Postpartum injection of human chorionic gonadotrophin: Effects on sow ovarian follicles

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Summary
Sows received 1000 IU human chorionic gonadotrophin at 24 or 48 hours after farrowing as controls. Ovaries were examined ultrasonically at 0, 24, 48, 72, 96, and 120 hours. No sows ovulated by 120 hours, although corpora lutea at 10 days indicated a later ovulation in some sows.

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In the immediate postpartum period, active luteinizing hormone (LH) pulsatility has been observed for up to 78 hours post farrowing, after which suckling-induced inhibition of LH pulsatility takes effect. Further, postpartum sow ovaries have potentially estrogenic medium follicles (4 to 5 mm) and some sows exhibit estrous behavior. However, the postpartum estrus observed at 2 to 4 days post farrowing is anovulatory, likely due to an inability to generate a preovulatory LH surge.

If litters are weaned immediately after farrowing (zero-weaning) so that the suckling-induced inhibitions are removed, estrogenic follicles may continue development, which may trigger estrus and ovulation. However, zero-weaning is also associated with development of cystic follicles and poor subsequent reproductive performance, a further effect of the inability to mount a preovulatory LH surge. Interestingly, previous workers have provided an exogenous postpartum ovulatory signal in attempts to induce ovulation, as the ovary is still receptive to exogenous gonadotrophins. Specifically, injection of 1000 IU human chorionic gonadotrophin (hCG) within 24 hours of farrowing induced ovulation in 75% and 41% of sows, respectively. In these earlier studies, determination of ovulation was based on detection of serum progesterone concentrations of ≥ 5 ng per mL at 7 to 10 days 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 postpartum sows have not been documented.

If inducing ovulation early in lactation initiates a normal estrous cycle followed by a secondary ovulation, it could result in a novel estrus synchronization protocol. If predictable ovulation can be achieved, it would have great utility as an inexpensive method of postpartum estrus suppression. Such activity would be invaluable under conditions of forced zero-weaning, eg, as a consequence of a disease such as herd infection with porcine epidemic diarrhea virus. It could also be employed to capture the potential for mating during lactation. 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 approximately 24 or approximately 48 hours post partum.

Material and methods
This study was approved by the University of Adelaide Animal Ethics Committee.

A total of 48 mixed-parity sows (mean ± standard error [SE], 2.5 ± 0.2) were selected for convenience and were housed in farrowing crates from 110 days of gestation until weaning. After farrowing, litter sizes were standardized to 10 or 11 (mean ± SE, 10.9 ± 0.2 piglets), and piglets were weaned at 28 days post farrowing. During lactation, sows were fed to appetite with a diet formulated to provide 14.3 MJ digestible energy per kg, 12.5% crude protein, and 0.9% total lysine.

Sows were each assigned to one of three treatments by parity. Treatments were intramuscular injection of 1000 IU hCG (Chorulon; MSD Animal Health, Bendigo, Australia) either 24 to 30 hours after farrowing (hCG24; n = 16), or 48 to 54 hours after farrowing (hCG48; n = 18), or no injection and serving as controls (Control; n = 14).

Sows farrowing overnight were treated at...
9:00 AM the day after farrowing (hCG24) or 2 days after farrowing (hCG48). Sows farrowing during the day (8:00 AM to 4:00 PM) were treated at 24 hours or 48 hours after the end of farrowing.

Transrectal real-time ultrasound (MyLabOne; Esaote Pie Medical, Maastricht, The Netherlands) with an 8-MHz transducer was used to examine ovarian follicle size and number. The ovaries of all sows were scanned at 0, 24, 48, 72, and 96 hours after farrowing, with the hCG48 sows also scanned at 120 hours, to monitor follicle development and determine ovulation. Sows were deemed to have ovulated when pre-ovulatory follicles observed on the previous scan had disappeared. The hCG24 sows were expected to ovulate between 48 and 96 hours after injection, and the hCG48 sows were expected to ovulate between 72 and 120 hours after injection. All sows were also scanned at 10 days of lactation to determine presence of corpora lutea (CLs). Sows were scanned between 7:00 AM and 11:00 AM. For each scan, one ovary was located and scanned from end to end. A video clip of the ultrasound was saved and analyzed for size and number of follicles and presence of CLs.

The data were analysed using SAS (SAS Institute Inc, Cary, North Carolina). In addition to follicle disappearance, evidence of ovulation included the presence of CLs at 10 days. Sows were retrospectively categorized as ovulating or non-ovulating, and the difference between treatments was tested using chi-square. Maximum follicle size was the diameter of the largest follicles at each time point. A generalized linear model was used to compare treatments, and those that ovulated versus non-ovulated in their maximum follicle size, using the following model: \( y = \mu + A + \text{day} + A\text{day} + e \), where \( A \) is either treatment or ovulation status, \( \mu \) is estimated overall mean, and \( e \) is unexplained error. To determine follicular dynamics, follicles were assigned into two classes: < 5 mm (small) and ≥ 5 mm (large), and number of follicles in each class was counted for each scan. Differences between treatments were considered significant when \( P < .05 \).

**Results**

There were no significant differences between control, hCG24, or hCG48 for parity (parities 3.1 ± 0.4, 2.4 ± 0.3, and 2.1 ± 0.2, respectively) or litter size suckled (10.7 ± 0.2, 10.8 ± 0.3, and 10.9 ± 0.2 piglets, respectively). On the basis of presence of CLs, none of the control sows ovulated, while five of the 16 hCG24 sows and four of the 18 hCG48 sows had CLs, indicating ovulation had occurred. However, ultrasound examinations showed that ovulation was not detected by 120 hours post farrowing, indicating that ovulation occurred later and was not directly induced by the hCG injection.

At the first ovarian scan immediately following farrowing, 21% of the sows had one or more follicles ≥ 5 mm. At 24, 48, 72, and 96 hours, the percentages of sows with follicles ≥ 5 mm were 38%, 32%, 29%, and 39%, respectively. The diameters of the largest follicles were between 4.9 and 9.0 mm at the first scan post farrowing. Sows in the Control group exhibited follicle growth of 0.68 mm during the 24 hours after farrowing, but then follicle size decreased by 0.4 mm between 24 and 96 hours post farrowing (Figure 1). In contrast, hCG24 sows experienced follicle growth of 1.0 mm from 48 to 96 hours, while hCG48 sows exhibited follicle growth of 1.2 mm from 72 hours to 120 hours. At 72 and 96 hours, follicle diameter was larger \((P < .05)\) in sows destined to ovulate than in sows that did not ovulate.

**Discussion**

Injecting sows with 1000 IU hCG in the immediate postpartum period resulted in eventual ovulation in 33% and 22% of hCG24 and hCG48 sows, respectively, as indicated by presence of CLs at 10 days. No control sows had evidence of ovulation. The current data differs from previous reports of 71%8 and 41%9 of sows ovulating in response to an injection of 1000 IU hCG within 24 hours of farrowing. Compared to the results of previous studies, hCG treatment in the present study was considerably less successful. Previous studies used only progesterone concentrations at 7 to 10 days after farrowing to determine whether ovulation had taken place or not. For the current study, ultrasound was employed to determine occurrence of ovulation on the basis of follicle disappearance and observation of luteal structures at 10 days post partum. Our failure to detect ovulation in the immediate postpartum period likely means that hCG is not capable of inducing ovulation at this time and that previous reports indicating ovulation based on blood progesterone concentrations were incorrect. The LH pulsatility immediately postpartum is very active, and it is possible that soon after farrowing, the follicular LH receptors may be down-regulated. This would preclude an ovulation in response to an exogenously supplied ovulatory LH surge.

The follicular dynamics in the control sows were similar to what has been found in CLs, none of the control sows ovulated, while five of the 16 hCG24 sows and four of the 18 hCG48 sows had CLs, indicating ovulation had occurred. However, ultrasound examinations showed that ovulation was not detected by 120 hours post farrowing, indicating that ovulation occurred later and was not directly induced by the hCG injection.
previous studies; those studies found that follicles were no bigger than 5 mm in early lactation,\textsuperscript{4} while others found that follicles did not exceed 3 to 4 mm in diameter.\textsuperscript{10-12} An average follicle diameter of 4.6 mm after farrowing has also been documented, which declined to 2.6 mm over a week.\textsuperscript{13} These data are consistent with the results seen in our control sows, which showed a decrease in follicle size over the 5 days following farrowing.

In cyclic gilts and sows, follicles of approximately 3 to 4 mm in size grow primarily in response to LH activity.\textsuperscript{14} Of the sows that had evidence of ovulation in the present study, all had follicles approximately 4 mm in diameter at the time of injection. We did observe follicle growth in hCG-treated sows, in contrast to controls. Therefore, the CLs observed at 10 days may be a result of hCG-induced follicle growth with subsequent spontaneous ovulation prior to 10 days. Of the 34 sows that received an injection, 23 were recorded as having an increase in follicle size, follicle numbers, or both. However, other sows had similar follicle size and number at the time of injection, but were unable to achieve ovulation. The factor(s) that determined why some ovulated but others did not remain unknown.

Implication
Under the conditions of this study, an immediate ovulatory response of farrowed sows to hCG cannot be confirmed, and therefore this treatment modality will not predictably control postpartum ovarian function.

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Conflict of interest
None reported.

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References