

# Effect of dose of equine chorionic gonadotrophin on the estrus responses of gilts and weaned sows and effect of the interval between equine chorionic gonadotrophin and luteinizing hormone injections on sow performance

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## Summary

**Objectives:** To determine estrus and ovulation responses of gilts and sows to different doses of equine chorionic gonadotrophin (eCG), with different intervals between eCG and luteinizing hormone (pLH) injections.

**Materials and methods:** Experiment One: gilts received 0 (Control; n = 20), 600 (n = 42), or 900 (n = 42) IU eCG, then were exposed to a boar daily for estrus detection. Experiment Two: weaned primiparous sows received 600 (n = 56) or 900 (n = 59) IU eCG, 2.5 mg pLH 80 hours later, and a single insemination 36 hours after pLH. Controls (n = 43) received no hormones and were inseminated at estrus detection and 24 hours later. Experiment Three: weaned mixed-parity sows received 600 IU eCG at weaning and 2.5 mg pLH 72 (n = 107) or 80 hours (n = 116) later, or served as Controls (n = 66). Sows were inseminated as for Experiment Two.

**Results:** Experiment One: rate of ovulation was greater at the higher eCG dose ( $P < .05$ ). Experiment Two: sows receiving 900 IU eCG had a higher farrowing rate than controls ( $P = .03$ ), with sows receiving 600 IU eCG being intermediate. Litter sizes were unaffected. Experiment Three: farrowing

rates were higher for the eCG treatment than for Controls ( $P < .05$ ). Litter sizes were unaffected.

**Implications:** These data suggest that the dose of eCG should be 900 IU for induction of estrus in young females. For controlled ovulation, the eCG-pLH interval can be reduced to 72 hours without affecting reproduction.

**Keywords:** swine, gonadotrophins, estrus, ovulation

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**Resumen - Efecto de la dosis de gonadotropina coriónica equina en la respuesta del estro de primerizas y hembras destetadas y el efecto del intervalo entre la gonadotropina coriónica equina y las inyecciones de hormona luteinizante en el desempeño de las hembras**

**Objetivos:** Determinar la respuesta de estro y de la ovulación en primerizas y hembras a diferentes dosis de gonadotropina coriónica equina (eCG por sus siglas en inglés), con diferentes intervalos entre eCG y las inyecciones de hormona luteinizante (pLH por sus siglas en inglés).

**Materiales y métodos:** Experimento Uno: las primerizas recibieron 0 (Control; n = 20), 600 (n = 42), ó 900 (n = 42) IU eCG, y luego fueron expuestas a sementales diariamente para detectar el estro. Experimento Dos: las hembras primerizas destetadas recibieron 600 (n = 56) ó 900 (n = 59) IU eCG, 2.5 mg pLH 80 horas más tarde, y una inseminación única 36 horas después del pLH. Los controles (n = 43) no recibieron hormonas y se inseminaron a la detección del estro y 24 horas más tarde. Experimento Tres: las hembras destetadas de paridad mixta recibieron 600 IU eCG al destete y 2.5 mg pLH 72 (n = 107) ó 80 horas (n = 116) más tarde, o sirvieron como Controles (n = 66). Las hembras se inseminaron como en el Experimento Dos.

**Resultados:** Experimento Uno: el índice de ovulación fue mayor en la dosis más alta de eCG ( $P < .05$ ). Experimento Dos: las hembras que recibieron 900 IU eCG tuvieron un porcentaje de fertilidad más alto que los controles ( $P = .03$ ), y las intermedias fueron las hembras que recibieron 600 IU de eCG. El tamaño de camada no se afectó. Experimento Tres: el porcentaje de fertilidad fue más alto para los tratamientos de eCG que para los Controles ( $P < .05$ ). El tamaño de camada no se afectó.

**Implicaciones:** Estos datos sugieren que la dosis de eCG debería ser de 900 IU para la inducción del estro en hembras jóvenes. Para la ovulación controlada, el intervalo de eCG-pLH puede reducirse a 72 horas son afectar la reproducción.

**Résumé - Effet du dosage de la gonadotrophine chorionique équine sur les réponses œstrales des cochettes et truies sevrées et effet de l'intervalle entre les injections de gonadotrophine chorionique équine et d'hormone lutéinisante sur les performances des truies**

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Drs Zak and Rogan were employed by Bioniche Animal Health during these studies.

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**Objectifs:** Déterminer les réponses œstrales et ovulatoires de cochettes et truies à différentes doses de gonadotrophine chorionique équine (eCG), avec différents intervalles entre les injections d'eCG et d'hormone lutéinisante (pLH).

**Matériels et méthodes:** Expérience Un: des cochettes ont reçu 0 (Témoin; n = 20), 600 (n = 42), ou 900 (n = 42) UI d'eCG, et ont par la suite été mises quotidiennement en présence d'un verrat pour détection de l'œstrus. Expérience Deux: des truies primipares sevrées ont reçu 600 (n = 56) ou 900 (n = 59) UI d'eCG, 2.5 mg de pLH 80 heures plus tard, et une insémination unique 36 heures après l'administration de pLH. Les truies témoins (n = 43) n'ont reçu aucune hormone et furent inséminées lors de la détection de l'œstrus et 24 heures plus tard. Expérience Trois: des truies sevrées de parités variées ont reçu 600 UI d'eCG au sevrage et 2.5 mg de pLH 72 (n = 107) ou 80 heures (n = 116) plus tard, ou ont servi de Témoins (n = 66). Les truies furent inséminées comme dans l'Expérience Deux.

**Résultats:** Expérience Un: le taux d'ovulation était plus grand au dosage le plus élevé d'eCG ( $P < .05$ ). Expérience Deux: les truies recevant 900 UI d'eCG avaient un taux de mise-bas plus élevé que les animaux témoins ( $P = .03$ ), et celles recevant 600 UI d'eCG étant intermédiaires. La taille des portées était non-affectée. Expérience Trois: les taux de mises-bas étaient supérieurs pour les animaux recevant le traitement eCG comparativement aux animaux Témoins ( $P < .05$ ). La taille des portées n'était pas affectée.

**Implications:** Ces données suggèrent que la dose d'eCG devrait être de 900 UI pour l'induction de l'œstrus chez les jeunes femelles. Pour une ovulation contrôlée, l'intervalle eCG-pLH peut être réduit à 72 heures sans affecter la reproduction.

Controlling gilt availability through puberty induction, and the creation of simultaneously synchronized gilt and sow breed groups, minimizes non-productive days, reduces the variation in piglet age at weaning, and enables precise scheduling of labor and resources. The seminal studies for the use of gonadotrophins in swine breeding management were reported by Tanabe et al<sup>1</sup> and Dziuk and Baker.<sup>2</sup> More recently, the East German pig industry routinely used protocols based on gonadotrophins to induce puberty, synchronize estrous cycles, and implement

fixed-time insemination strategies in the female.<sup>3,4</sup>

We have demonstrated that while 600 IU equine chorionic gonadotrophin (eCG) was adequate to induce estrus and ovulation in weaned sows,<sup>5</sup> this dose of eCG was relatively ineffective for inducing estrus and ovulation in prepubertal gilts.<sup>6</sup> Similarly, the estrus response to 600 IU eCG at weaning was lower in primiparous than in multiparous sows (L. J. Zak; unpublished data). The reason for the lower estrus responses to 600 IU eCG in younger females is unclear. It has been shown that porcine ovarian follicle development beyond 4 mm requires primarily luteinizing hormone (LH) activity.<sup>7</sup> Further, while eCG does have some LH-like activity,<sup>4,8</sup> it has been suggested that for gilts, the LH-like activity of 600 IU eCG may be inadequate for stimulating ongoing follicle development.<sup>9,10</sup> The relative follicle-stimulating hormone (FSH)-like and LH-like activities of the eCG molecule vary in different eCG preparations,<sup>8,11</sup> and this may affect the estrus responses of gilts to eCG treatment. Estrus responses of gilts to eCG were increased when higher doses were administered,<sup>12,13</sup> possibly due to an associated increase in LH-like activity, although there is a paucity of data concerning eCG dose comparisons within a single study.

When employing fresh extended semen, optimal sow fertility is achieved by insemination during the 24-hour period before ovulation.<sup>14</sup> However, there is considerable variation in the estrus-to-ovulation interval among sows.<sup>15</sup> Exogenous eCG followed by pLH can be employed to control onset of estrus and time of ovulation, allowing the application of a single fixed-time insemination.<sup>5,16,17</sup> The protocol employed in these latter studies involved the administration of 600 IU eCG to sows at weaning and treatment with pLH 80 hours later. Using this protocol, ovulation was shown to occur approximately 38 hours after pLH treatment,<sup>5,16</sup> and fertility was maintained if single inseminations were performed near the time of ovulation.<sup>5,17</sup> While the protocol has proven effective in the field, producers have voiced a desire to be able to modify the eCG-pLH interval. Although an 80-hour interval between eCG and hCG treatments is considered optimal,<sup>3</sup> others have shown shorter intervals to be effective.<sup>18,19</sup>

Therefore, we undertook studies to examine the hypotheses that the estrus responses of gilts and primiparous sows to eCG treatment would be improved at a higher dose

and that sow fertility after eCG and pLH treatments will remain unchanged when the pLH is injected at 72 or 80 hours after eCG.

## Material and methods

The protocols for these studies were approved by the University of Guelph Animal Care committee.

These studies were performed during 12 months preceding December 2007 at a commercial 600-sow farrow-to-finish facility near Guelph, Ontario.

For Experiment One, to examine gilt responses to different eCG doses, 104 Yorkshire × Landrace gilts were employed that were assumed to be prepubertal on the basis of their weights (average weight 96.4 kg) and lack of previously observed estrus. Gilts were housed at eight to 12 animals per pen (approximately 1.6 m<sup>2</sup> per gilt) and fed ad libitum a corn-soybean meal finisher diet formulated to provide 13.7 MJ metabolizable energy (ME) per kg, 14.0% crude protein, and 0.79% lysine. At selection, each gilt was assigned by weight to one of three treatment groups in such order as to minimize weight differences among groups. Group 1 (n = 42) received an intramuscular (IM) injection of 600 IU eCG (Pregnecol; Bioniche Animal Health, Belleville, Ontario), Group 2 (n = 42) received an IM injection of 900 IU eCG, and Group 3 (n = 20) were not injected, serving as nontreated controls. Fewer control gilts were employed because, on the basis of experience with this farm, we anticipated few, if any, would exhibit estrus during the 7-day observation period. The higher dose of eCG has been associated with relative efficacy in previous studies.<sup>12,13,20</sup> From 2 days until 7 days after eCG treatment, all gilts were subject to direct exposure to a mature boar for 15 minutes daily to facilitate detection of estrus. Estrus was defined as the expression of a standing reflex in the presence of the boar.

To characterize the ovulation responses to hormone treatment, blood samples were obtained from all gilts at 3 and 10 days after eCG injection. Serum samples were assayed for progesterone concentrations by established radioimmunoassay.<sup>21</sup> Assay sensitivity and intra-assay coefficient of variation were 0.2 ng per mL and 8.0%, respectively. An elevation of serum progesterone from < 1.0 ng per mL at 3 days to ≥ 5 ng per mL at 10 days was arbitrarily considered to indicate an ovulation response.

For Experiment Two, at weaning after a 24-day lactation, 158 primiparous

Yorkshire × Landrace sows received either 600 IU (n = 56) or 900 IU (n = 59) eCG followed by 2.5 mg pLH (Lutropin-V; Bioniche Animal Health) 80 hours later, and then a single insemination of pooled semen containing  $3 \times 10^9$  sperm at 36 hours after pLH treatment. The semen was collected on-farm and used within 48 hours of collection. The control group (n = 43) received no hormone treatments and were inseminated at detection of natural estrus and 24 hours later. Sows were assigned sequentially in weaning order to treatments without considering previous litter size. All treatments were represented in all breeding weeks, and within each breeding week all sows were inseminated in the presence of a boar with pooled semen from the same boars and containing  $3 \times 10^9$  sperm. Sires were equally represented among treatments. 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 digestible energy (DE) per kg and 15% crude protein. Farrowing rates and total born litter sizes were recorded.

For Experiment Three, after 24 days of lactation, 289 mixed-parity Yorkshire × Landrace sows were each assigned to one of three groups, ensuring groups were balanced on the basis of parity. All treatments were represented in all breeding weeks, and within each breeding week all sows were inseminated with pooled semen from the same boars. Sows in Group 1 (n = 116) received an IM injection of 600 IU eCG in the afternoon of the day of weaning (Thursday afternoon), then 2.5 mg pLH (Lutropin-V) 80 hours later (Sunday night),<sup>22</sup> and then a single insemination of pooled semen ( $3 \times 10^9$  sperm) at 36 hours after pLH treatment (Tuesday morning). Sows in Group 2 (n = 107) received 600 IU eCG at weaning (Thursday morning), 2.5 mg pLH 72 hours later (Sunday morning), and a single insemination of pooled semen ( $3 \times 10^9$  sperm) at 30 hours after pLH treatment (Monday afternoon). Sows in Group 3 (n = 66) served as controls in that they received no hormone treatment and were inseminated at detection of natural estrus and again 24 hours later. The interval between pLH treatment and insemination was shorter for Group 2 in order to allow the protocol to be completed during normal working hours. Sires were equally represented among treatments. Sows were managed during gestation as described for Experiment Two.

## Statistical analysis

Data recorded in Experiment One were gilt weight at time of selection onto experiment, proportion of gilts in estrus within 7 days after onset of treatment period, proportion of gilts ovulating, and proportion of gilts ovulating and expressing concomitant estrus behavior. The weight of gilts by treatment was tested for normality and homogeneity of variance and analyzed using a general linear model (SAS Institute, Inc, Cary, North Carolina). The percentages of gilts expressing behavioral estrus, ovulating, and concomitantly expressing behavioral estrus with ovulation were analyzed using a chi-squared test.

In Experiment Two, the effect of treatment on total litter size born was analyzed using a linear mixed effect model. All variables were tested for normality and homogeneity of variance. Treatment was considered a fixed effect. The percentages of sows farrowing after weaning were analyzed using a chi-squared test.

In Experiment Three, the effects of treatment, parity, week of selection onto experiment, and the effect of treatment-by-parity interaction on total litter size born were analyzed using a linear mixed-effect model. All variables were tested for normality and homogeneity of variance. Treatment, parity, and the interaction were considered fixed effects, while week was considered a random effect. The percentage of sows farrowing after weaning was analyzed using a chi-squared test. Parity  $\geq 4$  was analyzed as one group. In all analyses, significance was assumed at  $P < .05$ .

## Results

For Experiment One, there were no differences among treatment groups for initial body weight. Of the 42 gilts receiving 600 IU eCG (Group 1), 20 exhibited estrus and ovulated, four exhibited estrus but were anovulatory, and one was not detected as estrous but ovulated (Table 1). Of the 42 gilts receiving 900 IU eCG (Group 2), 24 were estrous and ovulated, three estrous gilts failed to ovulate, and six gilts were not detected in estrus but did ovulate (Table 1). During the study period, one gilt in Group 3 (control) exhibited estrus and ovulated, one was estrous but did not ovulate, and two gilts were not detected in estrus but did ovulate. Overall, rates of estrus were not affected by eCG dose, but rate of ovulation was higher ( $P < .05$ ) at the higher eCG dose.

In Experiment Two, the farrowing rate for control primiparous sows was relatively low and treatment with eCG at weaning was associated with higher farrowing rates (Table 2). The farrowing rate of sows receiving 900 IU eCG was higher ( $P = .03$ ) than that of controls, with that of the 600 IU-treated group being intermediate (Table 2). Litter sizes were not affected by treatment.

In Experiment Three, there was no effect of parity or a treatment × parity interaction detected. The farrowing rate for sows in Group 2 (pLH at 72 hours after eCG) was higher ( $P < .05$ ) than for controls, with the sows receiving pLH at 80 hours being intermediate. Litter size was not affected by treatment.

## Discussion

Although significant treatment effects on rates of estrus and ovulation in gilts were not detected in Experiment One, the present data do tend to support the findings of previous studies that found that, in the absence of additional LH-like activity such as that supplied by concurrently administered hCG, a dose of 600 IU eCG is relatively non-efficacious for induction of estrus and ovulation in prepubertal gilts.<sup>6,9,10,23,24</sup> In common with our previous observations,<sup>6,9,10</sup> there was a difference between estrus detection rates and the proportions of gilts ovulating, which may be due to an inadequate duration of sufficient LH-like activity to allow ovarian follicles to complete development and ovulate.<sup>10</sup> Indeed, we have recently noted improved ovulation responses to injection of a combination of eCG and hCG when an additional injection of 100 IU hCG was administered 24 hours later.<sup>25</sup>

In Experiments Two and Three, the total-born litter sizes were considered adequate. However, farrowing rates were quite low for the control sows in both experiments, but were better in sows treated with exogenous gonadotrophins. Since the hormone-treated sows received a single timed insemination, in contrast to the double insemination at estrus detection and 24 hours later of control sows, the lower farrowing rates of control sows implies a problem with their breeding management. It suggests that the timing of insemination relative to ovulation was less than optimal for control sows, an effect corrected by breeding at a hormonally controlled estrus and ovulation. We have previously

**Table 1:** Effect of equine chorionic gonadotrophin (eCG) dose on estrus expression and ovulation in prepubertal gilts (Experiment One)\*

	Control	600 IU eCG	900 IU eCG
Number of gilts	20	42	42
Weight (kg)†	97.2 ± 2.0	102.7 ± 1.4	100.3 ± 1.4
Gilts in estrus within 7 days (%)‡	2 (10.0) <sup>a</sup>	24 (57.1) <sup>b</sup>	27 (64.3) <sup>b</sup>
Gilts ovulating (%)§	3 (15.0) <sup>a</sup>	21 (50.0) <sup>bc</sup>	30 (71.4) <sup>bd</sup>
Estrus and ovulating (%)	1 (5.0) <sup>a</sup>	20 (47.6) <sup>b</sup>	24 (57.1) <sup>b</sup>

\* Gilts in a commercial herd were assigned by weight to treatments to minimize weight differences among groups. The two treated groups received either 600 IU or 900 IU of eCG (Pregnenol; Bioniche Animal Health, Belleville, Ontario, Canada) by intramuscular injection on the same day. Control gilts were not injected.

† Mean ± SE.

‡ Determined in the presence of a boar.

§ Determined on the basis of an increase in plasma progesterone from < 1 ng/mL on day 3 (after eCG injection) to > 5 ng/mL on day 10.

<sup>ab</sup> Means within a row with no common superscript differ ( $P < .01$ ; chi-squared test)

<sup>cd</sup> Means within a row with no common superscript differ ( $P < .05$ ; chi-squared test).

**Table 2:** Influence of dose of equine chorionic gonadotrophin (eCG) and timing of porcine luteinizing hormone (pLH) injections on sow reproductive performance (Experiments Two and Three)\*

	No. sows	Farrowing rate (%)	Litter size†
<b>Experiment Two</b>			
Control	43	55.8 <sup>a</sup>	10.9 ± 0.6
600 IU‡	56	67.9 <sup>ab</sup>	10.6 ± 0.5
900 IU‡	59	78.0 <sup>b</sup>	10.6 ± 0.4
<b>Experiment Three</b>			
Control	66	67.2 <sup>a</sup>	11.3 ± 0.4
72 hours§	107	79.4 <sup>b</sup>	10.5 ± 0.3
80 hours§	116	76.7 <sup>ab</sup>	11.0 ± 0.3

\* Experiment Two: primiparous sows were treated at weaning with 600 IU or 900 IU eCG (Pregnenol; Bioniche Animal Health, Belleville, Ontario) followed by 2.5 mg pLH (Lutropin-V; Bioniche Animal Health) 80 hours later, and then a single insemination of pooled semen containing  $3 \times 10^9$  sperm at 36 hours after pLH treatment. Experiment Three: mixed-parity sows received an IM injection of 600 IU eCG at weaning, then 2.5 mg pLH either 80 or 72 hours later,<sup>22</sup> followed by a single insemination of pooled semen ( $3 \times 10^9$  sperm) 36 or 30 hours after pLH treatment, respectively. Controls received no hormone treatment and were inseminated at detection of natural estrus and again 24 hours later.

† Mean ± SE for total litter size born.

‡ Dose of eCG.

§ Interval between eCG and pLH injections.

<sup>ab</sup> Means with no common superscript differ ( $P < .05$ ; linear mixed-effect model for litter size and chi-squared test for farrowing rate).

determined that the interval between LH injection and ovulation is approximately 38 hours.<sup>5,16</sup> In Experiment Three, the times of insemination relative to ovulation were different for each treatment group, but it was anticipated that treatment groups would be inseminated 2 or 8 hours prior to ovulation, a timing difference unlikely to impact fertility.<sup>26</sup> Further, although insemination of sows at estrus detection and 24 hours later is an accepted commercial practice, it is possible that many of the control sows in the present studies were late ovulators (prolonged estrus-to-ovulation interval) and that the breeding protocol should have been extended to include a possible third insemination. However, in the absence of ultrasound evidence of timing of ovulation, this suggestion remains speculative.

In Experiment Two, the 900-IU dose of eCG resulted in a significantly higher farrowing rate than for sows in the control group, with the sows receiving the 600-IU dose of eCG being intermediate. The tendency for a better response to the higher eCG dose may indicate that the ovaries of weaned primiparous sows are less sensitive to hormonal stimulation than those of older sows, and so may require a higher dose of eCG to stimulate the onset of estrus and ovulation. Alternatively, it is possible that primiparous sows would have lower circulating endogenous LH concentrations than those of older sows as a result of likely lower nutrient intakes during the previous lactation, a situation known to be associated with reduced circulating LH concentrations.<sup>27,28</sup> Since LH is required for follicular development beyond 4 mm,<sup>7</sup> a low circulating level of endogenous LH may limit sow responses to eCG, as has been suggested to occur in the prepubertal gilt.<sup>7,10</sup> Since eCG does have some LH-like activity,<sup>4,8,9</sup> the higher eCG dose may have provided sufficient LH-like activity to stimulate more sows to undergo follicle development to the point of estrus and ovulation. The present results further suggest that the interval between eCG and pLH treatments in the estrus-ovulation induction protocol for single timed insemination can be successfully reduced from 80 to 72 hours. This altered timing will allow producers to undertake treatments during normal working hours and so allow a more widespread adoption of the single timed insemination protocol.

## Implications

- On the basis of the present results, when treating gilts and primiparous sows with the eCG product Pregnenol, the dose should be 900 IU rather than the more commonly used 600 IU.
- A protocol of an eCG-induced estrus combined with a pLH-induced ovulation allows the maintenance of fertility following a single insemination, likely due to optimal timing of insemination relative to ovulation.
- The interval between eCG and pLH treatments can be reduced to 72 hours without detriment to sow fertility.

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