ORIGINAL RESEARCH PEER REVIEWED

Effect of single or double insemination on fertility of sows bred at an induced estrus and ovulation

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Summary

Objective: To determine the effect of porcine luteinizing hormone (pLH) on time of ovulation and subsequent fertility to single or double artificial insemination (AI).

Materials and methods: In Experiment One, 17 sows received equine chorionic gonadotropin (eCG; 600 IU) intramuscularly (IM) at weaning, then 5 mg pLH IM 80 hours later to induce ovulation. Time of ovulation was determined using transrectal real-time ultrasonography. In Experiment Two, 567 sows were assigned to five treatments: no hormone treatments, AI AM and PM day 5 after weaning; eCG at weaning; AI AM and PM day 5 after weaning; pLH 80 hours after weaning, AI 36 and 44 hours later; eCG at weaning, pLH 80 hours later, AI 36 and 44 hours after pLH; eCG at weaning, pLH 80 hours later, AI 36 hours after pLH.

Results: Time from pLH to ovulation was 38.2 ± 2.8 hours (range, 34.25 to 42.5 hours). Of the 567 weaned sows, 530 (93.5%) were bred and 403 (76.0%) farrowed. There was no effect of eCG on subsequent fertility. Farrowing rate was higher ($P < .01$) for sows receiving eCG followed by pLH at both insemination frequencies. Litter size was unaffected by treatment.

Implications: A protocol of eCG-induced estrus and pLH-induced ovulation allows for predictable timing of ovulation and optimal timing of insemination relative to ovulation. The degree of predictability allowed for maintenance of fertility to a single, fixed-time insemination.

Keywords: swine, fertility, ovulation, equine chorionic gonadotropin, porcine luteinizing hormone

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Resumen – Efecto de la inseminación sencilla o doble sobre la fertilidad de las hembras inseminadas con una ovulación y celo inducidos

Objetivo: Determinar el efecto de la hormona luteinizante porcina (pLH) en el tiempo de ovulación y la subsiguiente fertilidad en la inseminación artificial (AI por sus siglas en inglés) doble o sencilla.

Materiales y métodos: En el experimento Uno, 17 hembras recibieron gonadotropina coriónica equina (eCG por sus siglas en inglés; 600 IU) intramuscularmente (IM por sus siglas en inglés) al destete, luego 5 mg de pLH IM 80 horas después para inducir la ovulación. El tiempo de la ovulación se determinó usando ultrasonografía transrectal de tiempo real. En el experimento Dos, 567 hembras se asignaron a cinco tratamientos: tratamiento sin hormonas, AI AM y PM en el día 5 después del destete; eCG al destete; AI AM y PM en el día 5 después del destete; pLH 80 horas después del destete, AI 36 y 44 horas después; eCG al destete, pLH 80 horas después, AI 36 y 44 horas después de la pLH; eCG al destete, pLH 80 horas después, AI 36 horas después de la pLH.

Resultados: El tiempo de la pLH a la ovulación fue 38.2 ± 2.8 horas (34.25 a 42.5 horas de rango). De las 567 hembras destetadas, 530 (93.5%) fueron inseminadas y 403 (76.0%) parieron. El eCG no tuvo efectos en la fertilidad subsiguiente. El porcentaje de fertilidad fue más alto ($P < .01$) en las hembras que recibieron el eCG seguido de la pLH en ambas frecuencias de inseminación. El tamaño de la camada no fue afectado por el tratamiento.

Implicaciones: Un protocolo del celo inducido por la eCG y una ovulación inducida por la pLH permiten un tiempo predecible de ovulación y un tiempo óptimo de inseminación con relación a la ovulación. El grado de predicción permitió mantener la fertilidad con una inseminación dada en un tiempo fijo.

Résumé – Effet de l'insémination simple ou double sur la fertilité de truies sailles à un chaleur et ovulation induits

Objectif: Déterminer l'effet de l'hormone luteinizant porcine (pLH) à l'heure de l'ovulation et la fertilité subséquente dans l'insémination artificielle (AI par ses sigles en anglais) double ou simple.

Matières et méthodes: Dans l'Expérience Un, 17 truies ont reçu des gonadotropin chorionique chevalin (eCG par ses sigles en anglais; 600 IU) intramusculaire (IM par ses sigles en anglais) à sevrage, après pLH de 5 mg IM 80 heures après pour induire

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l'ovulation. Le temps de l'ovulation a été déterminé avec l'ultrasonographie transrectal de temps réal. Dans l'Expérience Deux, 567 truies ont été assignées à cinq traitements: traitement sans hormones, AI AM et PM le jour 5 après du sevrage; l'eCG à sevrage; AI AM et PM le jour 5 après du sevrage; la pLH 80 heures après sevrage, AI 36 et 44 heures après; l'eCG à sevrage, la pLH 80 heures après, AI 36 et 44 heures après du pLH; l'eCG à sevrage, la pLH 80 heures après, AI 36 heures après du pLH.

Résultats: Le temps du pLH à l'ovulation a été 38.2 ± 2.8 heures (34.25 à 42.5 heures de gamme). De les 567 truies sevrées, 530 (93.5%) ont été saille et 403 (76.0%) ont été mise bas. Il n'y a eu aucun effet de l'eCG sur la fertilité subséquente. Le pourcentage de gestation a été plus haut ($P < .01$) pour des truies qui ont reçu l'eCG suivi par le pLH aux deux fréquences d'insémination. La dimension de la portée n'a pas été affectée par le traitement.

Implications: Un protocole du chaleur induit par l'eCG et l'ovulation induite par la pLH permet un temps prévisible d'ovulation et un temps optimal d'insémination relatif à l'ovulation. Le degré de prévisibilité a permis l'entretien de la fertilité de une insémination simple, à un temps fixe.

Optimal sow fertility is achieved by insemination of fresh extended semen during the 24-hour period before ovulation.[1,2] However, among sows, there is a great variation in the wean-to-estrus interval, duration of estrus, and consequently also in the onset of estrus-to-ovulation interval (EOI).[3–6] This variability in EOI presents a challenge in determining a reliable AI schedule, since the onset of estrus is not a good predictor for the optimal time of insemination.[7] The lifespan of ova after ovulation and the lifespan of a sufficient number of sperm cells capable of fertilization within the oviduct define the time, relative to ovulation, during which inseminations can lead to successful fertilization.[1] The use of exogenous pharmaceutical products for synchronization of estrus and ovulation would allow for the application of targeted intensive detection of estrus and determination of the appropriate timing for successful AI.

The most common protocol for the induction of estrus in weaned sows is injection of 500 to 750 IU of equine chorionic gonadotropin (eCG) or a combination of 400 IU eCG and 200 IU of human chorionic gonadotropin (hCG). There is a wealth of literature demonstrating the efficacy of this approach for induction of a fertile estrus after weaning.[8–10] While efficacious for induction of estrus, injection of eCG alone or eCG plus hCG does not provide adequate synchronization of ovulation. Further, when an earlier onset of estrus is induced with either eCG or eCG plus hCG, the EOI may increase, [10,11] making the prediction of time of ovulation even more difficult. However, because gonadotropin treatment results in a sow population having longer EOI, this knowledge can be used in a protocol of induced ovulation to allow more precise timing of insemination relative to ovulation.

It is known that ovulation typically occurs at about 42 hours after hCG injection.[12] When ovulation is induced using gonadotropin-releasing hormone or porcine luteinizing hormone (pLH), the interval from injection to ovulation tends to be shorter, at 36 to 38 hours.[5,13,14] Therefore, if sows are expected to ovulate at > 36 hours after estrus detection, induction of ovulation using pLH should provide for a relatively predictable time of ovulation. Since the target is to inseminate sows during the 24-hour period before ovulation, if time of ovulation can be accurately predicted, then breeding management for optimal sow fertility will be relatively simple. Therefore, the objectives of this study were first to determine the time of ovulation in sows treated with eCG at weaning and pLH injected 80 hours later, and secondly, to examine sow fertility to fixed-time insemination following this hormonal regime.

Materials and methods

Animals, housing, and management

These studies were conducted on 25 weekly breeding groups between November 2003 and June 2004 on a commercial, 700-sow farrow-to-feeder pig operation. Management of the commercial facility and the study protocol were approved by the Animal Care Committee of the University of Guelph. The sows were Yorkshire × Landrace and lactation length was 26.3 ± 4.3 days. During gestation, sows were housed in individual gestation stalls and provided approximately 2.5 kg per day of a diet formulated to provide 14.2 MJ of digestible energy per kg and 15% crude protein.

Experiment One

For the first objective, 17 mixed-parity sows (average parity, 7.5) received an intramuscular (IM) injection of 600 IU eCG (Pregnecol 5000; Bioniche Animal Health, Belleville, Ontario, Canada) at weaning (Day 0) to induce synchronous ovarian follicular development. At 80 hours after eCG injection, sows received an injection (IM) of 5 mg pLH (Lutropin-V; Bioniche Animal Health) to induce ovulation. The doses of eCG and pLH were chosen on the basis of their known efficacies.[5,13] The time of ovulation was determined using transrectal real-time ultrasonography (RTU) using an Aloka SSD 500 (Aloka Inc, Wallingford, Connecticut) with a 7.5-MHz linear array transducer for visualization of the ovaries, as described by Knox and Althouse.[15] Starting at 16 hours after pLH injection, RTU was performed every 8 hours, and from 32 hours after pLH injection, RTU was performed at approximately 2- to 4-hour intervals until ovulation was complete. Ovulation was considered to be complete when there were fewer than four follicles of > 6.5 mm remaining on the ovaries.[11]

Experiment Two

To determine effects of gonadotropin-induced estrus and ovulation on fertility, 567 mixed-parity sows (average parity, 7.4 ± 4.7) were assigned by restricted randomization (distributing parities evenly among treatment groups as required), on the basis of number of piglets weaned and parity, to one of five treatments as described in Table 1. Pigs were weaned on Day 0 of the study. To confirm the efficacy of the protocol for induction of ovulation, RTU was performed as described on 20 sows per treatment at 8-hour intervals from 16 hours to 48 hours after the pLH injection.

Sows on Treatments 1 through 4 were inseminated only if exhibiting a standing estrus, and any sow not in estrus by Day 5 was excluded. Detection of estrus involved boar exposure once per day from Day 4. Only six sows exhibited estrus on Day 4, and these were removed from the experiment. Wean-to-estrus interval was 5 days in all other sows. Sows on Treatment 5 received their single insemination regardless of estrus status. Pooled semen from two proven boars was used for AI. Insemination doses were 80 mL fresh extended semen containing at least 3×10^9 live sperm. Pregnancy was determined by transabdominal RTU 30 days after the last insemination.

Table 1: Study design for postweaning (PW) treatment of sows (N = 567) with equine chorionic gonadotropin (eCG),[1] porcine luteinizing hormone (pLH),[2] or both, or no hormone treatment before insemination (Experiment Two)

Treatment group		n	eCG	pLH	Timing of AI
1	Control	131	None	None	Day 5 PW, AM and PM
2	eCG	111	At weaning	None	Day 5 PW, AM and PM
3	pLH	113	None	80 hours PW	36 and 44 hours after pLH[3]
4	eCG + pLH2	110	At weaning	80 hours PW	36 and 44 hours after pLH[3]
5	eCG + pLH1	102	At weaning	80 hours PW	36 hours after pLH

[1] eCG (Pregnecol 5000; Bioniche Animal Health, Belleville, Ontario, Canada) administered intramuscularly (IM) at a dose of 600 IU.

[2] pLH (Lutropin-V; Bioniche Animal Health) administered IM at a dose of 5 mg.

[3] 36 hours after pLH corresponds to AM, and 44 hours after pLH to PM, in Control group AI timing.

Statistical analysis

Data recorded for sows were parity, pretreatment litter size suckled, lactation length, 30-day pregnancy status, first-service farrowing rate, and subsequent total-born litter size. All analyses were performed using SAS software, Version 9.1 (SAS Institute Inc, Cary, North Carolina) and significance assumed at $P < .05$.

Binomial responses were analyzed by logistic regression model adjusted for the effect of centered parity (median parity, 7) and its quadratic effect. The overall effect of treatment was evaluated by Wald chi-squared test. After testing for overall significance, individual treatment effects were tested by a Wald chi-squared test. The fit of the model was assessed by Hosmer and Lemeshow goodness-of-fit test and $P > .10$ was considered indicative of appropriate fit. Total litter size was analyzed in a linear regression model with centered parity and its quadratic effect included as covariates. The overall statistical significance of treatments was tested by F test. Interaction between treatments and parity was tested in all models. Parity means among treatments were tested in a linear regression model containing treatments only as fixed effects.

Results

The time from pLH treatment to ovulation in Experiment One ranged from 34.3 to 42.5 hours, with a mean of 38.2 ± 2.8 hours (Figure 1). The mean parity of sows in this study was 7.5, with five sows of parities 1 to 3, four sows of parities 4 to 8, and eight sows of parities > 8. The mean times (± SE) from pLH treatment to ovulation for these three groups were 36.8 ± 1.7 hours, 40.8 ± 2.8 hours, and 37.8 ± 2.6 hours, respectively, which were not statistically different.

In the fertility study (Experiment Two), of the 20 sows per treatment subjected to RTU examination, none had ovulated at 32 hours after pLH injection. At 40 hours after pLH, approximately 20% and 40% of Control and eCG-treated sows, respectively, had ovulated, while nearly all sows receiving pLH had ovulated (Table 2).

Of the 567 sows weaned in the fertility study, 530 (93.5%) were bred. Of the sows bred, 403 (76.0%) farrowed. The mean parity of sows in each treatment group ranged from 6.7 to 8.0, but differences were not significant (Table 3). There was no effect of eCG on the percentages of sows exhibiting estrus by Day 5 or on subsequent fertility (Table 3). When sows received pLH in the absence of eCG, there was a higher farrowing rate and a tendency for a higher pregnancy rate, but litter size was not affected (Table 3). The pregnancy and farrowing rates were higher for sows receiving both eCG and pLH than for Controls, with either single or double inseminations (Table 3). Litter size was not affected.

Discussion

This study showed that, under the conditions in this herd, time of ovulation was more predictable and sow fertility was better when the eCG + pLH protocol was used. It must be noted that this was a very mature herd, but given the apparent lack of a parity effect on the time of ovulation, a similar response would likely be observed in a younger herd. However, it should also be noted that the use of exogenous pharmaceuticals merely serve to remove some uncertainty concerning the time of ovulation and so make it easier to appropriately time insemination. In herds with excellent husbandry and good fertility, a lower (or no)

Figure 1: Distribution of intervals to ovulation after injection of porcine luteinizing hormone (pLH) in 17 mixed-parity sows in a commercial 700-sow farrow-to-feeder pig operation (Experiment One). At weaning, 600 IU equine chorionic gonadotropin was administered intramuscularly (IM) to induce synchronous ovarian follicular development, and 80 hours later, 5 mg pLH was administered IM to induce ovulation. Time of ovulation was determined using transrectal real-time ultrasonography.

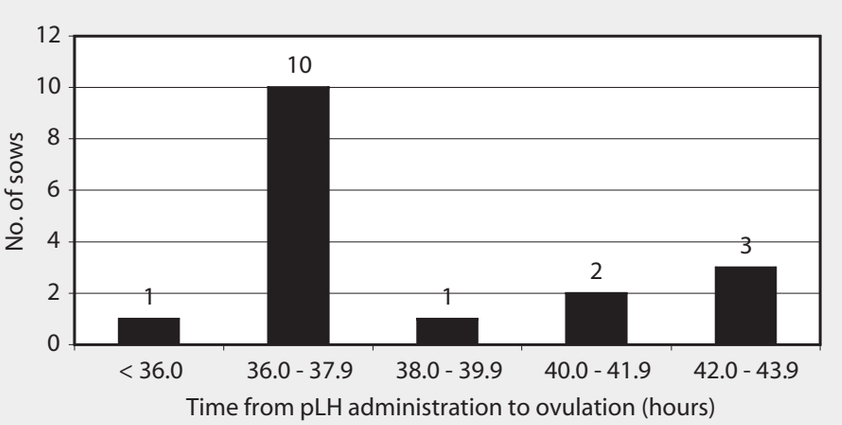

Table 2: Number of sows ovulating at different times[1] after injection of porcine luteinizing hormone (pLH) (Experiment Two)

Treatment group	No. of sows (%)		
	40 hours	48 hours	> 48 hours
Control	4 (20)	11 (55)	5 (25)
eCG	8 (40)	10 (50)	2 (10)
pLH	18 (90)	2 (10)	0 (0)
eCG + pLH2	20 (100)	0 (0)	0 (0)
eCG + pLH1	20 (100)	0 (0)	0 (0)

[1] 20 sows per treatment group (described in Table 1) were examined by real-time ultrasound at 8-hour intervals from 16 to 48 hours after injection of pLH (5 mg per sow, intramuscularly). No sows in any treatment group ovulated at < 32 hours after pLH injection.

effect could be expected in sows receiving multiple inseminations.

The lack of effect of eCG on the percent of sows exhibiting estrus by Day 5 indicates that the wean-to-estrus interval (WEI) was not likely a limiting factor for sow fertility. The observed lack of effect of eCG on fertility was not unexpected, since the WEIs were not different, and so the timing of insemination relative to ovulation would also likely be similar. Sows having relatively short WEIs tend to have longer EOIs,[1] and so would be more likely to ovulate at > 36 hours after onset of estrus. This would increase the proportion of the sow population available for an induced ovulation. Most sows in this study returned to estrus by Day 5, and the higher pregnancy and farrowing rates in response to pLH in the absence of eCG treatment indicated that, even with a natural onset of estrus, some sows likely had a controlled ovulation. Higher farrowing rates following insemination near a hormonally controlled ovulation has been demonstrated previously.[6,16] The enhanced efficacy of the combination of eCG followed by pLH, compared to pLH alone, likely resulted from the eCG inducing more late-ovulating sows, and these sows would be available for a controlled ovulation. Therefore, more sows would be inseminated at the optimal time relative to ovulation. That this is the case is supported by the observation that when both eCG and pLH were used, fertility was maintained at the maximum level even following a single insemination.

Implications

- Under the conditions of this study, a protocol combining an eCG-induced estrus with a pLH-induced ovulation allows for predictable timing of ovulation and optimal timing of insemination relative to ovulation.
- The degree of predictability provided by this protocol allows for maintenance of fertility to a single, fixed-time insemination.

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Table 3: Sow fertility to single or double insemination at an estrus induced by equine chorionic gonadotropin (eCG), with or without induction of ovulation by porcine luteinizing hormone (pLH)[1]

Variable	Control	eCG	pLH	eCG + pLH2	eCG + pLH1
Sows weaned	131	111	113	110	102
Litter size weaned (± SE)	8.6 ± 2.0	8.2 ± 2.2	8.4 ± 1.9	8.2 ± 2.1	8.6 ± 2.1
No. of sows bred (% bred)	119 (90.8)	103 (92.8)	103 (91.2)	103 (93.6)	102 (100)
Pregnant/sows weaned (%)[2]	65.6	68.5	75.2[a]	80.9[c]	89.2[c]
Pregnant/sows bred (%)[3]	72.3	73.8	82.5[a]	86.4[c]	89.2[c]
Farrowing rate (%)[4]	68.7	69.0	81.4[b]	84.2[c]	86.1[c]
Litter size (total)[5] (± SE)	11.1 ± 2.6	10.7 ± 3.2	10.3 ± 3.3	10.3 ± 3.1	10.6 ± 3.5
Mean parity[6] (± SE)	7.2 ± 4.8	6.7 ± 4.5	7.2 ± 4.8	7.8 ± 5.0	8.0 ± 4.5

[1] eCG injected at weaning, pLH injected 80 hours later. Controls received no hormone treatment. Double AI was performed AM and PM on postweaning day 5 (Control and eCG groups) or 36 and 44 hours after pLH (pLH and eCG + pLH2 groups), and single AI 36 hours after pLH (eCG + pLH1 group).

[2] Sows pregnant 30 days post insemination, based on number of sows weaned ($\chi^2_4 = 18.5$, $P = .001$).

[3] Sows pregnant 30 days post insemination, based on number of sows bred ($\chi^2_4 = 13.8$, $P = .01$).

[4] Farrowing rate adjusted for nonreproductive culls ($\chi^2_4 = 15.8$, $P = .003$).

[5] Born alive plus stillborn. Least square means ± SE ($F_{4,394} = 0.87$, $P = .48$).

[6] Least square means ± SE ($F_{4,564} = 1.25$, $P = .29$).

[abc] Means within a row differ from Control: a, $P < .1$; b, $P < .05$; c, $P < .01$.

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