Effects of exogenous gonadotrophins at farrowing on incidence of ovulation

by

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Declaration

I declare that this thesis is a record of original work and contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

Signature of Miss Jessica Zemitis

Miss Jessica Zemitis

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Literature Analysis

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).

During lactation, follicle growth is usually limited to the medium size (4-5mm), 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. This requires that piglets are weaned at 3 to 4 weeks of age because sows are not usually able to be bred until after weaning. 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 feeds and an improved immunocompetence (Vanbeersschreurs *et al.* 1992).

To uncouple weaning from oestrus and ovulation allows for ovulation and breeding to occur during lactation. Ovulation induced during lactation allows mating to take place while the piglets are still suckling. Concurrent pregnancy and lactation allows the piglets to suckle to an age which is more beneficial for the piglets without disrupting sow production efficiency.

There are currently few practices for inducing ovulation during lactation. Practices such as intermittent suckling and split weaning as described below, 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. The use of exogenous hormones is one way of reducing these labour costs.

This literature review will discuss the oestrous cycle of sows, particularly during and after lactation. Focus will be upon the potential of being able to induce ovulation soon after parturition, taking into account sow endocrinology, post-partum physiology and considering previous methods that have been successful in achieving lactation ovulation.

2. The Oestrous Cycle

The oestrous cycle in pigs is usually 18 to 24 days in length (Soede *et al*. 2011). In terms of endocrinology the oestrous cycle is controlled by the hypothalamic-pituitary-ovarian axis. During the follicular phase of the oestrous cycle (4-6 days preceding oestrus), the growing follicles release oestradiol. As these follicles approach maturity, the level of oestradiol in the circulation increases until it reaches a threshold concentration. This high oestradiol concentration has a positive feedback on the surge center of the hypothalamus, which releases a large quantity of Gonadotropin Releasing Hormone (GnRH). The GnRH stimulates the anterior pituitary gland to release a surge of Luteinising Hormone (LH). The LH is important in stimulating a number of intra-follicular biochemical changes that eventually lead to ovulation (Senger 2003a).

Following ovulation, the corpora lutea develop from the ovulated follicles and begin production of progesterone. Increased progesterone levels exert a negative feedback on the hypothalamus, reducing GnRH secretion. The low levels of GnRH still stimulate low frequency LH pulses, allowing follicles to develop to the 4mm (medium size) stage but not to a preovulatory stage (Senger 2003b). At about day 14, endometrial production of prostaglandin F2a initiates luteolysis with cessation of progesterone production, allowing and increased LH pulsatility and resumption of follicular growth to the ovulatory stage.

3. Follicle Development

There are four processes involved in follicle development: recruitment, selection, dominance and atresia. The recruitment process is the growth of small follicles (<3mm) in response high follicle stimulating hormone (FSH) levels. These follicles release some oestradiol and low levels of inhibin. As the follicles continue to grow the levels of inhibin being produced increase, sending a negative feedback to the anterior pituitary. The feedback decreases the amount of FSH produced and the lack of FSH prevents the recruitment of new follicles into the growing pool. At this point the follicles are approximately 4-6mm and have entered the selection process. When the follicles grow to a size of >6mm the dominance stage is reached. Follicle growth and development from 4mm to ovulation is controlled primarily by LH and as the follicles grow the increasing levels of oestradiol eventually trigger the preovulatory surge of LH (Senger 2003a).

4. Lactational Anoestrus

During Lactation sows remain anoestrus (Soede *et al.* 2012). This is due to the suckling of the piglets. The suckling stimulates the release of endogenous opiates which suppress the release of GnRH (Armstrong *et al.* 1988a; Langendijk *et al.* 2007). As the amount of GnRH released decreases so do circulating concentrations of LH. Sesti (1993) found that LH secretion was lower on day 7 of lactation than on day 1. However, the pituitary gland remained sensitive to GnRH and sensitivity increased as lactation continued (Barb *et al.* 1986). The concentration of LH present is also thought to be affected by the negative energy balance of the sow after farrowing (Soede *et al.* 2011). Within 24 hours of farrowing there are follicles present on the ovary that are gonadotropin sensitive, and suckling induced inhibition is not yet functional. Concurrently, there is active secretion of LH(De Rensis *et al.* 1993)., although an LH surge cannot be produced. These factors indicate that, at farrowing, GnRH production is limiting but that it may be possible to induce ovulation early in lactation using exogenous gonadotropins.

As lactation progresses the amount of GnRH and gonadotrophins (LH and FSH) available in the hypothalamus and pituitary, respectively, increase (Sesti and Britt 1993; Langendijk *et al.* 2007). At the same time, the positive feedback mechanism involving oestradiol is restored. These events indicate that the hypothalamic-pituitary-ovarian axis is relatively functional, and presents a second opportunity to induce ovulation in late lactation (Langendijk *et al.* 2009). The restoration of the hypothalamic-pituitary axis takes approximately 10-12 days (Armstrong *et al.* 1999).

In the early stages of lactation low levels of LH prevent the follicles present in the ovary from developing beyond about 4 mm and reaching preovulatory size (Kluivers-Poodt *et al.* 2010). Boar exposure in early lactation can stimulate sows to exhibit a behavioural oestrus as shown by van Wettere *et al.* (2013). This behavioural oestrus shortly after farrowing could be indicative of oestrogenic follicles present on the ovary. The With sufficient levels of FSH

still present, the follicles do continue to develop to medium size (<5mm)(De Rensis *et al.* 1993). At day of weaning the follicles present in the ovary are recruited and go on to become preovulatory follicles. Due to inadequate lactation nutrient intakes, particularly of primiparous sows, it is thought that the follicles may be of lower quality due to the energy demands placed on the sow during lactation. This, in turn, could negatively impact oocyte quality. To overcome this it may be beneficial to mate at the second ovulation after weaning, when follicles have developed in a less nutrient-stressed environment. The downside of this strategy, however, is the increase in non-productive days (NPD's) and associated decreased in production efficiency (Kemp and Soede 2012).

5. Post-Partum Physiological Changes

After farrowing, the ovary only requires a day to resume normal activity (Armstrong *et al.* 1999). Follicles at this stage of growth are still sensitive to LH. Shortly after farrowing the reproductive tract starts to undergo uterine involution and returns to normal reproductive function (in sows, however, ovarian activity is inhibited during lactation). Involution of the uterus involves myometrium contractions that expels fluid and debris and reduces the overall size of the uterus in preparation for the next pregnancy, as well as tissue repair. The time taken for complete uterine involution in sows is approximately 21 days (Armstrong *et al.* 1999). This limits the ability of sows to maintain a normal pregnancy in early lactation.

6. Lactation Oestrus

In modern management systems, piglets are usually weaned before they are 4 weeks old. This time period is not considered sufficient for an optimal change over from easily digestible sow milk to the solid weaner feed (Vanbeersschreurs et al. 1992; Kuller et al. 2004). This may result in reduced growth and diarrhoea, both undesirable from welfare and production standpoints. Increasing lactation length also has an effect on the number of piglets born alive in the next litter as shown in Figure 1 (Mabry et al. 1995) . Increasing the lactation length to a point when it is beneficial for the piglets can be achieved by mating during lactation, allowing the piglets to continue suckling. Mating during lactation requires an induced ovulation.

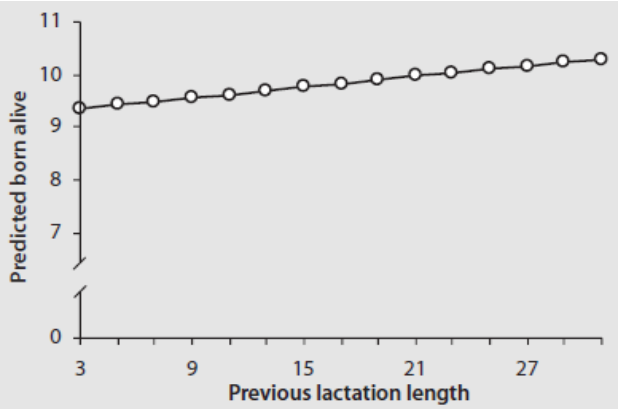

Figure 1. Effect of lactation length on subsequent litter born

As previously stated, there appear to be two time points during lactation at which ovulation can be induced. Immediately post farrowing, the hypothalamic-pituitary-ovarian axis is functional and the ovary is potentially sensitive to hormonal stimulation. The hypothalamus is yet to be inhibited by endogenous opioids allowing stimulation of LH secretion to continue. Additionally, the pituitary is still sensitive to gonadotropins (Barb *et al.* 1986). Thereafter, the inhibitory effects of the suckling stimuli on hypothalamic-pituitary function lessen after about 14 days.

7. Current Methods

7.1 Intermittent Suckling

Intermittent suckling involves the separation of the sow and litter for a designated number of hours a day for several days. This uses the reduction in suckling intensity to reduce the inhibition on GnRH. By allowing the unrestricted secretion of GnRH, the hypothalamic-pituitary axis is more likely to be able to mount a preovulatory LH surge. This permits ovulation and mating to take place during lactation, and suckling to continue throughout these events. By achieving this, lactation length can be increased and the weaning-to-oestrus interval removed, with no impact on sow productivity. An added benefit of intermittent suckling is the gradual weaning process that takes place in the sow's absence, reducing the harsh consequences of sudden weaning.

The downside of intermittent suckling is the labour required. As the sows must be removed and returned to their litters each day, as well as the boar contact that is usually also used, labour can become costly. The stress caused by the repeated separation may also have negative effects. However, Kluivers-Poodt (2010) found that cortisol levels in intermittently suckled sows exhibited no significant difference from those of acutely weaned sows.

Intermittent suckling has proven successful in inducing lactation ovulation. Studies have demonstrated that separating sows and litters for 12 hours per day yields a high incidence of ovulation (Langendijk *et al.* 2007; Kluivers-Poodt *et al.* 2010). However, there is some debate over when during lactation is the optimal time to begin intermittent suckling, the duration of intermittent suckling, and the use of boar contact. Langendijk (2007) had 90-100% of sows ovulating during lactation with 12 hours of separation starting at day 14 of lactation. However, in another study (Langendijk 2009) only 28% of sows ovulated and boar contact made no significant difference. It was observed, though, that those sows that did ovulate did so synchronously. Other studies have shown varying results with treatments of varying

lengths and start times. One study showed 50-64% lactational oestrus, with the regime starting in early lactation (day 19) and running for 2 weeks (Soede *et al.* 2012).

Under current weaning management practices, 80-90% of sows are expected to return within 7 days from weaning (Rossi *et al.* 2009). However, lactational oestrus induced by intermittent suckling shows much variation between results (Table 1). Unless intermittent suckling can consistently achieve and maintain a comparable rate of returns, its commercial viability is questionable.

Table 1. Oestrus response to intermittent suckling regimes (Taken forn Kemp and Soede, 2012)

Start (Day of lactation)	Duration of daily separation (h)	Lactation Length (Days)	Parity	Sows in oestrus during lactation %	Timing of lactational oestrus (Days after start)	Reference
4	12	28	Multiparous	22	ND	Kuller *et al.*2004
13-18	12	20-25	Multiparous	83	<7	Langendijk *et al.* 2007
14	12	~42	Multiparous	100	4.2 ± 1	Gerritsen *et al.* 2008
	2 x 6			92	4.9 ± 0.7	
14	12	~42	Multiparous	70	4.7 ± 0.3	Gerritsen *et al.* 2009
21				83	4.4 ± 0.1	
14	12	>21	Multiparous	25	5.3 ± 0.6	Langendijk *et al.* 2009
	12 + boar			19	5.8 ± 0.9	
19	10	26	Mixed	50	5.0 ± 0.1	Soede *et al.*2012
		35		64		
26		35		61		

7.2 Split Weaning

Split weaning involves weaning a varying number of piglets in the litter some days before the remaining piglets are fully weaned. This method works in much the same way as intermittent suckling, using a modification of the suckling load to restore GnRH secretion. Usually employed towards the end of lactation it is commonly used to stimulate a rapid return to oestrus after weaning (Zak *et al.* 2008) but can be used to induce lactation ovulation. Again, this can allow longer lactation length without affecting sow productivity. Split weaning has been shown an affects follicle, with follicle growth greater than that of the control sows (van Leeuwen *et al.* 2012). A study by Terry *et al.* (Unpublished) showed that any number of piglets weaned at day 18 of lactation elicits a greater exhibition of lactation oestrus than having no piglets removed. By weaning 3, 5 or 7 piglets, 90 % of the sows exhibited lactation oestrus. In contrast, control sows who had a standardised litter of 10, only 56% exhibited lactation oestrus. Piglets weaned at day 18 showed no significant difference in bodyweight gain between days 17 to day 40 post-partum to those that remained with the sow until day 30 of lactation. Piglet bodyweight gain, was unaffected by the weaning age in split weaning systems (Terry *et al.* Unpublished).

Although not seen consistently, the process of split weaning may result in poorer growth and compromised immunity in those piglets that are removed early. This method is also costly in terms of the labour that is required to remove the piglets and the boar contact if used.

7.3 Boar Contact

Boar contact is used to stimulate LH production in sows. Pheromones are released by the boar in the presence of the sow and these pheromones act on the olfactory system in the sow to stimulate the central nervous system. This causes the release of hormones and neuropeptides that are involved in the control of LH secretion. Boar contact increases the frequency of the low amplitude LH pulses that are required for ovulation to take place after weaning. Simultaneously, stimulation of LH promotes follicle growth (Kemp *et al.* 2005).

Boar exposure is commonly used in tandem with intermittent suckling, split weaning and other methods inducing ovulation. Boar contact is also useful in detecting oestrus, should it occur during lactation. It has also been successful in inducing ovulation during lactation either in a detection mating area or through fenceline contact (Van Wettere, 2013). However, Langendijk (2009) saw no difference in incidence of lactation ovulation between sows undergoing intermittent suckling with and without boar contact. Again, providing this contact daily for several days is costly in terms of labour.

7.4 Exogenous Hormones

Manipulation of the hypothalamic-pituitary-ovarian axis to induce ovulation can be achieved with the use of exogenous gonadotropins. These can be used in place of endogenous ones or to start biochemical events. By using these methods the labour required can be greatly reduced, lowering overall costs.

Human chorionic gonadotropin (hCG) ia an LH analogue that acts directly on the ovary, effectively bypassing the hypothalamic-pituitary axis. A lactational incidence of ovulation of 41% was noted by Kirkwood *et al.* (1999) after a 1000 IU hCG injection within 24 hours of farrowing. In other studies, response rates of 75 to 90% have been observed (Armstrong *et al.* 1999). In later lactation, Rossi *et al.* (2009) injected varying concentrations of equine chorionic gonadotropin (eCG) and hCG, with insemination taking place at regular intervals after. Even though oestrus or ovulation rates were not recorded, an average pregnancy rate of 77% was observed.

When injected in late lactation is effective in inducting, eCG ovulation and shorting the weaning-to-oestrus interval. When used earlier in lactation the treatment failed to be effective. However, a number of studies coupled an injection of eCG with an injection of hCG in mid to late lactation with positive results (Hausler *et al.* 1980). Hodson *et al.* (1981) found that a mixture of 1000IU PMSG/1,500IU hCG given as early as day 14 of lactation yielded a 76%

pregnancy rate. Other studies have shown oestrus response in 85-99.5% of sows treated with hCG and eCG close to or at weaning (Polanco *et al.* 1980; Kirkwood and Thacker 1998).

Administration of a combination of 400 IU eCG and 200 IU hCG (PG600) was shown to increase the rate of return to oestrus in sows when injected close to time of weaning (Estienne and Hartsock 1998; De Rensis *et al.* 2003). When used early in lactation, however, it resulted in only 6% of treated sows ovulating(Van Wettere, 2013).

The use of exogenous gonadotropins for inducing ovulation has potential, as shown in Table 2. However, the degree of success of the treatments relies on many factors such as the timing of administration, amount of hormone administered and the use of boar contact. Studies have also shown that the use of exogenous gonadotropins can have adverse effects on the quality of oocyte produced by the follicles (Hunter 2000).

Table 2. Effects of gonadotrophins on ovulation and pregnancy rates

Injection	**Time of injection (day of lactation)**	**Response**	**Reference**
1000IU hCG	Day 0	41% ovulation rate	Kirkwood *et al.*(1999)
1000IU hCG	Day 0	75% ovulation rate	Armstrong *et al.* (1999)
1500/1000IU eCG 1000/500IU hCG	Day 15/Day 20	77% ave. pregnancy rate	Rossi *et al.* (2009)
1000IU PMSG 1500IU hCG	Day 14	76% pregnancy rate	Hodson *et al.* (1981)
1000IU PMSG	Day 28	85% oestrus detected	Kirkwood (1998)
PG600	Day 0	6% ovulation rate	Van Wettere (2013)

8. Conclusion

With the public's interest in pig welfare, the need for practices that ensure better health and welfare are more important than ever. Shorter lactation lengths may compromise health and welfare of newly weaned piglets, but lactation oestrus is a plausible alternative. Current practices being used are effective but ultimately costly in terms of time and labour. Use of exogenous gonadotropins resolves this labour issue but a regime that results in a predictable and synchronous oestrus remains to be described. The current study hopes to use GnRH or hCG shortly after farrowing to stimulate the follicles present, resulting in an ovulation 2 to3 days later. The aim is to determine if an oestrus cycle of normal length (18-24 days) ensues, resulting in a second ovulation at which a mating can take place. This will result in mating taking place during lactation, allowing the piglets to continue to suckle until it is more beneficial for them to be weaned.

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Final Paper

1. Abstract

Shorter lactation lengths are causing concerns for piglet health and welfare. For longer lactation lengths to be achieved, a commercially viable way to uncouple the event of weaning and oestrus is required. The use of exogenous gonadotrophins as a easily applied management of lactational oestrus has yielded inconsistent results. The aim of the present study was determine if the timing of post-partum injection of human chorionic gonadotrophin (hCG) had an effect on the incidence of ovulation. As well as documenting follicular and corpora lutea changes during the immediate post-partum period. Sows were injected with 1000IU hCG at approximately 24 h (n = 16) or 48 h (n = 18) post farrowing. All sows were subjected to transrectal ultrasound examination of their ovaries at 0, 24, 48, 72 and 96 h post farrowing. Sows in the 48 h group also received a scan at 120 h. Blood samples were taken on day 10 for progesterone concentrations to confirm if ovulation had taken place. Ovulation experienced during early lactation was 33% and 22%, for 24 h and 48 h respectively. Of the 9 sows that were thought to have ovulated only 6 had elevated progesterone levels. Post-partum ovulation rates were not significantly affected by the timing of the hCG injection.

2. Introduction

Pork production practices are being scrutinised for welfare concerns. While the pork industry has been proactive in improving welfare for sows and piglets, it has to remain conscious of maintaining production efficiency. Since lactation anoestrus in sows usually prevents mating until after weaning, lactation lengths are relatively short in order to maintain production efficiency. Shorter lactation lengths have potentially negative effects on piglet health and recent research suggests that extending the length of lactation would be beneficial for the piglet (Kuller *et al.* 2004; Berkeveld *et al.* 2007; Berkeveld *et al.* 2009). Lactation anoestrus is a result of piglets suckling, which inhibits release of gonadotrophin releasing hormone (GnRH) from the hypothalamus and thus the release of the gonadotrophin luteinizing hormone (LH). Management practices which reliably stimulate lactation oestrus would allow mating to take place during lactation, and piglets to be weaned at an older age which would benefit their health and post-weaning performance.

Methods to induce lactation oestrus include intermittent suckling, which involves periods of daily separation of the sow and litter (Soede *et al.* 2012) and split weaning, which involves the full weaning of the heavier piglets a few days prior to their litter mates (Zak *et al.* 2008). Both of these modified weaning practices reduce suckling intensity and so promote increased LH release. However, these practices are labour intensive. 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, thereafter suckling induced inhibition 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 anovular (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 at least 5 ng/mL 7 to 10 d after injection. Although the reasons for these 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 the 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 effect on these changes of timing of postpartum hCG injection.

3. Materials and methods

3.1 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 days post farrowing. During lactation, sows were fed to appetite a ration 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 the 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 were treated at 24 h or 48 h after end of farrowing. All sows received boar exposure 3 days prior to weaning, and then daily following weaning to determine oestrus status and the weaning-oestrus intervals were recorded. 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 detected oestrus with commercially sourced semen doses having 3 x 10^9 sperm in 80mL extender (SABOR, Clare, SA).

3.2 Blood samples

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.

3.3 Ovarian Ultrasound

Transrectal real time ultra-sound was used to examine ovarian follicle size and number. Sow ovaries were scanned at 0, 24, 48, 72 and 96 h after farrowing. Additionally, sows in the 48h 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). 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 follicle's and CL.

3.4 Data analysis and 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 $p < 0.05$.

4. Results

4.1 Litter statistics

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

Table 3. Reproductive characteristics (mean ± SEM) of sows receiving hCG at 24 h or 48 h after farrowing or serving as non-injected controls

	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

4.2 Post-partum ovulation

The detection of corpora lutea (CL) at the day 10 scan across the three treatments is shown 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 indicating ovulation. Both 24 h and 48 h had higher a high proportion than the control group ($P < 0.09$).

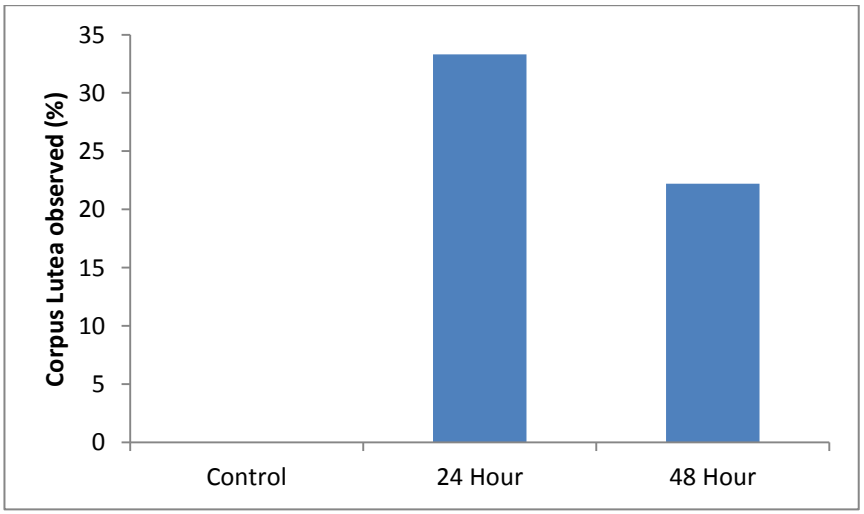

Figure 1. Percentage of sows from each treatment group that had corpus lutea (CL) present at day 10 of lactation

4.3 Weaning-to-oestrus interval

The average weaning-to-oestrus (WOI) for all sows was 3.5 ± 0.3 days with 81.5% of the sows expressing oestrus within 7 days post weaning. The majority of these sows had a WOI of 4 to 5 days. As seen in Figure 2, there was little variation in the timing of oestrus post-weaning between the three treatment groups. Two sows in the 48 h group experienced oestrus two 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.

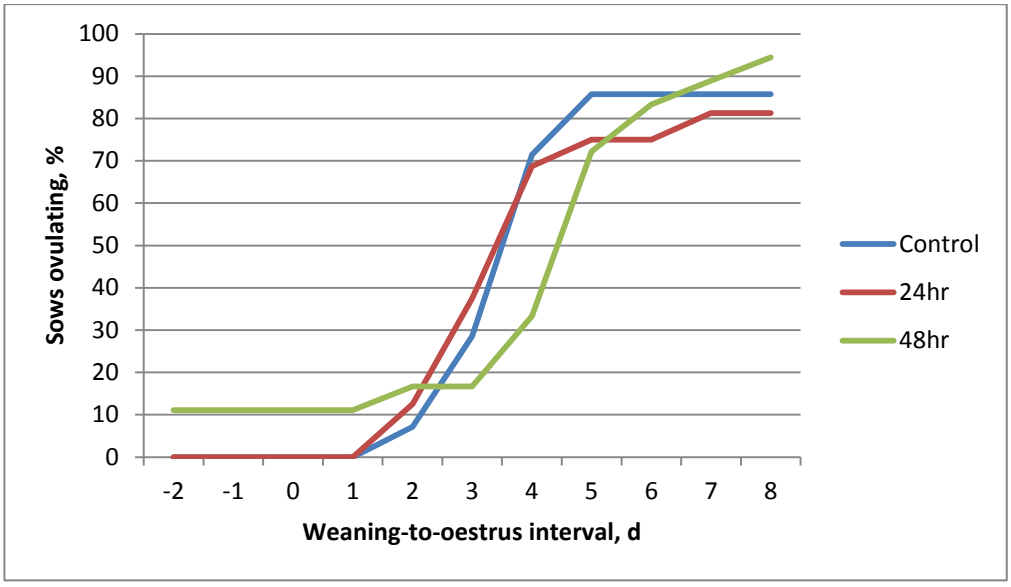

Figure 2. Cumulative percentage of sows ovulating relative to the day of weaning

4.4 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 0.11 to 30.1 ng/ml. Even though there were more sows from the 24 h group with significant progesterone concentrations, those in the 48 h group had a numerically higher average

concentration. The average concentrations in sows having significant progesterone were 4.5 ± 3.8 ng/ml for 24 h and 8.6 ± 12.5 ng/ml for 48 h.

4.5 Post-partum follicular dynamics

At the first ovarian scan immediately following farrowing, 73% of the 30 sows that were scanned 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 (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 48 h exhibited follicle growth of 0.6 mm from 72 h to 120 h (Fig 3).

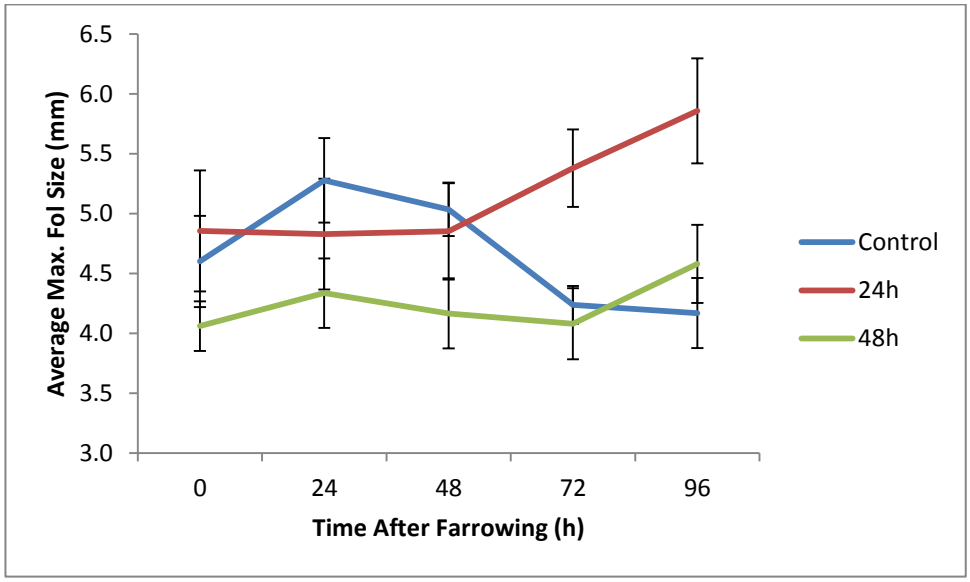

Figure 3. Average maximum follicle size (mm) in the 96 hours post farrowing for each treatment.

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 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.

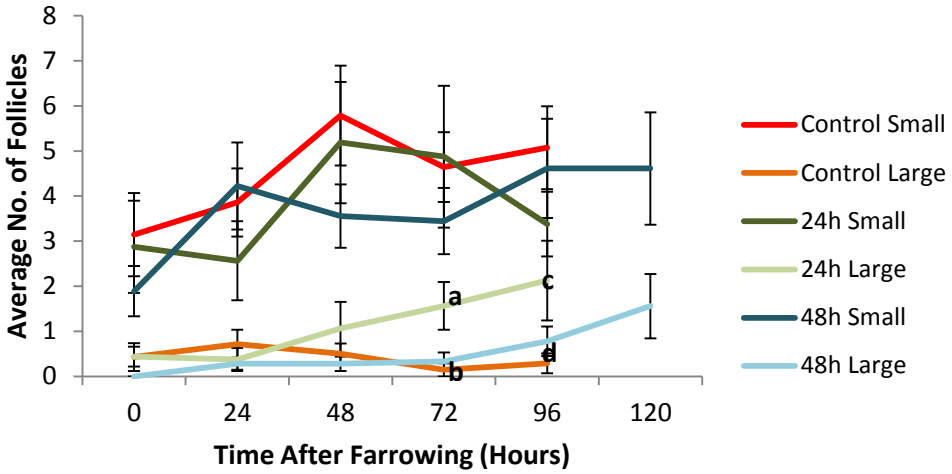

Figure 4. Average number of small and large follicles per treatment

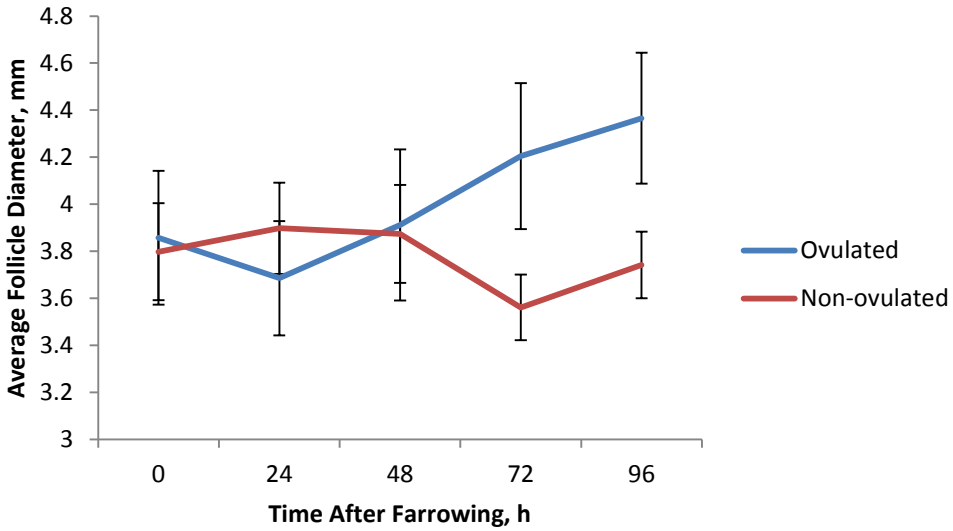

Figure 5. Average follicle diameter following farrowing for sow having different ovulation status's

Figure 5. describes the average follicle diameter in the 96 h following farrowing for ovulated and non-ovulated sows. As expected, follicle diameter in those sows who ovulated increases from 24 h onwards, while the sows that did not ovulate showed no consistent changes in diameter.

4.6 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 treatment with the average CL diameter at 96 h being 5.5 ± 0.2 across all three treatment groups.

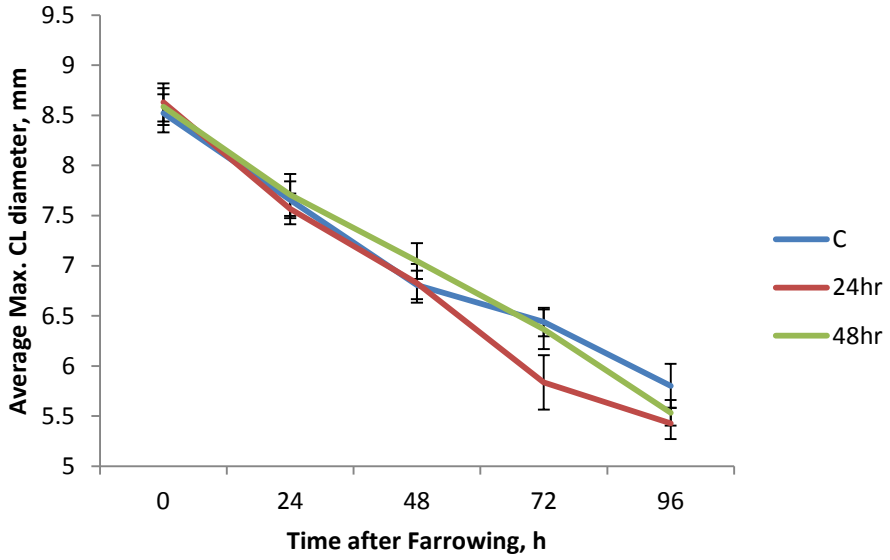

Figure 6. Average corpora luteal diameter during 96 hours post farrowing.

5. *Discussion*

Injecting sows with hCG 24 or 48 h after farrowing resulted in 33% and 22% of sows, respectively, showing evidence of ovulating by day ten of lactation. The current data differed from previous reports of 71% and 41% of sows ovulating in response to an injection of 1000IU of hCG within 24 h of farrowing (Armstrong *et al.* 1999; Kirkwood *et al.* 1999). These varying results have also been seen with eCG (Cole and Hughes 1946) with only one sow injected with the first 5 d of lactation ovulating. 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 is less successful. Similar methods were used to those with previous reasonable success. For example, 1000 IU of hCG was used for most studies. 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 (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.

These previous studies solely used progesterone concentrations to determine whether ovulation had taken place or not. For this study as well as the progesterone concentrations, the ultrasound data, specifically the CL data, was used to determine ovulation. When using just progesterone as a determinant, only 8 sows would have been classified as ovulated, 5 from the 24 h group and 3 from the 48 h group. Interestingly of the sows that had CLs present, 5 had progesterone readings between 2 – 4 ng/ml. These levels in many papers have been classed as insignificant readings or only as a ‘partial effect’. Similarly there sows with readings between 1 – 2 ng/ml that do not have CLs present. These readings are the results of follicle luteinisation. When follicles are exposed to hCG prematurely or in an insufficient amount,

luteinisation can occur (Einspanier *et al.* 1993). Interestingly, three sows had progesterone concentration less than 1 ng/ml, yet CLs were present at their day 10 ultrasound. One sow had CLs 5.2 mm in size at d 5, and CLs 3.9 mm in size on d 10. It is possible that these CLs are still present from farrowing.

The large variations in progesterone concentrations of those sows that ovulated suggest that the ovulation may not have been synchronous among the 10 sows as progesterone concentration after ovulation increases over time (Gerritsen et al. 2008a). Gerritsen et al. (2008b) found that the amount of feed eaten during lactation did not have an effect on the progesterone levels, and that sows experience lower progesterone during lactation.

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 5mm 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 that 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 growth 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). At 24 h after farrowing the control groups 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 an improvement in follicle from growth 24 h after the injection was administered, while the control group showed a decrease in overall follicle size

following farrowing. This comparable to previous studies were ovulation resulting from the administration of hCG has taken place approximately 40 hours later (Soede *et al.* 1998). 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 ovulating, 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 10 sows that had CL's present at d 10, none showed a standing heat response when boar exposure was given. The lack of secondary oestrus could be due to the lack of boar exposure used during the later stages of lactation. Using extended boar exposure is a crucial factor in stimulating the induced oestrous cycle to continue (Bartlett *et al.* 2009). Wettere (2013) showed that regular boar exposure during lactation can stimulate a high proportion of sows to express oestrus. In gilts, boar exposure is needed to maintain oestrous cyclicity when induced to ovulate with exogenous gonadotrophins (Bartlett *et al.* 2009).

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 were 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.

6. 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.

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