

Improving reproductive performance during seasonal infertility: Identification of “at-risk” sows and the role of oocyte quality

2D-108

Report prepared for the
Co-operative Research Centre for an Internationally
Competitive Pork Industry

Dr Christopher Grupen, Dr Patricia Holyoake and Mr Michael Bertoldo

Faculty of Veterinary Science
University of Sydney
JL Shute Building, 425 Werombi Road
Camden, NSW 2570

May 2010



Established and

Executive Summary

Late pregnancy loss (LPL) is a common syndrome of seasonal infertility resulting in reduced farrowing rate. The factors that lead to late pregnancy loss during the summer and autumn period remain to be clearly established, making it difficult to implement cost effective intervention strategies, particularly as only a proportion of sows and gilts are affected. The project analysed the risk factors associated with late pregnancy loss during the seasonal infertility period, and investigated the effects of season on ovarian properties and oocyte quality in sows.

The initial work in the project involved retrospective analyses of the reproductive performance of some 13,122 sows and gilts from three herds known to be affected by farrowing rate declines in summer. The initial evaluation investigated possible links between late pregnancy loss and factors such as parity, weaning to oestrus interval, lactation length, and the number of piglets weaned. Subsequent studies examined the effect of season on ovarian properties in sows. The assessments included follicle distribution, follicular fluid steroid content and oocyte quality. First, sows culled for non-reproductive reasons were slaughtered in summer or winter 4 days after weaning. Next, sows classified as “pregnancy test negative” at day 35 post-mating were slaughtered following matings in summer or winter. Finally, sows classified as “not-in-pig” were slaughtered in summer or winter and grouped according to the season in which they were mated.

The results of the initial study showed that the factors affecting the probability of late pregnancy loss (LPL) in summer varied with the herd involved, as did the severity of seasonal infertility. Across all three herds the probability of LPL increased with:

- Increasing parity
- Increasing weaning to oestrus interval
- Decreasing lactation length
- Decreasing litter size weaned.

To avoid reductions in sow reproductive performance during the seasonal infertility period, recommendations for producers include practicing longer lactations, ensuring that the first oestrus after weaning is identified in a higher proportion of animals, and culling higher order parity sows earlier.

In sows culled for non-reproductive reasons 4 days after weaning, the effect of season on follicle distribution varied between farms, but was consistent with the weaning to oestrus interval being shorter in winter than in summer. Results obtained following ovary collections in two summers and the two corresponding winters showed two clear effects of season:

- The concentration of progesterone in follicular fluid from both small and large follicles was about 50% lower in summer compared with winter
- The quality of oocytes recovered from large follicles, as measured by their capacity to develop to the blastocyst stage of embryonic development, was severely reduced in summer (21%) compared with winter (55%).

This is the first study to demonstrate that oocyte quality in pigs is compromised during the summer months. The findings support the hypothesis that suppressed endocrinological control mechanisms during lactation in summer adversely affect oocyte quality at the post-weaning oestrus. Further studies are needed to determine whether strategies that increase the concentration of follicular progesterone during lactation improve oocyte quality and alleviate seasonal infertility.

In sows culled for being “not-in-pig”, an effect of season on oocyte quality was also observed. The oocytes of NIPs culled in winter (mated in summer) had significantly greater developmental potential than those of NIPs culled in summer (mated in winter) (64% vs 34%). Since NIPs culled in winter displayed good oocyte quality, the cause of late pregnancy loss in NIPs is not due to inherently poor oocyte quality in this group of sows.

In sows culled for being “pregnancy test negative”, there was also an effect of season on oocyte quality. However, a summer-autumn induced reduction in oocyte quality was not observed. PTN sows culled in late-spring displayed poorer oocyte developmental potential (43%) compared with PTN sows culled in summer (63%). A possible explanation for this result is that PTN sows have inherently poor oocyte quality compared with the rest of the herd. In late-spring, all PTN sows may have comprised this group of animals. In summer, when the number of PTN sows is greatest, perhaps some PTN sows also had poor oocyte quality, but many had good oocyte quality and failed to maintain pregnancy due to other summer-autumn related factors, such as heat stress.

It should be noted that interpretation of the NIP and PTN findings is complicated by the fact that the ovaries were collected at unknown stages of the oestrous cycle. Further studies are needed to determine the underlying cause(s) of pregnancy loss in NIP and PTN sows. Nevertheless, oocyte quality undoubtedly contributes to the losses in a proportion of these animals following mating during the seasonal infertility period.

Table of Contents

- Executive Summary i
- 1. Introduction 1
- 2. Methodology 2
- 3. Outcomes 3
- 4. Application of Research 12
- 5. Conclusion 12
- 6. Limitations/Risks 15
- 7. Recommendations 15
- 8. References 16

1. Introduction

During seasonal infertility (SI), farrowing rate typically declines between 5 and 15%. Pregnancy loss during seasonal infertility may occur early and result in return to oestrus about 21 days after mating, be detected at the first pregnancy test at 4-6 weeks post-mating (resulting in a delayed return to oestrus), or it may occur after this pregnancy test (referred to as late pregnancy loss hereafter). Late pregnancy loss has been recognised as the most costly part of SI due to an inability to forecast volume (Seasonal Infertility Workshop, 2006). A preliminary study conducted on two neighbouring large commercial pig farms in Victoria with the same genetics (Spicer et al., personal communication) found that 10% of gilts on one farm lost their pregnancies after 6 weeks gestation.

The sow-specific and environmental risk factors that contribute to late pregnancy loss (often coined "autumn abortion syndrome") have not been determined. Various authors blame temperature fluctuations and cold stress (Almond et al., 1985), low progesterone concentrations, combined with inadequate nutrition, genetic predisposition and social deprivation (Wrathall, 1982) and a combination of low environmental temperatures and inadequate energy intake (Sanford, 1982). Certainly, on most farms in Australia, low environmental temperatures are unlikely to be the major predisposing factor for late pregnancy loss in January and February. It is recognised that pregnant sows have lower circulating progesterone concentrations in summer/autumn than winter/spring (Peacock, 1991) and it is likely that this makes sows more vulnerable to other environmental stressors that result in pregnancy loss. Group-housing conditions appear to exacerbate the problem (Seasonal Infertility Workshop, 2006). In agreement to this, Spicer et al. (personal communication) found that pregnancy loss was higher in group-housed sows (8%) than individually-housed sows (1%) during SI.

The early disruption of pregnancy during SI may be due to embryonic defects that result in an inadequate signal from the conceptus for the maternal recognition of pregnancy. Aberrant formation of oocytes and/or spermatozoa causes the production of defective embryos. Oocyte quality, as measured by their ability to form normal embryos, has been correlated with levels of circulating and follicular progesterone in preceding oestrous cycles. Previous studies in gilts have shown that oocyte quality increases from the first to the third oestrus, concomitant with progressive increases in follicular fluid steroid concentrations. Similarly, oocyte quality is proposed to decline during the non-breeding season in ewes as a consequence of reduced progesterone production. The effect of season on endocrine function, and the resultant impact of this on oocyte quality, has not been explored in pigs.

The objectives of this study were to determine:

- i) the sow-specific risk factors that contribute to late pregnancy loss during the seasonal infertility period
- ii) the effect of season on ovarian properties, including the distribution of antral follicles and follicular fluid (FF) steroid content, and oocyte quality in sows culled for non-reproductive reasons (i.e. having normal reproductive function)
- iii) whether sow-specific risk factors of seasonal infertility are associated with seasonal effects on ovarian properties and oocyte quality in sows culled for reproductive reasons (i.e. having impaired reproductive function).

2. Methodology

Experiment 1

Reproductive performance records of 3,901 gilts and 10,123 sows were obtained from three farms (Farms A, B and C) in Australia. The specific seasonal infertility period for each farm was defined as the period in which matings resulted in a farrowing rate more than 5% lower than the farrowing rate for the rest of the year. Sow-specific factors were used in the analysis to determine if they contributed to late pregnancy loss (LPL) during the seasonal infertility period. The selected parameters have previously been shown to be good indicators of reproductive performance. A "case" sow was defined as one that was recorded as pregnant by ultrasound examination at approximately 30 d post-mating but failed to farrow. "Control" sows were those that farrowed as a result of the recorded mating.

Experiment 2

Ovaries were recovered in pairs from sows culled for reasons unrelated to fertility 4 days post weaning during winter and summer. Sows culled for reasons unrelated to fertility were used in the study to determine whether the seasonal changes in ovarian physiology occurred in reproductively normal females (summer: n=372; winter: n=366). The number of ovarian structures was measured for each sow. Follicular fluid was also collected and assayed for steroid content (summer: n=40; winter: n=40). The developmental competence of oocytes recovered from small and large follicles was assessed. Recovered oocytes were subjected to established protocols for IVM/IVP of porcine embryos.

Experiment 3

"Not-In-Pig" (NIP) Sows

Ovaries were collected in winter from sows that were mated in summer, diagnosed pregnant by ultrasound at ~35 days after mating, but found to lose their pregnancies after Day 35 post-mating. The same was repeated for ovaries collected in late-spring from sows mated in winter. Sows mated in summer and then slaughtered in winter were termed summer mate/winter culls (SuMWC). Those sows mated in winter and subsequently slaughtered in late-spring were referred to as winter mate/spring culls (WMSC).

"Pregnancy Test Negative" (PTN) Sows

Ovaries were collected from sows following two negative pregnancy diagnoses at 30 and 35 days post mating in both summer and spring. Sows mated in summer and then slaughtered in summer were called summer mate/summer cull (SuMSuC). Those sows mated in late-spring and subsequently slaughtered in late-spring were called spring mate/spring cull (SMSC).

For both groups of animals, sows were grouped according to their previous WSI (≤ 6 days or > 6 days) and the presence (CL) or absence (NCL) of CL. The numbers of CLs were counted, follicular fluid was collected from 3 to 8 mm follicles and assayed, and recovered oocytes were subjected to established IVM/IVP protocols. Table 1 summarises the number of NIP and PTN sows categorised into each group.

Table 1 - Numbers of animals in each group of Experiment 3.

WSI ^a	CL ^b	NIPs		PTNs	
		WMSC ^c	SuMWC ^d	SMSC ^e	SuMSuC ^f
≤6d	+	40	37	33	33
	-	21	25	18	35
>6d	+	17	25	16	19
	-	6	7	9	21

^aWean-to-service interval; ^bPresence or absence of corpora lutea; ^cWinter mate/Spring cull sows; ^dSummer mate/Winter cull sows; ^eSpring mate/Spring cull sows; ^fSummer mate/Summer cull sows

3. Outcomes

Experiment 1

Age at first service

Age at first service was found to be a risk factor for LPL during seasonal infertility on only one farm. Gilts on Farm C with an increased risk of LPL were those either mated before 200 d or after 240 d of age (Fig. 1).

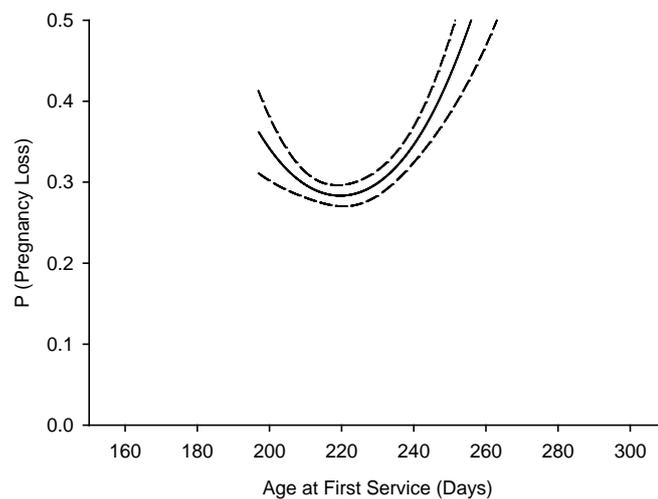


Fig.1 - The effect of age at first service on the probability of late pregnancy loss (P(Pregnancy loss)) on Farm C ($P < 0.05$). The period of seasonal infertility on Farm C was defined as 10/23/05 - 03/12/06.

Parity

There was a significant effect of parity on LPL in the combined analysis of data from the three farms, but these effects differed across the three farms (Fig. 2; $P < 0.001$). The probability of LPL increased with increasing parity on Farms A and C only. The effect of parity was substantially larger on Farm C than Farm A.

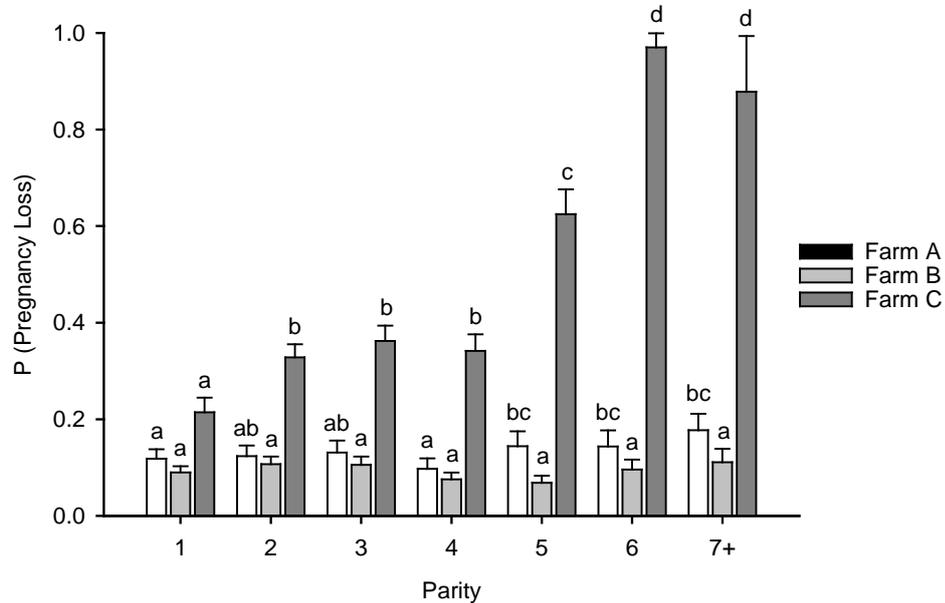


Fig. 2 - The effect of parity on the probability of late pregnancy loss on farms A, B and C during the seasonal infertility period. The seasonal infertility period was identified to be between October and April. Farm groups with common scripts are not significantly different.

Wean to service interval

The incidence of LPL was considerably higher for Farm C than Farms A and B. There was a significant Farm \times WSI interaction ($P < 0.001$), indicating a different effect of WSI among the three farms (Fig. 3). As WSI increased, the chance of LPL also increased for Farms A and B, but decreased for Farm C. But note that the incidence of LPL on Farm C was unusually high, particularly in older sows.

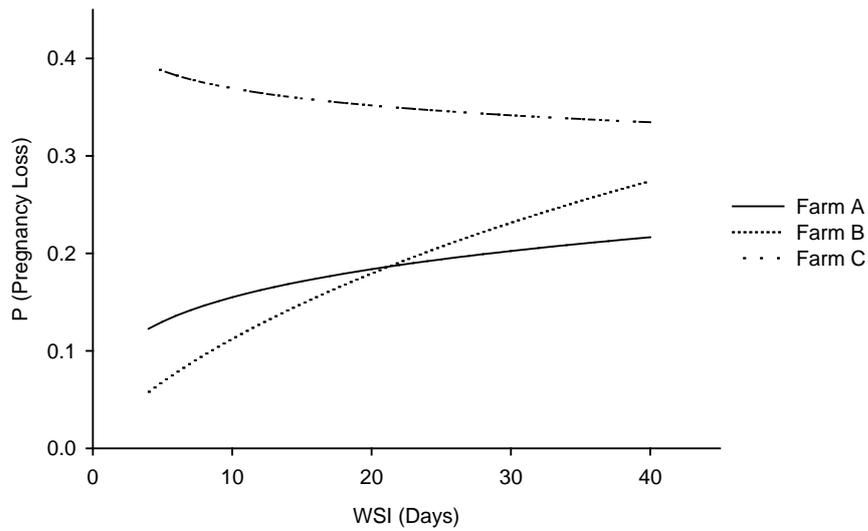


Fig. 3 - The effect of wean to service interval on the probability of late pregnancy loss on farms A, B and C ($P < 0.001$) during the seasonal infertility period. The seasonal infertility period was identified to be between October and April.

Lactation length

The mean lactation length among sows in this period was 28, 20 and 21 d on Farms A, B and C respectively. Lactation length had a constant significant effect on LPL across all three herds ($P < 0.05$), with a lower probability of late pregnancy loss in sows with longer lactations (Fig. 4). With every incremental day increase in lactation length, the risk of LPL decreased by 2%.

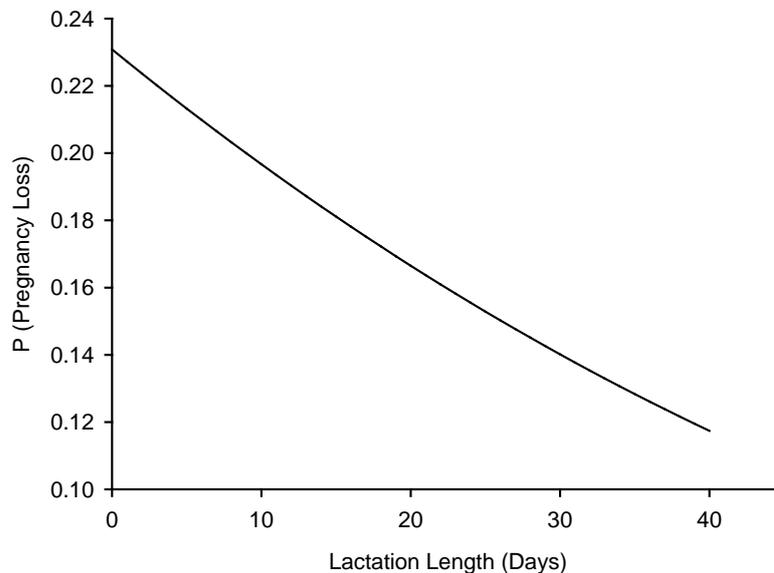


Fig. 4 - Effect of lactation length on the probability of late pregnancy loss on Farms A, B and C combined during the seasonal infertility period ($P < 0.05$). The seasonal infertility period was identified to be between October and April.

Number of piglets weaned

The mean number of piglets weaned per litter was 9.7, 9.6 and 9.7 on Farms A, B and C respectively. The number of piglets weaned per litter was shown to have a significant effect on LPL ($P < 0.05$). Sows that weaned 7 piglets had the highest chance of LPL (22%) at their subsequent mating. Those sows weaning litters of between 8 to 12 piglets had approximately 17% chance of losing their pregnancy, while those sows weaning 13+ piglets had approximately 14% chance of losing their pregnancy. If a sow weaned less than 6 piglets, the probability of losing her pregnancy would be 16%.

Experiment 2

The effect of season on ovarian morphology

The mean parity of sows did not differ between the summer (5.88 ± 0.11) and winter (6.00 ± 0.09) groups. The mean numbers of 3 to 4 mm (small), 5 to 8 mm (large) and 8 to 12 mm (preovulatory) follicles per sow from Farms A and B are presented in Table 2. The proportion of sows that had ovulated during lactation or within 4 days following weaning was significantly higher in winter compared with summer on Farm A ($16.5 \pm 1.6\%$ v. $6.0 \pm 2.7\%$; $P < 0.001$) but not on Farm B ($12.5 \pm 1.7\%$ v. $8.9 \pm 2.2\%$; $P > 0.05$). The mean number of corpora lutea (CL) per sow that had ovulated was significantly higher in winter compared with summer from sows on Farm B (20.4 ± 0.1 v. 8.7 ± 0.2 ; $P < 0.001$) but not on Farm A (27.4 ± 4.2 v. 27.3 ± 5.3 ; $P > 0.05$). The mean ovarian weight was heavier in winter than in summer (20.1 ± 0.8 g v. 8.7 ± 0.7 g; $P < 0.001$).

Table 2 - The effect of season on the number of small, large and preovulatory follicles on ovaries of sows from Farms A and B.

Follicle type	Farm A		Farm B	
	Winter	Summer	Winter	Summer
Small	4.6 ± 0.5^a	8.5 ± 0.7^b	10.2 ± 1.5^a	6.5 ± 1.0^b
Large	15.4 ± 0.5^a	17.8 ± 0.6^b	17.6 ± 1.0^a	19.8 ± 0.9^a
Preovulatory	2.9 ± 0.4^a	2.9 ± 0.3^a	1.2 ± 0.5^a	0.5 ± 0.3^b
Total	23.0 ± 0.5^a	29.0 ± 0.6^b	29.0 ± 1.2^a	26.8 ± 1.9^a

Values are the mean \pm s.e.m. Values across rows and within farm groups with different superscripts differ significantly ($P < 0.05$).

The effect of season on ovarian steroidogenesis

The effect of season on the concentrations of progesterone in FF of Farm B sows collected from small and large antral follicles is shown in Fig. 5. The mean concentration of progesterone (P_4) in FF of small follicles was greater in winter (1235.55 ± 164.47 nmol L⁻¹) than in summer (701.3 ± 115.54 nmol L⁻¹; $P < 0.001$). Similarly, mean progesterone concentration in FF of large follicles was greater in winter (2470.9 ± 169.13 nmol L⁻¹) than in summer (1469.2 ± 156.51 nmol L⁻¹; $P < 0.001$).

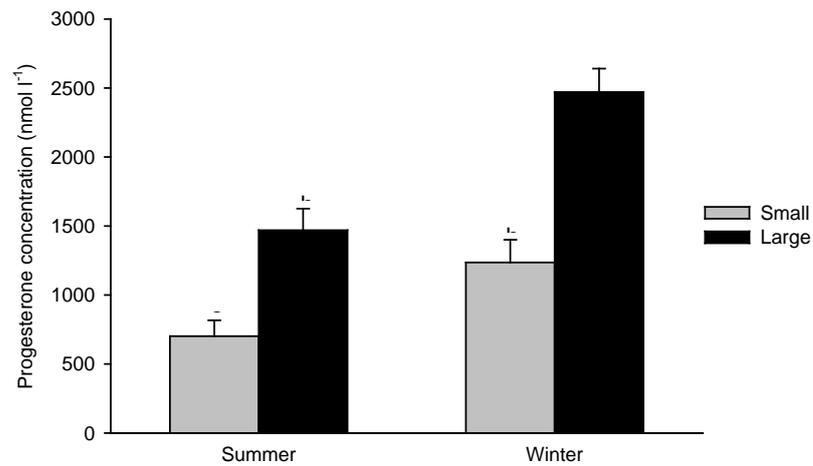


Fig. 5 - Concentrations of progesterone in follicular fluid aspirated from small (3 to 4 mm) and large (5 to 8mm) surface antral follicles during summer and winter. Ovaries were recovered 4 days after weaning. Values with different subscripts differ significantly ($P < 0.001$).

Oocyte quality

The rates of oocyte maturation and cleavage were not affected significantly by season. Blastocyst formation rates are shown in Figure 6. The proportion of oocytes from large follicles that formed blastocysts was greater in winter than in summer ($P < 0.05$). During winter, oocytes from large follicles displayed significantly higher blastocyst formation rates than oocytes from small follicles. In contrast, during summer, oocytes from large follicles displayed a similar blastocyst formation rate to oocytes from small follicles. There was no effect of season on the proportion of oocytes from small follicles developing to the blastocyst stage.

The mean blastocyst cell number is presented in Figure 7. Blastocysts derived from oocytes of small follicles during summer had fewer cells (24.25 ± 1.48) than those derived from oocytes of small follicles during winter (32.0 ± 3.45 ; $P < 0.05$).

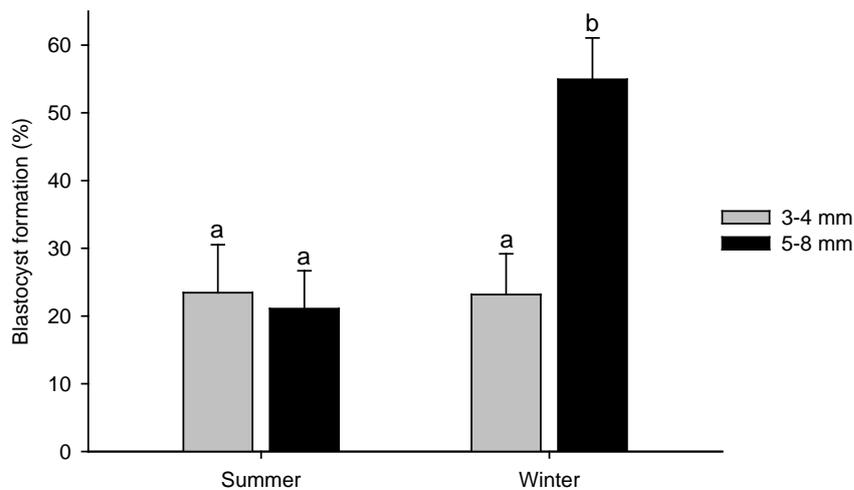


Fig. 6 - Comparison between seasons and follicle sizes of developmental competence of sow IVM oocytes following parthenogenetic activation. Oocytes were recovered from sows 4 days after weaning. Different subscripts indicate significant differences ($P < 0.05$).

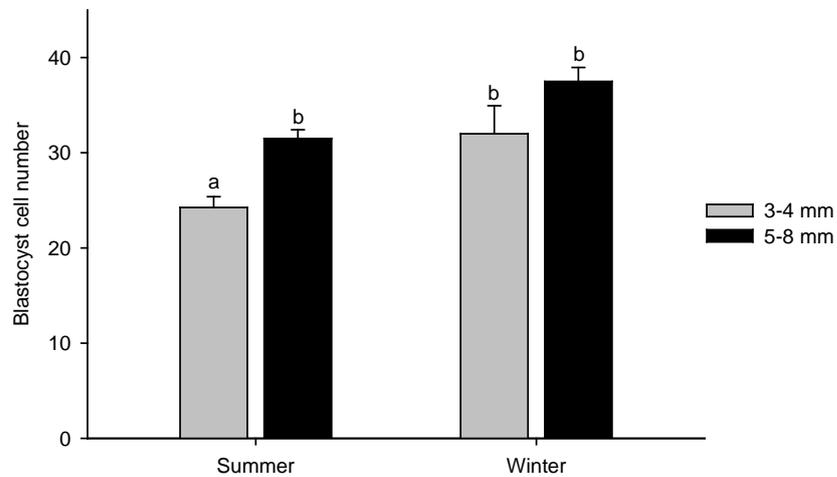


Fig 7 - Comparison between seasons and follicle sizes of number of cells in blastocysts obtained from small and large follicles. Different subscripts indicate significant differences ($P < 0.05$).

Experiment 3

Assessment of ovarian morphology

NIP sows: There was a significant interaction between season mated and WSI for the number of CL per sow with those animals expected to have the lowest fertility having the lowest number of CL (Figure 8).

PTN sows: There was an effect of season mated on the mean number of CL per sow. The mean number of CL was greater (11.6 ± 3.3) per SMSC sow than in SuMSuC sows (9.3 ± 1.0 ; $P < 0.05$).

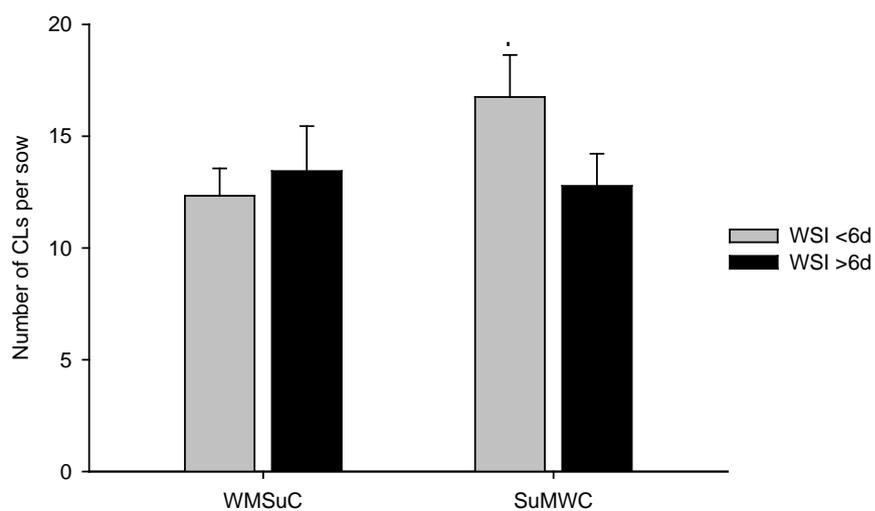


Fig. 8 - Comparisons between seasons and WSI on the number of CL per winter mate/summer cull (WMSuC) and summer mate/winter cull (SuMWC) sow. Different subscripts indicate significant differences ($P < 0.05$).

Assessment of follicular fluid progesterone content

NIP sows: There were no differences in mean concentrations of progesterone in follicular fluid (FF) between summer ($1840 \pm 280 \text{ nmol L}^{-1}$) and winter ($1307 \pm 201 \text{ nmol L}^{-1}$; $P > 0.05$) mated sows. However, ovaries without CLs contained more FF progesterone in summer ($2414 \pm 412 \text{ nmol L}^{-1}$) than in winter ($1656 \pm 377 \text{ nmol L}^{-1}$; $P < 0.05$) mated sows. Ovaries without CLs had greater concentrations of FF progesterone ($2077 \pm 290 \text{ nmol L}^{-1}$) than those with CLs ($1082 \pm 126 \text{ nmol L}^{-1}$; $P < 0.05$). In ovaries with CLs, there was no difference in FF progesterone levels between summer ($1123 \pm 150 \text{ nmol L}^{-1}$) and winter ($1053 \pm 194 \text{ nmol L}^{-1}$; $P > 0.05$) mated sows. There was no effect of WSI on follicular progesterone concentrations.

PTN sows: There were no effects of season mated, WSI or CL status on FF progesterone concentration in ovaries collected from PTN sows.

Assessment of oocyte developmental competence

NIP sows: Rates of oocyte maturation and cleavage did not differ between season, WSI or CL status ($P > 0.05$). Blastocyst formation rates are shown in Fig. 9. The proportion of oocytes forming blastocysts by Day 5 and Day 7 was higher coming from SuMWC sows compared to WMSuC sows ($P < 0.05$). The proportion of oocytes developing to the blastocyst stage from animals with a WSI ≤ 6 days was lower than those oocytes from sows with a WSI > 6 days ($P < 0.05$; Fig. 10). There was no effect of CL status on the proportion of oocytes developing to the blastocyst stage. There was no effect of season mated, WSI or CL status on blastocyst cell number.

PTN sows: Rates of oocyte maturation and cleavage did not differ between season mated, WSI or CL status ($P > 0.05$). There were significant seasonal and CL status effects on the proportion of oocytes developing to the blastocyst stage by Day 5 and Day 7 (Figs. 11 and 12). Oocytes recovered in summer had greater developmental competence than those recovered in late-spring, regardless of physiological state of the ovary. Oocytes derived from ovaries with CLs also had greater developmental competence than those from ovaries without CLs. Oocytes recovered from NCL ovaries during summer had superior developmental competence (48.62 ± 5.45) compared to those recovered from NCL ovaries during late-spring (16.54 ± 53.80 ; $P < 0.05$; Fig. 9). Oocytes derived from ovaries with CL in the summer also had superior developmental competence (63.26 ± 8.14) compared to oocytes recovered from ovaries with CL in late-spring (42.89 ± 9.12 ; Fig. 10). There was no effect of WSI on oocyte developmental competence ($P > 0.05$).

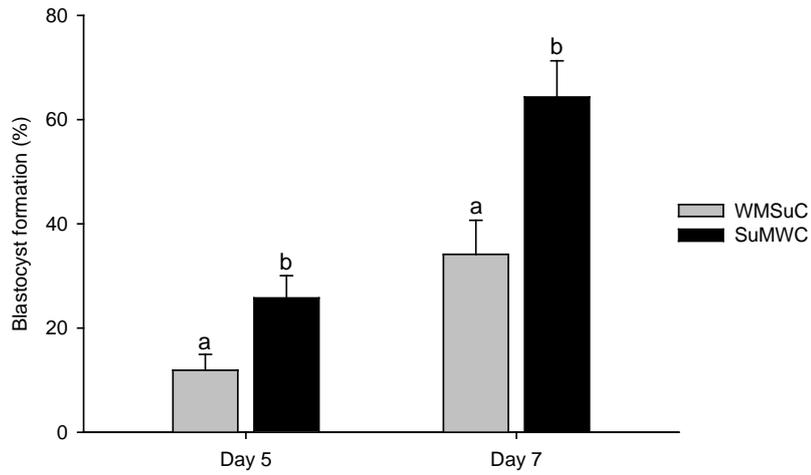


Fig. 9 - Effect of season on developmental competence of sow IVM oocytes on Days 5 and 7 of culture following parthenogenetic activation. Oocytes were recovered from winter mate/spring cull (WMSuC) and summer mate/winter cull (SuMWC) NIP sows. Different subscripts indicate significant differences ($P < 0.05$).

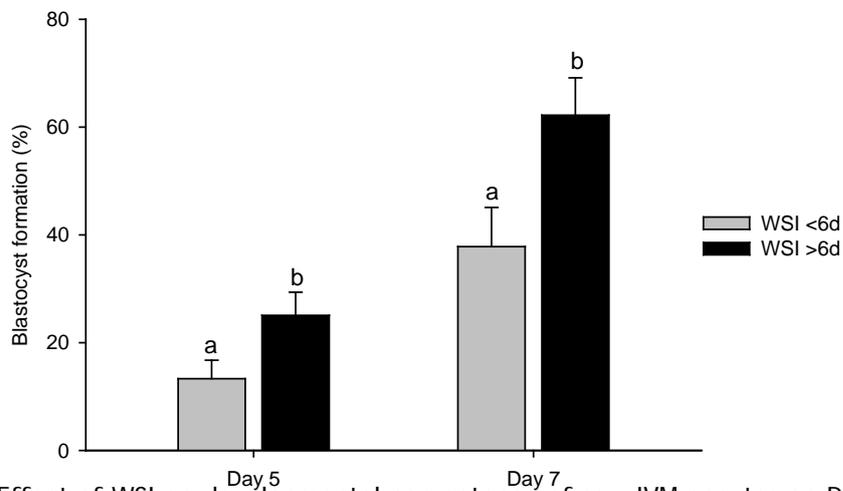


Fig. 10 - Effect of WSI on developmental competence of sow IVM oocytes on Days 5 and 7 of culture following parthenogenetic activation. Oocytes were recovered from NIP sows. Different subscripts indicate significant differences ($P < 0.05$).

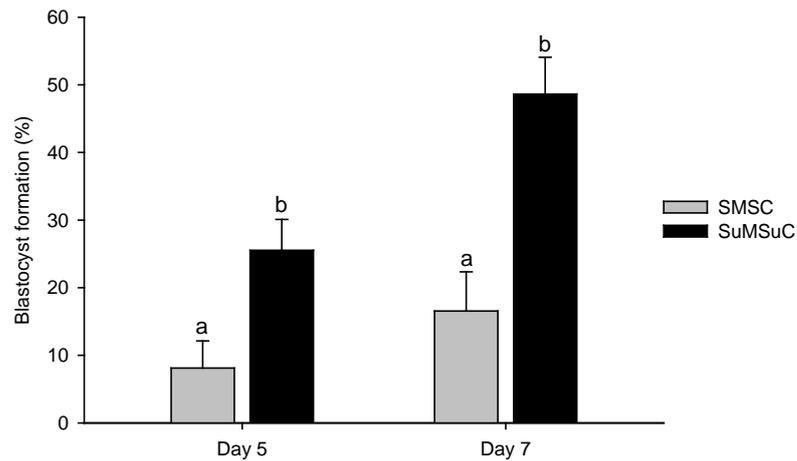


Fig. 11 - Effect of season on developmental competence of oocytes from ovaries without CLs. Oocytes were recovered from spring mate/spring cull (SMSC) and summer mate/summer cull (SuMSuC) PTN sows. Different subscripts indicate significant differences ($P < 0.05$).

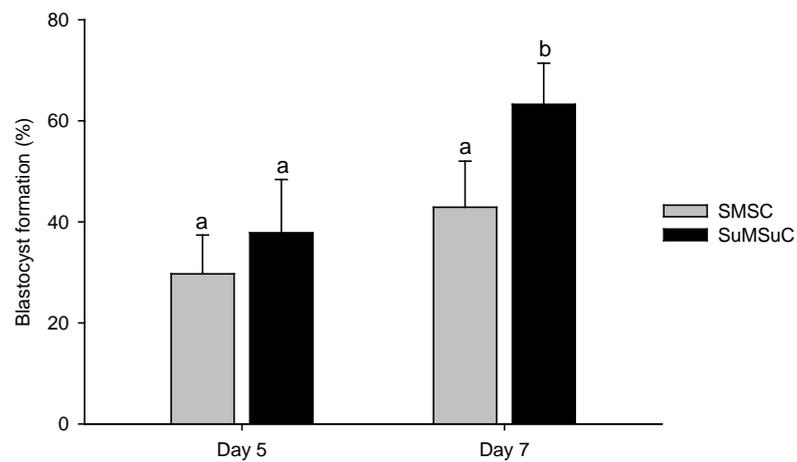


Fig. 12 - Effect of season on developmental competence of oocytes from ovaries with CLs. Oocytes were recovered from spring mate/spring cull (SMSC) and summer mate/summer cull (SuMSuC) PTN sows. Different subscripts indicate significant differences ($P < 0.05$).

There were also independent seasonal and CL status effects on the mean blastocyst cell number. Blastocysts derived from SuMSuC sows had a greater number of cells (46.6 ± 1.2) than blastocysts derived from SMSC sows (43.2 ± 1.0 ; $P < 0.05$). The effect of CL status on blastocyst cell number was more distinct. Those blastocysts derived from oocytes of ovaries with CL (47.1 ± 1.1) had more cells than those blastocysts derived from oocytes of ovaries without CL (42.9 ± 0.8 ; $P < 0.05$).

4. Application of Research

A number of sow-specific factors associated with late pregnancy loss in the seasonal infertility period were identified, including age at first service, parity, lactation length, number of piglets weaned per litter and wean-to-service interval. Producers may be able to maintain production volumes during the seasonal infertility period by replacing breeding herd sows that have an increased risk of late pregnancy loss. Farm management practices also appeared to exert a substantial influence on the manifestations of seasonal infertility. Further studies are needed to assess the effects of specific practices, such as the duration of lactation, on late pregnancy loss during the seasonal infertility period.

The ability to maintain production volume can be extremely challenging during the seasonal infertility period. The additional sows that need to be mated to make up for the reduction in farrowing rate represents a significant cost to the producer. Also, maintaining sows that experience late pregnancy loss has a major economic impact. By implementing the recommendations resulting from this research, the costs associated with seasonal infertility will be reduced. The recommended breeding management practices can be readily implemented by producers.

The results also demonstrated that follicular fluid progesterone content was reduced during the seasonal infertility period. The reduction in progesterone concentration was correlated with a significant reduction in oocyte quality. This apparent relationship between progesterone and oocyte development was also evident in NIP sows. These results suggest that it may be possible to improve oocyte quality by using strategies that increase follicular progesterone levels, at least during lactation. Clearly, further research is needed to test such strategies. The impact of this research extends well beyond the potential to alleviate seasonal infertility in the pig industry. If a causal association between progesterone concentration in follicular fluid and oocyte quality is confirmed, this has the potential to increase the efficiency of in vitro embryo production systems in pigs as well as in other species, and improve the pregnancy outcomes of sub-fertile females in production animal breeding programs and women undergoing infertility treatments.

5. Conclusion

Experiment 1

This study identified age at first service, parity, lactation length, number of piglets weaned per litter and WSI as risk factors for LPL during the period of seasonal infertility, highlighting the multifactorial nature of the seasonal infertility problem. Given that the genetics of the 3 herds examined were similar, the large differences observed between farms demonstrate that LPL is affected by farm management practices and/or relatively small variations in environmental conditions. Our findings suggest that not all sows are equally susceptible to LPL during the seasonal infertility period indicating that breeding herd efficiency can be improved by tailoring management interventions and/or culling protocols to target animals with an increased risk of LPL. Our recommendations include

practicing longer lactations, ensuring that the first oestrus after weaning is identified in a higher proportion of animals, and culling higher order parity sows earlier, to avoid reductions in sow reproductive performance during this time of the year.

Although real-time ultrasounds were used to diagnose pregnant animals on Farm C, it is possible that there was some degree of inaccuracy by the operators. Management had attempted to reduce the risk of inaccuracy by repeat pregnancy testing of any "test negative" animals. However, they did not re-check "test positive" animals. As a result, it is possible that some animals were falsely recorded as being pregnant at 30-d of gestation. However, these results support the work of Koketsu et al. (1997) that showed a larger proportion of higher-parity sows failed to maintain pregnancy compared to mid-parity sows. Takai and Koketsu (2007) also found farrowing rate decreased as parity increased from 1 to more than 6. In addition, Anil et al. (2005) found that sows of parity 2 to 5 appeared to be protected against breeding failure in summer.

Experiment 2

This study demonstrated that seasonal changes in ovarian steroidogenesis and ovarian morphology exist in weaned sows. We observed reductions in follicular progesterone levels in both small and large follicles during summer, indicating reduced ovarian activity. We also observed farm-specific seasonal differences in the type and number of surface antral follicles. The greater proportion of sows ovulating during lactation or after weaning on Farm A during winter, compared to summer, suggests animals are at more advanced stages of the oestrous cycle 4 days post-weaning during the winter months. On Farm B, the greater number of CLs per sow during winter, compared to summer, suggests that gonadotrophin release is greater during the winter months resulting in more prolific follicular growth and therefore greater numbers of CL. The reduced reproductive performance observed in sows during the seasonal infertility period may in part be attributed to these seasonal changes in follicular progesterone and ovarian dynamics.

We hypothesised that the impaired reproductive performance observed during the late summer and early autumn months could be attributed to a reduction in oocyte developmental competence. The results show that oocyte developmental competence was reduced during the period of seasonal infertility and this coincided with a reduction in follicular progesterone. We observed distinct differences in the ability of oocytes to form blastocysts between winter and summer and these differences were follicle size-specific. In summer, a reduced proportion of oocytes recovered from large follicles developed to the blastocyst stage, and oocytes recovered from small follicles produced blastocysts with fewer cells, indicating a reduction in developmental competence. It is possible that at least part of the observed reduction in fertility during summer in sows is due to a reduction in gonadotrophin-stimulated, progesterone-dependent support to oocytes. This finding has greatly advanced our understanding of the mechanisms leading to pregnancy loss during seasonal infertility.

Experiment 3

The results demonstrate that WSI is not a reliable indicator of oocyte developmental competence. The oocyte quality data from NIP sows supports the

results of Experiment 2 in that oocytes collected from sows in winter months displayed greater rates of blastocyst formation than those collected from summer culled sows. This is not the case for the oocyte quality data from PTN sows, where blastocyst formation rates were higher in summer compared to late-spring. Surprisingly, the developmental competence of oocytes recovered from NIP sows with a WSI >6 days was greater than those recovered from NIP sows with a WSI of ≤6 days.

In the present study, the numbers of CLs on the ovaries of PTN and NIP sows were considerably lower than that previously reported in sows culled for reasons unrelated to fertility (Bertoldo et al., 2009). Sows of compromised fertility may be expected to have a lower number of CLs. NIP SuMWC sows with a WSI ≤ 6 days had the greatest number of CL on their ovaries compared with the other groups. Animals with shorter WSI are considered to have higher fertility than those with longer WSI (Varley and Foxcroft, 1990; Tummaruk et al., 2000). The results show that in those animals that would be expected to have compromised fertility, CL number was lowest. Lower numbers of pregnancy supporting CL may increase the incidence of pregnancy loss during the seasonal infertility period.

In Experiment 3, sows were culled for reasons related to infertility and ovaries were collected at unknown stages of the oestrous cycle, either containing CL or having no CL. The interval between pregnancy loss and slaughter was also unknown and undoubtedly varied between animals; the interval may have been several weeks for those sows losing their pregnancy early compared to months for those sows experiencing late-pregnancy loss. It is possible that at least part of the observed reduction in fertility during summer in NIP sows was due to a reduction in oocyte developmental competence. The results of the present study suggest that the relationship between WSI and late pregnancy loss does not involve oocyte quality. Therefore, the increase in late pregnancy loss associated with long WSI may involve impaired CL function or other pregnancy maintenance mechanisms.

Final conclusions

In closing, the findings of this project have greatly advanced our understanding of the effects of season on sow reproductive performance. Sow-specific factors associated with late pregnancy loss in the seasonal infertility period include age at first service, parity, lactation length, number of piglets weaned per litter and wean-to-service interval. Farm management practices also appear to exert a substantial influence on the manifestations of seasonal infertility.

The results of the ovarian morphology assessments indicate that season does not dramatically alter the numbers and distribution of antral follicles. However, there was a clear reduction in follicular fluid progesterone levels during the summer months. Importantly, the concentration of progesterone in follicular fluid was positively associated with oocyte quality. This is the first study to demonstrate a reduction in sow oocyte quality during the summer months. Together the findings support the hypothesis that elevated progesterone levels prior to final oocyte maturation are needed to ensure the needs of the developing oocytes are met.

Finally, reduced oocyte quality during the summer months may contribute to the late pregnancy loss observed in NIP sows. In PTN sows, a reduction in oocyte quality was observed that was not consistent with the previous findings, suggesting the involvement of other factors in the pregnancy loss seen in this group of sows.

6. Limitations/Risks

The application of the research findings is primarily limited by the capacity to accurately assess the effectiveness of any adopted procedures.

Management practices, which often vary from farm to farm, exert an influence on the severity of seasonal infertility. Without a better understanding of the influence of specific practices on reproductive processes, it will be difficult to determine the efficacy of any adopted procedures.

The severity of seasonal infertility can vary considerably from year to year, even on the same farm. Clearly, any adopted procedures would need to be tested over several years in a stably managed environment before a definitive result is achieved.

As the improvement resulting from any adopted procedures is likely to be relatively small (eg. a 5% increase in farrowing rate), a large number of animals must be assessed in order to demonstrate a significant effect.

The risks associated with the application of the research findings are minimal. The preferential culling of sows identified as having an increased risk of late pregnancy loss is extremely unlikely to exacerbate the reduction in farrowing rate observed during the seasonal infertility period.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

Reductions in farrowing rate during the seasonal infertility period may be avoided by i) practicing longer lactations, ii) ensuring that the first estrus after weaning is identified in a higher proportion of animals, and iii) culling higher-order-parity sows earlier.

Strategies to increase oocyte quality during the seasonal infertility period, is certainly warranted. The positive relationship between follicular fluid progesterone level during lactation and oocyte quality needs to be further investigated.

If late pregnancy loss is high in commercial piggeries, greater emphasis should be given to pregnancy diagnosis by re-checking animals that do not return to oestrus at about 21 days after mating, and also test positive to an initial pregnancy diagnosis by ultrasound at day 30-35.

Further studies are needed to assess the effects of progesterone on oocyte maturation.

8. References

- Almond G, Friendship R, Bosu W (1985) Autumn abortions in sows, *Canadian Veterinary Journal*, 26:162-163.
- Anil SS, Larriestra A, Deen J, Anil L (2005) A path analysis of the factors associated with seasonal variation of breeding failure in sows, *Can J Anim Sci*, 85:317-25.
- Bertoldo M, Holyoake PK, Evans G, Grupen CG (2009) The effect of season on sow ovarian morphology, *Manipulating Pig Production XII*, 146 (abstract).
- Koketsu Y, Dial GD, King VL (1997) Returns to service after mating and removal of sows for reproductive reasons from commercial swine farms, *Theriogenology*, 47:1347-63.
- Peacock A (1991) Environmental and social factors affecting seasonal infertility of pigs, PhD thesis, University of Sydney.
- Sanford S (1982) Fall Abortions in sows, *Canadian Veterinary Journal*, 23(36):36.
- Takai Y, Koketsu Y (2007) Identification of a female-pig profile associated with lower productivity on commercial farms, *Theriogenology*, 68:87-92.
- Tummaruk P, Lundeheim N, Einarsson S, Dalin A-M (2000) Reproductive performance of purebred Swedish Landrace and Swedish Yorkshire sows: I. Seasonal variation and parity influence, *Acta Vet Scand*, 50:205-16.
- Varley MA, Foxcroft GR (1990) Endocrinology of the lactating and weaned sow, *J Reprod Fertil Suppl*, 40:47-61.
- Wrathall A (1982) The autumn abortion syndrome, *Proceedings of the 7th International Pig Veterinary Society Conference, Mexico City*, 205 (abstract).