

# Ovulation induction protocol using equine chorionic gonadotropin and porcine luteinizing hormone in the weaned sow

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## Summary

**Objective:** To determine the lowest dose of porcine luteinizing hormone (pLH) that induces ovulation in a predictable manner in mixed-parity sows 80 hours after treatment with equine chorionic gonadotropin (eCG) at weaning.

**Materials and methods:** All sows were injected intramuscularly (IM) with 600 IU eCG at weaning. At 80 hours post weaning, sows were treated IM with pLH at doses of 0.625 mg (n = 5), 1.25 mg (n = 14), 2.5 mg (n = 21), and 5.0 mg (n = 5). The 15 controls were untreated. Approximate time of ovulation was determined using transrectal realtime ultrasound 8 hours before and 16

hours post pLH injection, and thereafter at 8-hour intervals until ovulation was confirmed.

**Results:** Administration of pLH was effective in inducing ovulation in weaned sows. The pLH-to-ovulation interval was shorter in sows given pLH at 1.25 mg ( $P < .05$ ), 2.5 mg ( $P < .01$ ), and 5.0 mg ( $P < .05$ ) than in controls. Variance associated with the pLH-to-ovulation interval tended to be less in treated than in control sows ( $P < .10$ ). More sows administered either 2.5 or 5.0 mg pLH ovulated by 40 hours post pLH administration than all other treatments ( $P < .01$ ).

**Implications:** Doses of 2.5 and 5.0 mg of pLH did not differ in their effect of induc-

ing ovulation in an estrus-synchronization program initiated with 600 IU of eCG at weaning. Controlling the time of ovulation allows insemination to occur at the time of optimal fertilization.

**Keywords:** swine, equine chorionic gonadotropin, porcine luteinizing hormone, ovulation, ultrasound

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## Resumen - Protocolo de inducción de la ovulación utilizando gonadotropina coriónica equina y hormona luteinizante porcina en cerdas destetadas

**Objetivo:** Determinar la dosis mínima de hormona luteinizante porcina (pLH por sus siglas en inglés) que induzca la ovulación de forma predecible en grupos de hembras de paridad variada, 80 horas después del tratamiento con gonadotropina coriónica equina (eCG por sus siglas en inglés) al destete.

**Materiales y métodos:** Todas las hembras fueron inyectadas intramuscularmente (IM por sus siglas en inglés) con 600 IU de eCG al destete. Ochenta horas post destete, las hembras se trataron IM con pLH con dosis de 0.625 mg (n = 5.0), 1.25 mg (n = 14), 2.5 mg (n = 21), y 5.0 mg (n = 5). Las 15 hembras control no fueron tratadas. La hora aproximada de ovulación se determinó utilizando ultrasonido transrectal de tiempo real 8 horas antes y 16 horas post inyección de pLH, y a partir de entonces a intervalos de 8 horas hasta que se confirmó la ovulación.

**Resultados:** La administración del pLH fue eficaz en inducir la ovulación en hembras destetadas. El intervalo de pLH a ovulación fue más corto en las hembras a las que se les administró pLH a 1.25 mg ( $P < .05$ ), 2.5 mg ( $P < .01$ ), y 5.0 mg ( $P < .05$ ) comparado con los controles. La variación asociada al intervalo de pLH a ovulación tendió a ser menor en las hembras tratadas que en las control ( $P < .10$ ). Un mayor número de hembras a las que se les administró ya sea 2.5 ó 5.0 mg de pLH ovularon a las 40 horas post la administración de pLH en comparación con los otros tratamiento ( $P < .01$ ).

**Implicaciones:** Las dosis de 2.5 y 5.0 mg de pLH no difirieron en su efecto de inducción de ovulación en un programa de sincronización de estro iniciado con 600 IU de eCG al destete. El control del tiempo de la ovulación permite que la inseminación ocurra en el tiempo óptimo de fertilización.

## Résumé - Protocole d'induction de l'ovulation utilisant la gonadotrophine chorionique équine et l'hormone lutéinisante porcine chez la truie sevrée

**Objectif:** Déterminer la plus faible concentration d'hormone lutéinisante porcine

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CP: Genesafe Technologies Ltd, Guelph, Ontario.

Dr Bennett-Steward was a consultant to Bioniche Animal Health while this study was being conducted. Dr Zak became a Bioniche Animal Health consultant after the study had been conducted, and contributed to data analysis and manuscript preparation.

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(pLH) induisant l'ovulation de manière prévisible chez des truies de parité mélangée 80 heures après un traitement avec de la gonadotrophine chorionique équine (eCG) au sevrage.

**Matériels et méthodes:** Toutes les truies ont été injectées par voie intramusculaire (IM) avec 600 UI d'eCG au sevrage. Au temps 80 heures post-sevrage, les truies ont été traitées IM avec de la pLH à des doses de 0.625 mg (n = 5), 1.25 mg (n = 14), 2.5 mg (n = 21), et 5.0 mg (n = 5). Un groupe de 15 animaux non traités a servi de témoin. Le temps approximatif de l'ovulation a été

déterminé au moyen d'une échographie en temps réel par voie trans-rectale effectuée 8 heures avant et 16 heures post-injection de pLH, et par la suite aux 8 heures jusqu'à ce que l'ovulation soit confirmée.

**Résultats:** L'administration de pLH a été efficace à induire l'ovulation chez les truies sevrées. L'intervalle pLH-ovulation était plus court chez les truies ayant reçu la pLH à un dosage de 1.25 mg ( $P < .05$ ), 2.5 mg ( $P < .01$ ) et 5.0 mg ( $P < .05$ ) comparativement aux animaux témoins. La variance associée avec l'intervalle pLH-ovulation avait tendance à être moindre chez les

truies traitées comparativement aux truies témoins ( $P < .10$ ). Plus de truies ayant reçu 2.5 ou 5.0 mg de pLH ont ovulé au maximum 40 heures post administration de pLH comparativement à tous les autres traitements.

**Implications:** L'administration de doses de 2.5 et 5.0 mg de pLH a eu des effets similaires sur l'induction de l'ovulation dans un programme de synchronisation de l'oestrus initié avec 600 UI d'eCG au sevrage. Une surveillance du temps de l'ovulation permet de faire l'insémination au temps optimal de fertilisation.

**A**rtificial insemination is the predominant method of breeding in the swine industry.<sup>1</sup> Optimal fertilization rates are achieved when insemination occurs within a period of 4 to 24 hours before ovulation. However, timing of ovulation is often difficult to predict accurately when it is based on the first observed behavioural estrus post weaning,<sup>2</sup> which depends upon the estrus-detection protocol utilized,<sup>3</sup> wean-to-estrus interval, and duration of estrus.

The best estimate of the time from onset of estrus to ovulation is based on the knowledge that for most weaned sows, ovulation occurs two-thirds of the way through estrus.<sup>4</sup>

Hormones administered at weaning, eg, equine chorionic gonadotropin (eCG)<sup>5</sup> or eCG in combination with human chorionic gonadotropin (hCG),<sup>6</sup> are effective in reducing the wean-to-estrus interval, and more treated sows exhibit estrus behavior than naturally cyclic sows. However, even with eCG or eCG-hCG treatment, the time of ovulation cannot be accurately predicted in relation to the onset of estrus.<sup>7,8</sup>

Sequential administration of exogenous hormones, eg, eCG followed by porcine luteinizing hormone (pLH), induce ovulation.<sup>9,10</sup> A commonly used protocol includes administration of 600 IU eCG at weaning, followed up to 80 hours later by 5.0 mg of pLH, which induces ovulation approximately 40 hours later.<sup>9</sup> Using this protocol, the optimal timing of insemination<sup>11</sup> (ie, within 24 hours of ovulation) can be accurately predicted. The objective of this field trial was to determine the lowest efficacious dose of pLH to induce a predictable ovulatory response in weaned sows.

## Materials and methods

A dose-titration study was conducted to establish the lowest effective dose of pLH (Lutropin-V; Bioniche Animal Health Canada Inc, Belleville, Ontario, Canada) necessary to predictably induce ovulation in mixed-parity sows. The trial was conducted on a 650-sow farrow-to-grower commercial unit and performed using two replicates of 30 sows each, conducted in August 2004 and January-February 2005. Treatment groups were equally represented in each replicate. The animals were cared for and managed with due regard for their welfare.

The study included 60 mixed-parity sows (parity range 1 to 17) weaned 21 to 28 days after farrowing (average lactation length 26 days). Sows were assigned alternately in order of weaning to five treatment groups (Table 1). All sows received 600 IU eCG (Pregneol 5000; Bioniche Animal Health Canada Inc) IM in the neck at weaning, followed 80 hours later by an IM injection of either saline (Control) or pLH. The approximate time of ovulation was determined using realtime transrectal ultrasound (RTU) (5mHz linear probe; Falco Vet 100 ultrasound machine, Pie Medical, Maastricht, Holland), performed 8 hours before and 16 hours post pLH injection, then at 8-hour intervals until ovulation was confirmed, ie, less than four follicles > 6.5 mm in diameter remaining on the ovaries.<sup>7</sup> On this commercial farm, as is usual practice, estrus detection was enhanced by once-a-day boar contact in front of the stalls.

Treatment effect on time to ovulation after pLH administration (pLH-to-ovulation interval) was analysed using the General

Linear Model procedure of SAS (SAS Institute Inc, Cary, North Carolina). In the event of a significant treatment effect, differences among treatment means were determined using probability for differences of the least squared means (SAS, PROC PDIF). Chi-squared analysis (SAS, PROC CATMOD) was used to determine the differences among treatment groups in the number of sows ovulating 24 to 40 hours after pLH or saline injection. Bartlett's test was used to determine the homogeneity of variance, ie, to measure the dispersion associated with pLH-to-ovulation interval. A 5% significance level was applied for all analyses.

## Results

The mean pLH-to-ovulation interval differed among treatment groups ( $P < .001$ ). Mean pLH-to-ovulation interval was shorter in sows administered the combination of eCG and 1.25, 2.5, or 5.0 mg pLH than in sows treated with eCG and saline only (Table 1). The mean pLH-to-ovulation interval in sows treated with 2.5 mg pLH was shorter than that in sows treated with 1.25 mg pLH (Table 1), but did not differ from that in sows treated with 5.0 mg pLH (Table 1).

There was a significant effect of treatment ( $P < .01$ ) on the number of sows ovulating within 24 to 40 hours after pLH or saline injection. Fewer control sows (two of 15; 13%;  $P < .01$ ) and more sows treated with 2.5 mg pLH (17 of 21; 81%;  $P < .05$ ) or 5.0 mg pLH (four of five; 80%;  $P < .01$ ) also ovulated in this period (Table 2). The standard deviations for the pLH-to-ovulation interval tended to differ among treatments (Table 1).

## Discussion

In this study, the wean-to-ovulation interval was shorter in sows treated with 1.25 mg to 5.0 mg of pLH than in control sows. These observations are in agreement with results of other studies in which the time interval from pLH administration to ovulation was shortened as the dose of pLH was increased up to 2.5 mg pLH,<sup>12</sup> or when weaned sows given eCG at weaning

followed 80 hours later by 5.0 mg pLH were compared to sows given only eCG at weaning.<sup>13</sup>

Although we and others<sup>10,12,14-16</sup> found that the pLH-to-ovulation interval was shorter when 1.25, 2.5, or 5.0 mg pLH was administered after eCG given at weaning, the average pLH-to-ovulation interval was still within the physiological time frame observed in weaned sows ovulating

after an endogenous LH surge, following a 14-day or 24-day lactation period.<sup>17</sup>

This suggests that the ovulation-induction protocol used in this study allows follicles to ovulate within a period that is not dissimilar to that for ovulation after an endogenous LH surge.

Other studies have shown that the timing of ovulation can be made more predictable and that variation in the timing of ovulation can be significantly reduced in sows treated with 2.5 or 5.0 mg of pLH.<sup>10,12,14-16</sup> Although we did not observe a significant increase in the homogeneity of the pLH-to-ovulation interval in this study, we did find that more sows ovulated within 24 to 40 hours after administration of 2.5 or 5.0 mg pLH, compared to sows given 1.25 mg or no pLH, which suggests a more precise timing of ovulation in response to pLH.

Together, these findings show that the time from pLH administration to ovulation is shorter in sows treated with 1.25, 2.5, or 5.0 mg pLH, and more sows given 2.5 or 5.0 mg pLH ovulate within 24 to 40 hours after administration of pLH.

## Implications

- 2.5 and 5.0 mg of pLH do not differ in their effect of inducing ovulation in an estrus-synchronization program initiated with 600 IU of eCG at weaning.
- Knowing when the majority of sows will ovulate may enhance reproductive performance and reduce the number of nonproductive sow days by allowing insemination at an optimal time relative to ovulation.

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**Table 1:** Mean time from luteinizing hormone administration to ovulation for 60 sows treated with different doses of pLH\*

Treatment	Dose	No. of sows	Average parity	pLH to ovulation (hours)	SD†
Saline (mL)	0.5	15	5	55.5 <sup>a</sup>	11.1
pLH (mg)	0.625	5	6	52.8 <sup>ab</sup>	4.4
	1.25	14	7	48.0 <sup>b</sup>	8.3
	2.5	21	5	39.2 <sup>c</sup>	7.1
	5.0	5	6	41.6 <sup>bc</sup>	3.6

\* All sows were treated IM at weaning with 600 IU equine chorionic gonadotrophin, then with an IM injection of saline (Control) or porcine luteinizing hormone (pLH) 80 hours later.

<sup>ab</sup> Values within a column with no common superscript differ ( $P < .05$ ; analysis of variance).

<sup>ac, bc</sup> Values with no common superscript differ ( $P < .01$ ; analysis of variance).

† Bartlett's test for homogeneity of variance ( $P < .10$ ).

**Table 2:** Number of sows ovulating at specific time intervals after administration of different doses of porcine luteinizing hormone (pLH)\*

Ovulation time	Dose of pLH (mg)				
	0 (n = 15)	0.625 (n = 5)	1.25 (n = 14)	2.5 (n = 21)	5.0 (n = 5)
24 hours	0	0	0	1	0
32 hours	1	0	0	5	0
40 hours	1 <sup>a</sup>	0 <sup>a</sup>	6 <sup>b</sup>	11 <sup>b</sup>	4 <sup>b</sup>
<b>24-40 hours</b>	<b>2<sup>c</sup></b>	<b>0<sup>c</sup></b>	<b>6<sup>c</sup></b>	<b>17<sup>d</sup></b>	<b>4<sup>d</sup></b>
48 hours	2	2	3	3	1
56 hours	7 <sup>b</sup>	3 <sup>b</sup>	4 <sup>b</sup>	1 <sup>a</sup>	0
64 hours	3	0	1	0	0
72 hours	0	0	0	0	0
80 hours	1	0	0	0	0

\* Treatment groups are described in Table 1. A total of 60 mixed-parity sows weaned 21 to 28 days after farrowing (average 26 days) were treated at weaning with 600 IU equine chorionic gonadotrophin IM in the neck, and 80 hours later, with an IM injection of saline or pLH. Sows were examined by realtime transrectal ultrasound 8 hours before and 16 hours after pLH injection, and then at 8-hour intervals, to determine time of ovulation.

<sup>ab</sup> Values within a row with different superscripts differ ( $P < .05$ ; chi-square analysis).

<sup>cd</sup> Values within a row with different superscripts differ ( $P < .01$ ; chi-square analysis).

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