pregnancy (Ferguson *et al*., 2003; Novak *et al*., 2002). However, no clear relationship in these studies was established. Bovine (Einspanier *et al*., 1990) and porcine (Ptak *et al*., 2003) luteal cells, for example, produce more progesterone when stimulated with IGF-1 in vitro, and Ptak *et al* (2004) reported reduced apoptosis in luteal cells treated with IGF-1 in vitro. Especially during the early luteal phase bovine luteal tissue contains increased numbers of IGF-1 receptors (Schams *et al*. 1999). The influence of IGF-1 on luteal function in the pig has not only been established in vitro, but also in vivo by Miller *et al*. (2003), who found an acute increase in progesterone production when IGF-1 was infused in the ovarian vasculature. Langendijk *et al*. (2008) reported an in vivo relationship between IGF-1 and progesterone shortly after ovulation in primiparous sows. Despite these relationships, there have hardly been any studies that aimed to use dietary manipulations to alter IGF-1 level and luteal function. An increase in feed allocation generally increases IGF-1 in the circulation (Barb *et al*., 2001; Ferguson *et al*., 2003). Nevertheless, Novak *et al*. (2003) did not find a higher progesterone in oviduct veins at 72 h after ovulation in gilts on a high post mating feed level. However, these samples were taken under general anaesthesia, which may have affected the progesterone values. Samples taken from the caudal vena cava close to where the utero-ovarian veins drain, showed an increase in the number of progesterone pulses and in mean progesterone in gilts fed a high feed level (Athorn *et al*., 2012b). Manipulation of feed ingredients (starch or sugars replacing fat) has only marginal effects (Wientjes *et al*., 2011) on IGF-1. In this respect it is interesting to note that in gilts on a high feed level, replacement of starch in the diet with a fat source increased progesterone secretion (at d15 of pregnancy), whilst luteal tissue mass (at d35) seemed to be unaltered (Athorn *et al*., 2012a). No difference in IGF-1 was reported between these diets. More work is required with models that manipulate insulin, IGF-1, fats in the diet, and other potential modulators of luteal function like PGE2.

The role of LH in mediating *direct* effects of nutrition on luteal function is not clear. As mentioned, blockage of LH with Antarelix resulted in less luteal mass and lower systemic progesterone but this may also be a long term effect (Quesnel *et al*., 2000). In the same study, feed restriction did not affect LH or progesterone. Ferguson *et al*. (2003), however, reported a decrease in LH in feed restricted gilts, but an increase in systemic progesterone. The effects of a GnRH antagonist on progesterone secretion during the luteal phase of cyclic gilts does indicate that, although during this window corpora lutea are independent of LH, they are still sensitive to LH, unless other mediators of feed restriction are involved. At day 6 and day 10 after ovulation, feed restriction close to maintenance level, from mating onwards, does reduce progesterone secretion by the ovaries, based on measurements in the caudal vena cava (Athorn *et al*., 2012b). This reduction in progesterone appears to be independent from LH, since only chronic feed restriction for months (Prunier *et al*., 1993), and not short term restriction during the luteal phase (Quesnel *et al*., 2000) affected LH secretion.

As pointed out above, moderate feed restriction has equivocal effects on LH and unfortunately some of the studies mentioned did not assess progesterone. There is some indication for a direct stimulatory effect of gonadotrophins in studies with GnRH agonists (Peltoniemi *et al*., 1995) or hCG (O'Leary *et al*., 2011). However these studies do not reflect the magnitude of impact nutritional manipulations have on LH. More extreme nutritional manipulations such as fasting have rarely been studied. Razdan *et al*. (2004) reported an increased systemic progesterone

during a 2-d fasting period (d13-14 of gestation), whereas Langendijk *et al*. (2012, unpublished data), observed a drop in systemic progesterone in gilts in the days following fasting on day 10 and 11 of gestation, as well as a smaller litter size at farrowing. The increase in progesterone during fasting was thought to be originating from the adrenal glands as part of an acute stress response to the feed deprivation (Razdan *et al*. 2004) but will to some extent have been a consequence of reduced clearance by the liver. The reduction in progesterone after fasting is probably not a consequence of altered LH secretion, since LH secretion (in prepubertal gilts) does not seem to be affected by a two day fast (Barb *et al*., 2001) .

The group of Peltoniemi *et al*. have examined the effects of feeding strategies on LH secretion during early pregnancy in the gilt and sow (Virolainen *et al*., 2004; Virolainen *et al*., 2005b). Three feeding regimens from mating through day 34 of pregnancy, high, low and modified (low until day 14, high until day 21 and low thereafter), were applied to explore effects of feeding on LH secretion and fertility. Modified feeding, which was thought to combine the benefits of restricted feeding after ovulation and abundant feeding during implantation, were first tested on three groups of eight gilts in autumn. The pregnancy rate was higher in the high group (100%) than in the low (25%) or modified (38%) groups, although the high group had significantly lower systemic progesterone concentrations on days 9 and 12. The basal and mean LH levels were higher in the high group than in the modified group. The LH amplitude tended to be higher for gilts in the high group. The modified feeding strategy failed to provide the benefits anticipated. Instead, the high feeding strategy provided a distinct advantage in pregnancy rate.

The same regimens were tested during early pregnancy in the multiparous sow (Virolainen *et al*., 2005b). In this experiment, dietary treatment did not significantly affect LH characteristics (mean, pulse amplitude, pulse frequency and basal level). However, systemic progesterone concentration tended to be lower in the high group than in the low group. In the modified group, progesterone concentration seemed to be associated with the level of feeding. Neither high feeding nor modified feeding provided any benefits for reproductive performance in multiparous sows.

In summary, effects of normal range feed levels on LH and progesterone secretion (not systemic concentration) seem mild, with a higher plane of nutrition increasing progesterone secretion and positively affecting embryo survival and the maintenance of pregnancy. Extreme variations in feed level such as temporary fasting or feed incidents may have more profound effects but have hardly been investigated. Especially effects of (longer term) extreme variations in feed intake on luteal failure are of interest, but also effects of short term variations on progesterone variation prior to implantation. There are also indications for mild effects of other nutritional manipulations on progesterone secretion, such as increasing dietary fat content and ingredients that stimulate IGF-1. Increased feed level does seem to increase luteal tissue mass which may represent more indirect effects of even mild nutritional manipulations on progesterone secretion.

Even though variation in feed intake may only mildly affect LH and progesterone, there are some indications that during periods of seasonal infertility, these effects may be involved in pregnancy failure. Some reported data suggest there are seasonal effects on progesterone supply towards the uterus (Virolainen *et al*., 2005a). It has been proposed that LH secretion may be weak under long days combined with restricted feeding during implantation, resulting in decreased progesterone secretion by the ovaries, and affecting oestradiol signalling by the embryos around day 17 of pregnancy, causing interruption of pregnancy and return to oestrus between d 25-35, typical for seasonal infertility (Tast *et al*., 2002). In contrast, short days in combination with high plane feeding stimulated pineal gland secretion of melatonin and LH from the pituitary; and maintained pregnancy (Peltoniemi and Virolainen, 2006). These

observations are supported by the beneficial effects of abundant feeding on pregnancy rates in gilts (Virolainen *et al*., 2004) and sows (Virolainen *et al*., 2005a).

Local *vs* systemic progesterone

Effects of nutrition on progesterone secretion are complicated by the metabolisation and clearance of progesterone from the systemic circulation by the liver. The liver is the primary organ responsible for clearance of progesterone, and the metabolisation rate increases with feeding level (Prime and Symonds, 1993).

It is important to note that supply of progesterone to the uterine horns does not only rely on systemic progesterone. There is also transfer of progesterone through a counter-current transfer and through lymphatic pathways, and anastomoses between ovarian veins and uterine arteries. These pathways may shunt progesterone directly from ovarian venous blood to arterial blood supplying the oviducts and uterine horns (Krzymowski *et al*. 1990). As this local supply of progesterone is direct, it is not subject to hepatic metabolism like that of systemic progesterone. Therefore an increase in feeding hypothetically increases progesterone secretion by the ovaries and supply to the uterus, whereas clearance from the systemic circulation is increased at the same time. The net effect of these processes eventually determines the available progesterone. Thus, the influence of a high feeding level on progesterone secretion and luteal function can only be truly assessed when taking into account the transfer at a local level, or at least by measuring progesterone as close to the ovaries as possible, unaltered by systemic clearance.

It is not known to what extent the shunting of progesterone by counter-current transfer and other mechanisms, affects the supply of progesterone to the oviduct and uterus. Pharazyn *et al*. (1991) reported that progesterone in both ovarian and oviductal venous drainage was higher than systemic but found no difference in progesterone concentrations between uterine venous drainage and the jugular vein up to Day 16 of gestation. This suggests that ovarian progesterone may only have an effect on progesterone supply to the oviduct and that the effects of elevated plasma progesterone are limited to the period in which the embryos are in the oviduct. However, Stefanczyk-Krzymowska *et al*. (1998) measured progesterone concentrations in blood plasma from branches of the uterine artery supplying both the ovarian and cervical ends of the horn, and found that progesterone was higher in both those arteries compared with the jugular vein. Stefanczyk-Krzymowska *et al*. (1998) also noted that progesterone concentrations in the uterine artery distal to the ovary were significantly lower than in the uterine artery proximal to the ovary. Pharazyn *et al*. (1991) may have found no difference in progesterone concentrations in uterine veins because a vast majority of the hormone (20 to 30% of systemic progesterone) is taken up by uterine tissues (Magness *et al*., 1986), with less progesterone passing through the uterine veins. In a previous study conducted in our laboratory, ovaries were removed from one uterine horn, whilst leaving the opposite horn intact. Higher embryo numbers were found in the horn that was exposed to both local (i.e. direct ovarian supply) and systemic progesterone, compared with the contralateral horn (6.3 vs 5.0 embryos), which was only exposed to systemic progesterone. This difference was more evident in those animals on a high feeding level (Athorn *et al*. 2011).

There are a small number of studies that provide some indication of the magnitude and variation in progesterone when measured close to the source (see Figure 2). Athorn *et al*. (2012 and Virolainen *et al*. (2005) showed that secretion by the ovaries is extremely pulsatile and the concentration in the utero-ovarian vein is much higher (mean 88 ng/ml vs 19 ng/ml; Athorn *et al*., 2012) than in the systemic circulation. The number of pulses, based on the limited data available, does seem to increase with establishment of luteal function (Athorn *et al*., 2012), from

7 pulses per 12 h at day 6 of pregnancy to 10 pulses per 12 h at day 10 (Athorn *et al*., 2012), and decrease again to 6 to 8 pulses per 12 h between day 11 and 17 (Brussow *et al*., 2011), and 0 to 5 pulses by day 22 (Virolainen *et al*., 2005). The release of progesterone seems to be a fairly autonomous process, since the progesterone pulses correlate poorly with LH pulses (Virolainen *et al*., 2005; Brussow *et al*., 2011). A high (2.4M vs 1.2M) feed level did have a mild positive effect on progesterone in the vena cava in the early embryonic phase (d6-9; Athorn *et al*., 2012), but not in established pregnancy (Virolainen *et al*., 2005, d20-24). Novak *et al*. (2003) also provide some insight in the magnitude of progesterone in the oviductal and ovarian vein very early after ovulation. Although these authors did not take serial samples to establish time variation, they did show a much higher progesterone in the ovarian vein, and also that a higher post-mating feed level resulted in a higher concentration of progesterone in that vein although this was not significant.

Feed level and embryo survival

There is no doubt that progesterone is important for remodelling the endometrium to allow successful implantation of, and supply of nutrients to the conceptuses, and hence, systemic progesterone during early gestation (72 h post ovulation) correlates with embryo survival (Foxcroft, 1997, r = 0.48; Zak *et al*., 1998, r = 0.72). Beyond this window embryo survival is not always reported to be correlated to progesterone (Athorn *et al*., 2012, Gerritsen *et al*., 2008). Providing greater amounts of feed reduces systemic concentrations of progesterone due to increased hepatic clearance (Prime and Symonds, 1993). Therefore, feeding a high amount of energy is generally believed to reduce embryo survival (Jindal *et al*., 1996). However, results of studies on nutritional state during early pregnancy are equivocal (e.g. Quesnel *et al*., 2010). Figure 3 shows a number of studies that compared a range of feed levels. There is a wide variation in the feed levels that have been tested. The lower feed level generally ranges between 1.2 and 2.5 kg, which is still higher than some field recommendations that go as low as maintenance. Clearly, the study by Jindal *et al*. (1996) showed that a high feed level in the early 3d-window after ovulation had a negative impact on embryo survival. Two studies that reported a negative effect of a high plane of nutrition during the entire embryonic phase also reported a positive effect of the same high plane on pregnancy rate (Dyck and Strain, 1983; Virolainen *et al*., 2004). Most other studies, however, do not find a negative effect of a high feed level beyond day 3 on embryo survival, despite a consistent reduction in systemic progesterone, and a number of studies even find a positive effect of a high feed level on embryo survival (e.g. Quesnel *et al*, 2010; Athorn *et al*., 2011).

It has to be pointed out that the earlier study by Jindal *et al*. (1996) focused on the period shortly after ovulation. In later studies, this feed level paradigm has subsequently been translated, and maybe wrongly so, to the entire embryonic period. It also has to be noted that in older studies plane of nutrition is applied during the premating as well as the post-mating period. This has effects on ovulation rate and therefore complicates the separation of post mating effects from effects established earlier on (Foxcroft, 1997).

Embryo survival data obtained during early gestation (up to day 15) have to be interpreted with caution. Efficiency of flushing methods (morulas and blastocysts), and the fragility of embryos and morphological aspects once embryos start to elongate, can complicate the assessment of the number of embryos (Jindal *et al*., 1997; Ashworth *et al*, 1999; Athorn *et al*., 2012a).

The paradox between effects of feed level on systemic progesterone and embryo mortality in some studies may be due to the fact that these studies focused on the relationship between embryo survival and progesterone concentrations in the systemic blood circulation, rather than

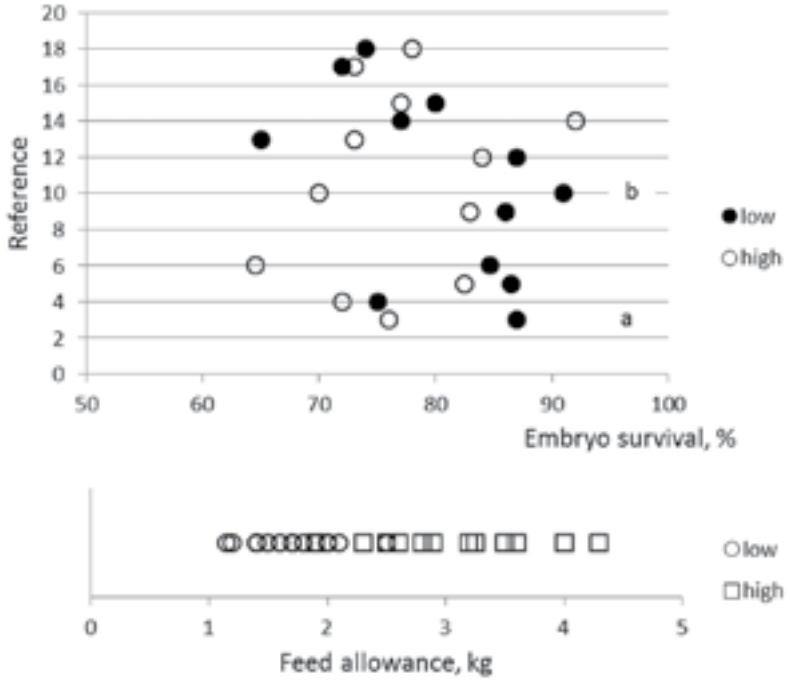

Fig. 3 Feed allowance in different studies with low and high feed level (bottom panel), and embryo survival in gilts (or sows) fed a low or high feed level during early gestation (upper panel). In the upper panel, each number on the y-axis represents one reference with one treatment on a high and one treatment on a low feed level. [a]Pregnancy rate for this reference was 64% on the low and 87% on the high feed level. [b]Pregnancy rate for this reference was 25% on the low and 100% on the high feed level. References: 3 Dyck and Strain (1983) ; 4 Toplis *et al*. (1983); 5 Pharazyn *et al*. (1991); 6 Jindal *et al*. (1996); 9 Ashworth *et al*. (1999); 10 Virolainen *et al*. (2004); 12 Virolainen *et al*. (2005); 13 Quesnel *et al*. (2010); 13 Athorn *et al*. (2011); 14 Athorn *et al*. (2012); 15 Athorn *et al*. (2012); 17 Hoving *et al*. (2012); 18 Soede *et al*. (1999)

concentrations in the blood supply to the uterus. As explained before, the supply to the uterus is not synonymous with systemic supply, because progesterone is 'locally' supplied directly from the ovary to the uterus, via counter current exchange and venous–arterial anastomoses (Krzymowski *et al*. 1990). Therefore, while a high feed intake decreases systemic concentrations of progesterone, it may actually increase the local supply of progesterone to the uterus via an increase in the secretion of progesterone by the ovaries. The amount of progesterone that is supplied to the uterus, and the effect of feeding level on this supply, will ultimately depend on the balance between the systemic clearance of progesterone and the ovarian production of progesterone.

Dynamics in progesterone secretion

The confusing effects of feed level on progesterone measurement in the systemic circulation and effects of feed level on embryo survival may be better understood if the dynamics of progesterone secretion throughout the first weeks of pregnancy and the above distinction between systemic and local circulation are taken into account. The secretion of progesterone by the ovaries is very low immediately after ovulation, but increases rapidly during the first 10 to 13 days thereafter. Early after ovulation, therefore, the gradient between local and systemic blood

is still low, and metabolisation by the liver at this stage will have a relatively large impact on systemic progesterone. With increasing secretory capacity of the ovaries, the gradient between local and systemic concentration will increase. Systemic clearance may then have less impact on the contribution of systemic progesterone. Models that incorporate these dynamics have hardly been explored, and studies that assessed progesterone in the vena cava (Athorn *et al*., 2012, Virolainen *et al*., 2005) provide some insight in the dynamics of secretory capacity, but so far these studies are limited.

Another factor that has to be taken into account, is the dynamics in blood flow to the utero-ovarian complex. In sheep, the blood flow to the ovaries basically follows the rapid increase in luteal tissue mass in early pregnancy (Niswender *et al*., 2000), and because also the concentration of progesterone in utero-ovarian venous blood increases, the total input of progesterone to the circulation, as well as the local transfer to the uterus increases dramatically. These dynamics have to be taken into account when considering the above mentioned windows of nutritional effects on uterine progesterone supply.

Conclusion

It is clear that porcine corpora lutea develop to full functional size at around day 12 of pregnancy, independent of gonadotrophic input, and therefore nutritional manipulations before this stage are not controlled by LH. However, premating and post mating plane of nutrition, and possibly some dietary (fat or IGF-1 promoting) ingredients can influence luteal function early after ovulation through other mechanisms, which require more study. Even after luteal tissue becomes LH dependent, a prolonged (3-5 days) and severe suppression of LH is required to cause luteal failure and loss of pregnancy, and moderate feed restriction is not likely to have such an effect, although again data in this area is lacking. There are however, some indications that moderate feed restriction around the time of implantation may impede the second embryonic oestrogen signal for maternal recognition by modulation of the uterine environment, which may be an explanation for delayed type returns during seasonal infertility. More severe feed restriction such as total deprivation during high ambient temperatures or socially caused feed incidents, may have a profound effect on LH and pregnancy, but there are hardly any studies to support this.

The paradigm of high feed levels causing more embryo mortality may be true for the first few days after ovulation, but the existing evidence does not support the extrapolation of that paradigm later into the embryonic phase. The magnitude and pulsatile secretion of progesterone by the ovaries, as well as "shunting" of progesterone from the ovaries directly to the uterus has probably been overlooked. This may explain why a high plane of nutrition after the first week of pregnancy may actually increase progesterone secretion and supply to the uterus, and benefit embryo survival and maintenance of pregnancy, even though systemic progesterone may be reduced by a higher clearance rate. This hypothesis needs more investigation, taking into account the dynamics of progesterone secretion during early luteal tissue formation and the dynamics of blood flow to and from the utero-ovarian circulation.

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