Reduced labour costs and increasing synchrony and predictability of lactation oestrus

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Report prepared for the

Co-operative Research Centre for High Integrity Australian Pork

By

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Executive Summary

The major objective of Program 1A in the CRC for High Integrity Australian Pork is to develop novel techniques for inducing ovulation in sows during lactation and establish new subsequent breeding strategies that are consistent with a reduction of sow confinement during lactation and a later and more gradual weaning of their litters. The desired overall outcome from this work is simple, commercially applicable methods of stimulating oestrus expression in lactating sows whilst maintaining fertility and subsequent litter sizes, comparable to conventionally weaned and inseminated sows. The target is for 85% of sows to respond to lactation induction procedures within 7 days, with the remaining 15% exhibiting a fertile oestrus within 7 days after subsequent weaning.

Pork CRC has already invested in several projects to study the response of sows to a wide range of approaches to induce oestrus and ovulation during lactation. The aim of this Project was to determine whether exogenous gonadotropins, specifically 1,000 IU hCG injected either 24 hours or 48 hours after farrowing, can be used to stimulate an ovulation immediately post-farrowing. The project also examined whether an induced ovulation was followed by an oestrous cycle of normal length, resulting in a predictable second ovulation about 21 days after the induced ovulation but still within the lactation period.

The results of this study showed that only 26% sows that were injected with hCG either 24 or 48 hours after farrowing responded by ovulating within 5 days of farrowing. However, none of these sows appeared to exhibit oestrus and ovulate 21 days after this first, post-farrowing, induced ovulation. All sows were only exposed to fenceline contact on one day (about day 25 of lactation) and this minimal boar stimulation may have contributed to the lack of the subsequent and predictable second oestrus and ovulation during late lactation in those sows that had responded to hCG treatment soon after farrowing.

Studies similar to the one conducted in this Project, using exogenous gonadotrophins to induce lactation ovulation, have also produced inconsistent results. Together, these results indicate that post-partum administration of hCG is not an effective means to stimulate an ovulation and subsequently a consistent oestrus cyclicity in the later lactation period. Thus it is unlikely that hCG treatment in early lactation can be used as a strategy to induce a fertile oestrus in lactating sows.

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1. Introduction

The pig industry is currently facing a push to adopt high welfare production practices, which not only maximize sow and piglet welfare but also maintain production efficiency. During the production cycle, one of the most stressful events for piglets is weaning. The abrupt change in environment, feed and social interactions can have negative effects on piglet health, welfare and growth performance. In current practices weaning age is determined by the need to wean the litter before mating of the sow can take place. Potentially, these shorter lactation lengths may negatively affect piglet health, and there is evidence that extending lactation lengths would benefit the piglet (Kuller et al. 2004; Berkeveld et al. 2007).

In conventional management systems during later lactation, follicle growth is usually limited to the medium size (4-5mm) follicles, and, therefore, the sow is unable to achieve oestrus and ovulation (Barb et al. 1986; Soede et al. 2012). Inhibition of the later stages of follicle development due to piglets suckling normally remains until weaning takes place and the inhibition is removed (Soede et al. 2011). However, spontaneous oestrus and ovulation will occur if lactation is prolonged (eg. >5 weeks) with the likelihood increasing with increasing duration of lactation. Following weaning of the litter, oestrus is expected to occur 4 to 5 days later, with ovulation occurring approximately 40 hours after oestrus onset (Armstrong et al. 1988b; Kluivers-Poodt et al. 2010).

Current management protocols are designed to maximise sow productivity while attempting to minimise adverse effects on their litters and piglets are therefore weaned at 3 to 4 weeks of age. However, under natural conditions pigs are weaned gradually over many weeks and piglet health and performance is improved by older weaning ages. Indeed, piglets weaned after short lactations may experience problems such as diarrhoea caused by the sudden diet shift and changed environments (Gerritsen et al. 2008). Extending lactation lengths gives piglets a longer period to acclimatise to the impacts of weaning, including adapting to new feed and an improved immunocompetence (Vanbeersschreurs et al. 1992).

Uncoupling weaning from oestrus and ovulation would allow for ovulation and breeding to occur during lactation. Concurrent pregnancy and lactation allows the piglets to suckle to an age which is more beneficial for the piglets without disrupting sow production efficiency. A number of practices are reported to induce ovulation during lactation, such as intermittent suckling and split weaning which use modification of the suckling load to reduce the suckling induced inhibition on follicle growth and maturation (Gerritsen et al. 2008; van Leeuwen et al. 2012). Boar exposure is often employed in conjunction with lactation oestrus-induction practices and has proven beneficial for the oestrus responses (van Wettere et al. 2013). These management practices, however, require high levels of labour which can be costly. Other research has shown that the use of exogenous gonadotrophins, such as equine chorionic gonadotrophin (eCG) combined with human chorionic gonadotrophin (hCG) (Hausler et al. 1980; Hodson et al. 1981) and eCG alone (De Rensis et al. 2003), are effective for inducing lactation oestrus, although the timing of treatment is a limitation leading to inconsistent results.

In the immediate postpartum period, active LH pulsatility has been observed up to until 78 h post farrowing, after which suckling induced inhibition of LH pulsatility takes effect (De Rensis et al. 1993). Further, post-partum sow ovaries have

medium follicles (4-5 mm) and some sows exhibit oestrous behaviour (De Rensis et al. 1993; Sesti and Britt 1994; Langendijk et al. 2007). However, due to an inability to generate a preovulatory GnRH surge, the postpartum oestrus is always anovulatory (Baker et al. 1953; van Wettere et al. 2013). Interestingly, previous workers have provided an exogenous post-partum preovulatory signal in an attempt to induce ovulation, as the ovary is still receptive to exogenous gonadotrophins (Barb et al. 1986). Specifically, injection of 1,000 IU hCG within 24 hours of farrowing induced ovulation in 75% (Armstrong et al. 1999) and 41% (Kirkwood et al. 1999) of sows. Determination of ovulation was based on detection of serum progesterone concentrations of ≥5 ng/mL at 7 to 10 d after injection. Although the reasons for the different responses are unknown, an influence of timing of injection cannot be discounted. Similarly, to our knowledge, direct serial observations of ovarian follicular dynamics in individual sows in the post-partum period have not been documented.

If inducing ovulation early in lactation initiates a normal oestrous cycle followed by a secondary ovulation, it could result in novel oestrus synchronisation protocols with the potential for mating during lactation. An ovulation induced immediately after farrowing would not be suitable for mating due to a need for prior uterine involution. This requires about 21 days and limits sow fertility until complete (Armstrong et al. 1999). The objective of the current study was to determine ovarian follicular dynamics in the immediate postpartum period and the relationship between ovarian follicular status and the response to hCG injection at either 24 or 48 hour postpartum.

2. Methodology

This study was approved by the University of Adelaide Animal Ethics Committee (project number S-2013-016).

Animals and treatments

A total of 48 mixed parity sows (2.5 ± 0.2; range 1 to 5) were used across two replicates. Sows were housed in farrowing crates from 110 d of gestation until weaning. After farrowing, litter sizes were standardised to 10 or 11 (average 10.9 ± 0.2) and piglets were weaned at 28 d post farrowing. During lactation, sows were fed to appetite with a diet formulated to provide 14.3 MJ DE/kg, 12.5% crude protein and 0.9% total lysine.

Within each replicate, sows were assigned to one of three treatments by parity. Treatments were intramuscular injection of 1,000 IU hCG within 24 h after farrowing (n=16), or within 48 h after farrowing (n=18), or no injection and serving as Controls (n=14). For sows farrowing overnight, hCG injection was administered at 09:00 h on the day after farrowing completion or approximately 24 h after farrowing completion (24 h and 48 h treatments, respectively). Sows farrowing during the day (0800 to 16:00 h) were treated at 24 h or 48 h after the end of farrowing. All sows were taken to an oestrus detection and mating pen for fenceline boar exposure daily following weaning to determine weaning-oestrus intervals. Because of the batch farrowing management employed on this farm, any sows failing to express oestrus by 7 d after weaning were deemed anoestrus and were culled. Sows were inseminated at their first postweaning oestrus with commercially sourced semen doses having 3×10^9 sperm in 80 mL extender (SABOR, Clare, SA).

Blood sampling

Based on a very low incidence of ovulation in early lactation, the intended serial blood sampling blood sampling was reduced to a single sample on day 10 post-farrowing. Blood samples were collected by jugular venepuncture into a heparinised vaccutainer tube and the plasma harvested and stored at -20C until assayed for progesterone content using a commercial radioimmunoassay kit (Beckman Coulter, Brea, CA, USA). Assay sensitivity and intra- and inter-assay coefficients of variation were 1 ng/mL, <10% and <15%, respectively.

Ovarian ultrasound

Transrectal real time ultra-sound was used to examine ovarian follicle size and number. The ovaries of all sows were scanned at 0, 24, 48, 72 and 96 h after farrowing to monitor follicle development and determine ovulation. Sows were deemed to have ovulated when pre-ovulatory follicles observed on the previous scan had disappeared. Sows treated within 24 h after farrowing were expected to ovulate between 72 and 96 h after injection, and sows treated within 48 h after farrowing were expected to ovulate between 96 and 120 h after injection. Hence, sows in the 48 h treatment group were also scanned 120 h after farrowing. All sows were scanned at day 10 of lactation coincident with blood sampling to determine presence of corpora lutea (CL) and their function as indicated by serum progesterone concentrations.
Sows were scanned between 7:00 and 11:00 in the morning. For each scan one ovary was located and scanned from end to end. A video clip of the ultrasound was saved and analysed for size and number of follicles and presence of CLs.

Statistics

The data were analysed using SAS (SAS Inst. Inc., Cary, NC, USA). Evidence of ovulation was determined by the sow's individual day 10 ultrasound. Sows were categorised into two groups, ovulated (CL's present on day 10) and non-ovulated (no CL's present on day 10) and the difference between treatments was tested with a Chi-square. Follicle growth was expressed as mean follicle size or maximum follicle size. The mean was defined as the mean diameter of all follicles >1.5 mm diameter that were measured on one ovary. This cut-off was the smallest measurable size. Maximum follicle size was the diameter of the largest follicle at each time point. PROC GLM was used to compare treatments and those that ovulated vs non-ovulated in their mean and max follicle size using the following model: $y = \mu + A + day + A*day + e$, with A either treatment or ovulation status. To determine follicular dynamics, follicles were assigned into two classes; $\leq$5mm (small) and >5mm (large) and number of follicles in each class counted for every scan. Treatment effects and ovulatory status (ovulated vs non-ovulated) on follicle count (small and large follicles) were determined using a general linear model similar to mean and max follicle size. Differences between treatments were considered significant when $p < 0.05$.

3. Outcomes

Litter statistics

There were no differences between treatments for mean parity, lactation length, litter size suckled or subsequent weaning-to-oestrus intervals (Table. 1).

	Control	24h	48h
N	14	16	18
Parity	3.1 ± 0.4	2.4 ± 0.3	2.1 ± 0.2
Litter Suckled	10.7 ± 0.21	10.8 ± 0.3	10.9 ± 0.19
Lactation Length, d	28.8 ± 0.4	28.1 ± 0.2	27.8 ± 0.2
Wean-oestrus interval, d	4.1 ± 0.4	3.7 ± 0.3	4 ± 0.6

Table 1 - Reproductive characteristics (mean ± SEM) of sows receiving hCG at 24 h or 48 h after farrowing or serving as non-injected controls.

Post-partum ovulation

The presence of corpora lutea (CL) at the day 10 scan across the three treatments is illustrated in Figure 1. None of the control sows ovulated, while in the treated groups, 5 of the 16 sows treated at 24 h (33%) and 4 of the 18 sows treated at 48 h (22%) did have corpora lutea at day 10 indicating ovulation had occurred. In none of these sows was ovulation detected within 120 h post farrowing, indicating that any ovulation occurred later and was not induced by the hCG injection.

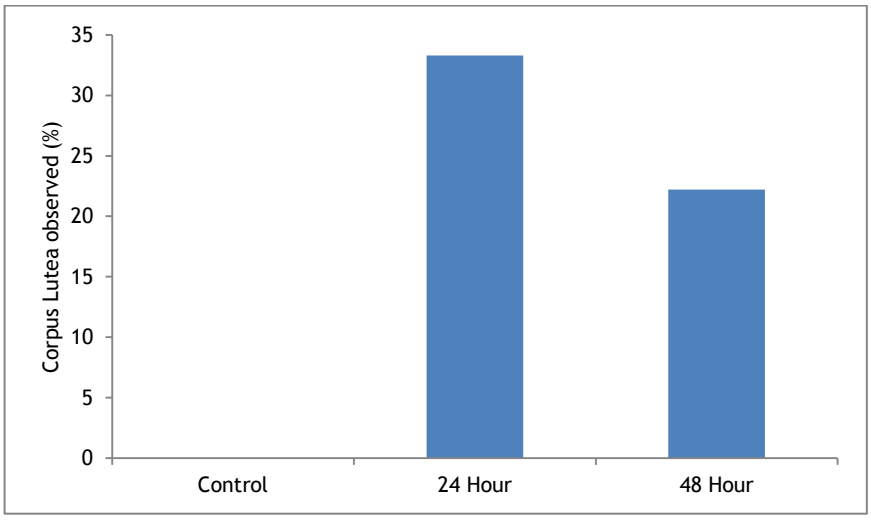

Figure 1 - Percentage of sows from each treatment group having corpora lutea at day 10 of lactation

Weaning-to-oestrus interval

The average weaning-to-oestrus (WOI) for all sows was 3.5 ± 0.3 d with 81.5% of the sows expressing oestrus within 7 d post weaning. The majority of these sows had a WOI of 4 to 5 d. As seen in Figure 2, there was little variation in the timing of oestrus post-weaning between the three treatment groups. In addition to those sows showing oestrus post weaning, 2 sows in the 48 h group that did not ovulate in response to hCG experienced oestrus 2 days prior to weaning. Sows that did not show oestrus by 7 days after weaning were declared anoestrus. Sows that remained anoestrus were 14%, 19%, and 6% for Control, 24 h and 48 h, respectively.

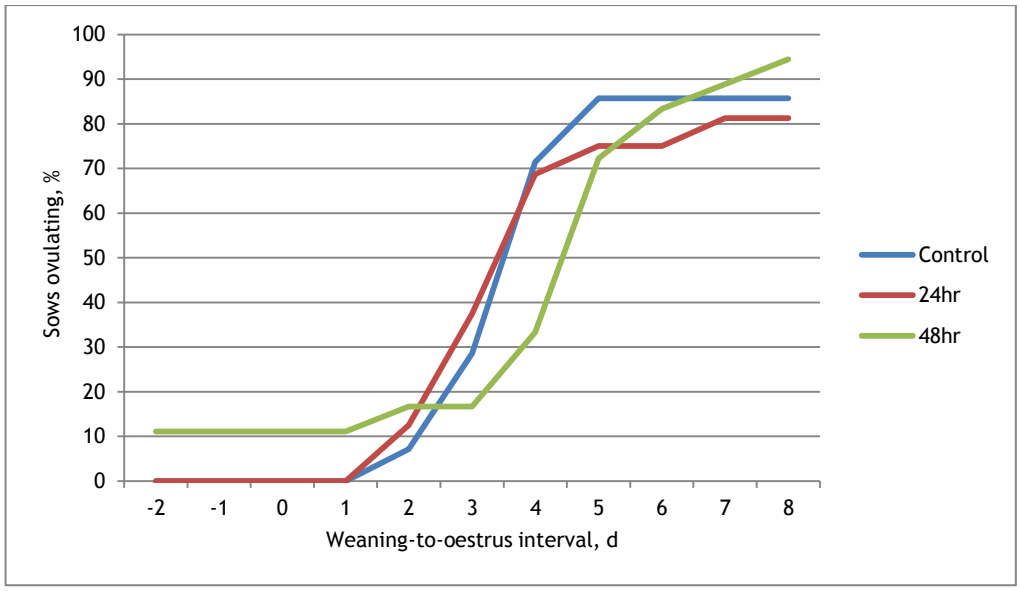

Figure 2 - Cumulative percentage of sows ovulating relative to the day of weaning

Progesterone concentrations

Of the 8 sows that had concentrations of progesterone >2.0 ng/ml, seven had CLs observed on day 10 (one sow had no day 10 data recorded). However, 3 sows had CLs on day 10 but progesterone concentrations less than 1 ng/ml. The progesterone concentrations ranged from 0.1 to 30.1 ng/mL. The average concentrations in sows having significant progesterone were 4.5 ± 3.8 ng/mL for the 24 h group (n=5) and 8.6 ± 12.5 ng/mL for the 48 h group (n=3).

Post-partum follicular dynamics

At the first ovarian scan immediately following farrowing, 73% of the sows had one or more follicles ≥4 mm in diameter, while 96.7% had one or more follicles ≥3 mm in diameter. The diameter of the largest follicles was between 4.9 and 9 mm (mean 7.0 ± 0.2 mm).

Sows in the control group exhibited follicle growth of 0.68 mm during the 24 h after farrowing, but then follicle size decreased by 0.4mm/d between 24 and 96 h post farrowing. In contrast, sows in the 24 h treatment group experienced follicle growth of 0.5 mm/d from 48 to 96 h, while sows at treated at 48 h exhibited follicle growth of 0.6 mm from 72 h to 96 h (Fig 3).

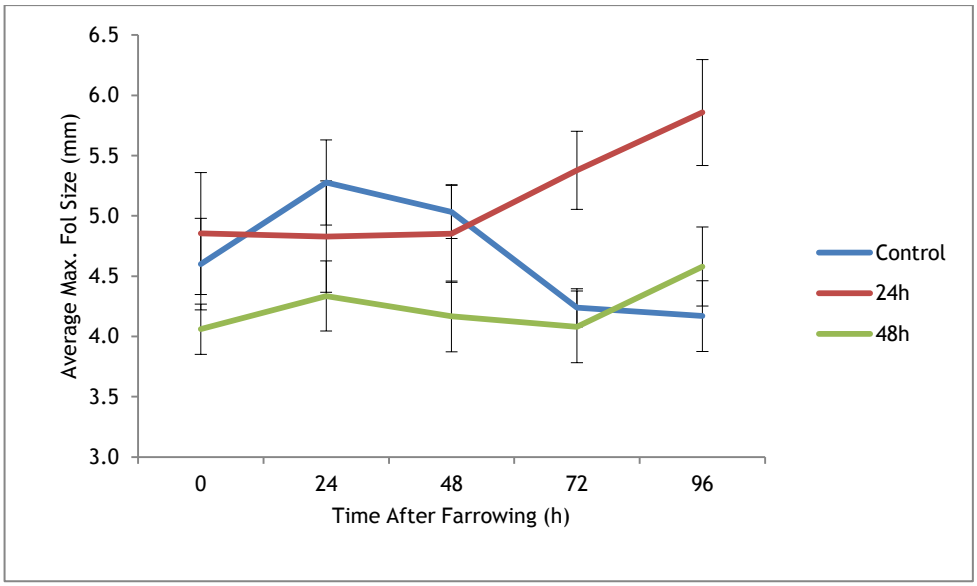

Figure 3- Average maximum follicle size (mm) in the 96 hours post farrowing for each treatment.

There was no significant effect of treatment on average numbers of small follicles detected to 120 h post-partum (Fig. 4). The average number of large follicles at 72 or more hours after farrowing was greater ($P<0.02$) in the sows treated at 24 h compared to the other treatments (Fig. 4). Follicle growth continued in the sows treated at 48 h resulting in follicle sizes eventually very similar to those in sows treated at 24 h. However, a statistical comparison was not made.

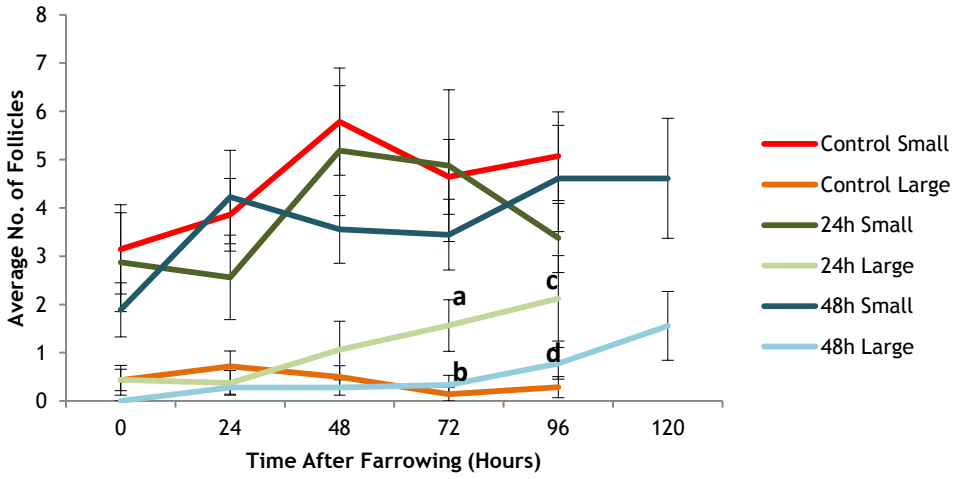

Figure 4 - Average number of small and large follicles per treatment

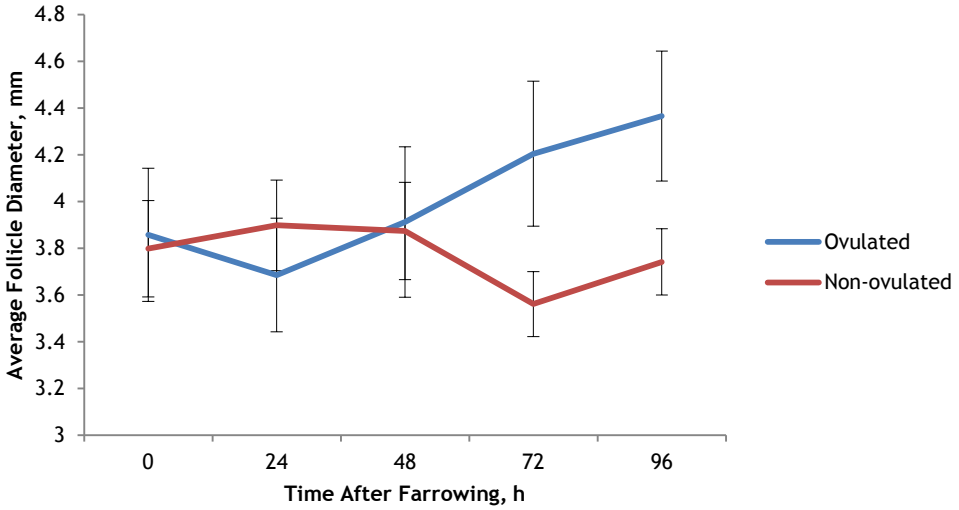

Figure 5. Average follicle diameter following farrowing for sow having different ovulation status

Data in Figure 5 describes the average follicle diameter in the 96 h following farrowing for sows that did or did not ovulate based on detection of corpora lutea on day 10. Follicle diameter in those sows that ovulated increased from 24 h onwards, while the sows that did not ovulate showed no consistent changes in diameter.

Corpora lutea regression

Immediately following farrowing the average CL diameter was 8.5 ± 0.2 mm (Figure 7). There was no variation between the three treatment groups. Thereafter, the pattern of CL regression was similar amongst treatments, with the average CL diameter at 96 h being 5.5 ± 0.2 across all three treatment groups.

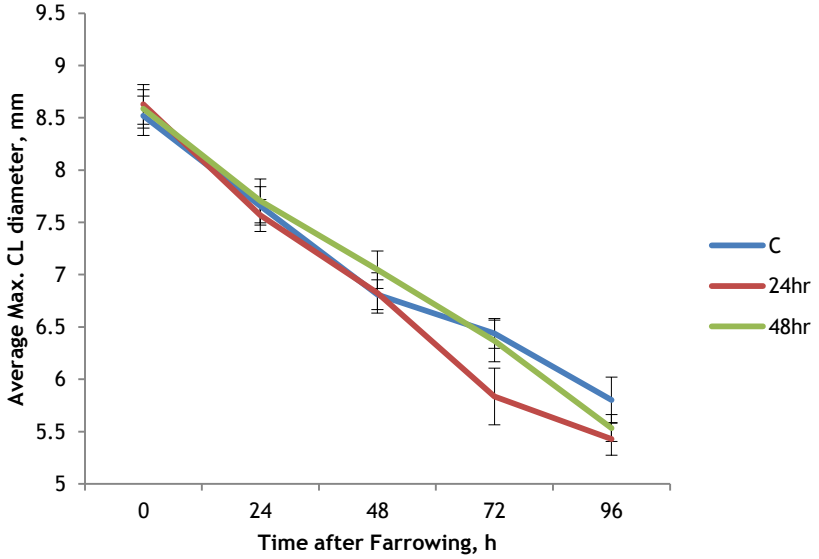

Figure 6 - Average corpora luteal diameter during 96 hours post farrowing.

4. Application of Research

Injecting sows with 1,000 IU hCG 24 or 48 h after farrowing resulted in 33% and 22% of sows, respectively, showing evidence of having ovulated by 10 d after farrowing. The current data differs from previous reports of 71% and 41% of sows ovulating in response to an injection of 1000 IU hCG within 24 h of farrowing (Armstrong *et al*. 1999; Kirkwood *et al*. 1999). Other studies have found success using exogenous gonadotropins but these are usually administered in late lactation (Hausler *et al*. 1980; Hodson *et al*. 1981; Armstrong *et al*. 1999).

When compared to previous studies, the response in the present study was less successful. The timing of the injections for both the Armstrong et al. (1999) and Kirkwood et al. (1999) studies occurred within 24 h after the completion of farrowing. In terms of Kirkwood et al. (1999) where injections were given at varying intervals within 24 hours post farrowing, it is possible that the injections administered in this study at approximately 24 h after farrowing and 48 h after farrowing were too late into lactation. Therefore, the results may have been influenced by the suckling induced inhibition.

Previous studies used solely progesterone concentrations to determine whether ovulation had taken place or not. For this study, in addition to progesterone concentrations, ultrasound was employed to determine ovulation on the basis of observed luteal structures at d10 post partum. If using just progesterone as a determinant of active CL function, one of the 4 sows from the 48 h group would not have been classified as having ovulated. Interestingly, of the sows that had CLs present, 5 had plasma progesterone concentrations between 2 – 4 ng/ml, which in many papers would be considered insignificant or only as evidence of 'partial" responses in terms of active luteal function.. Similarly there were sows with readings between 1 – 2 ng/ml that did not have CLs present. These readings are likely the result of follicle luteinisation. When follicles are exposed to hCG prematurely or in an insufficient amount, luteinisation can occur (Einspanier *et al*. 1993). Interestingly, 3 sows had progesterone concentration less than 1 ng/ml, yet CLs were evident at their day 10 ultrasound. The reason for the lack of progesterone is unknown. The follicular dynamics in the control sows were similar to what has been found in previous studies. De Rensis *et al*. (1993) found that follicles were no bigger than 5 mm in early lactation. Similarly, other studies have found that follicles did not exceed 3-4 mm in diameter (Kunavongkrit *et al*. 1982; Lucy *et al*. 2001; Soede *et al*. 2011). Palmer *et al*. (1965) noted an average follicle diameter of 4.6 mm after farrowing which declined to 2.6 mm over a week. This is consistent with the results seen in our control sows which showed a decrease in size over the five days following farrowing.

The decrease in follicle size seen in the control group is consistent with previous reports of the suckling induced inhibition being established with 24 – 48 h after farrowing (Sesti and Britt 1993). From 24 h after farrowing the control group's average follicle size for the largest follicles gradually decreased. Follicles of approximately 3 – 4 mm in size respond primarily to LH or hCG. Of the sows that had evidence of ovulation, all had follicles approximately 4 mm in diameter at the time of injection.

Overall, the two treatments, when compared to the control sows, had an effect on follicle growth. Both treatments showed follicle growth 24 h after the injection was administered, while the control group showed a decrease in overall follicle size following farrowing. Of the 34 sows that received an injection, 23 were recorded as having an increase in follicle size and/or number. The 24 h group had

a higher proportion of sows that ovulated, however, the difference between the two injection times was not enough to choose one as optimal.

After the injection was administered, follicle number and size increased. However, there were other sows that had similar follicle size and number at the time of injection but were unable to achieve ovulation. The factor that determined why some ovulated but others didn't is currently unknown.

After farrowing all sows that received an ultrasound (39) had CL's over 7 mm in diameter. The days following showed a pattern of regression that was similar across all treatments and remained unaffected by the injections given.

It was expected that after an initial ovulation in early lactation, a regular oestrous cycle would follow and a second oestrus would presumably be seen in late lactation. However, of the 9 sows that had CL's present at d 10, none showed a standing heat response when fenceline boar exposure was given.

WOI in the treatment sows that responded to boar exposure did not differ from the control sows. The lack of late responders to boar exposure indicates that there was no abnormal cyclic activity among those sows.

Of the two sows that stood at day 25, neither had an elevated progesterone concentration at day 10 nor any significant follicle growth in the 5 days after farrowing. This indicates that these sows exhibited a behavioural oestrus in late lactation through other means, since the hypothalamic-pituitary-ovarian axis usually begins to recover from approximately day 14 of lactation (Sesti and Britt 1993; Langendijk et al. 2009; Soede et al. 2011).

While many studies in this area are currently focusing on intermittent suckling and the effects of such a regime of reproductive parameters, commercial viability will always be an influence on its success. Studies similar to this one, using exogenous gonadotrophins to induce lactation ovulation are producing results that are inconsistent. Further study is required into the factors that limit induced ovulation during lactation such as the effect that the suckling induced inhibition has on exogenous gonadotrophins.

5. Conclusion

In summary, the timing of the hCG injection at 24 or 48 hours post farrowing is not a limiting factor for inducing lactational oestrus. However, the size of the follicles at the time of injection and the ability to delay the effects of suckling induced inhibition after an induced ovulation may prevent a consistent result from this method. Our hypothesis was disproven, as neither timing option of the injection yielded a greater degree of success over the other. However, both treatments had an effect of follicle growth and number.

6. Limitations/Risks

The data from this study indicated that there is a serious limitation to our ability to induce a post-partum ovulation with hCG administration. The results of this study showed that only 26% sows that were injected with hCG within 48 hours after farrowing responded by ovulating within about 5 days of farrowing. However, none of these sows appeared to exhibit normal oestrus cyclicity after this induced ovulation. More intense boar stimulation around the time of the expected second oestrus and ovulation may have improved the oestrus response, but other strategies offer greater promise to induce a fertile oestrus during late lactation.

7. Recommendations

Studies similar to the one conducted in this Project, using exogenous gonadotrophins to induce lactation ovulation, have also produced inconsistent results. Together with data from this Project, these results indicate that post-partum administration of hCG is not an effective means to stimulate an ovulation and subsequently a consistent oestrus cyclicity during the later lactation period. Thus it is unlikely that hCG treatment in early lactation can be used as a strategy to induce a fertile oestrus in lactating sows.

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