Dietary manipulation of local versus systemic progesterone and effects on embryo survival and litter size in gilts

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ABSTRACT

Progesterone is an important driver of endometrial function and as such plays an important role in early embryo development, implantation and survival. High feeding levels during early pregnancy in gilts usually result in a decrease in the concentration of progesterone in systemic blood circulation, through an increase in hepatic metabolism, and have as a consequence been associated with a reduction in embryo survival. However, effects of feeding level on embryo survival have been equivocal with some studies finding no, or even, positive effects of an increased feeding level on embryo survival. This paradox may be due to an underestimated supply of 'local' progesterone (directly from the ovary to the uterus), which may be enhanced at a higher feed level. This thesis proves the importance of the contribution of this local source of progesterone for embryo survival, using a unilateral ovariectomy model, and that progesterone concentrations in the venous drainage from the reproductive organs actually increases at higher feeding levels.

Across all studies presented in this thesis a high feed level was not detrimental to embryo survival and was actually beneficial in some studies. Ovarian production of progesterone may also be increased in animals on a high feed level, and therefore progesterone transferred directly from the ovary to the uterus may add considerably to systemic progesterone supplied to the uterus, and counteract a reduction in systemic progesterone when gilts are fed at a high feed level. Furthermore, a high feeding level also seemed to be beneficial in terms of growth rate which is important in gilts as they are still growing towards their mature body weight and a higher growth rate was also positively correlated to pregnancy rate.

DECLARATION

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CHAPTER 1

Literature Review

1.1 Introduction

Like most other domestic mammals the domestic pig (*Sus scrofa*) has a reproductive cycle consisting of both a follicular phase, where oocytes develop under the influence of systemic factors, such as gonadatrophins, and local intra-ovarian factors, in preparation for ovulation and a luteal phase, during which the ruptured follicle forms the corpus luteum (CL) which produces the hormone progesterone. Progesterone is commonly termed the 'pregnancy hormone' because it plays a pivotal role in endometrial development as well as early embryo development and survival (Foxcroft 1997). As progesterone is one of the primary hormones involved in the maintenance of pregnancy the formation of functional and efficient corpora lutea is essential for a successful pregnancy. The formation and maintenance of functional corpora lutea is governed by certain factors, such as hormones and growth factors, from before ovulation until the end of pregnancy and subsequent regression of the CL. These factors influence each other and have differing roles depending on the stage of the animal's reproductive cycle. This review aims to highlight some of these factors and elaborate on their role in the porcine reproductive cycle. Furthermore, the role of nutrition and how it can be used to manipulate these factors in order to positively influence pregnancy outcomes is also explored.

1.2 Development of the corpus luteum

The follicular phase of the porcine oestrous cycle ends when there is a sharp rise in luteinising hormone (LH) which is released from the pituitary gland resulting in ovulation of oocytes from the follicles. The second stage of the oestrous cycle is known as the luteal phase and this is where, through the process of luteinisation, the theca interna cells and

granulosa cells of the follicle transform into the small (SLC) and large luteal (LLC) cells of the corpus luteum, respectively (Wuttke *et al.* 1997).

1.2.1 Control

As mentioned previously, the process of ovulation is initiated by a short sharp rise in systemic LH. This surge in LH, which lasts around 20 hours, induces luteinisation of the follicular tissue. For the first 10-12 days after ovulation, further development of the CL occurs independently of gonadatrophic input, or at least gonadatrophins are not essential in this process (Brinkley *et al.* 1964; Peltoniemi *et al.* 1995; Tast *et al.* 2002). Hypophysectomy on the day after oestrus or mating does not prevent the development of normal, progesterone secreting corpora lutea by day 12 after oestrus (Anderson *et al.* 1967). However, between days 16 - 20 corpora lutea do start to regress in hypophysectomised mated sows (Brinkley *et al.* 1964; Anderson *et al.* 1967). Meduri *et al.* (1996) showed that at 48 hours after follicle rupture, there is a marked decrease in the density of LH receptors in luteal cells, and that 6 days after ovulation the receptor density seems to increase again. Therefore, beyond day 10-12 of the luteal phase, support of the corpora lutea by LH does become important, although it seems that a reduction in gonadatrophic support has to be severe and chronic to result in luteal regression and pregnancy failure. Single treatment with a GnRH antagonist between days 14 and 19 after ovulation resulted in disruption of LH secretion for a period of 2.7 days, on average, and loss of pregnancy in only three of 15 sows (Virolainen *et al.* 2003). Whereas, Peltoniemi *et al.* (1995) reported that chronic treatment with a gonadatrophin-releasing hormone (GnRH) agonist from day 14 or day 21 of pregnancy resulted in a short rise in plasma LH for 38h, followed by a reduction in LH for 6 – 9 days resulted in the initiation of regression of the CL. Progesterone concentrations also increased transiently in conjunction with LH following GnRH implant insertion and also followed a downward trend with a steep

decline 2 days before or on the day of abortion 15 days after treatment. Furthermore, active booster immunisation or passive immunisation against GnRH resulted in luteal failure by 3-5 days after the increase in GnRH antibody titre. Gilts immunised via either active or passive immunisation on days 10 and 12 of pregnancy, respectively, seemed to not establish implantation (based on ultrasound), did not signs of abortion, and were not pregnant on day 18 after mating. Gilts immunised via active booster immunisation at day 20 of pregnancy also showed luteal failure (decline in progesterone) 3-5 days after the increased in GnRH antibody titre, and did show signs of abortion 10 days after booster immunisation (Tast *et al.* 2000). Unlike treatment with a GnRH agonist, active or passive immunisation against GnRH did not result in an initial rise in LH and progesterone, but did result in quicker luteal failure than that of the GnRH agonist model. This may be because the initial rise of LH seen in the GnRH agonist model may boost CL function before the negative effects of the GnRH agonist starts to affect luteal function.

1.2.2 Angiogenesis

The formation of the CL in the pig requires a significant remodelling of the vascular system, termed angiogenesis, within the ovary (Boonyaprakob *et al.* 2003). The success of the angiogenic process is of vital importance as it is essential for the development of mature corpora lutea capable of producing high levels of progesterone. The process of angiogenesis starts during the follicular phase resulting in a complete remodelling of the vasculature within the ovary (Martelli *et al.* 2006). After ovulation occurs, capillary endothelial cells located in the vessels of the internal theca rapidly proliferate and invade avascular granulosa layers, forming a widespread capillary network to supply the proliferating luteal cells which will form the CL (Martelli *et al.* 2006). Regulation of this process is markedly dependent on the secretion of vascular endothelial growth factor (VEGF) (Mattioli *et al.* 2001; Boonyaprakob *et al.* 2003).

1.2.3 Function of the corpus luteum

Once formation of a functional CL is achieved there are many regulatory factors involved in its function throughout the early, mid and late periods of the luteal stage. Many of these factors play a supporting role in the secretion of progesterone, and are vital for embryo survival and the maintenance of pregnancy.

1.2.4 Luteinising hormone

From about day 12 of pregnancy in the pig LH produced by the pituitary gland is one of the most important hormones in the stimulation of progesterone secretion by the small luteal cells of the corpus luteum (Virolainen *et al.* 2003). Luteinising hormone release from the pituitary is controlled by GnRH which is secreted episodically from the hypothalamus (Peltoniemi *et al.* 1995; Virolainen *et al.* 2005b). However, the way in which LH stimulates the production and secretion of progesterone is not entirely clear. In a study conducted by Virolainen *et al*. (2005b) in which progesterone pulses were measured conjunction with LH pulses in the vena cava on day 22 of gestation, pulses were shown to correspond approximately 50% of the time suggesting that LH pulses leads to progesterone release from the ovary. However, Brüssow *et al*. (2011) did not find any relationship between LH pulses and progesterone pulses in blood measured in the vena cava of gilts treated with a GnRH analogue on days 11, 13, 15, or 17 of gestation. Peltoniemi *et al.* (1995) also reported that only supraphysiological exogenous stimulation of LH secretion directly influenced progesterone concentrations. Easton *et al*. (1993) suggested that it may be that only a few pulses of LH are needed to maintain luteal function, rather than a certain base level of LH . Moreover, only severe, long-term abolishment of LH secretion results in regression of corpora lutea and subsequent pregnancy failure (Peltoniemi *et al.* 1995; Tast *et al.* 2000; Virolainen *et al.* 2003).

1.2.5 Insulin like growth factor - 1

Insulin like growth factor – 1 (IGF-1) has been shown to affect early luteal function in cattle (Schams and Berisha 2002; Webb *et al.* 2002) and pigs (Ptak *et al.* 2003). *In vitro* studies have not only shown a positive effect of IGF-1 on progesterone secretion by luteal cells (Ptak *et al.* 2003), but also an anti-apoptotic effect on luteal cells (Ptak *et al.* 2004), indicating a role of IGF-1 on both the formation and secretory function of luteal cells, especially during the first 12 days of gestation when the CL is insensitive to LH input. Furthermore, Miller *et.al*. (2003) demonstrated an acute increase in progesterone production when IGF-1 was infused in the ovarian vasculature in the pig and Langendijk *et al.* (2008) also reported a positive relationship *in vivo* between systemic IGF-1 concentrations and progesterone concentrations in the first few days after ovulation in primiparous sows

1.2.6 Prostaglandins

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2), secreted by the endometrium, are important hormones that play a role in both the maintenance and the regression of the CL. $PGF_{2\alpha}$ is essentially luteolytic and is secreted by the glandular epithelium, whereas PGE_2 is anti-luteolytic or luteotrophic and secreted by the stromal cells of the endometrium (Davis and Blair 1993). In the non-pregnant pig pulsatile secretion of $PGF_{2\alpha}$ begins to increase markedly on around day 13 of the oestrous cycle and continues to increase up to about day 16. At the same time PGE_2 increases, but concentrations of PGE_2 are at least 3 times lower than those of $PGF_{2\alpha}$ (Christenson *et al.* 1994). However, in the pregnant pig prostaglandin secretion peaks earlier (day11-12), and PGE_2 now becomes the predominant prostaglandin (Christenson *et al.* 1994). Viable conceptuses secrete oestradiol as a signal for the maternal recognition of pregnancy which leads to an increase in endometrial PGE_2 secretion and a change the ratio of PGE/PGF. Once the establishment of a successful pregnancy occurs

two mechanisms by which the CL is protected against the luteolytic actions of $PGF_{2\alpha}$ have been proposed: The first one is the redirection of $PGF_{2\alpha}$ secretion from endocrine (uterine venous drainage) to exococrine (into the uterine lumen) (Bazer and Thatcher 1977), and the second, the retrograde transfer of $PGF_{2\alpha}$ from venous and lymphatic vessels to the uterine lumen (Krzymowski *et al.* 1990). Furthermore, Christenson *et al.*, (1994) showed that an increase in PGE_2 in utero-ovarian venous blood in the gravid versus non-gravid uterine horn in unilaterally pregnant sows was associated with enhanced CL function on the ipsilateral ovary as evidenced by an elevated progesterone content and concentration as well as increased CL weights.

1.2.7 Other factors

Epidermal growth factor (EGF) stimulates embryonic growth and development but is also thought to be luteotrophic through its action on PGE_2 synthesis, which is, in addition to its secretion by the endometrium, secreted by the amnion (Ziecik *et al.* 2006). TNF-α, a pro-inflammatory cytokine produced luteal macrophages which accumulate in the CL towards the end of the oestrous cycle, in conjunction with its action on endothelin (ET)-1 induces luteolytic sensitivity in the porcine CL, decreasing progesterone secretion (Gadsby *et al.* 2006).

1.3 Nutritional effects on corpus luteum function, progesterone and embryo survival

1.3.1 Pre–mating nutrition

There is ample evidence to show that increased nutrition, through an increase in feed level or certain dietary energy sources, during the phase of follicle recruitment and development promotes follicle development and oocyte maturation (Almeida *et al.* 2000; Ferguson *et al.* 2003; Ferguson *et al.* 2006; Ferguson *et al.* 2007). An increase in feeding level during the pre-ovulatory period leads to a reduction in circulating steroid hormones, such as progesterone and oestradiol, due to an increase in metabolic clearance by the liver. This

reduction in circulating steroid concentrations results in a negative feedback on the hypothalamus-pituitary axis leading to an increase in gonadatrophin release which may improve follicle development and oocyte maturation and thus embryo survival through a change in follicular fluid composition (Ferguson *et al.* 2003). An increase in pre-mating feed intake, even as far back as the previous luteal phase, can also have positive effects on subsequent luteal function. Almeida *et al*. (2000), reported higher progesterone concentrations in systemic circulation in gilts fed a high feed level during the previous luteal phase, suggesting that follicle quality was improved leading to enhanced subsequent luteal function during early pregnancy. Furthermore, Chen *et al*. (2012) reported lower systemic progesterone concentrations after ovulation in gilts fed a low feeding level throughout the previous luteal and follicular phases.

1.3.2 Post–mating nutrition

In contrast to pre–mating feed intake, an increase in feeding level post-mating is generally believed to be detrimental to embryo survival, especially in gilts. High feed levels during early pregnancy usually result in a decrease in the concentration of progesterone in systemic blood circulation, through an increase in hepatic metabolism, and have therefore been associated with a reduction in embryo survival (Prime and Symonds 1993). However, there is surprisingly little evidence to support this paradigm, with only a few reporting detrimental effects of a high feed level on embryo survival with others reporting no or even positive effects on embryo survival and even pregnancy rate even though the effects on systemic progesterone concentrations are quite consistent in those studies that measure it (for a summary of these studies see Table 1.1). Dyck and Strain (1983) and Jindal *et al.* (1996) both reported a decrease in embryo survival during early pregnancy in gilts fed at a high feed level compared to gilts fed at a low feed level with Jindal *et al*. (1996) also reporting a decrease in systemic progesterone concentrations in those animals on the high

feed level. However, in the Dyck and Stain (1983) study pregnancy rate was negatively affected by a low feed level, compared to the high feed level during early pregnancy. Virolainen *et al*. (2004) also reported a positive effect of a high feed level on pregnancy rate compared to gilts fed at either a low or a varying feed level up to day 34 of gestation. The high pregnancy rate was seen in those gilts on the high feed level even though they had significantly lower systemic progesterone concentrations on both days 9 and 12 of gestation. In a later study with multiparous sows, Virolainen *et al*. (2005b), again found that sows that were fed at a high feed level tended to have lower systemic progesterone concentrations compared to sows fed at a low feed level, but there was no effect on embryo survival at day 35. Studies such as those conducted by Jindal *et al*. (1997) and Ashworth *et al*. (1999) assess embryo survival at very early stages during gestation, days 3-5 and 11-12 respectively, which may have confounded their findings. During these early stages of pregnancy embryo recovery is highly dependent on uterine flushing efficiency and is further complicated by the fact that at day 12 of gestation the embryos have started to elongate and become entangled making it impossible to discern the actual number of embryos recovered and Ashworth *et al*. (1999) do note that this did occur in their study.

Table 1.1 Comparison of past studies measuring effects of feed level on systemic progesterone, pregnancy rate and embryo survival

Study	Feed levels[#]	Treatment and day of gestation applied	Progesterone (ng/ml[1])	Pregnancy rate (%)	Embryo survival (%)
Dyck and Strain (1983)	H: 2.5 kg L: 1.5 kg	HH: H (0-35) HL: H (0-11), L (11-35) LL: L (0-35) LH: L (0-11), H (11-35)	Not measured	HH=87[a] HL=86[a] LL=64[b] LH=87[a]	HH=76[a] HL=77[a] LL=87[b] LH=85[b]
Toplis *et al*. (1983)	L: 2 kg H: 4 kg	L: 2-30 H: 2-30	Not measured	L=100 H=100	L=79 H=76
Pharazyn *et al*. (1991)	H: 2.6 M* L: 1.6 M	H: 3-15 L: 3-15	No difference	Not measured	H=82 L=88
Jindal *et al*. (1996)	H: 2.6 kg N: 1.9 kg	H: 1-15 N: 1-15 N3: 3-15	H=4.5[b] N=10.5[a] N3=3.7[b]	Not measured	H=67[a] N=86[b] N3=77[ab]
Jindal *et al*. (1997[a])	H: 2 M N: 1.5 M	H: 0-3/5 N: 0-3/5	H=1[a] N=0.9[a]	Not measured	H=74[a] N=86[a]
Jindal *et al*. (1997[b])	H: 2 M N: 1.5 M	H: 0-12 N: 0-12	H=11[a] N=15[b]	Not measured	H=72[a] N= 81[a]
Ashworth *et al*. (1999)	H: 3.5 kg N: 1.5 kg	H: 0-12 N: 0-12	H=17 N=18	H=92 N=100	H=83 N=86
Virolainen *et al.* (2004)	H: 4.3 kg L: 2.1 kg	LLL: (0-34) HHH: (0-34) LHL: L (0-10), H (10-17), L (17-34)	LLL=17[a] HHH=12[b] LHL=14[ab]	LLL=25 HHH=100 LHL=38	LLL=75[a] HHH=67[a] LHL=69[a]
Virolainen *et al.* (2005)	H: 4 kg L: 2 kg	LLL: (0-35) HHH: (0-35) LHL: L (0-11), H (11-21), L (21-35)	LLL=14 HHH=10 LHL=14	LLL=83 HHH=100 LHL=67	LLL=69 HHH=45 LHL=55
Quesnel *et al*. (2010)	H: 4 kg L: 2 kg	H: 0-7 L: 0-7	Not measured	H=100 L=87	H=84 L=87

[#] H = high feed level; N = normal feed level; L = low feed level.

*M = Maintenance requirement

[a,b,c]Different superscripts indicate significant differences between treatments within a row (at least $P < 0.05$).

While the effect of high feed levels on systemic progesterone concentrations is indeed evident, the relationship between feed level, systemic progesterone and embryo survival is more complex and may be confounded by the fact that a 'local' supply of progesterone directly from the ovary to the uterus occurs. This direct or local supply of progesterone is transferred directly from the veins draining the ovary to the arteries supplying the uterus through counter current transfer and anastomoses, as well as via lymphatic drainage (Krzymowski *et al.* 1990). This local supply of progesterone is direct and therefore not subject to hepatic metabolism and an increase in feed level may actually lead to an increase in secretion of progesterone by the ovary and whilst this increase may not be reflected in systemic progesterone concentrations it may very well be influencing embryo survival through its direct transfer to the uterus.

Even though a high feeding level increases the rate of metabolism of progesterone by the liver (Prime and Symonds 1993), there are indications that a high nutritional status may increase progesterone secretion by luteal cells. IGF-1 increases with a high feeding level, especially so in starch-rich diets, and as mentioned previously in this review, may influence early luteal function and thus progesterone secretion (Schams and Berisha 2002; Miller *et al.* 2003; Ptak *et al.* 2003; Langendijk *et al.* 2008). According to Jindal *et al.* (1996), an increase in feed level during the very early embryonic stage (days 1-3) is detrimental to embryo survival. However, in other studies high feed levels are imposed immediately after mating without any detrimental effects on embryo survival (Virolainen *et al.* 2004; Quesnel *et al.* 2010). Whilst it is possible that the high feeding level during the very early embryonic stage (day 1-3) might have a negative effect on embryo survival, due to a delay in the rise of post ovulatory progesterone, increases in nutritional mediators such as IGF-1 may prevent further losses by actually increasing luteal progesterone, which would only be discernable at a local level, and promoting the uterine environment during

the later stages of the embryonic phase. Furthermore, restricted or low feeding levels can have negatively impact gonadatrophins, such as LH, which support luteal function. Peltoniemi *et al*. (1997) reported that LH pulse frequency was significantly reduced due to feed restriction in gilts during early gestation. Restrict feeding or situations where individual feed intake may be sub-optimal, such as in group housing, may result in reduced LH support for corpus luteum function.

1.4 Conclusions and aims of the thesis

Inconsistent findings between studies in the relation to effects of feed level during early pregnancy on embryo survival in the gilt may be due to fact that most studies focus on the relationship between systemic progesterone and feed level. However, as mentioned in this review a 'local' supply of progesterone directly from the ovary to the uterus via counter current transfer does occur. This local transfer is not taken into consideration when assessing the effects of feed level on progesterone and may explain the equivocal findings between these studies. Due to this lack of understanding in regards to the role of 'local' progesterone in embryo survival the objectives of the studies outlined in this thesis were to:

1) Establish the role of local progesterone in embryo survival.

2) Establish the effect of feed level on ovarian output of progesterone.

3) Investigate if progesterone secretion can be manipulated by different dietary energy sources to positively influence reproductive outcomes.

CHAPTER 2

Direct ovarian uterine transfer of progesterone increases embryo survival in gilts

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2.1 Abstract

This study employed a unilateral ovariectomy model in order to investigate the relevance of the local supply of progesterone (ovary) compared to the systemic supply of progesterone, in terms of embryo survival in the ipsilateral uterine horn as opposed to the contralateral uterine horn Thirty gilts were unilaterally ovariectomised (ULO) during the luteal stage of their first oestrous cycle. Half of the ULO gilts were fed at 1.2 maintenance requirement (M), while the other half were fed at 2.4 M. Across ULO gilts 0.8 more embryos survived in the ipsilateral horn compared to the contralateral horn at day 35 of gestation ($P < 0.05$). In ULO gilts on the 2.4 M feed level the difference (+1.3; $P < 0.05$) between the ipsi- and contralateral horn was more pronounced than on the 1.2 M feed level (+0.4; n.s.). The higher feed level reduced circulating levels of systemic progesterone on day 5 of pregnancy but not embryo survival at day 35. However, post-implantation embryo survival was lower on the low feed level. In conclusion, these data indicate that local progesterone supply from the ovaries to the uterus contributes to the probability of embryo survival.

2.2 Introduction

In pigs, ovulation rate sets the maximal obtainable litter size, but is not usually matched by the number of pigs born at the end of pregnancy (Pope 1994). Embryonic mortality is known to contribute significantly to the loss of potential offspring during early pregnancy and can result in losses ranging from 10 to 40% (Kemp *et al.* 2006). Progesterone is known as an important driver of endometrial function and as such is important for early embryo development, implantation and survival of embryos (Foxcroft 1997; van den Brand *et al.* 2000; Spencer *et al.* 2004). High feeding levels have been shown to reduce systemic progesterone levels during early pregnancy (Prime and Symonds 1993), and have been linked to a decrease in embryo survival in some studies (Dyck and Strain 1983; Jindal *et al.* 1996). However effects of feeding level on embryo survival have been equivocal with other studies finding no or even positive effects of an increased feeding level on embryo survival (Toplis *et al.* 1983; Ashworth *et al.* 1999; Virolainen *et al.* 2004; Quesnel *et al.* 2010). It may be that measurement of systemic progesterone provides an imperfect assessment of the influence of feeding level on progesterone and embryo survival, because in addition to progesterone supply from systemic circulation, a local supply of progesterone directly from the ovary to the uterus occurs.

Progesterone entering the systemic circulation is subject to metabolism in the liver, which is increased when the animal is fed a higher feed level (Prime and Symonds 1993). However, the local supply of progesterone is direct and hence not modulated by hepatic metabolism and moreover, concentration of progesterone in the local circulation is much higher compared to that in systemic circulation (Stefanczyk-Krzymowska *et al.* 1998). Measurement of uterine responses to the local supply of progesterone therefore would be a more perfect assessment of the influence of feeding level on progesterone supply and its effect on uterine function. Therefore, we hypothesised that a uterine horn exposed to a

local source of progesterone as well as a systemic supply would have a higher survival of the contained embryos as opposed to a uterine horn that is only exposed to a systemic supply of progesterone. To test this we employed the use of a unilateral ovariectomy model (ULO) in order to compare embryo survival between one uterine horn that relies on both local (ovarian) and systemic progesterone (ipsilateral) and one horn that relies solely on systemic progesterone supply (contralateral) within the same gilt. Furthermore, feed level was also included in the model to see what effect it would have on embryo survival between the horns.

2.3 Materials and methods

This experiment was conducted during 2009 at the Pig and Poultry Production Institute (PPPI) at the Roseworthy campus of the University of Adelaide, South Australia with approval from the Animal Ethics Committee of The University of Adelaide. Forty-two Large White x Landrace gilts, housed in groups of ten, were induced into puberty at 24 - 26 weeks of age by a single intramuscular injection (IM) of PG600® (400 IU of PMSG and 200 IU of hCG; Intervet, Holland). Their first oestrus was monitored by boar exposure for 20 minutes daily from the time of PG600 injection until the end of their first oestrus. After their first oestrus the gilts were housed in individual slatted floor stalls. At their second oestrus the gilts were 28 - 30 weeks of age and weighed 127 ± 3 kg at the start of treatments (day 0 of pregnancy).

2.3.1 Unilateral ovariectomy

After their first oestrus 32 gilts were subjected to unilateral ovariectomy (ULO) on days 5 or 6 of the luteal phase. The other 10 gilts did not undergo surgery and were kept intact. Gilts were fasted for 24 hrs prior to surgery. The animals were anaesthetised by thiopentone sodium at a dose rate of 10mg/kg of body weight administered by injection via an ear vein. Anaesthesia was maintained using a combination of isoflurane and oxygen.

ULO was performed by mid-ventral laparotomy. Ovarian blood supply was ligated and secured with vicryl suture. The right ovary was removed in half of the gilts and the left ovary was removed in the other half. The wound was closed using vicryl absorbable sutures. Animals were given 250 mg IM (intramuscular) of Flunixil (Flunixin-Meglumine; Norbrook Laboratories, N. Ireland) as an analgesic and 1050 mg IM of Moxylan (amoxicillin; Jurox, Australia) as an antibiotic. Gilts then received 1050 mg IM per day of Moxylan for 2 days post surgery.

The surgery was performed on day 5 or 6 of the luteal phase to allow the remaining ovary time for compensatory follicular growth so that the number of ovulations would be equal to that of the number of ovulations in intact gilts (Kramer and Lamberson 1991). In the ULO gilts therefore, the number of ovulations and the potential number of embryos were similar to that of an intact animal, but with one uterine horn relying solely on systemic supply of progesterone, as opposed to the other horn also having a local supply of progesterone from the ovary. Distribution of embryos was also assumed to be equal across horns as pig embryos are known to migrate throughout the entire uterus before implantation irrespective of in which oviduct fertilization occurs (Dhindsa *et al.* 1967).

2.3.2 Treatments

From 10 days after first oestrus all animals including the intact gilts were fed 3 kg of a commercial gilt developer diet (13.2 MJ of ME/kg; 145 g/kg CP) per day. From day 14 after their first oestrus gilts were subjected to once daily boar exposure. From day 19 onwards gilts were then subjected to boar exposure twice a day (0800 and 1900 hrs) in order to determine the duration of their second oestrus and to estimate the time of ovulation (2/3 of oestrus duration). At the first sign of standing oestrus gilts were artificially inseminated (AI) with 3×10^9 sperm cells less than 48 hrs old. Subsequent inseminations occurred every 24 hrs until no further standing response was observed. After

the first AI ULO gilts were assigned to either a high (2.4 x maintenance requirement, ULO 2.4 M; n=17) or low (ULO 1.2 M; n=15) feeding regime, in order of showing oestrus and distributing body weights equally over treatments. The rationale behind the ULO model was to compare embryo survival between uterine horns (one with both a local and systemic supply of progesterone, the other that relied solely on the systemic supply of progesterone) within an animal. An extreme difference in feeding levels was included in the model (high – 2.4 M vs. low – 1.2 M) in order to exaggerate the systemic clearance of progesterone on the high feeding level when compared to the low feeding level. Our hypothesis was that despite an increased clearance of systemic progesterone on the high feeding level a decrease in embryo survival would not occur, particularly in the uterine horn ipsilateral to the remaining ovary, due to the local transfer of progesterone directly from the ovary to the uterus via counter current exchange. In the contralateral horn, progesterone supply would depend on systemic supply, which is lower on a high feed level, thus exaggerating the difference with the ipsilateral horn. Another ten gilts did not undergo surgery and were kept intact (INT) and served as reference animals in order to ascertain that there were no adverse effects of the ULO surgery on reproductive parameters and that the remaining ovary in the ULO gilts compensated in terms of the number of ovulations to equal that of INT gilts. The INT gilts were fed at 1.8 M in order to mimic normal gilt feeding levels during early gestation in commercial production. Maintenance requirement was calculated as ($BW^{0.75}$ * 460)/MJ DE. Rations were evenly distributed over two meals daily (0800 and 1600 hrs). From day 26 after first AI all animals then received a ration of 1.8 M, again distributed over two meals daily until slaughter at days 35 - 37 of gestation. Animals had unlimited access to water throughout the experiment.

2.3.3 Measurements

Animals were weighed prior to induction of first oestrus, prior to second oestrus, once weekly during the trial (to monitor weight gain) and just before slaughter. Jugular blood samples, representing systemic blood, were taken at days 5, 10, 15 and 30 of pregnancy to assess circulating systemic progesterone levels. Two blood samples were taken on these days, one pre-prandial (morning) and one three hours postprandial. At days 35-37 of pregnancy gilts were slaughtered at a local abattoir and reproductive tracts were collected and assessed while fresh. Number and weight of corpora lutea, total weight of gravid uterus, full and empty weight of each uterine horn, length of each uterine horn, number of placentations and embryos per horn, distance between placentations and the weight and crown rump length of each embryo were measured. Size of individual placentas was measured using planimetric assessment. The placentas were carefully separated from the endometrial lining of the uterus and spread out on a piece of baking paper. Using a pencil the outside of the placenta was traced onto the paper. After allowing the paper to dry the tracing was cut out and weighed. The weight of the tracing was compared to the weight of a 'standard', which had a known area. The area of the tracings was then calculated by correlating the weight of the tracing to that of the 'standard'. Allantoic fluid was collected and pooled for analysis of progesterone content from the first three viable embryos closest to the tubal end of each uterine horn. The number of corpora lutea on each (remaining) ovary was counted in order to determine ovulation rate. Embryo survival was calculated as the proportion of viable embryos to the number of corpora lutea, expressed as a percentage. Embryos were deemed not viable if they weighed more than two standard deviations below the average embryo weight or were obviously degenerated. Post implantation survival was calculated by the percentage of implantation sites that contained viable embryos.

2.3.4 Progesterone assay

Plasma progesterone was determined by radio-immuno-assay in 25ul of a 1:10 dilution of plasma in duplicate by double antibody RIA according to the manufacturer's instructions (DSL-3400; DSLabs, Webster, TX, USA). The intra assay CV was always less than 10%. The inter assay CV was 9.6% at 40pg/tube; 4.9% at 402pg/tube and 11.5% at 654pg/tube. The sensitivity of the assay was 0.10 ng/ml.

2.3.5 Statistical analysis

Data were analysed using SAS (SAS/STAT 1990). Differences between treatments were tested by univariate analysis using PROC GLM. Differences between treatments in progesterone were compared separately for each day of sampling. Differences in uterine and embryonic characteristics between the ipsi- and contralateral horn were analysed within animal using a paired T-test. Pearson correlations where mentioned were calculated using the PROC CORR statement in SAS.

2.4 Results

2.4.1 Animals

The mean duration of the second oestrus was 64 ± 2 h (24 to 96 h) and did not differ significantly between treatments. Of the 42 gilts that were mated at their second oestrus, 40 were pregnant at the time of slaughter (d35 of pregnancy). The 2 gilts that were not pregnant at slaughter lost their pregnancies at d19 and d21 after mating, presumably (based on the observation of a drop in feed intake prior to pregnancy loss) due to illness.

2.4.2 Feed level

During the first 25 days of pregnancy growth rates of the gilts differed between treatments, as expected, based on the different feed levels allocated to the gilts (614 ± 84; 1019 ± 77 and 243 ± 57 g/d for INT, ULO 2.4 M and ULO 1.2 M gilts respectively ($P < 0.05$). The

total number of embryos at d35 of pregnancy was lowest for ULO gilts on the 1.2 M feed level (10.3 vs. 11.3 (ULO 2.4 M) and 11.7 (INT)) (n.s.). There was also no significant difference in embryo survival between feed levels (74, 73 and 65 % for INT, ULO 2.4 M and ULO 1.2 M, respectively). However, post implantation survival of embryos, defined by the number of implantation sites that that contained viable embryos, was significantly lower ($P < 0.05$) for ULO gilts on the 1.2 M feed level (85% vs. 95 (ULO 2.4 M) and 97% (INT).

2.4.3 ULO model

Removal of one ovary in the ULO gilts resulted in the remaining ovary compensating in terms of follicle development and ovulation making the ovulation rate similar to that of the INT gilts: 15.9 ± 0.8, 15.7 ± 0.6 and 15.9 ± 0.4 for CON, ULO 2.4 M and ULO 1.2 M gilts respectively. In ULO gilts the mean distance of all embryos from the tip of the ovariectomised horn was 68 ± 1.7 cm, and in INT gilts it was 77.5 ± 4.2 cm (n.s.). In ULO gilts the mean number of embryos in the proximal 60cm of the ovariectomised horn was 2.5 ± 0.14, and in INT gilts it was 2.4 ± 0.18 (n.s.).

The number of implantations and embryos in the ipsilateral horn of ULO gilts (intact side) was not different from the number in intact gilts (Table 2.1). However in ULO gilts the number of implantation sites and the number of embryos in the uterine horn on the side of the remaining ovary (ipsilateral) was 0.8 higher than in the other, contralateral horn ($P<0.05$) across treatments. For ULO gilts on a low feed level (1.2M), the differences between the ipsilateral and contralateral horn was +0.4 (n.s.). However for ULO gilts on a high (2.4M) feed level the difference between ipsi- and contralateral horns in terms of the number of implantations (+1.2; $P < 0.05$) and embryos (+1.3; $P < 0.05$) was more pronounced (Table 2.1).

Table 2.1 Implantations and embryos per horn (mean ± s.e.m) in intact (INT) and unilaterally ovariectomised (ULO) gilts

		Number of implantations per horn			Number of embryos per horn		
Treatment	n	Ipsi	Cont	Ipsi-cont[A]	Ipsi	Cont	Ipsi-cont[A]
INT	10	6.1 ± 0.4			5.9 ± 0.3		
ULO, 2.4M	15	6.5 ± 0.3	5.3 ± 0.4	1.2 ± 0.5*	6.3 ± 0.4	5.0 ± 0.4	1.3 ± 0.4*
ULO, 1.2M	15	6.3 ± 0.5	5.8 ± 0.4	0.5 ± 0.7	5.3 ± 0.5	4.9 ± 0.5	0.4 ± 0.7

*Significant difference between horns within treatment ($P < 0.05$).
1.2 M, and 2.4 M = feeding level.
Ipsi = horn ipsilateral to the ovary. Cont = horn contralateral to the ovary.
[A]Difference between the ipsilateral (with ovary) and the contralateral (without ovary) horn.

There was no significant difference between feed levels in terms of macroscopic uterine characteristics. However for ULO gilts on the 2.4 M feeding level embryo weight, placental weight and implantation area were smaller in the ipsilateral horn compared to the contralateral horn (Table 2.2). Uterine space available per embryo also differed significantly between contralateral and ipsilateral horns in gilts on the ULO 2.4 M feed level (Table 2.2). This was related to the difference in embryo number between the ipsi- and contralateral horn, the difference in embryo weight between horns was correlated ($r=0.46$; $P<0.05$) to the difference in number of embryos between the horns.

Table 2.2 Ovarian, uterine and embryonic characteristics (mean ± s.e.m.) for unilaterally ovariectomised (ULO) gilts at day 35 of pregnancy

	High feed level (2.4M)			Low feed level (1.2 M)		
Characteristic	Ipsi	Ipsi-cont[A]	*P*[C]	Ipsi	Ipsi-cont[A]	*P*[C]
Uterine horn full (g)	1316 ± 64	+1	NS	1249 ± 73	-37	NS
Uterine horn empty (g)	736 ± 25	+57	0.12	710 ± 33	0	NS
Uterine Length (cm)	155.2 ± 4.08	-6.4	ns	151.6 ± 5.52	+3.8	NS
Conceptus (g)[B]	579 ± 49	-55	ns	540 ± 64	-37	NS
Embryo length (cm)	4.4 ± 0.1	-0.03	ns	4.3 ± 0.1	0	NS
Embryo weight (g)	5.1 ± 0.2	-0.6	0.004	5.4 ± 0.2	+0.1	NS
Placental surface (cm^2)	489 ± 26	-73	0.001	501 ± 22	-12	NS
Placental weight (g)	25 ± 2	-5	0.001	26 ± 2	-2	NS
Length implantation site (cm)	15 ± 1	-2	0.03	15 ± 1	-0.3	NS
Uterine space available/embryo (cm)	26.17 ± 1.89	-6.4	0.03	31.87 ± 2.97	-6.84	NS
Embryo weight/placental surface (g/embryo/100 cm^2)	1.1 ± 0.06	+0.02	NS	1.1 ± 0.04	+0.04	NS
P4 concentration in pooled allantoic fluid (ng/ml)	4.02 ± 0.6	-0.9	NS	4.23 ± 0.5	+0.73	NS

Ipsi, horn ipsilateral to the ovary; Cont, horn contralateral to the ovary; NS, not significant; P4 progesterone in pooled allantoic fluid.
[A]Difference between the ipsilateral (with ovary) and the contralateral (without ovary) horn.
[B]Embryos, placenta, embryonic sacs and fluids.
[C]P = P-value for intra-animal contrast.

2.4.4 Progesterone

Systemic plasma progesterone increased throughout the first ten days of gestation and dropped off again between days 10 and 30 of gestation (Figure 2.1). There was a correlation of 0.71 ($P < 0.05$) in postprandial systemic plasma samples, within gilts, between d5 and d10 of gestation, showing that gilts that had a high postprandial plasma progesterone concentration on day 5 also had high postprandial plasma progesterone concentrations on d10. When analysed by day there was an effect of feed level on d5, but not on the other days. On d5 the gilts on the 1.2 M feed level had a 5.5 ng/ml higher plasma progesterone concentration compared to gilts on the 2.4 M feed level. On d10, d15 and d30 the plasma progesterone concentration was similar for all of the feeding levels.

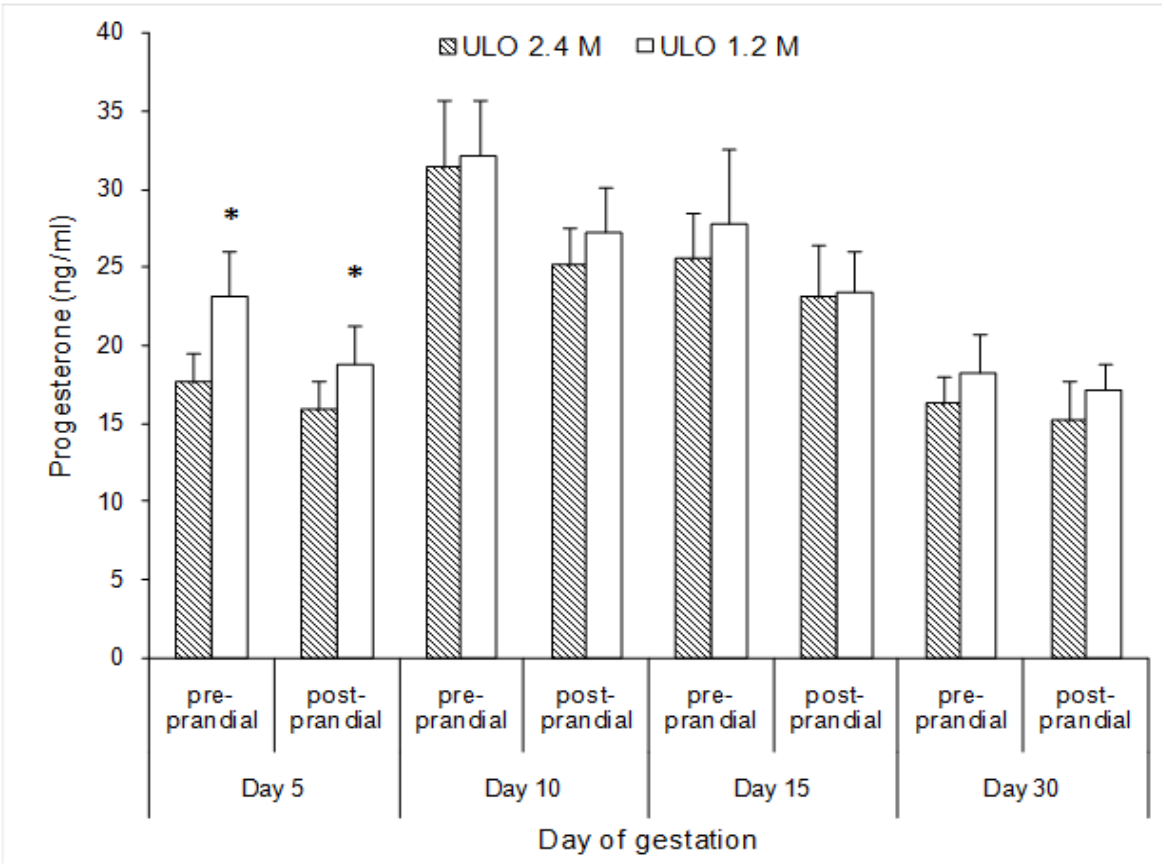


Figure 2.1 Progesterone concentration in systemic plasma on Days 5, 10, 15 and 30 of gestation in unilaterally ovariectomised gilts (ULO) on a 2.4 M and 1.2 M feed level.

Total luteal weight differed significantly ($P < 0.05$) between INT (6.49 ± 0.37 vs. 7.74 ± 0.29 (ULO 2.4 M) and 7.50 ± 0.3 g (ULO 1.2 M)). Average luteal weight per CL was also significantly lower ($P < 0.05$) for INT gilts (0.42 ± 0.02 g) compared to both the ULO 2.4 M and ULO 1.2 M treatments (0.50 ± 0.02 and 0.47 ± 0.02 g, respectively). However, there was no significant difference in these parameters between gilts on the ULO 2.4 M and ULO 1.2 M feeding levels. A positive correlation between total luteal weight and ovulation rate was seen across all treatments ($r= 0.46$; $P < 0.01$). No relationship between total luteal weight and systemic progesterone levels was seen on any of the sampling days.

Throughout the whole period of early gestation systemic progesterone dropped after feeding with the average drop being 2.89 and 3.69 ng/ml for the ULO 2.4 M and ULO 1.2 M groups respectively. Interestingly, between individual animals the change in systemic progesterone around feeding showed a great range, with some gilts even showing an increase in systemic progesterone postprandially (Figure 2.2). For example, on d5, the change in systemic plasma progesterone ranged from -20 ng/ml (drop) to 10 ng/ml (increase), however this variation was not related to treatment. Gilts that had a higher systemic progesterone content before feeding also showed a greater drop in systemic progesterone after feeding.

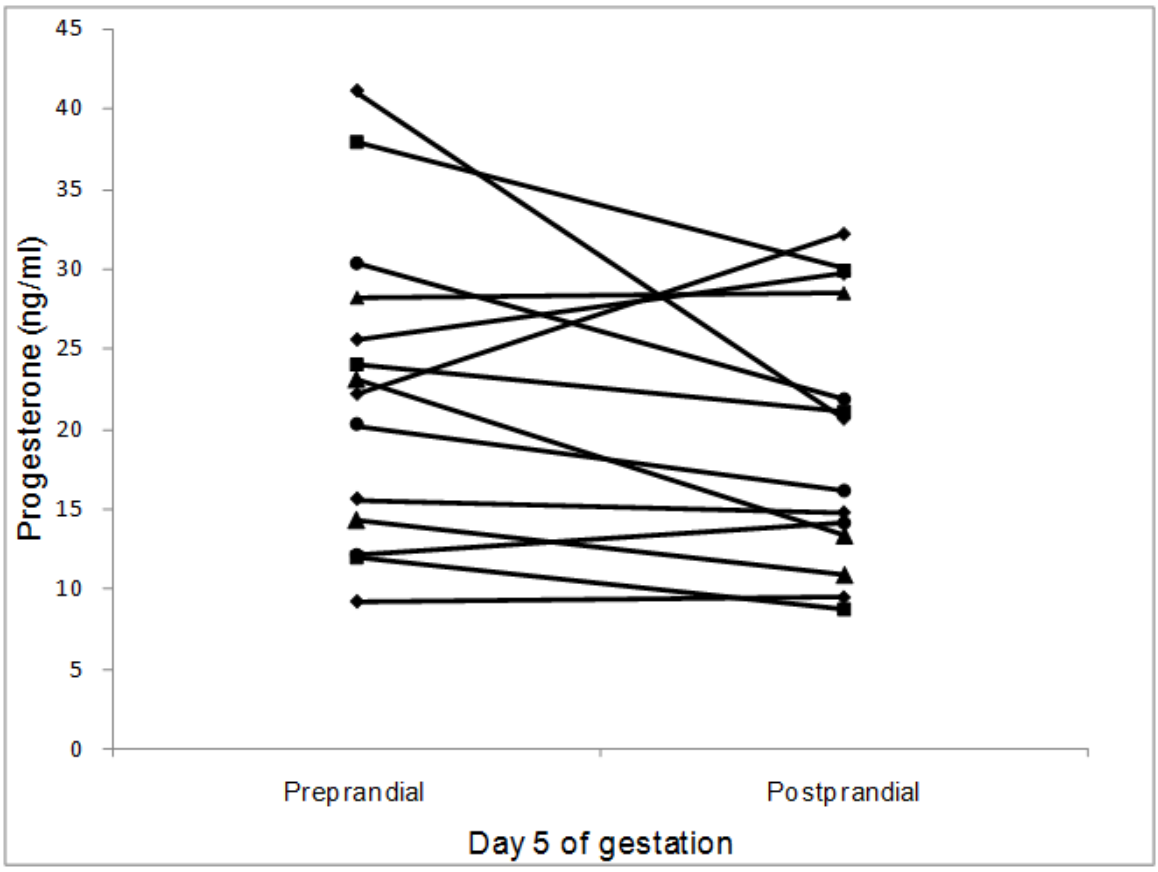
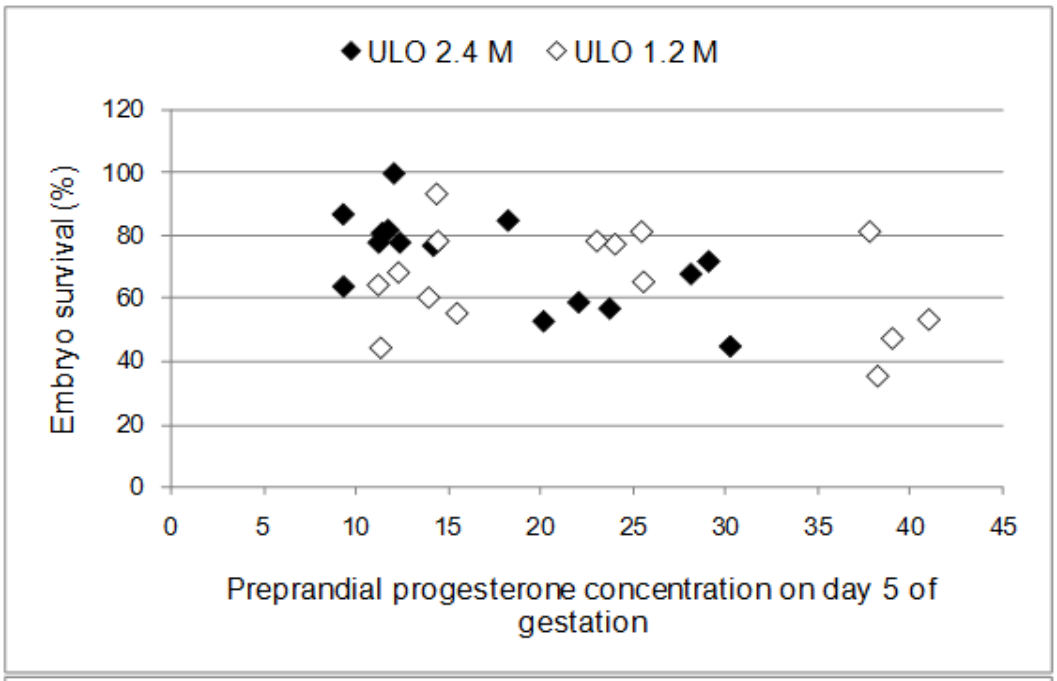


Figure 2.2 Systemic progesterone concentration in 14 individual gilts, selected randomly, before (preprandial) and after feeding (postprandial) on d5 of pregnancy.

No positive relationship between systemic plasma progesterone concentration on day 5 of pregnancy and embryo survival at d35 of gestation was seen for any of the treatments (Figure 2.3). Gilts that were deemed to have high systemic progesterone (> 20 ng/ml) on day 5 of gestation had a lower embryo survival than those gilts that had low systemic progesterone levels (<20 ng/ml) (62 ± 0.3 vs. 75 ± .04 %, $P < 0.05$). Nevertheless there was a positive correlation between plasma progesterone concentration and embryo weight in ULO gilts in the contralateral horn ($r = 0.43$, $P < 0.05$), although this was not the case in the ipsilateral horn. ULO gilts had 0.8 more placentas in the ipsilateral horn, resulting in less available space per embryo (Table 2.2). Despite having less available space, placentas in the ipsilateral horn in ULO gilts with low systemic progesterone were +0.1 g/embryo/100cm^2 more efficient than in the contralateral horn (P=0.08). In ULO gilts with high systemic progesterone (> 20 ng/ml on day 5) there was no such difference between

horns (-0.02 g/embryo/100cm^2, n.s.). Placental efficiency (embryo weight per area of placenta) was correlated to progesterone at d5 for the ipsilateral horn (r = 0.30; P=0.06), and this relationship was even stronger for the contralateral horn (r = 0.49; P=0.001).

(a)



(b)

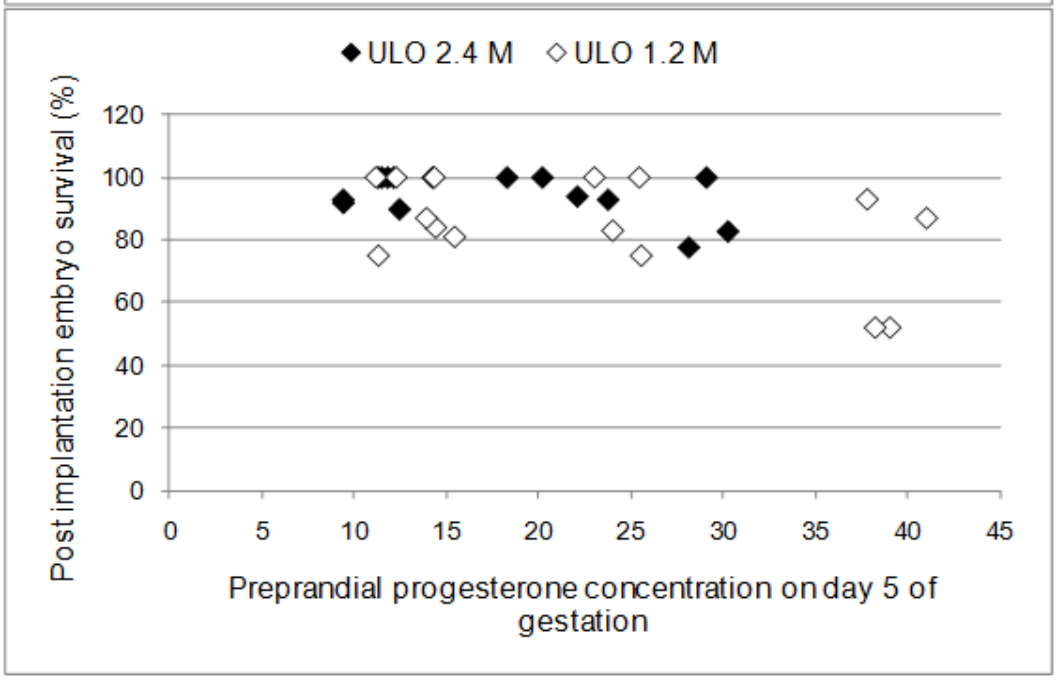


Figure 2.3 (a) Relationship between systemic progesterone concentration in plasma in ULO gilts on d5 of gestation and embryo survival at d35 of gestation, and (b) percentage of embryos surviving after implantation.

There was no correlation between the level of progesterone in allantoic fluid from embryos in the contralateral horn and the level of progesterone in systemic blood across all sampling days for any of the treatments. A positive relationship between the level of progesterone in allantoic fluid taken from embryos in the ipsilateral horn and embryo weight in the same horn was seen for both the high and low feeding levels ($r = 0.48$; $P < 0.01$). However, there was no such relationship between progesterone content in allantoic fluid and placenta surface area for either horn across treatments.

2.5 Discussion

This study demonstrated that the presence of a local supply (from the ipsilateral ovary) of progesterone promotes embryo survival. The higher survival of embryos in the ipsilateral horn (+0.8) compared to that of the contralateral horn of unilaterally ovariectomised gilts (ULO) indicates that the uterine environment is more favourable for implantation of embryos and better able to support these embryos after implantation has occurred. This result is consistent with the notion that local transfer of progesterone from the ovary to its corresponding uterine horn, through counter current transfer from ovarian veins to uterine arteries as well as by transfer through the lymphatic system (Stefanczyk-Krzymowska *et al.* 1998), contributes significantly to the progesterone supplied to the uterus and thus positively affects embryo survival.

Interestingly, this difference in the number of embryos was even more pronounced when feeding level was taken into consideration. In our study ULO gilts fed the 2.4 M feed level had significantly more embryos (+1.3) in the ipsilateral horn compared to the contralateral horn, whereas there was no significant difference (+0.4) in the number of embryos between horns in ULO 1.2 M gilts. This difference in embryo survival occurred between the horns in the ULO 2.4 M gilts probably due to the fact that circulating levels of systemic progesterone were lower in these gilts compared to those on the 1.2 M feeding ration,

significantly so on day 5 of pregnancy ($P < 0.05$), exaggerating the difference between the ipsi- and contralateral horn in progesterone supply. It has been shown that an increased feeding level negatively affects progesterone levels in systemic blood circulation due to an increased metabolism by the liver of systemic progesterone (Prime and Symonds 1993). It is this reduction in systemic progesterone that has led some to suggest that a high feeding level negatively effects embryo survival (Dyck and Strain 1983; Jindal *et al.* 1996), whereas other studies have found conflicting evidence on feed level effects and embryo survival (Toplis *et al.* 1983; Ashworth *et al.* 1999; Virolainen *et al.* 2004; Quesnel *et al.* 2010). It is also interesting to note that not all animals respond equally, in terms of circulating levels of systemic progesterone, to a feeding event. In this study, some gilts showed a small rise in postprandial levels of systemic progesterone irrespective of the feeding level they were on. One explanation for this rise in postprandial progesterone in systemic circulation may have been due to the fact that progesterone levels in systemic circulation follow a slight pulsatile pattern (Virolainen *et al.* 2005a), and that these particular animals happened to be sampled during a pulse. Stefanczyk-Krzymowska (1998) showed that concentration of progesterone in the uterine arteries can be 20-70% higher than in systemic circulation, especially in the arteries supplying the tubal end of the uterine horns which is close to the ovarian vasculature. Virolainen *et al*. (2004) suggested that higher feeding levels may have a positive effect at the utero-ovarian level via uterine secretions and progesterone synthesis and secretion. These positive effects may negate any negative effects on embryo survival of a rise in progesterone clearance by the liver. It could be this countervailing effect between ovarian progesterone production and systemic progesterone clearance that is the cause of conflicting results amongst these studies.

In ULO gilts on the low feed level, the difference between the ipsi- and contralateral horns was only 0.4 embryos, and not significant. Due to a lower feed level, hepatic activity is

lower and hence the rate of clearance of progesterone in these gilts is lower, resulting in a higher systemic concentration of progesterone, at least during early pregnancy. On the other hand, the ovarian secretion of progesterone in gilts on the lower feed level may have been lower as well. Other studies have shown that progesterone concentration is related to IGF-1 in the circulation (Langendijk *et al.* 2008), and Gadsby *et al*. (2006), have shown that infusion of IGF-1 directly into the ovarian vasculature increases the secretion of progesterone by the ovaries in pigs. These findings suggest that IGF-1, which is an indicator of nutritional status, may increase the production and/or secretion of progesterone by the ovary. Also, progesterone concentration in the vena cava draining the uterus in gilts has been shown to be significantly higher in gilts fed on a high feed level compared to a low feed level (Athorn *et al.* 2010). A high feed level, therefore, may increase the rate of metabolism of progesterone in the systemic circulation, but at the same time increase the secretion of progesterone by the ovaries. Therefore, in this study the total supply of progesterone to the ipsilateral horn comprised both the systemic and local supplies, but the local supply would not be affected by hepatic metabolism. Consequently, the difference in the total concentration of progesterone supplied to the ipsi- vs. the contralateral horn may have been greater in gilts on the high feeding level. If so, then the smaller difference in progesterone supply to the two horns in gilts on a low feeding level may explain the smaller difference in embryo number between the two uterine horns of these gilts.

In gilts on the higher feeding level, embryo weight, placental weight, placental surface area and length of implantation site were smaller in the ipsilateral horn compared to the contralateral horn. Uterine space available per embryo was also smaller in the ipsilateral compared to the contralateral horn, indicating that there was more competition for endometrial space in this horn. Despite having less available space, placentas in the ipsilateral horn of ULO gilts with low levels of systemic progesterone on day 5 of

pregnancy were more efficient than in the contralateral horn. This again supports the notion that a local supply of progesterone may play a vital role in uterine environment, even after implantation has occurred.

In this study we found no effect of feeding level on overall embryo survival across the treatments. ULO gilts on the 1.2 M feed level did have a lower embryo survival than both the ULO 2.4 M and INT feeding levels, although this difference was not significant. However, this difference in embryo survival did become significant when post implantation embryo survival was taken into account, with gilts on the ULO 1.2 M treatment having a significantly lower post implantation embryo survival compared to that of the other feeding levels. These findings are inconsistent with the generally accepted notion that a high feeding level is detrimental to embryo survival (Dyck and Strain 1983; Jindal *et al.* 1996), but they do concur with other studies that have found no effects of an increased feeding level on embryo survival, even though the expected effect of feeding level on Our study indicates that a low feeding level during early pregnancy can have negative effects on embryo survival, even after implantation, as the development of the uterine environment may have been compromised during the very early days of pregnancy, impairing its capacity to support a large number of embryos after implantation. This detrimental effect of a low feeding level on embryo survival may have occurred due to limited progesterone production by the ovary. A decrease in feed level may have an effect on metabolic parameters, such as IGF-1, which in turn may affect progesterone production by the ovary. A high feeding level may have increased the capacity of the ovary to produce progesterone at a local level and promoted the survival of more embryos. Therefore, contrary to general belief, a high feeding level during early gestation may not negatively affect embryo survival, and may well have a positive effect. Thus, the effect of an increased feeding level, not only on systemic levels of progesterone, but also on local

supply of progesterone, needs to be taken into consideration when assessing its effect on embryo survival as this paradigm seems to be more complex than previously assumed.

Studies using sheep have found that in conjunction with progesterone the pre ovulatory release of oestradiol also plays an important role in the development of a favourable uterine environment for implantation of embryos through oestradiol priming of the uterus (Wilmut *et al.* 1985). Oestradiol levels were not measured in this experiment, however, it is reasonable to assume that oestradiol is also transferred directly from the ovary to the uterus via the counter current pathway and in lymph and that in an ULO animal the horn ipsilateral to the ovary would be exposed to both local and systemic oestradiol, whilst the horn contralateral to the ovary would only be exposed to systemic oestradiol. Systemic levels of oestradiol would not be affected by ULO when compared to an intact animal due to ovarian compensation of follicle growth. Kramer and Lamberson (1991), demonstrated that oestradiol levels can be even higher in ULO gilts when compared to intact gilts due to follicles growing larger on the compensating ovary and therefore having the capacity to produce greater quantities of oestradiol. Like progesterone, systemic levels of oestradiol would be reduced in animals on a high feeding level due to an increase in the metabolism of steroid hormones by the liver. However, despite this systemic decrease in oestradiol levels the local ovarian-uterine transfer of oestradiol, which is not subject to hepatic metabolism, may contribute to embryo survival thus supporting the hypothesis that the local supply of hormones to the uterus plays an important role in embryo survival.

No positive relationship between systemic progesterone at day 5 of pregnancy and embryo survival at d 35 of pregnancy was seen in this study. In contrast we found that gilts with higher systemic levels of progesterone on day 5 of gestation had a lower embryo survival than those gilts with lower progesterone levels. We did, however, find a positive correlation between systemic progesterone at day 5 of pregnancy and mean embryo weight

in the contralateral horn at day 35 of pregnancy. This positive correlation between embryo weight in the contralateral horn, which was only exposed to systemic progesterone, and progesterone level shows that progesterone plays an important role in the development of embryos. The reason that we found no correlation between systemic progesterone levels at day 5 of pregnancy and embryo survival at day 35 of pregnancy may be that systemic progesterone is not indicative of what is occurring in terms of progesterone supply at the local level.

In conclusion, this study shows that a local supply of progesterone to a uterine horn had a positive effect on embryo survival compared to a uterine horn that relied solely on a systemic supply of progesterone, even after implantation had occurred. Furthermore, the effect of feeding level, not only on systemic levels of progesterone but also on the local supply of progesterone, needs to be taken into consideration when assessing its affect on embryo survival.

CHAPTER 3

Effect of feeding level on luteal function and progesterone concentration in the vena cava during early pregnancy in gilts

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3.1 Abstract

This study assessed the effect of feeding level on progesterone concentration in the caudal vena cava during early pregnancy in gilts. Twenty four Landrace gilts were allocated to either a High (2.8 ± 0.02) or a Low (1.5 ± 0.01 kg/d) feeding level at day 0 of pregnancy. Serial blood samples were collected every 15 minutes for 3 hours before and 3 hours after feeding on days 6 and 9 of pregnancy. Embryo survival and development as well as in vitro luteal progesterone production were assessed at day 10 of pregnancy. Progesterone concentration in the vena cava was pulsatile with gilts on the High feeding level having more pulses compared to Low gilts on day 9 of pregnancy ($P < 0.05$). On day 6 the number of pulses did not differ significantly between treatments, however, the average progesterone concentration in the vena cava tended to be higher in the gilts on the High feeding level ($P < 0.10$). Embryo survival at day 10 was 92 ± 3 % for High gilts compared to 77 ± 3% for Low gilts ($P < 0.05$). No difference in embryo development between the treatments was seen. There was no difference between treatments in *in vitro* secretion of progesterone by luteal tissue. In conclusion, a high plane of nutrition positively affects progesterone secretion by the ovaries in early pregnancy.

3.2 Introduction

Progesterone concentration in systemic blood circulation is related to embryo survival during early pregnancy in gilts, underlining the importance of this hormone for maintenance of pregnancy and litter size (Foxcroft 1997). It has been repeatedly reported that a high feeding level reduces systemic progesterone concentration, through an increase in hepatic metabolism, resulting in a decrease in embryo survival in some studies (Prime and Symonds 1993; Jindal *et al.* 1996). However, other studies have reported no such effects of feeding level on embryo survival, with some studies finding no or even positive effects of an increased feeding level on embryo survival (Virolainen *et al.* 2004; Quesnel *et al.* 2010). This paradox may be explained by the transfer of progesterone through counter-current transfer and lymphatic pathways 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 and therefore a high feeding level could be beneficial if it increases progesterone production by the ovaries. Results from a previous study conducted in our laboratory show that removal of an ovary from one uterine horn, whilst leaving the opposite horn intact, resulted in higher embryo numbers in the horn which was exposed to both local (i.e. ovary) and systemic progesterone, compared to the other horn which was only exposed to systemic progesterone, and this difference was especially evident in those animals on a high feeding level (Athorn *et al.* 2011a). Therefore the influence of a high feeding level on progesterone production and luteal function is only discernable at a local level.

Virolainen *et al.* (2005a) measured the progesterone concentration on day 22 of pregnancy in the vena cava, close to where the utero-ovarian vein drains into the vena cava, as a measure of ovarian secretion. They found that progesterone is secreted by the ovaries in a

pulsatile manner, and that the concentration of progesterone measured in the vena cava is considerably higher than in systemic circulation. The local transfer of progesterone ensures a higher than systemic concentration of progesterone in arterial blood supplying the uterus, since it adds to the progesterone supplied by the systemic circulation. Therefore, this study investigated the concentration of progesterone in the vena cava during early pregnancy, before implantation occurs (days 6 and 9 of pregnancy), to identify the magnitude and nature of secretion of progesterone by the ovary during this period. Furthermore, it was hypothesised that an increased feeding level would result in higher concentrations of progesterone in the vena cava compared to a low feeding level. In addition, in vitro luteal development was analysed on day 10 of pregnancy as a means of measuring the potential secretory capacity of luteal tissue during the early stage of the luteal cycle in gilts on differing feed levels (Green *et al.* 2007). Embryo survival and early embryo development were also assessed at day 10 of pregnancy to assess what effect feeding level has an on these parameters during the pre-implantation period.

3.3 Materials and methods

This experiment was conducted between March 2009 and May 2010 at the Pig and Poultry Production Institute (PPPI) at the Roseworthy campus of the University of Adelaide, South Australia with approval from the Animal Ethics Committee of The University of Adelaide. Twenty four Landrace gilts were induced into puberty at either 23 or 24 weeks of age by a single intramuscular (IM) injection of PG600® (400 IU of PMSG and 200 IU of hCG; Intervet, Holland). Their first oestrus was monitored by boar exposure for 20 min daily from the time of PG600 injection until the end of their first oestrus. The gilts were housed in individual slatted floors immediately after their first oestrus and remained in these stalls until the end of the experimental period.

Gilts were fed 2 kg of a commercial gilt developer diet (13.2 MJ of digestible energy kg $^{-1}$; 145 g kg $^{-1}$ crude protein) per day, from the end of their first oestrus until approximately 14 days later. From this time until the first standing response of their second oestrus gilts were fed 3 kg of the same commercial gilt developer diet per day. Daily boar exposure commenced again 14 days after their first oestrus. From Day 17 onwards gilts were boar exposed twice a day (0800 and 2000 hours). Ovarian activity was monitored by transcutaneous ultrasound using an ultrasonic device with a 5 MHz sector probe (Aquila Pro Vet. Esaote Europe B.V., Maastricht, The Netherlands) once every 12 hours after the first standing response in order to determine time of ovulation. Time of ovulation was defined as the average of the last time preovulatory follicles were detected and the first time no follicles could be detected any more on the ovary. At the first instance of standing oestrus gilts were artificially inseminated (AI) with 3 x 10^9 sperm cells (terminal sire line pooled semen) of less than 48 hrs old. Subsequent inseminations occurred once every 24 hrs until ovulation was determined by ultrasound.

3.3.1 Treatments

After the first AI gilts were assigned to either a High (n=11; maintenance requirement (M) + 1.5 kg/d) or a Low (n =13; M + 0.2 kg/d) feed level of the same commercial gilt developer diet. Gilts were assigned to either the Low or the High treatment according to their body weight, ensuring both treatments had similar average body weights at the start of the treatments (126 ± 2 kg; $P > 0.05$). Maintenance requirement was calculated as 460kJ digestible energy × bodyweight$^{0.75}$, and therefore High gilts received 2.78 ± 0.02 kg/d and Low gilts 1.51 ± 0.01 kg/d on average. Rations were equally distributed over two meals daily (0900 and 1600 hours). Animals had unlimited access to water throughout the experiment.

3.3.2 Vena cava cannulation

On either day one or day two after their second oestrus gilts underwent surgery to have a catheter inserted into the caudal vena cava close to where the utero-ovarian vein drains into the vena cava. Gilts were fasted for 24 hrs prior to surgery, and the hair on the lateral aspect of the right hind leg was shaved using electric clipper. Anaesthesia was induced by thiopentone sodium at a dose rate of 10mg/kg of body weight administered by injection via the ear vein. Anaesthesia was then maintained through inhalation using a combination of isoflurane and oxygen. Gilts were laid on their left side and the lateral aspect of the right rear leg that was previously shaved was disinfected with povidone iodine and 70% alcohol (EtOH). Catheterisation of the vena cava via the lateral saphenous vein was performed in accordance with (Benoit and Dailey 1991) and Virolainen *et al.* (2005a). In short, 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 separated, and the lateral saphenous vein isolated by blunt dissection. A haemostat was placed under the vessel, and two loose ligatures were placed approximately 1 cm apart. Slight traction was maintained on the distal ligature, a small transverse incision made in the vein, and a catheter (1.0 mm i.d., 1.5 mm o.d.) was inserted into the vessel. The catheter had been gas sterilised before surgery. Prior to insertion a mark was made on the catheter 52 cm from the end which was to be inserted into the vein as this was determined as the optimum position for placement of the catheter in gilts of a comparable size to the ones used in this study (Benoit and Dailey 1991). Once the catheter was inserted up to the 52 cm mark, a 10 ml syringe was placed on the open end of the catheter and approximately 5ml of blood was withdrawn to ensure that collection of the sample at this point was not impeded. If the blood moved freely through the catheter the catheter was flushed with heparinised saline to clear the line. If blood did not flow freely, the catheter was retracted slightly (as the end of the catheter may have been sitting up against the vessel wall or a valve) until blood could be withdrawn easily

and then again advanced to the 52cm mark, which usually resulted in a successful withdrawal of blood through the catheter. The catheter was then secured with the two ligatures around the vein. Before the catheter was secured the distal ligature was used to tie off the vein. After the catheter was secured the incision was sutured. Antibacterial cream (cetrimide) and gauze were placed over the wound and the leg was bandaged, with care being taken not to bend or kink the catheter. The free end of the catheter was placed in a Velcro pouch which was secured to the inside of the leg with the bandage. Three sutures were placed dorsally along the caudal surface of the rear leg lateral to the tail to form loops, and the catheter was fed through these when sampling occurred to prevent kinking of the catheter. Catheters were flushed on the second day after surgery so as to remain patent for the first sampling period which took place on days 3 or 4 after surgery. Animals were given 250 mg intramuscular (i.m.) of Flunixil (Flunixin-Meglumine; Norbrook Laboratories, N. Ireland) as an analgesic and 1050 mg i.m. of Moxylan (amoxicillin; Jurox, Australia) as an antibiotic. Gilts then received 1050 mg i.m. per day of Moxylan for 2 days post surgery.

3.3.3 Blood sampling

Concurrent blood samples were collected at 15 min intervals for 6 h (0900 to 1500 h) on day 6 and day 9 of pregnancy (day 0 is day of ovulation). Gilts were fed half of their daily feed ration 3 h into the sampling period (1200 h) to assess if feeding had any immediate stimulatory effect on local progesterone concentrations. Approximately 2 ml of blood was withdrawn and discarded before the collection of a 5 ml sample. After each sample the line was cleared by flushing with 2 ml of saline. Catheter patency was maintained by flushing the catheter with a heparinised saline solution (100 iμ/ml) once every hour. On both days, at the end of the sampling period, a systemic blood sample was collected via jugular

venipuncture. Blood samples were centrifuged at 2500 rpm and plasma separated and stored at -20°C until assayed for progesterone.

3.3.4 In vitro culture of luteal tissue and embryo development

On day 10 of pregnancy gilts were fed half of their daily ration 2 hrs prior to slaughter. At approximately 0800 h gilts were humanely slaughtered on site by captive bolt and exsanguinated. Reproductive tracts were collected immediately and ovaries were placed in phosphate buffered saline (PBS) and put on ice, whilst the uterine tract was placed in a Styrofoam box for transport to the laboratory. Individual corpora lutea (CL) were dissected out and weighed, during this process the CLs were kept moist with PBS. The method for in vitro luteal tissue was adapted from the methods described by (Green *et al.* 2007). Six individual CLs were mashed thoroughly, taking care to exclude any haemorrhagic CLs, and placed in a 10 ml plastic tube. The mash was weighed and then 3.5 ml of PBS was added to the tube. After gentle agitation, the tube containing the mash was placed in the centrifuge for 5 minutes at 1500 rpm. The supernatant was then discarded and the remaining mash weighed again. The amount of mash required for *in vitro* culture (IVC) was determined by the weight of the mash after washing/ amount of mash before washing and multiplying it by 0.1, and as a result 100 mg of luteal tissue was always used. For each individual gilt 100 mg of luteal tissue was added to each of 5 petri dishes using aseptic techniques. In a fume hood cabinet 3.5 ml of the media M199 (300 µl glutamine + 100 µl gentamycin + 1 ml penicillin: 100 ml of M199) was added to each petri dish. One petri dish was left to stand in the fume hood cabinet for 10 minutes (0 hour incubation). Two of the petri dishes were placed in the incubator (38.5 °C, 5% CO_2) for 2 and 3 hours respectively. The remaining 2 petri dishes were refrigerated (4°C), again for 2 and 3 hours respectively (as a measure of passive diffusion). At the end of the allocated incubation time the media and mash were placed into a 10 ml plastic tube and centrifuged at 1500 rpm for

5 minutes. The supernatant (1 ml) was then collected and stored at -20°C for later analysis of progesterone.

In order to flush the uterus for collection of embryos uteri were hung vertically on a stand, and the mesometrium removed. An incision was made in the tip of the uterine horn and another just below the cervix. The right horn was clamped off and 50 ml of PBS was flushed, from cervix to tip, whilst gently rocking the horn back and forth. The flushings were collected into a clean beaker, and the horn was flushed once more. The process was then repeated for the right horn. The embryos were then removed from the beaker and placed in a petri dish with fresh PBS. The remaining fluid from the uterine flush was put through a filter and the fluid put under a dissecting microscope to check for oocytes. Using a dissecting microscope the number embryos and surface area (calculated as $\pi \times$ (width/ magnification) $\times$ (length/ magnification)) of each embryo was measured. Presence of unfertilised oocytes if any was also recorded.

3.3.5 Progesterone assay

Plasma progesterone was determined by radio-immuno-assay (RIA) in 50µl of a 1:10 dilution of plasma in duplicate (vena jugular) or singularly (vena cava) by coated tube RIA according to the manufacturer's instructions (IM1188; Beckman Coulter, Brea, CA, USA). Progesterone from IVC medium was determined by RIA in 50µl of a 1:100 dilution of the medium in duplicate with the same assay. The lowest detectable concentration was 0.5 pg/tube (equivalent to 1 ng/ml for plasma and 10 ng/ml for culture medium). The intra assay coefficient of variation was less than 10% and the inter assay coefficient of variation was 13.7% at 57 pg/tube.

3.3.6 Statistics

Data were analysed using SAS (SAS/STAT 1990). Normally distributed data such as weight gain and systemic progesterone were analysed using PROC GLM with treatment (High, Low) as the independent variable. Some characteristics of the vena cava profiles were not normally distributed (for example, the number of pulses per 6 h), and were analysed using non-parametric tests (rank test or median test) in the NPAR1WAY procedure; where relevant this is indicated in the tables. Vena cava progesterone characteristics for day 6 and day 9 were compared within sow using a paired sample t-test. Pearson correlations where mentioned, were calculated using the PROC CORR statement in SAS. All data are presented as least square means + or – standard error of the mean (±SEM). A pulse was defined as a peak which consisted of at least two consecutive samples, one of which was greater than 100ng/ml (this threshold was arbitrarily chosen from preliminary analyses using differing thresholds) and the other that was greater than basal concentration. The basal progesterone concentration in the vena cava was defined as the mean of three consecutive samples preceding each identified pulse. If there was more than one possible pulse, the basal level was calculated using the basal value of progesterone that gave the lowest progesterone concentrations.

In vitro culture parameters are described as 'total in vitro secreted progesterone' (the amount of progesterone measured in the culture medium × 3.5 ml × total luteal weight per sow / 0.1), and 'total actively secreted progesterone' (total in vitro secreted progesterone at 38.5 °C, corrected by subtracting the total in vitro secreted progesterone in the culture medium at 4 °C (the latter as a measure of passive diffusion and luteal tissue content).

Twenty two out of the twenty four gilts had a cannula inserted into the vena cava. In the analysis of vena cava characteristics two of the cannulated gilts were not included in the analysis due to one gilt having very low progesterone levels, probably due to incorrect

placement of the catheter, and the other because the catheter did not remain patent. Two gilts were not cannulated and were only used in the analysis of ovarian and embryo characteristics and IVC. Five of the cannulated gilts were not included in the analysis of ovarian and embryo characteristics due to three of the gilts from the Low treatment group having elongated embryos at the time of slaughter (embryos could not be counted or measured), one gilt from the High treatment with a case of endometritis and another gilt from the Low treatment with a very low embryo survival.

3.4 Results

Weight gain in the period from mating until slaughter at day 10 of pregnancy differed significantly between treatments with gilts on the High feeding level gaining 1029 ± 60 g/d compared to 370 ± 40 g/d for gilts on the Low feeding level ($P < 0.01$). Ovulation rate, total luteal weight and average luteal weight did not differ between treatments (Table 3.1). An average of 2.4 more embryos were recovered from gilts on the High feed level compared to the Low, and embryo survival was 15% higher for High gilts ($P < 0.05$, Table 3.1). Embryo age at time of recovery also differed between the treatments, with those embryos recovered from the High feed level gilts being younger than those of the Low gilts ($P < 0.05$). However, no difference in embryo surface area (size) was seen between the treatments.

Table 3.1 Ovulation rate, corpora lutea and embryo characteristics at day 10 of pregnancy for gilts fed a High or Low feeding level from ovulation

Parameter	High	Low
n	11	13
Ovulation rate	14.9 ± 0.6	15.1 ± 0.6
Total luteal weight, (g)	8.2 ± 0.6	7.9 ± 0.5
Luteal weight, g per (CL)	0.55 ± 0.03	0.53 ± 0.03
Total embryos[A,B] *n*	14 ± 0.5[a]	11.6 ± 0.5[b]
(range)	(11-17)	(10-17)
Embryo survival[A] %	92 ± 0.03[a]	77 ± 0.03[b]
(range)	(80-100)	(67-94)
Embryo age (days)	9.9 ± 0.1[a]	10.3 ± 0.1[b]
Embryo surface area[A] (mm^2)[#]	11.9 ± 5.1	8.8 ± 2.7

[A] *n*=10 (High) and *n*=8 (Low)
[B] LS means corrected for ovulation rate
[a,b] Different superscripts indicate significant difference between treatments (P < 0.05)

Across all gilts, progesterone concentration in blood sampled from the vena cava followed a pulsatile pattern (Figure 3.1). The number of progesterone pulses increased from an average of 3 pulses on day 6, to an average of 4.5 pulses on day 9 (P < 0.05; Table 3.2). On day 9, gilts on the High feeding level had a greater number of pulses during the 6 h sampling period than those gilts on the Low feeding level (4.9 vs. 3.8; P < 0.05). Both mean basal and mean pulse amplitude in the vena cava did not differ between treatments on either day of pregnancy. The mean basal level of progesterone in the vena cava was higher on day 9 compared to day 6 (45 ± 4.9 vs. 37 ± 4.3 ng/ml; P < 0.05). However, mean pulse amplitude did not differ between day 6 (160 ± 10.8 ng/ml) and day 9 (176 ± 15 ng/ml). The mean progesterone concentration of all samples taken per sow during the 6 h sampling period tended to be higher (P < 0.10) for gilts on the High feeding level on day 6. Mean progesterone concentration in systemic circulation was 20 ± 1.3 ng/ml on day 6 of pregnancy and increased to 33 ± 2.4 ng/ml on day 9 (P < 0.01). No correlation was seen between progesterone concentrations in the systemic circulation and the vena cava progesterone characteristics on either of the sampling days.

Table 3.2 Progesterone characteristics in the vena jugular and the vena cava in gilts fed a High or Low feeding level on days 6 and 9 of pregnancy

Parameter	Feeding level	
	High (2.8 kg^{-1})	Low (1.5 kg^{-1})
n	8	11
Day 6		
Progesterone in vena jugular (ng/ml)	19.6 ± 1.6	20.9 ± 1.9
Average progesterone in vena cava (ng/ml)[A]	88 ± 11[x]	70 ± 8[y]
Average progesterone in vena cava before feeding (3 h)	88 ± 13	69 ± 5
Average progesterone in vena cava after feeding (3 h)	88 ± 14	70 ± 11
Basal (ng/ml)	38 ± 6	37 ± 6
Pulses per 6 h	3.6 ± 0.6	2.7 ± 0.7
Pulse amplitude (ng/ml)	173 ± 19	149 ± 11
Day 9		
Progesterone in vena jugular (ng/ml)	31.3 ± 3.2	35 ± 3.4
Average progesterone in vena cava (ng/ml)[A]	102 ± 14	85 ± 11
Average progesterone in vena cava before feeding (3 h)	108 ± 14	92 ± 12
Average progesterone in vena cava after feeding (3 h)	96 ± 16	78 ± 10
Basal (ng/ml)	45 ± 7	45 ± 7
Pulses per 6 h*	4.9 ± 1.1[a]	3.8 ± 0.7[b]
Pulse amplitude (ng/ml)	179 ± 22	174 ± 21

[A]Difference between high and Low feed levels tested with a non parametric test.
[x,y]$P < 0.10$; [a,b]$P < 0.05$.

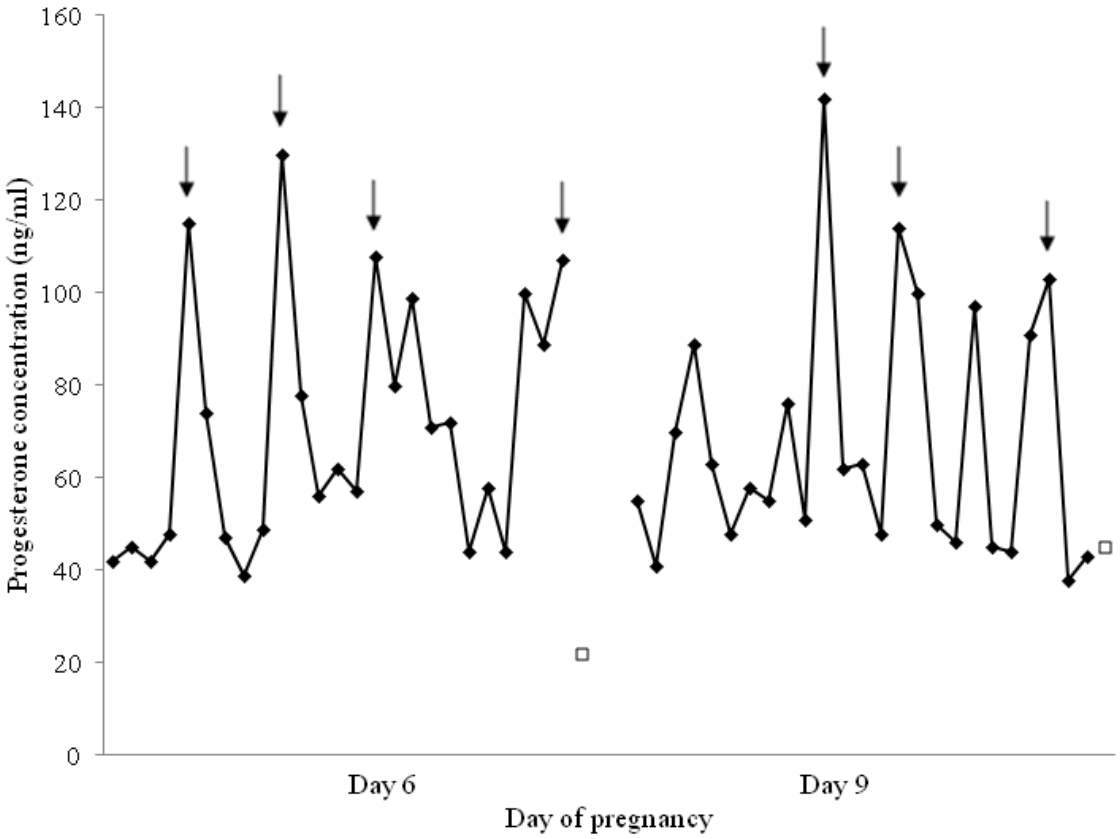

Figure 3.1 Vena cava progesterone profile collected over a 6 h sampling period on both days 6 and 9 of pregnancy and progesterone concentration in the vena jugular (□) taken at the end of the sampling period in an individual gilt. Arrows (↓) indicate pulses as identified by criteria.

Incubation of luteal tissue at 4 °C resulted in a plateau in the progesterone released into the culture medium at 2 h of incubation. Incubation at 38.5 °C, however, resulted in a progressive release of progesterone into the culture medium when cultured for 3 h vs. 2 h ($P < 0.01$; Figure 3.2). The rate of progesterone release was 4.3 ± 1.3 µg and 6 ± 2.9 µg per h for the first 2 h of incubation, increasing to 6.5 ± 2.9 µg and 6.5 ± 3.4 µg in the third hour, for the Low and High treatments, respectively. Total actively secreted progesterone after 3h of incubation at 38.5 °C tended to be positively correlated to basal progesterone concentration on day 9 of pregnancy (Table 3.3). Total actively secreted progesterone after both 2 and 3 h of incubation also tended to be positively correlated to systemic progesterone levels on day 6 of pregnancy as did total in vitro secreted progesterone after 3 hours of incubation at 38.5° C.

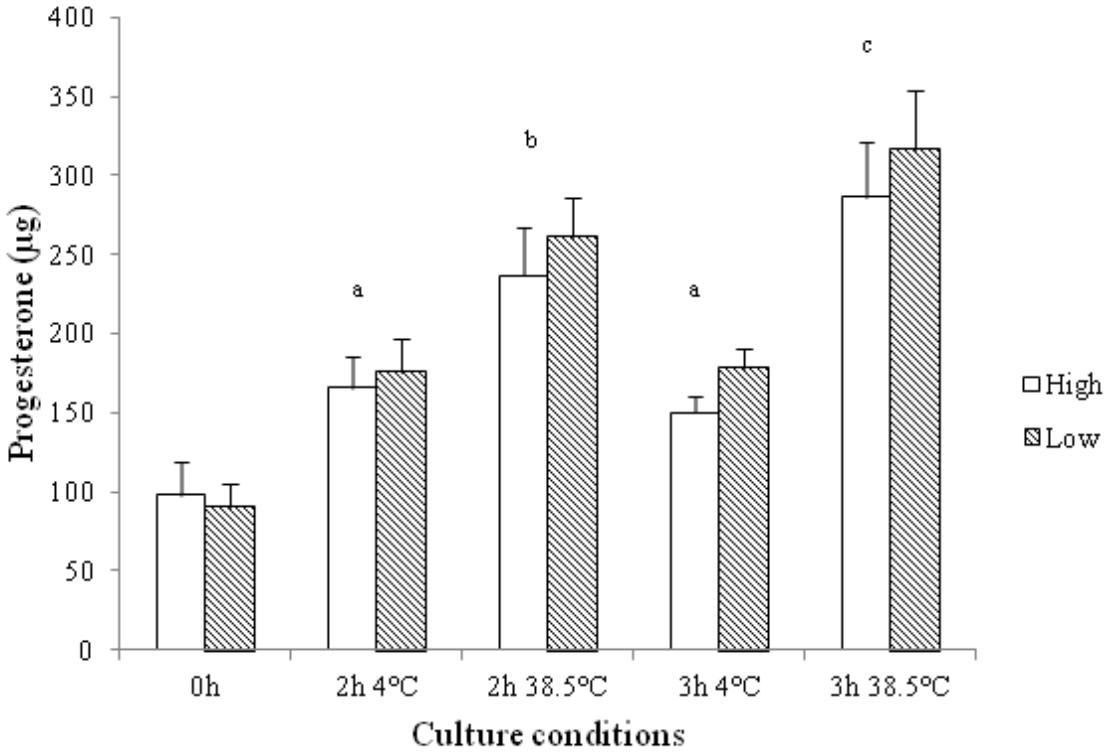

Figure 3.2 Progesterone secreted into in vitro culture medium after 0, 2 or 3 h of incubation at 4° C or 38.5° C, estimated for total luteal tissue mass. Total luteal progesterone after 0, 2, or 3 h of incubation at 4 °C or 38.5 °C of 100 for gilts fed at a High (n=12) or Low (n=10) feeding level.

[a,b,c]Different superscripts indicate significant differences between treatments ($P < 0.01$).

Table 3.3 Correlation coefficients between systemic and vena cava progesterone characteristics and in vitro progesterone production

Parameter	Total in vitro secreted[A] progesterone	*P*-value	Total actively secreted[B] progesterone at 2 hours	*P*-value	Total actively secreted[B] progesterone at 3 hours	*P*-value
Day 6						
Systemic progesterone in vena jugular (ng/ml)	0.44	0.07	0.47	0.08	0.46	0.08
Average progesterone in vena cava (ng/ml)	-0.11	0.66	0.17	0.56	0.11	0.70
Basal progesterone in vena cava (ng/ml)	0.08	0.77	0.19	0.52	0.14	0.63
Pulses per 6 h	-0.12	0.63	0.29	0.31	0.27	0.34
Pulse amplitude (ng/ml)	-0.30	0.32	-0.07	0.84	-0.33	0.35
Day 9						
Systemic progesterone in vena jugular (ng/ml)	0.05	0.84	-0.28	0.30	-0.23	0.41
Average progesterone in vena cava (ng/ml)	0.12	0.63	0.23	0.42	0.27	0.35
Basal progesterone in vena cava (ng/ml)	0.35	0.17	0.28	0.32	0.49	0.08
Pulses per 6 h	0.37	0.14	0.24	0.41	0.38	0.18
Pulse amplitude (ng/ml)	-0.11	0.67	0.02	0.93	-0.03	0.91

[A]Total in vitro secreted progesterone after 3 h of incubation at 38.5° C measured as the concentration of progesterone present in the in vitro culture medium × 3.5 ml × total luteal weight per sow / 0.1.
[B]Total actively secreted progesterone after 2 or 3 h of incubation measured as the total in vitro secreted concentration of progesterone at 38.5° C, corrected for total in vitro secreted concentration of progesterone at 4° C (the latter as a measure of passive diffusion and luteal tissue content)

3.5 Discussion

This study demonstrated that progesterone concentrations in local blood circulation (represented in blood collected from close to where the utero-ovarian vein drains into the vena cava) can be up to four times higher than that of progesterone concentrations in systemic circulation during early pregnancy. These findings are in accordance with those of Virolainen *et al.* (2005a) who demonstrated that on day 22 of pregnancy, blood sampled from the vena cava had a considerably higher progesterone concentration than that of blood collected simultaneously from the systemic circulation. Virolainen *et al.* (2005a) also reported that progesterone concentrations in local blood follows a pulsatile pattern, when compared to systemic concentrations which are fairly stable and not pulsatile. Again, this pulsatile release pattern was seen in the current study on both days 6 and 9 of pregnancy, indicating that even during the very early stages of pregnancy clear differences between local and systemic progesterone concentrations are evident.

A high feeding level is presumed to be detrimental to embryo survival due to the decrease in systemic progesterone levels as a result of increased hepatic metabolism (Prime and Symonds 1993; Jindal *et al.* 1996). However, in the current study no decrease in systemic progesterone concentrations was seen in those gilts on the High feeding level compared to those on the Low feeding level. Novak *et al*. (2003) and Gerritsen *et al*. (2008) also reported no effect of an increase in feeding level on systemic progesterone levels in gilts up to 72 hours after ovulation or in multiparous sows up until day 6 after ovulation, respectively. Novak *et al.* (2003) suggested that the lack of difference between feed levels in their study may have been due to the fact that gilts on the high feed level did not experience a change in feed intake after mating. This was also the case in the current study, as the gilts were flush fed at a level of 3 kg/d during the week prior to ovulation and then those on the High feeding level were fed an average of 2.8 kg/d after mating. Unlike

systemic progesterone concentrations, progesterone concentration in local blood circulation would not be expected to be negatively affected by an increase in feed level as it is not subject to hepatic metabolism. Conversely, it is assumed that progesterone concentrations in local blood circulation would be higher in those animals fed on a higher feed level compared to a lower feed level due to an increase in the capacity of the ovaries to produce progesterone. Evidently, in the current study gilts on the high feeding level tended to have a higher progesterone concentration in the vena cava on day 6 of pregnancy compared to those animals on the low feed level. Moreover, on day 9, gilts on the High feeding level had 1.1 more pulses (+29%) during the 6 h sampling period than those on the Low feeding level. This means that progesterone transferred directly from the ovarian veins to the uterus through counter-current transfer and lymphatic pathways would be higher on the high feed level, adding to the systemic progesterone supplied to the uterus.

It is not known to what extent the ovarian counter current transfer of progesterone has on the supply of progesterone to the oviducts and uterus. (Pharazyn *et al.* 1991b) reported that progesterone was higher in both ovarian and oviductal venous drainage 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 concentration in blood plasma from branches of the uterine artery supplying both the ovary and the cervix and found that progesterone was higher in both compared to 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 and this may explain why (Pharazyn *et al.* 1991b) found no difference in progesterone

concentrations in venous drainage from the uterus as the lower arterial supply may have resulted in a vast majority of the hormone being taken up by the tissues and therefore less passing through.

In the current study a positive effect of the High feeding level was seen on embryo survival at day 10 of pregnancy, with 15% more embryos surviving on the High feed level compared to the Low feed level. In a study conducted by Soede *et al.* (1999), no difference in embryo survival between days 5 and 12 of pregnancy was seen when gilts were fed either a high (4kg/d) or normal (2.5 kg/d) feeding level, again contradicting the argument that high feeding levels are detrimental to embryo survival. Also, in the current study embryos recovered from the gilts on the High feeding level were actually younger in age than those on the Low feeding level, however their development did not differ from that of the Low treatment. In fact, embryos from the High treatment were numerically greater in size, indicating that even though those embryos were actually younger in age they were at least as or even more developed than embryos from the Low gilts.

Blood samples were taken at day 6 of pregnancy, as it is assumed that during the first week of pregnancy the ovaries are not yet sensitive to the input of luteinising hormone (LH), and function autonomously and independent of gonadatrophins (Anderson *et al.* 1967). An increase in feeding level at this early stage of pregnancy may therefore increase luteal capacity through an increase in 'nutrition' leading to an increase in nutritional mediators such as IGF – 1 (Ferguson *et al.* 2003). Langendijk *et al.* (2008) showed a positive relationship between IGF-1 early after ovulation and systemic progesterone in first parity sows. By day 9 of gestation LH may have started to play a stimulatory role in the secretion of progesterone by the ovaries due to the small LH receptor population present on the ovaries in conjunction with nutritional mediators (Meduri *et al.* 1996). During the luteal phase, LH is released in a pulsatile pattern and these pulses were shown to correspond

approximately 50% of the time to that of progesterone pulses in the vena cava at day 22 of pregnancy (Virolainen *et al.* 2005a). However, LH was not measured in this study so it is unclear if there was any relationship between progesterone pulses and LH pulses in this study and if in fact an increase in feeding level led to an increase in LH secretion which may have in turn led to an increase in progesterone. Brüssow *et al. (2011)* found no relationship between LH pulses and progesterone pulses in blood measured in the vena cava of gilts treated with a GNRH agonist on days 11, 13, 15 or 17 of gestation. Also, Quesnel *et al*. (2010) did not find an effect of feeding level on LH secretion in cyclic gilts fed at either 240 or 80% of maintenance requirements during the luteal phase.

In vitro incubation of luteal tissue did not result in any difference in progesterone secretion between treatments. Nevertheless, there was a progressive increase in the release of progesterone in vitro with time when cultured at 38.5 °C, which was not observed in culture at 4 °C, indicating active secretion of progesterone by the luteal tissue. Also, secretion of progesterone by luteal tissue in vitro seemed to be related to systemic progesterone, suggesting that in vitro release of progesterone does provide some measure of the capacity of luteal tissue to secrete progesterone and therefore provides a measure of luteal function. However, there were no correlations between total in vitro secreted progesterone or total actively secreted progesterone and any of the vena cava progesterone characteristics on either day 6 or 9 of pregnancy. Results from earlier work in our laboratory have also shown that total luteal mass is related to systemic progesterone concentration at day 5 of pregnancy, therefore it seems that both the amount of luteal tissue as well as the secretory capacity of that tissue is important for the secretion of progesterone into the system(Athorn *et al.* 2013). Also, other work from our laboratory has shown that a high feeding level resulted in an increase in total luteal mass, which provides another

argument as to how a high feed level may actually promote luteal tissue function (Athorn *et al.* 2013).

In conclusion, from the present study it is clear that the secretion and concentration of the progesterone in the vena cava follows a pulsatile pattern and even basal levels are much higher than progesterone in systemic circulation. At day 6 of pregnancy, progesterone concentration in the vena cava tended to be higher in those gilts on the High feed level compared to those animals on the Low feed level. Also, a greater number of pulses were seen during a 6 hour sampling period on day 9 of pregnancy in those gilts on the High feeding level. Over a 24 hour period the uterine environment in the gilts on the High feed level would be exposed to significantly more progesterone pulses which may affect embryo survival. Indeed, at day 10 of pregnancy embryo survival was 15% higher in gilts on the High feeding level. Therefore, progesterone transferred directly from the ovary to the uterus may add considerably to systemic progesterone supplied to the uterus, and counteract a reduction in systemic progesterone when gilts are fed a high feeding level.

CHAPTER 4

Feeding level and dietary energy source have no effect on embryo survival in gilts despite changes in systemic progesterone levels

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4.1 Abstract

This study was designed to assess the effect of feeding level and dietary energy source on luteal function, systemic progesterone concentration and embryo survival in gilts during early gestation. At day 0 of pregnancy, 104 gilts were allocated to one of four experimental diets (LStarch: 1.2 × maintenance requirement (M) Starch diet (43.3% starch), n = 31; HStarch: 2.4 M Starch diet (43.3% starch), n = 21; HFat: 2.4 M Fat diet (13.5% fat), n = 23; and HFibre: 2.4 M Fibre diet (7.2% fibre), n = 23). On day 5 of gestation, no significant difference in circulating concentration of systemic progesterone was seen between the treatments. However, on day 15 of pregnancy, gilts on the HStarch diet had a significantly lower concentration of systemic progesterone when compared to gilts on both the LStarch and HFat diets ($P < 0.05$; 24.8 ± 2.4 versus 32.7 ± 2.4 and 36.1 ± 2.1 ng/ml, respectively). At day 35 of gestation, there was also a tendency for gilts on the HStarch and HFat diets to have a higher total luteal weight compared to gilts on the LStarch diets (7.2 ± 0.2 and 7.1 ± 0.2 versus 6.7 ± 0.2 g ($P <0.05$)). No difference in embryo survival was seen between the treatments. From the present study we can conclude that altering feeding level and dietary energy source did not affect embryo survival, despite the fact that systemic progesterone concentrations were affected on day 15 of gestation. Also, luteal weight was greater for those gilts on the high feeding level compared to those gilts on the low feeding level when fed the same energy source.

4.2 Introduction

In pigs, embryonic mortality can reach up to 40%, severely reducing the potential litter size and, ultimately, the number of pigs weaned per sow per year (Pope 1994; Kemp *et al.* 2006). Progesterone is known to be an important driver of endometrial function and, as such, is important for early embryo development, implantation and survival of embryos (Foxcroft 1997; van den Brand *et al.* 2000; Spencer *et al.* 2004). High feeding levels have been shown to reduce systemic progesterone concentrations during early pregnancy (Prime and Symonds 1993), and therefore have been linked to a decrease in embryo survival in gilts (Dyck and Strain 1983; Jindal *et al.* 1996). However, the effects of feeding level on embryo survival have been equivocal, with other studies finding no (Pharazyn *et al.* 1991a; Quesnel *et al.* 2010) or even positive (Virolainen *et al.* 2004) effects of an increased feeding level on embryo survival and pregnancy rate (Dyck and Strain 1983; Virolainen *et al.* 2004). This paradox may be due to the fact that these studies focus on the relationship between embryo survival and progesterone concentrations in the systemic blood circulation, rather than concentrations in the blood supply to the uterus. The supply to the uterus is not synonymous with systemic supply, because a 'local' supply of progesterone directly from the ovary to the uterus, via counter current exchange and venous-arterial anastomoses, occurs (Krzymowski *et al.* 1990). Therefore, whilst a high feed intake decreases systemic levels 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. Therefore, 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.

Even though a high feeding level increases the rate of metabolism of progesterone by the liver (Prime and Symonds 1993), there are indications that a high nutritional status may

increase progesterone secretion by luteal cells. Insulin-like growth factor 1 (IGF-1) increases with a high feeding level, especially so in starch rich diets, and it has been suggested may influence early luteal function and thus progesterone secretion (Schams and Berisha 2002; Ptak *et al.* 2003). Miller *et al*. (2003) showed that infusion of IGF-1 directly into the ovarian pedicle increased secretion of progesterone by the ovary and Langendijk *et al*. (2008) established a positive correlation between endogenous IGF-1 levels after mating and the systemic concentration of progesterone in primiparous sows. In a previous study from our laboratory in which gilts were unilaterally ovariectomised, a significant difference in embryo survival was found between uterine horns with and without a local source of progesterone (ovary), with more embryos surviving in the uterine horn ipsilateral to the remaining ovary, even though a decrease in systemic concentration of progesterone was seen on day 5 of pregnancy (Athorn *et al.* 2011a). Also, progesterone concentration in blood from utero-ovarian circulation tended to be higher in gilts on a high feeding level on day 9 of pregnancy compared to those on a low feed level (Athorn *et al.* 2012). So, essentially, what we see in terms of systemic circulating progesterone concentration on a high feeding level is not necessarily indicative of the amount of progesterone being supplied to the uterus at the local level.

Besides feeding level, dietary energy sources such as fat or fibre may also influence luteal production of progesterone. Diets with a high fibre content are getting increasing attention in terms of sow welfare as producers move to group housing of pregnant sows. Diets with fibre added have been shown to reduce aggression, through increased satiety, amongst sows in group housing situations, but the effects of a high fibre diet on embryo survival have not been investigated (Stewart *et al.* 2010). A high fibre content in the diet may result in a decrease in progesterone production due to binding of cholesterol, the precursor of steroid hormones, to the fibre source and subsequent excretion from the body.

It was hypothesised that an increase in feeding level would have no negative effects on embryo survival, and that a high feeding level may even have a positive effect on embryo survival. This study therefore compares two different feeding levels and their effects on systemic progesterone and embryo development and survival. Furthermore, the dietary energy source was also varied in the high feeding level diets. It was hypothesised that not only an increased feeding level but an increase in a particular energy source, such as starch, which would increase circulating levels of IGF-1, would be beneficial to embryo survival, as opposed to a diet high in fat which would be less favourable to embryo survival. Also, a diet high in fibre in conjunction with an increased feeding level would increase the removal of progesterone from systemic circulation, resulting in increased progesterone production by the ovary and, consequently, an improvement in embryo survival, even though systemic concentrations of progesterone would decrease.

4.3 Materials and methods

4.3.1 Animals and housing

This experiment was conducted during 2009 at the Pig and Poultry Production Institute at the Roseworthy Campus of the University of Adelaide. Large White x Landrace gilts (n = 104) were induced into puberty at 23 - 24 weeks of age by a single intramuscular injection of PG600® (400 IU of PMSG and 200 IU of hCG; Intervet, Holland). Their first oestrus was monitored by boar exposure for 20 min daily from the time of PG600® injection until the end of their first oestrus. After detection of their first oestrus, gilts were housed in groups of ten until approximately one week prior to their second oestrus when they were housed in individual semi-slatted floor stalls. At their second oestrus, the gilts were 27 - 28 weeks of age and weighed 126 ± 1 kg at the start of treatments (day 0 of pregnancy).

4.3.2 Treatments

From 14 days after their first oestrus, gilts were fed 3 kg of a commercial gilt developer diet (13.2 MJ of DE/kg; 145 g/kg CP) per day. Once-daily boar exposure was resumed 14 days after their first oestrus. From 19 days after their first oestrus, gilts were boar exposed twice daily (at 0800 and 1900 hours) in order to determine the duration of their second oestrus and to estimate the time of ovulation (2/3 of oestrous duration). At standing oestrus, gilts were artificially inseminated (AI) with 3×10^9 sperm cells less than 48 h old. Subsequent inseminations occurred every 24 h until no further standing response was observed. After first AI, gilts were assigned to 1 of 4 diets: either a base diet with starch as the primary energy source at a low feeding level (LStarch, 1.2 × maintenance requirement (M), n = 31); base diet with starch as the primary energy source at a high feeding level (HStarch, 2.4 M, n = 21); a diet with partial replacement of starch by fat at a high feeding level (HFat, 2.4 M, n = 26); a diet with a high fibre content at a high feeding level (HFibre, 2.4 M, n = 25), in order of showing oestrus and distributing body weights equally over treatments (Table 4.1). The low feeding level (1.2 M) was calculated as M (maintenance requirement) + 2.65 MJ DE (0.2 kg) and the high feeding level (2.4M) as M + 19.88 MJ DE. Maintenance requirement was calculated as ($BW^{0.75}$ * 460) MJ DE/day. The gilts on the high feeding levels (HStarch, HFat and HFibre) were fed isocaloric and isonitrogenous rations. Rations were distributed over two meals daily (0900 and 1600 hours). From day 26 after first AI, all animals received 2.5 kg/day of a standard gilt developer diet, again distributed over two meals daily, until slaughter at days 34 - 37 of gestation. Animals had unlimited access to water throughout the experiment.

Table 4.1 Composition of the diets (g/kg)

Diet	Starch (g/kg)	Fat (g/kg)	Fibre (g/kg)
Ingredient			
Barley	475	550	177
Lupins	-	46	-
Millmix	100	115	341
Wheat Starch	160	-	168
Sugar	50	-	44
Oat hulls	-	-	75
Canola	80	46	71
Soyabean meal	65	75	35
Meatmeal	30	34	26
Tallow	-	110	-
Molasses	20	-	18
Salt	2	2	2
Limestone-	12	14	11
Kynophos (mono-calcium phosphate)	2	3	2
Lysine sulphate	1	1.6	1.6
Bentonite	-	-	26
Choline chloride – 75%	0.2	0.2	0.2
Phyzyme XP. 5000L	0.1	0.1	0.1
Mineral-vitamin premix	2.75	3	2.45
Energy and nutrient content as analysed			
	Starch (g/1000 g)	Fat (g/870 g)	Fibre (g/1130 g)
Digestible energy for pigs (MJ)	13.3	13.3	13.3
Lysine	6.6	6.6	6.6
Protein	156.3	154.2	158.3
Starch	432	272	401
Fat	25	117	31.5
Fibre	44.9	44.9	81.4

1000 g of the Starch diet, 870 g of the Fat diet and 1130 g of the Fibre diet are isocaloric.

4.3.3 Measurements

Animals were weighed prior to induction of first oestrus, prior to second oestrus, once weekly during the trial (to monitor weight gain) and just prior to slaughter. Jugular blood samples were taken 3 h postprandial (after the morning feed) on days 5 and 15 of pregnancy to determine concentrations of systemic progesterone and IGF-1. Gilts were

also pregnancy checked at day 21 of gestation by transcutaneous ultrasound. At days 34 - 37 of pregnancy, gilts were slaughtered at a local abattoir and their reproductive tracts were collected. Total weight of gravid uterus, full and empty weight of each uterine horn, length of each uterine horn, number of placentations and embryos per horn, distance between placentations and the weight and crown rump length of each embryo were measured. Size of individual placentas was measured using planimetric assessment. The placentas were carefully separated from the endometrial lining of the uterus and spread out on a piece of baking paper. Using a pencil, the outside of the placenta was traced onto the paper. After allowing the paper to dry the tracing was cut out and weighed. The weight of the tracing was compared to the weight of a 'standard', which had a known area. The area of the tracings was then calculated by correlating the weight of the tracing to that of the 'standard'.

The corpora lutea on each ovary were excised and counted in order to determine ovulation rate and weight of individual corpora lutea. Embryo survival was calculated as the proportion of viable embryos to the number of corpora lutea, expressed as a percentage. Embryos were deemed not viable if they weighed more than two standard deviations below the average embryo weight or were obviously degenerate. Post implantation survival was calculated by the percentage of implantation sites that contained viable embryos.

4.3.4 Progesterone

Plasma progesterone was determined by radio-immuno-assay in 50µl of a 1:10 dilution of plasma in duplicate by double antibody RIA according to the manufacturer's instructions (IM1188; Beckman Coulter, Brea, CA, USA). The intra assay CV was always less than 10%. The inter assay CV was 9.6% at 40 pg/tube; 4.9% at 402 pg/tube and 11.5% at 654 pg/tube. The limit of detection was <1 ng/ml when using 1:10 diluted samples.

4.3.5 Igf-1

Plasma IGF-I was assayed in duplicate by double-antibody radioimmunoassay with human recombinant IGF-I (ARM4050, Amersham-Pharmacia Biotech, Buckinghamshire, England) and antihuman IGF-I antiserum (AFP4892898, National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases, California, USA) following acid-ethanol extraction and cryoprecipitation (Breier *et al.* 1991). All samples were processed in a single assay with a limit of detection of 0.04 ng mL^{-1} and intra-assay coefficient of variation of 4.00% at 1.98ng/ml and 6.90% at 0.35ng/ml.

4.3.6 Statistics

Data were analysed using SAS (SAS/STAT 1990). Differences between treatments were tested using PROC GLM for body weight gain, ovarian, uterine, and embryonic characteristics, and progesterone and IGF-1. These variables were all distributed normally. Where relevant, ovulation rate was included in the model as an independent variable (covariate). Gilts with embryo survival lower than 30% were excluded from the analysis. Pregnancy rates were compared using the χ^2 test in the PROC FREQ procedure. Pearsons correlations (Fig. 2 to 4) were calculated using PROC CORR. Differences between treatments in progesterone were compared separately for each day of sampling.

4.4 Results

During the first 25 days of pregnancy, growth rate differed significantly ($P < 0.05$) between gilts on the LStarch diet (320 ± 35 g/d) and gilts on the HStarch, HFat and HFibre diets (1000 ± 55; 919 ± 53 and 1055 ± 55 g/d, respectively). Pregnancy rate did not differ significantly between the four treatment groups (94%). Ovulation rate was slightly higher (n.s.) for the gilts in the HFibre treatment group, but as treatments were imposed after ovulation this difference was not due to a treatment effect. Therefore, the data presented in

Table 4.2 are corrected for ovulation rate. At day 35 of pregnancy, the total number of embryos, number of viable embryos and viable embryo survival did not differ between treatments (Table 4.2). When the data were pooled across the high feeding level treatment groups (HStarch, HFat and HFibre), there was also no difference between feeding levels for the number of viable embryos (11.1 versus 11.7 for high and low, respectively) or the viable embryo survival (72 versus 77% for high and low, respectively). The number of total embryos and the number of viable embryos was positively related to ovulation rate ($r = 0.35$; $P < 0.01$), indicating that at this stage of gestation uterine capacity did not limit the number of embryos at higher ovulation rates. Pre–implantation survival, calculated by the percentage of corpora lutea represented by an implantation, ranged from 79 to 87% across treatments, and the percentage of implantations represented by a viable embryo (post–implantation survival) ranged from 89 to 93% across treatments (n.s.), indicating that loss of embryos predominantly occurred before implantation.

Table 4.2 Embryo survival (means ± SE) in gilts fed different diets (Starch, Fat or Fibre) and allowances (L = 1.2 M[A] and H = 2.4 M) during the first 25 d of pregnancy

Diet	LStarch	HStarch	HFat	HFibre
n	31	21	23	23
Average daily feed allowance (kg/d)	1.5	2.8	2.4	3.2
Pregnancy rate (%)	94 (31/33)	91 (21/23)	96 (23/24)	96 (23/24)
Ovulation rate	15.3 ± 0.4	14.9 ± 0.5	15.3 ± 0.5	16.3 ± 0.4
Total luteal weight, (g)[B]	6.7 ± 0.2[a]	7.2 ± 0.2[b]	7.1 ± 0.2[b]	6.8 ± 0.2[a,b]
Luteal weight, g per (CL)[B]	0.44 ± 0.01[x]	0.47 ± 0.01[y]	0.46 ± 0.01[y]	0.45 ± 0.01[x,y]
Total embryos[B]	12.2 ± 0.5	11.9 ± 0.6	11.6 ± 0.6	11.6 ± 0.7
Viable embryos[B]	11.8 ± 0.5	11.2 ± 0.6	10.9 ± 0.5	11.1 ± 0.6
Embryo survival (%)[B]	80 ± 3	77 ± 4	76 ± 4	76 ± 4
Viable embryo survival (%)[B]	77 ± 3	73 ± 4	72 ± 3	72 ± 3
IGF-1 (ng/ml)	55.2 ± 6.2	54.2 ± 6.6	51.7 ± 5.9	52.9 ± 5.7

[A]M=Maintenance requirement, calculated as ($BW^{0.75}$ * 460)/MJ DE.
[B]LSmeans corrected for ovulation rate and gilts with embryo survival < 30% (n=3) are not included. Viable embryos were those weighing more than 2.17g (2*SD below the mean).
[a,b]Within rows, different superscripts indicate significant difference between diets ($P < 0.05$).

There was a tendency for gilts on the HStarch and HFat treatments to have higher total luteal weight and a higher average weight of single corpora lutea ($P < 0.10$). Total luteal weight was positively related to ovulation rate ($r = 0.62$; $P < 0.01$), meaning that the total luteal weight increased with ovulation rate but that individual corpora lutea and, therefore, total luteal weight, was higher for gilts on a high feed level, except for those on the HFibre treatment (Figure 4.1).

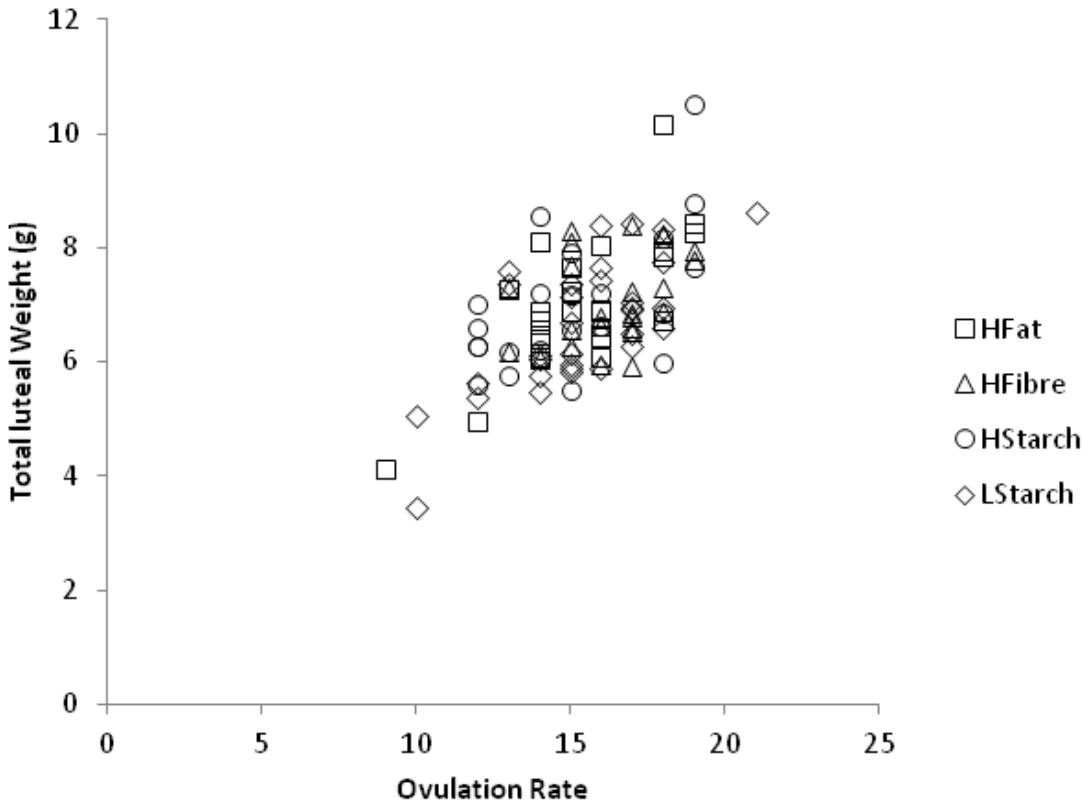

Figure 4.1 Relationship between ovulation rate and total luteal weight (g) in gilts on low, 1.2 M (LStarch) and high, 2.4 M (HStarch, HFat and HFibre) feeding levels. r = 0.62; P < 0.01.

There was no significant difference between feeding levels in terms of macroscopic uterine, embryo, implantation or placenta characteristics (Table 4.3). Only placenta surface area was larger in gilts fed the HFibre diet than in gilts fed the LStarch diet.

Embryo weight was not related to implantation length, again indicating that at this stage the available implantation sites did not limit the growth of embryos. The average available uterine horn length per ovulation was 21 cm, and did not differ between treatments. There was no correlation between uterine horn length and the number of embryos at day 35, or the percentage of viable embryos surviving. Interestingly, however, there was a correlation between available uterine length per ovulation (r = 0.25; P = 0.01) and embryo survival, although the regression coefficient was not very high (b = 1.5% per cm: ovulation).

Table 4.3 Macroscopic uterine and placental characteristics (mean ± SE) in gilts[A] on either a low, 1.2 M (Lstarch) or high, 2.4 M (HStarch, HFat and HFibre) feeding level

Treatment	LStarch	HStarch	HFat	HFibre
n	31	21	23	23
Embryo age (days)	35.5 ± 0.2	36.0 ± 0.2	35.3 ± 0.2	35.1 ± 0.2
Uterine length (cm)	322.8 ± 5.75	306.9 ± 7.02	321.9 ± 6.67	330.2 ± 6.78
Uterine weight full (kg)	2.62 ± 0.11	2.62 ± 0.14	2.63 ± 0.13	2.80 ± 0.13
Uterine weight empty (kg)	1.34 ± 0.04	1.32 ± 0.04	1.39 ± 0.06	1.44 ± 0.04
Implantation length (cm)	16.8 ± 0.56	16.5 ± 0.69	16.5 ± 0.65	17.5 ± 0.66
Mean placenta weight (g)	30.6 ± 1.73	33.3 ± 2.11	30.1 ± 2	33.1 ± 2
Mean placenta surface area ($cm^{2)}$	490.5^{a} ± 17	535.8^{ab} ± 21	494^{ab} ± 20	549^{b} ± 20
Embryo weight corrected for age (g)	5.1 ± 0.2	5.1 ± 0.2	4.9 ± 0.2	5.1 ± 0.2

[A]Gilts with embryo survival < 30% are not included.
Viable embryos were those weighing more than 2.17g (2*SD below the mean).
[a,b] Within rows, different superscripts indicate significant difference between diets ($P < 0.05$).

4.4.1 Igf-1

On day 5 of gestation, no significant difference was seen in IGF-1 levels in gilts on any of the diets. There was also no correlation between IGF-1 and concentration of systemic progesterone on day 5 of gestation.

4.4.2 Progesterone

On day 5 of gestation, no significant difference in concentration of systemic progesterone was seen in gilts on any of the diets (24.3 ± 1.5; 21 ± 1.9; 24. 8 ± 1.8 and 18.5 ± 1.8 ng/ml for LStarch, HStarch, HFat and HFibre diets, respectively) (Figure 4.2). On day 15 of gestation, the concentration of systemic progesterone was significantly lower ($P < 0.05$) for gilts on the HStarch diet when compared to those on the LStarch and HFat diets (24.7 ± 2.4 versus 32.7 ± 2.4 and 36.1 ± 2.1 ng/ml, respectively). Concentration of systemic progesterone also tended to be lower ($P < 0.10$) for gilts on the HStarch diet when compared to those on the HFibre (30 ± 2.3 ng/ml) diets.

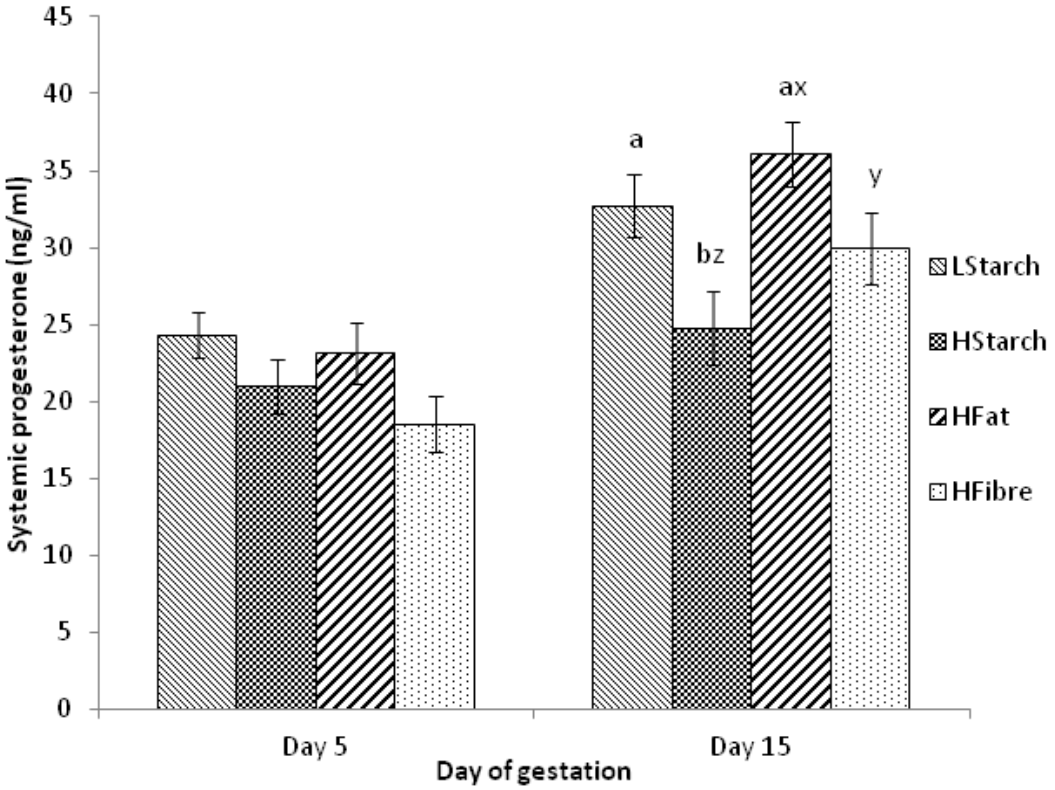

Figure 4.2 Systemic progesterone concentration in plasma on days 5 and 15 of gestation in gilts on low, 1.2 M (Lstarch) and high, 2.4 M (HStarch, HFat and HFibre) feeding levels. Values are LSmeans corrected for ovulation rate a,b Different superscripts indicate significant difference between diets ($P < 0.05$). ^{x,y,z} Different superscripts indicate significant difference between diets ($P < 0.10$).

Circulating concentration of systemic progesterone on day 5 was positively related to ovulation rate ($r = 0.33$; $P = 0.001$) and total luteal weight ($r = 0.26$; $P = 0.01$) (Figure 4.3) across all treatments. However, on day 15 a positive correlation was only seen between systemic progesterone and total luteal weight ($r = 0.45$; $P < 0.001$). No positive relationship between plasma progesterone concentration on day 5 of pregnancy and viable embryo survival at day 35 was seen for any of the treatments (Figure 4.4).

Gilts that were deemed to have high systemic progesterone (> 20 ng/ml) on day 5 of gestation had placentas that were +0.1g/embryo/100cm^2 more efficient than placentas in animals with low systemic progesterone (<20 ng/ml).

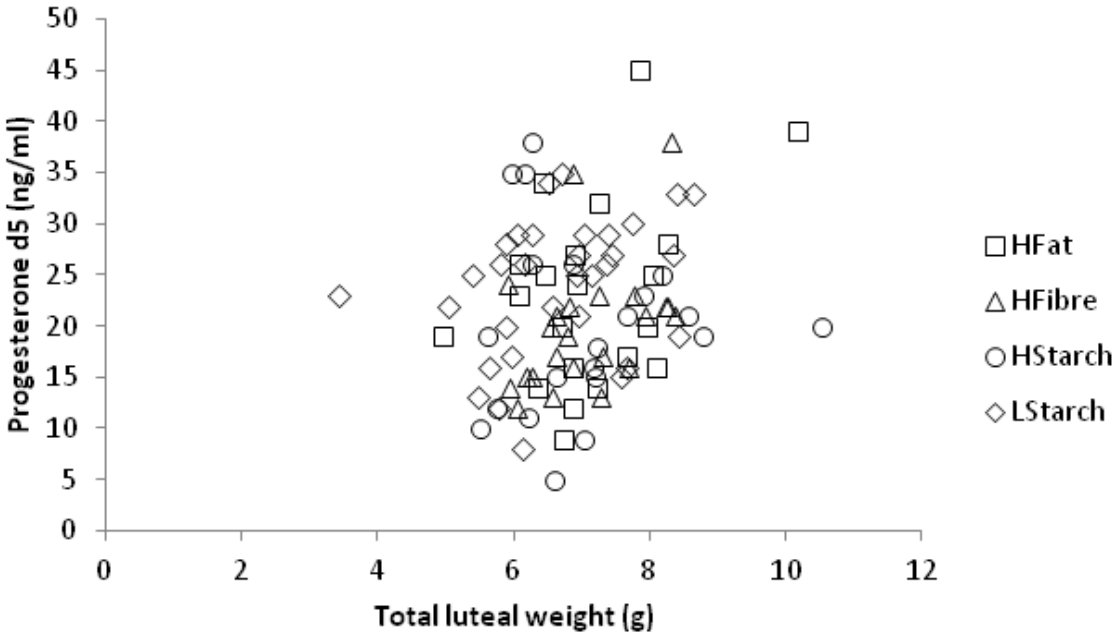

Figure 4.3 Relationship between total luteal weight (g) and systemic progesterone (ng/ml) on day 5 of gestation in gilts on low, 1.2 M (LStarch) and high, 2.4 M (HStarch, HFat and HFibre) feeding levels. r = 0.26; P = 0.01.

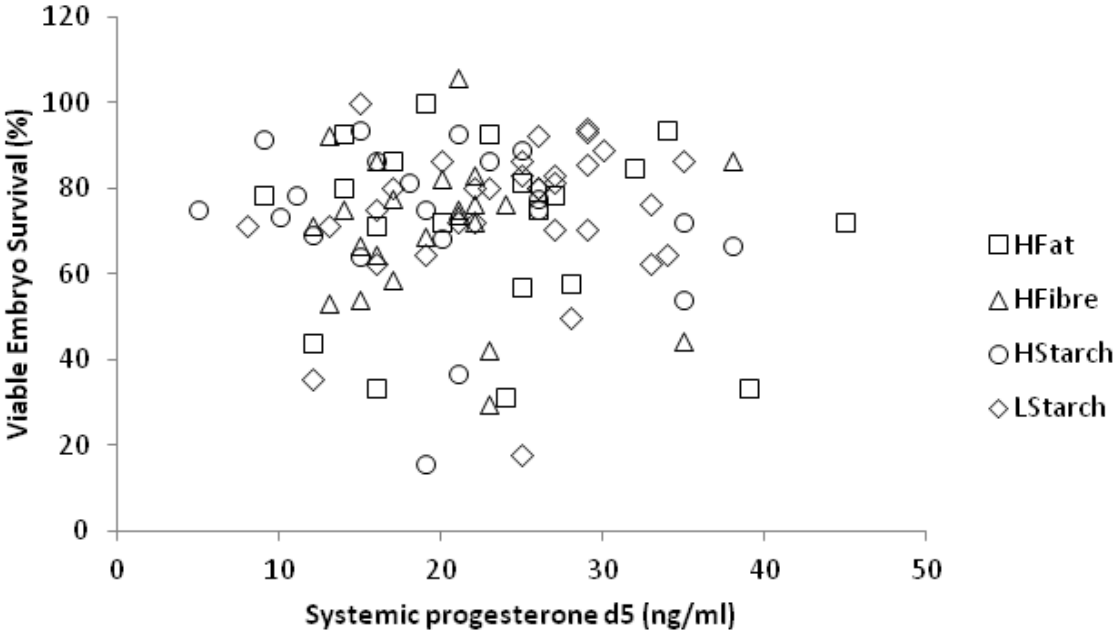

Figure 4.4 Relationship between systemic progesterone (ng/ml) on day 5 of gestation and viable embryo survival (%) at day 35 of gestation in gilts on low, 1.2 M (LStarch) and high, 2.4 M (HStarch, HFat and HFibre) feeding levels.

4.5 Discussion

The first aim of this study was to investigate the effects of a high versus a low feeding level on embryo survival in gilts. The low (1.5 kg/day) and high (2.8 - 3.2 kg/day) feeding levels used in this study were at either end of the range in normal feeding levels, to create a powerful contrast between treatments. This extreme difference in feeding levels resulted in profound differences in average daily weight gain, with gilts on the low feeding level gaining an average of 320 g/day compared to gilts on the high feeding level which had an average daily weight gain of approximately 1000 g/day. This approach differs from other studies where a moderate (1.6 – 1.9 kg/day) feeding level is referred to as 'low' (Jindal *et al.* 1996). In fact, there is a considerable range in 'low' or 'high' feeding levels across studies, which should be taken into account when comparing studies.

It was hypothesised that a high feeding level would result in a decrease in concentration of systemic progesterone, through an increase in portal blood flow and thus metabolism of progesterone by the liver (Prime and Symonds 1993). In addition, it was also hypothesised that embryo survival would not be negatively affected by a decrease in systemic progesterone concentration due to a local transfer of progesterone directly from the ovary to the uterus via counter current transfer (Stefanczyk-Krzymowska *et al.* 1998). Whilst a high feeding level may result in a decrease in systemic progesterone concentration, it may also result in an increase in progesterone secretion by the ovaries and thus increase the local transfer of progesterone from the ovaries to the uterus. Indeed, our results show that, despite a reduction in systemic concentration of progesterone in those animals fed the HStarch and HFibre diets, there was no difference in pregnancy rate, embryo survival, embryo development or any macroscopic uterine characteristics at day 35 of pregnancy when compared to the LStarch diet. These findings are in agreement with those other studies that have found no negative effect of a high feeding level on embryo survival and

contradict the current paradigm that a high feeding level has a negative effect on embryo survival (Pharazyn *et al.* 1991b; Virolainen *et al.* 2004; Quesnel *et al.* 2010).

The second aim of this study was to investigate the effect of different dietary energy sources (starch, fat and fibre) fed at a high feeding level on luteal function and embryo survival, compared to a low feeding level with starch as the main energy source. It has been shown that exogenous IGF-1 has a direct effect on in vivo luteal secretion of progesterone (Miller *et al.* 2003; Gadsby *et al.* 2006), and that endogenous IGF-1 levels are positively related to progesterone concentration soon after ovulation (Langendijk *et al.* 2008). This could be one of the mechanisms to explain the increased luteal weight and progesterone secretion (at the ovarian level) for gilts on high feeding levels compared to those on low feeding levels, regardless of systemic clearance. Ferguson *et al*. (2003) reported that IGF-1 levels were higher in cycling gilts fed at a high feeding level compared to those fed at a low feeding level on day 12 of the luteal stage. However, in the present study no difference was seen in IGF-1 levels in gilts on high or low feeding levels, or between dietary energy sources at day 5 of gestation, and no relationship was found between IGF-1 and progesterone. Nevertheless, despite there being no difference between IGF-1 levels, total luteal weight and average individual corpora lutea weight was greater for the gilts on the HStarch and HFat diets compared to the LStarch diet, suggesting that these diets had a positive effect on luteal weight, proliferation and function. Our data also show that progesterone was positively related to total luteal weight. This means that actual secretion of progesterone by the ovaries in gilts on the higher feeding level may have been higher, but did not result in a higher level of systemic progesterone due to an increased clearance by the liver. Therefore, local transfer of progesterone from the ovaries to the uterus may have been increased in gilts on the high feeding level, resulting in a net supply of progesterone to the uterus equal to or higher than that in gilts on a low feeding level.

On day 15 of gestation, the concentration of systemic progesterone was numerically higher in the gilts fed the HFat diet compared to those on the LStarch diet. Furthermore, systemic progesterone concentration was significantly higher when compared to gilts on the HStarch diet, despite the fact that the HFat diet was also fed to gilts at a high feeding level. This increased concentration of progesterone in the HFat diet group is in contrast to the findings of Kemp *et al.* (1995) who reported that the concentration of systemic progesterone was significantly greater in multiparous sows fed a starch rich diet compared to those fed a fat rich diet during early pregnancy. In a similar study conducted by van den Brand *et al.* (2000) using primiparous sows, no difference in systemic progesterone concentration was seen in gilts fed fat rich and starch rich diets at the same feeding level during early pregnancy. However, in these two studies, the diets were also fed to gilts during the previous lactation, which was not the case in our study. This may have had some influence on progesterone concentrations during early pregnancy due to the dietary effects on follicular development and hormone concentrations during lactation, the post weaning period and/or ovulation. Van den Brand *et al*. (2001) found that primiparous sows fed a fat rich diet during early pregnancy had a tendency for greater luteal weight when compared to gilts fed a starch rich diet at a relatively low feeding level (1.25 M), which may have led to enhanced luteal function and production of progesterone. Also, it may be that in the present study progesterone concentrations were higher in gilts on the HFat diet, as fat is a precursor to cholesterol and therefore steroid production, and this may have enhanced the secretion of progesterone by increasing the availability of its precursors.

No difference in embryo survival, embryo development or any of the uterine macroscopic characteristics, with exception of mean placental surface area, was seen in gilts fed the different dietary energy sources, suggesting that altering the dietary energy source of the diet does not influence the net supply of progesterone to the uterus, or at least not enough

to influence embryo survival. Alternatively, the extent to which the different energy sources were altered may not have been sufficient to result in any obvious effects. Also, in the current study the HFibre diet did not have any negative effect on embryo survival suggesting that the inclusion of fibre in the diets of group housed sows may result in the desired behavioural effects without compromising embryo survival.

In this study, average embryo survival rate was 72 and 77% for the high and low feeding levels respectively, indicating that there was room for improvement in all treatments. Uterine capacity did not seem to be limiting survival, as the number of viable embryos was positively related to ovulation rate, and not limited at higher ovulation rates. Similarly, embryo weight was not reduced for smaller implantation sites, indicating that available placental area was not limiting nutrient supply to embryos at this stage of gestation. Nevertheless, the available uterine length per ovulation was correlated to embryo survival, although the regression coefficient was not very strong. This may suggest that, in litters where there were more embryos after mating in relation to the total uterine length, the chance of survival was slightly lower, but that the competition between embryos was not one for nutrient supply limited by placental area. In fact, the loss of embryos before implantation (13 - 21%) was higher than the loss after implantation (7 - 11%).

According to Jindal *et al*. (1996) an increase in feeding level during the very early embryonic stage (days 1-3) is detrimental to embryo survival. However, in the current study high feeding levels were imposed immediately after mating without any negative effects on embryo survival up to day 35 of pregnancy. Whilst it is possible that the high feeding level during the very early embryonic stage (day 1-3) might have had a negative effect on embryo survival, due to a delay in the rise of post ovulatory progesterone, increases in nutritional mediators such as IGF-1 may prevent further losses by actually increasing luteal progesterone secretion and promoting uterine environment during the

later stages of the embryonic phase. Interestingly, Virolainen *et al*. (2004) reported a significantly higher pregnancy rate in gilts fed on a high feeding level from mating until day 34 of pregnancy (HHH) compared to gilts fed on either a low feed level (LLL) or gilts fed on a low fed level from mating until day 7, and then a high feed level from day 8 to day 17 and then again a low feed level from day 18 -34 of pregnancy (LHL). However, at day 19 of pregnancy there was no significant difference in pregnancy rates between the HHH and LHL treatments meaning that a significant percentage of gilts on the LHL treatment lost their pregnancies between day 19 and day 34. Therefore, further studies are required in order to determine the optimal timing of changes in feeding levels not only in order to improve embryo survival but also other factors such as pregnancy rate.

4.6 Conclusions

From the present study we can conclude that a high feeding level did not have a negative effect on embryo survival up to day 35 of gestation when compared to a low feeding level. In fact, a high feeding level, for gilts fed the HStarch and HFat diets, resulted in an increase in luteal tissue mass which may have led to an increase in progesterone secretion by the ovaries and therefore an increase in local supply of progesterone to the uterus despite, in the case of the HStarch diet, a decrease in systemic concentration of progesterone. Energy source (starch, fat or fibre) did not affect embryo survival in this study, although more extreme variations in energy source may. The inclusion of energy sources such as fibre during early pregnancy in group housed gilts may improve welfare without negatively affecting embryo survival.

CHAPTER 5

Feeding level and dietary fibre content during early pregnancy in gilts does not affect litter size but may have implications for pregnancy rate

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Feeding level and dietary fibre content during early pregnancy in gilts does not affect litter size but may have implications for pregnancy rate

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Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author
Certification that the statement of contribution is accurate

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

5.1 Abstract

This study was designed to assess the effect of feeding level and fibre on pregnancy rate, litter size and systemic progesterone concentration in gilts during early gestation. At day 0 of pregnancy, 233 gilts were allocated to one of four treatments and were maintained on these treatments until day 25 of pregnancy (Low: 1.6 kg day^{-1} of a standard diet, 21 MJ DE/d, n = 46; Medium: 2.35 kg day^{-1} of a standard diet, 31 MJ DE/d, n = 39; High: 3.2 kg day^{-1} of a standard diet, 41 MJ DE/d, n = 45; and Fibre (100 g/kg crude fibre): 2.85 kg day^{-1} of a fibrous diet, 31 MJ DE/d, n = 42). Gilts on the fibre diet were fed isocaloric to the Medium treatment. Pregnancy rate, and litter size were not affected by feed level or fibre. When gilts were pooled across treatments, the 25% of gilts with the highest growth rate (average 1003 g/d) had a higher pregnancy rate (92%) than the 25% of gilts with the lowest growth rate (average 216 g/d; 85% pregnant), and medium growth rate (average 573 g/d; 80 % pregnant; $P<0.09$). Progesterone in systemic circulation was significantly lower in gilts on the Medium feed level compared to those on the Low feed level. However, there was no significant difference between either the Low or the Medium diets when compared to the High and the Fibre diets. This study shows that a high feeding level during early pregnancy in gilts is not at all detrimental to pregnancy and litter size, and that the fibrous diet used in this experiment can be used without impacting on pregnancy or litter size.

5.2 Introduction

A high feed level during early pregnancy is assumed to reduce embryo survival, due to the increase in hepatic metabolism and thus the increase in progesterone clearance from the systemic circulation (Prime and Symonds 1993). Effects of feed level on systemic progesterone are indeed consistent, however, there is little and inconsistent evidence for a negative effect of high feeding levels on embryo mortality (Dyck and Strain 1983; Jindal *et al.* 1996) and there is an equal amount of evidence to suggest that low or even moderate feeding levels during early pregnancy can have an adverse effect on pregnancy rate (Dyck and Strain 1983; Virolainen *et al.* 2004). This paradox may be due to the previous focus on progesterone concentrations in systemic blood circulation, as opposed to actual progesterone supply to the uterus. Progesterone supply to the uterus is a net result of progesterone in the systemic circulation perfusing the uterine horns and supply of progesterone directly from the ovaries through counter current transfer and anastomoses between ovarian veins and uterine arteries, providing a higher than systemic concentration of progesterone in the uterine arteries (Krzymowski *et al.* 1990). The significance of this local supply of progesterone has been shown by unilateral ovariectomy (Athorn *et al.* 2011a).

Growth factors related to nutritional status, such as IGF-1, may increase progesterone secretion, and in fact there is *in vivo* and *in vitro* evidence of a positive effect of IGF-1 on progesterone secretion by luteal tissue (Miller *et al.* 2003; Langendijk *et al.* 2008). IGF-1 increases with a high feed level, especially in diets that are rich in starch. High feed levels may also have an indirect effect on progesterone secretion, as previous studies have shown that higher feed levels increase the amount of total luteal tissue on the ovaries and therefore their total progesterone secreting capacity (Gerritsen *et al.* 2008; Athorn *et al.* 2013). Additionally, diets with a high fibre content are getting increasing attention in terms

of sow welfare as producers move to group housing of pregnant sows. Diets with fibre added have been shown to reduce aggression, through increased satiety, amongst sows in group housing situations (Stewart *et al.* 2010). In terms of progesterone, a fibre rich diet may be expected to reduce progesterone as it has been reported that the steroid hormone oestrogen binds to certain fibres in the gut and therefore it is possible that progesterone, which is also a steroid hormone, may also bind to fibre in the gut leading to the interruption of entero-hepatic circulation (Arts *et al.* 1991). A higher clearance of progesterone from the system may result in a lower systemic concentration of progesterone, but if this reduces the negative feedback of progesterone on the hypothalamus-pituitary system, LH may be increased and therefore secretion of progesterone may actually be increased (Ferguson *et al.* 2007).

Therefore, it was hypothesised that a high feeding level would not be detrimental to pregnancy rate and litter size and may even increase them. In addition, it was also hypothesised that a diet rich in fibre would have no negative effects on pregnancy rate and litter size, and provide a management tool by which the welfare of sows in group housing can be improved.

5.3 Materials and methods

5.3.1 Animals and housing

This experiment was conducted during 2010 at the Research and Innovation unit at Rivalea Australia in Corowa, New South Wales. Two hundred and thirty three gilts (Large White x Landrace F1 gilts, PrimeGro^{TM} Genetics, Rivalea Australia) were housed in individual slatted floor stalls from day 1 until day 25 of pregnancy, whereafter they were moved to eco shelter accommodation as per normal commercial production until they were due to farrow. At the start of treatments (day 1 of pregnancy) gilts weighed 153 ± 17 kg.

5.3.2 Treatments

At their first standing oestrus, gilts were artificially inseminated (AI) with 3 x 10^9 sperm cells less than 48 h old. After their second and final mating 24 hrs later (day 1 of pregnancy) gilts were assigned to 1 of 4 dietary treatments until d 25 of pregnancy, the treatments being 1) Low: standard diet at maintenance requirement (M) + 0.1 kg day^{-1}, n = 46; Medium: standard diet at M + 0.85 kg day^{-1} n = 39; High: standard diet at M + 1.7 kg day^{-1}, n = 45; and Fibre: fibre diet at M + 1.35 kg day^{-1}, n = 42. Maintenance requirement was calculated as 460 kJ digestible energy × $bodyweight^{0.75}$. Dietary specifications are shown in Table 5.1. The gilts were allocated to the treatments in order of showing oestrus and body weights were equally distributed over treatments. Animals had unlimited access to water.

Table 5.1 Composition of the diets (g/kg)

Diet	Standard (g/kg)	Fibre (g/kg)
Ingredient		
Wheat	512	229
Barley	300	70
Millmix	122	477
Oat hulls		143
Meatmeal	10	16
Water	10	10
Natuphos 5000	0.1	0.1
Molasses	10	10
Tallow-mixer	5.3	20
Salt	3	3
Limestone	13	14
Di-calcium phosphate	9	5
Lysine-HCl	2	1
Threonine-	0.1	0.1
QAF breeder premix	1	1
QAF lac sow plus	1	1
Rumensin 100	1	1
Red micro-grits	1	1
Mycosorb	0.5	0.5
Energy and nutrient content as analysed		
	Standard (g/1000g)	Fibre (g/1000g)
Digestible energy for pigs (MJ)	13	10.9
Lysine	6	6
Protein	138.5	129
Starch	499	270
Fat	21	41.7
Fibre	38.9	99.8

5.3.3 Measurements

Animals were weighed after their second mating (prior to treatment allocation) and at day 25 of pregnancy. Jugular blood samples were taken 3 h postprandial (after the morning feed) on either days 7 or 8 of pregnancy in a subset of sows (n = 54) to determine

concentrations of systemic progesterone. Gilts were also pregnancy checked at day 28 of gestation by transcutaneous ultrasound. When the gilts farrowed total litter size and born alive were recorded.

5.3.4 Progesterone

Plasma progesterone was determined by radio-immuno-assay in 50µl of a 1:10 dilution of plasma in duplicate by double antibody RIA according to the manufacturer's instructions (IM1188; Beckman Coulter, Brea, CA, USA). The intra assay CV was always less than 10%. The inter assay CV was 9.6% at 40 pg/tube; 4.9% at 402 pg/tube and 11.5% at 654 pg/tube. The limit of detection was <1 ng/ml when using 1:10 diluted samples.

5.3.5 Statistics

Data were analysed using SAS (SAS/STAT 1990). Differences between treatments were tested using PROC GLM for body weight gain and progesterone concentration. Pregnancy rates were compared using the χ^2 test in the PROC FREQ procedure.

5.4 Results

There was a clear difference in weight gain between the Low versus the High feed level ($P < 0.01$), although gilts on the Medium feed level and the Fibre diet did not differ significantly from those on the Low feed level (Table 5.2). Pregnancy rate, litter size, total born and born alive were not affected by feed level or fibre. Interestingly though, when gilts were pooled across treatments, the 25% of gilts with the highest growth rates (mean 1003 g/d) tended to have a higher pregnancy rate (92%) than gilts with the 25% lowest growth rates (mean 216 g/d; 85% pregnant), and medium growth rates (mean 573 g/d; 80% pregnant; $P < 0.10$). In terms of systemic progesterone concentrations, gilts on the Medium feeding level had significantly lower systemic concentrations compared to those on the Low feed level ($P < 0.05$) (Table 5.2). There was no difference in systemic progesterone

concentration between either the Low or the Medium feed levels when compared to the High feed level and Fibre diet.

Table 5.2 Effects of dietary treatments during d 0-25 of gestation on weight gain reproductive performance and systemic progesterone

	Low (21 MJ DE/d)	Medium (31 MJ DE/d)	High (41 MJ DE/d)	Fibre (31 MJ DE/d)[A]
n	46	39	45	42
ADFA[B] (kg/d)	1.6	2.35	3.2	2.8
Weight gain (g/d)	421 ± 41[a]	495 ± 45[a]	912 ± 40[b]	569 ± 34[a]
Pregnancy rate d28 (%)	83 (50/60)	81 (44/54)	91 (53/58)	82 (50/61)
Total born	12.5 ± 0.4	12.2 ± 0.2	11.8 ± 0.4	12.3 ± 0.4
Born alive	11.5 ± 0.4	11.3 ± 0.4	11.2 ± 0.4	11.3 ± 0.4
Average progesterone[C] (ng mL^{-1})	17.1 ± 1[a]	13.1 ± 1[b]	14.9 ± 0.9[ab]	15.6 ± 1.1[ab]

[ab]Values in a row with different superscripts differ significantly ($P < 0.05$).
n, number at farrowing; DE, digestible energy.
[A]100 g/kg crude fibre versus 3 g/kg in other three treatments.
[B]Average daily feed allowance.
[C]Least-square means corrected for day of sampling.

5.5 Discussion

Despite considerable differences in feed levels between treatments there were no differences in pregnancy rate, litter size, total born or born alive. Prior to the study, the expectation was that gilts on the High feed level would have lower systemic concentrations of progesterone due to an increase in hepatic metabolism of progesterone. However, due to the same high feed level, secretion of progesterone by the ovaries would increase, due to the stimulatory effects of IGF-1 (which would be increased at a higher feeding level) on ovarian luteal tissue (Miller *et al.* 2003; Langendijk *et al.* 2008). Furthermore, low feed levels have been associated with a decrease in pulsatile secretion of LH which may have negative implications for progesterone secretion by the ovaries, especially beyond day 10 of gestation when the CL becomes sensitive to LH input (Anderson *et al.* 1967). During the luteal phase, LH is released in a pulsatile pattern and Virolainen *et al*. (2005a) showed that in the vena cava on day 22 of gestation LH pulses corresponded approximately 50% of

the time to that of progesterone pulses. However, Brüssow *et al.* (2011) found no relationship between LH pulses and progesterone pulses in blood measured in the vena cava of gilts treated with a GNRH agonist on days 11, 13, 15 or 17 of gestation. Also, Quesnel *et al*. (2000) did not find an effect of feeding level on LH secretion in cyclic gilts fed at either 240 or 80% of maintenance requirements during the luteal phase. Therefore, it is not entirely clear how LH affects progesterone secretion, but it is clear that a prolonged cessation of LH will result in luteal failure (Peltoniemi *et al.* 1995).

When gilts were pooled across treatments pregnancy rate tended to be higher for those animals with a higher weight gain. This may reflect a positive effect of feed level on the establishment and maintenance of pregnancy, not to mention the positive effects on the growth rate of the gilts (who are still growing towards their mature body size) which is undoubtedly restricted at lower feed levels. It is not clear however, why this effect was not expressed between treatments, although numerically animals on a high feed level had a higher pregnancy rate. In a similar study to this one conducted using first parity sows rather than gilts growth rate was again positively related to pregnancy rate (Athorn *et al.* 2011b). Furthermore, Hoving *et al*. (2011) reported an increase in both sow body weight recovery after lactation and subsequent litter size in first and second parity sows fed at an increased feed level during early pregnancy.

The combined, counteracting effects, of an increase in the concentration of progesterone transferred directly from the ovarian vasculature to the uterine arteries, and the decreased supply of systemic progesterone to the uterus, may result in a net zero effect or even an increased supply of progesterone to the uterus. These effects may explain why gilts on the High feed level had similar litter sizes to those on the Low feed level. These findings are consistent with those reported previously by our laboratory where embryo survival was not negatively affected at day 35 of gestation by a high feed level (Athorn *et al.* 2012), with

one study even finding an increase in post-implantation survival of embryos in gilts fed a high feed level (Athorn *et al.* 2011a). There was no difference in systemic concentrations of progesterone between the Low and the High feed level, however we also reported this in an earlier study (Athorn *et al.* 2012).

A diet rich in fibre was included in this study since there is an increasing interest for fibre rich diets to be used as a management tool in order to alleviate stress and improve welfare of group housed pregnant sows. In the current study the inclusion of a high (10%) concentration of fibre in the diet had no negative effects on pregnancy rate and litter size. This suggests that using high fibre diets as a management tool in group housed sows would have no negative effects on reproduction.

5.6 Conclusions

In gilts a higher feeding level did not affect pregnancy rate or litter size when compared to a low or medium feeding level. Interestingly, however, a higher weight gain irrespective of feeding level tended to be related to higher pregnancy rates. Providing gilts with medium to high feeding levels post mating allows animals to grow at a rate that does not compromise skeletal development and conformation whereas a low feeding level could lead to a restriction in the growth of these animals. Replacing a proportion of the starch with fibre in the diet did not have any effect on pregnancy rate or litter size, suggesting, that for welfare related reasons, specifically in group housing, diets with a high fibre content may be used without necessarily affecting reproductive performance.

CHAPTER 6

General Discussion

The number of pigs born (and subsequently weaned) per sow per year is one of the most important factors driving profitability in the pig industry. There are many variables that impact on the number of pigs born and weaned per sow per year; these include genetics, age of the sow, gestation management, farrowing management and pre-weaning survival. Another variable is embryonic mortality. Embryonic mortality is known to contribute significantly to the loss of potential offspring during early pregnancy and can result in losses ranging from 10 - 40% (Kemp *et al.* 2006). Embryo survival is driven by progesterone which is produced by the corpora lutea that form on the ovaries after ovulation (Foxcroft 1997). Over the past 30 years studies have looked into ways of improving embryo survival through the manipulation of progesterone by feeding different feed levels during early gestation. These studies vary considerably in their findings with some suggesting that high feed levels are detrimental to embryo survival due to a decrease in progesterone concentrations in the blood as a result of an increase in hepatic metabolism (Dyck and Strain 1983; Jindal *et al.* 1996), with others reporting no detrimental effects of high feed levels on embryo survival (Toplis *et al.* 1983; Pharazyn *et al.* 1991a; Jindal *et al.* 1997; Ashworth *et al.* 1999; Virolainen *et al.* 2004; Quesnel *et al.* 2010). Whilst all these studies look at feed level, not all measure progesterone in circulation, but those that do, invariably measure it in systemic circulation. However, in addition to systemic supply of progesterone to the uterus, a direct supply of progesterone from the ovary to the uterus occurs. This direct or 'local' supply of progesterone is transferred directly from the veins draining the ovary to the arteries supplying the uterus through counter current transfer and anastomoses, as well as via lymphatic drainage (Krzymowski *et al.* 1990). This local

supply of progesterone is therefore not subject to hepatic metabolism and an increase in feed level may actually lead to an increase in the secretion of progesterone by the ovary and, whilst this increase may not be reflected in systemic progesterone concentrations, it may very well be influencing embryo survival through its direct transfer to the uterus. Differential effects of feeding level on local versus systemic progesterone may be the reason for the inconsistent findings between the previous studies in this area. Therefore, this thesis investigated the role of local progesterone in embryo survival and how it may be manipulated by feeding level and/or dietary energy source to further improve embryo survival.

In order to quantify the role local progesterone plays in the probability of embryo survival a unilateral ovariectomy model (ULO) was used (Chapter 2). The ULO model involved the removal of one ovary prior to mating to ensure that during early pregnancy one uterine horn would be exposed to both local and systemic progesterone, whilst the other horn would only be exposed to systemic progesterone. The removal of an ovary resulted in 0.8 less embryos surviving in the horn without the ovary (contralateral) compared to horn where the ovary was still intact (ipsilateral), indicating that the ipsilateral horn provided a more favourable environment for the implantation and survival of embryos. So, it seems that local progesterone does play a role in the probability of embryo survival along with that of systemic progesterone, but the next question was whether these two sources of progesterone differed and if so, how?

The theory behind high feed level and a reduction in embryo survival is supported by only a limited number of studies, but it has become common practice throughout the world to restrict feed gilts during early pregnancy in order to increase embryo survival (Dyck and Strain 1983; Jindal *et al.* 1996). The danger behind this, beside the fact that there is not much evidence to support it (more to oppose it), is that these young gilts are still growing

toward their mature body size and restricting their feed intake may have consequences for their overall growth and longevity. In Chapter 2, along with the ULO, gilts were also fed at either a high or a low feeding level in order to see if effects on embryo survival differed between the horns. Systemic blood samples were collected on days 5 and 15 of gestation and in accordance with studies such as those conducted by Jindal *et al*., (1996) and Virolainen *et al*., (2004), gilts on a high feed level did have significantly lower systemic progesterone concentrations compared to gilts on the low feed level, at least on day 5 of gestation. However, when embryo survival was assessed there was no significant difference in embryo survival between feed levels, contradicting the paradigm that high feed levels are detrimental to embryo survival. Furthermore, when embryo survival was compared between uterine horns in relation to feeding level, gilts on a high feed level had 1.3 more embryos in the ipsilateral compared to the contralateral horn, whereas, in gilts fed at a low feed level there was only a difference of 0.4 embryos between the horns. This provides more evidence supporting the theory that local progesterone is important for embryo survival and that a high feeding level can even help to increase its beneficial effects.

In order to measure the effect of feeding level on progesterone in local circulation a catheter was placed in the vena cava, close but proximal, to where the uterine-ovarian vein drains into the vena cava (Chapter 3). Again, gilts were fed at either a high or a low feeding level and blood samples were collected on days 6 and 9 of gestation. On day 6 of gestation, progesterone concentrations in the vena cava tended to be higher in those gilts on a high feed level, which is opposite to the effect you would usually see in systemic circulation. Furthermore, progesterone concentrations in the vena cava followed a pulsatile pattern and there were significantly more pulses during a 6 hour sampling period in those gilts on the high feed level compared to the low feed level on day 9 of gestation (4.9 ±1.1

vs. 3.8 ± 0.7). Again, as with the previous study (Chapter 2), embryo survival (at day 9) was significantly higher for those gilts on the high feed level, compared to those on the low feed level (92 vs. 77%).

The first two studies presented in this thesis (Chapter 2 and Chapter 3) helped to establish the role that local progesterone plays in relation to embryo survival, and proved that it should not be overlooked when undertaking investigations into progesterone and embryo survival especially in regards to the influence of feeding level on circulating levels of progesterone in both systemic and local blood circulation. The final two studies presented in this thesis (Chapter 4 and Chapter 5) went on to see if and how progesterone concentrations could be manipulated in order to provide maximum benefit to embryo survival and litter size. In the third study (Chapter 4) gilts were again fed at either a high or a low feeding level, but this time different energy sources (starch, fat or fibre) were used in order to try to manipulate progesterone production by the ovaries. It was hypothesised that a starch rich diet would lead to an increase in insulin growth factor -1 (IGF-1) which in turn would influence early luteal function and thereby result in an increase in progesterone output by the ovaries. On the other hand, the inclusion of fat was not expected to yield any positive effect while fibre was also included as an energy source due to an increase in its use as a feeding management tool in group housed sows. On day 15 of pregnancy systemic progesterone concentrations were significantly lower in gilts fed the starch rich diet at a high feed level compared to those fed the starch rich diet at a low feed level. Again, however, despite this difference in systemic progesterone concentrations there was no difference in embryo survival between feed levels at day 35 of gestation. Furthermore, luteal tissue weight was positively affected by feed level with gilts fed the starch rich diet at a high feeding level having a greater luteal weight than those on the starch rich diet fed at the low feed level. In the fourth and final study presented in this thesis (Chapter 5)

feeding level and fibre effects on pregnancy rate and litter size were assessed. Gilts were fed either a high, medium or low feed level of a standard gestation diet, or a fibre diet (100g/kg crude fibre) at a medium feed level from mating until day 25 of gestation. Progesterone in systemic circulation was highest for gilts on the low feed level, but only significantly different from the medium feed level. Again, there was no difference between the feed levels or the high fibre diet in regards to pregnancy rate or litter size. Both studies showed that the inclusion of fibre had no negative effects on embryo survival and could be a possible management tool for group housed sows.

Another important aspect to take into consideration when managing the gilt during early gestation is the fact that she is still growing towards her mature body size and any severe to moderate restriction in feed intake whilst she is still growing may impact on her longevity in the breeding herd. Across all the studies presented in this thesis those gilts on the high feed level had significantly higher weight gains throughout early pregnancy compared to those on the low or medium feed levels. It is also interesting to note that in Chapter 5 when gilts were grouped according to their growth rate (irrespective of dietary treatment), those gilts with the highest daily growth rate (1003 g/d) had the highest pregnancy rate (92%) compared to those gilts with the lowest daily growth rate (216 g/d; 85% pregnant) and medium growth rate (573 g/d; 80% pregnant). This is in agreement with Dyck and Strain 1983, Virolainen *et al*. 2004, Hoving *et al*. 2011 and Athorn *et al*. 2011bb who all reported positive effects of a higher feed level on pregnancy rate or litter size.

Overall, this thesis proves that local progesterone supply to the uterus plays an important role in embryo survival in conjunction with that of systemic progesterone. Furthermore, feeding level has a differential effect on progesterone concentrations in local blood circulation compared to systemic circulation with benefits of a high feeding level only discernable at a local level. From an industry point of view this means that gilts can be fed

at feed levels that still promote growth and development leading to increased longevity without any detrimental effects on embryo survival and possibly positive effects on pregnancy rates which further help to increase the number of pigs born per sow per year. Further work in this area is needed to discern what effect feed levels have during the different windows of early gestation especially as changes in LH sensitivity and the dynamics of progesterone secretion by the CL may alter the ratio between systemic and local contributions of progesterone.

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