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Synchronization of ovulation in cyclic gilts with porcine luteinizing hormone (pLH) and its effects on reproductive function

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Abstract

The overall objective was to evaluate the use of porcine luteinizing hormone (pLH) for synchronization of ovulation in cyclic gilts and its effect on reproductive function. In an initial study, four littermate pairs of cyclic gilts were given altrenogest (15 mg/d for 14 d). Gilts received 500 μg cloprostenol (Day 15), 600 IU equine chorionic gonadotropin (eCG) (Day 16) and either 5 mg pLH or saline (Control) 80 h after eCG. Blood samples were collected every 4 h, from 8 h before pLH/saline treatment to the end of estrus. Following estrus detection, transcutaneous real-time ultrasonography and AI, all gilts were slaughtered 6 d after the estimated time of ovulation. Peak plasma pLH concentrations (during the LH surge), as well as the amplitude of the LH surge, were greater in pLH-treated gilts than in the control ($P = 0.01$). However, there were no significant differences between treatments in the timing and duration of estrus, or the timing of ovulation within the estrous period. In a second study, 45 cyclic gilts received altrenogest for 14–18 d, 600 IU eCG (24 h after last altrenogest), and 5 mg pLH, 750 IU human chorionic gonadotropin (hCG), or saline, 80 h after eCG. For gilts given pLH or hCG, the diameter of the largest follicle before the onset of ovulation (mean ± S.E.M.; 8.1 ± 0.2 and 8.1 ± 0.2 mm, respectively) was smaller than in control gilts (8.6 ± 0.2 mm, $P = 0.05$). The pLH and hCG groups ovulated sooner after treatment compared to the saline-treated group (43.2 ± 2.5, 47.6 ± 2.5 and 59.5 ± 2.5 h, respectively; $P < 0.01$), with the most synchronous ovulation ($P < 0.01$) in pLH-treated gilts. Embryo quality (total cell counts and embryo diameter) was not significantly different among groups. In conclusion, pLH reliably synchronized ovulation in cyclic gilts without significantly affecting embryo quality.

Keywords: Cyclic gilts; pLH; Estrus synchronization; Ovulation; Embryo quality

1. Introduction

Control of ovarian follicular development and ovulation offers practical advantages in livestock management and for development of reproductive technologies. Variation in the duration of standing estrus, which has direct implications for timing of ovulation, has been well established in swine [1]. Although a relationship between the time of ovulation after the onset of estrus, and estrus duration, has been reported in both sows [2] and gilts [3], no other factors seem to relate to the timing of ovulation [4]. Even though this relationship exists, Soede et al. [5] reported that substantial variation in the time of ovulation occurs during estrus (range of 39–133% in the duration of estrus). The recorded onset and duration of estrus also depends on the frequency of estrus detection [3] and the observational skills of the stockperson [5]. Therefore, the appropriate timing of insemination relative to ovulation can be problematic, yet has a large influence on subsequent fertility [5].

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Recent research has demonstrated that porcine luteinizing hormone (pLH) can reliably synchronize ovulation in weaned sows. These studies, typically involving a synchronization protocol with a 600 IU equine chorionic gonadotropin (eCG) on the day of weaning and 5 mg exogenous pLH 72–80 h later, resulted in average pLH treatment to ovulation intervals of 35.7 h [6], 38.2 h [7], and 39.2 h [8]. To date, little research has been conducted on the use of pLH in gilts. Furthermore, the work that has been published involved the use of this hormone for the induction of puberty [9].

It has been suggested that pLH improves the quality of the CL produced following ovulation stimulated by the pLH treatment in cattle [10]. In swine, an earlier rise in plasma progesterone concentrations following the onset of the LH surge was reported to be characteristic of the prolific Meishan sows when compared to Large White females [11]. Furthermore, metabolic effects on the timing of the rise in plasma progesterone concentrations in gilts and weaned sows have also been associated with reduced embryo survival [12]. Therefore, progesterone concentrations may be influenced by LH treatment, as in cattle, and this could influence embryonic survival.

In swine, the most established treatment protocol for controlling ovulation involves use of a combination of eCG to stimulate follicular growth, followed by human chorionic gonadotropin (hCG) to trigger ovulation [13]. However, a review of the literature revealed many variations on such procedures [14], suggesting that no single approach has produced consistent optimal results. As well, there is evidence that exogenous ovarian stimulation can be detrimental to reproductive function in gilts. In that regard, prepubertal gilts treated with eCG and hCG had decreased pregnancy rates [13]. Furthermore, it has been suggested that oocyte maturation may be influenced by exogenous hormones, such as hCG [15], which could reduce embryo quality and Ziecik et al. [16] reported that hormonally treated gilts had higher proportions of degenerated embryos. Given the promising results that have been obtained using pLH in weaned sows [6–8,17], the objective of the present research was to evaluate the efficacy of exogenous pLH for ovulation induction in cyclic gilts and its effects on subsequent reproductive function.

2. Materials and methods

2.1. Gilts

These studies were conducted in accordance with Canadian Council on Animal Care guidelines and were approved by the University of Alberta Faculty Animal Policy Welfare Committee. Littermate gilts (Hypor, Landrace × Large White) were selected based on their weight (difference of ≤15 kg) and the synchrony of their first estrus. Gilts were housed in individual crates and fed at 1.5× maintenance, with maintenance calculated as 112× kcal ME kg^{-1} $BW^{0.75}$ [18], where ME is metabolic energy in the diet and BW is body weight in kilograms. Gilts were exposed to 12 h light and 12 h dark each day.

2.2. Experiment 1

2.2.1. Altrenogest treatment and surgery

Four littermate pairs of gilts, that had completed their first estrous cycle, were given oral altrenogest treatment (15 mg/d; Regu-mate™, Intervet Canada Inc., Whitby, ON, Canada) for 14 d to synchronize their subsequent estrus. Upon withdrawal of altrenogest, gilts were checked for estrus twice a day (approximately 08:00 and 16:00), using 5-min periods of fence-line contact with two mature boars. Following altrenogest treatment, on Day 10 of the estrous cycle (Day 0 = onset of standing estrus), gilts underwent surgery for the insertion of an indwelling jugular catheter via the cephalic vein, as previously described [19].

2.2.2. Hormonal stimulation

Gilts within a littermate pair were randomly assigned to either control or pLH treatments. After a 4 d post-surgery recovery period, gilts received two im treatments of 500 μg cloprostenol (Estrumate, Schering-Plough Animal Health, Point-Claire, QC, Canada), 8 h apart. Twenty-four hours after the first cloprostenol treatment (Day 15), gilts received 600 IU eCG im (Folligon, Intervet Canada Inc., Whitby, ON, Canada). Then, 80 h after the eCG treatment (Day 18), gilts received either 5 mg pLH im (Lutropin-V, Bioniche Animal Health Canada Inc., Belleville, ON, Canada) or saline, depending on their assigned treatment.

2.2.3. Blood sampling and luteinizing hormone assay

Initially blood samples (3 mL) were collected, every 12 h from surgery (Day 10) until Day 18 of the estrous cycle. Then, 8 h prior to pLH treatment, sampling frequency increased to every 4 h until the end of standing estrus. Samples were centrifuged for 15 min at 2500 × *g*, the plasma transferred into separate tubes, and stored at −30 °C until analysis.

Plasma pLH concentrations were assayed in triplicate using 200 μL samples and a double antibody radioimmunoassay (RIA), as described by Wellen [20].

A second assay, in which high potency samples were diluted 5- or 10-fold, was also performed. The intra-assay coefficient of variance was 9.8 and 8.1% for the original and the rerun assays, respectively, and the inter-assay variance was 13.6%. The average sensitivity across the two assays, defined as 84% of total binding, was 0.10 ng/mL. Plasma LH concentrations are expressed as nanogram equivalents of the purified pLH preparation AFP12389A (A.F. Parlow, Harbor UCLA Medical Center, 1000 West Carson Street, Torrance, CA, USA) with a stated potency of 2.2 × NIH S1.

Amplitude of the LH secretory episodes was defined as the relative increase from baseline LH concentrations. To calculate the point at which the LH surge had commenced, a method similar to that described by Roski [21] was used. Briefly, the average LH baseline concentration was calculated for each gilt using all samples taken at 12-h intervals, and the standard deviation around the mean was calculated. The occurrence of consecutive samples that were two standard deviations above the LH baseline was then considered to be the point at which the LH surge had begun. Once LH concentrations had consistently declined below this level, the LH surge was deemed to be complete.

2.2.4. Determination of ovulation timing and breeding

Estrus detection and transcutaneous real-time ultrasonography was performed every 8 h (08:00, 16:00, and 24:00) beginning 8 h before pLH/saline treatment. Estimated time of ovulation was determined as described by Almeida et al. [22] adjusted for frequency of ultrasonography. The relationship between duration of estrus and timing of ovulation was determined by dividing the time between onset of estrus and ovulation by the total duration of estrus (% estrus). Gilts treated with pLH were inseminated 32 and 40 h after treatment with pooled Duroc semen processed on-site. Insemination doses contained 3×10^9 motile and morphologically normal spermatozoa, extended in 50 mL Beltsville thawing solution (Minitube of America, Verona, WI, USA). Control gilts were inseminated 16 h following initial detection of standing estrus, and then every 24 h with the same source and dose of semen, until the gilt was no longer exhibiting estrus. All inseminations were performed with semen that was <3 d old.

2.2.5. Embryo collection and classification

At Day 6.4 (±0.3) of gestation (ovulation = Day 1), gilts were euthanized on site by captive bolt stunning, followed by exsanguination. Reproductive tracts were recovered and ovulation rate was determined by the number of CL observed. The broad ligament was cut away from the uterus, starting at the cervix and moving towards the oviduct. An incision was made at the bottom of the uterine body near the intersection with the cervix, and a modified 10 mL syringe cylinder was inserted into the incision and secured in place. A 25 mL volume of a pre-warmed (∼35 °C) phosphate buffered saline (PBS) containing 5 mg/mL of bovine serum albumin (BSA) was injected into each uterine horn via the oviduct at the utero-tubal junction. The PBS solution was then massaged through the uterus until it drained out through the modified syringe into a 50 mL plastic conical tube (Falcon tube, Becton Dickinson Labware, Franklin Lakes, NJ, USA). This procedure was performed twice per uterine horn. The flush solution was kept in a warmed, insulated box to maintain its temperature at ∼35 °C. Embryos were identified under a dissecting microscope (Leica MZ 12_5, Leica Microsystems Ltd., Switzerland), harvested and classified by developmental stage at 25–50× magnification. Embryo classification was based on criteria described by Machaty et al. [23]. Morula-stage embryos were those with many blastomeres, but no blastocoel. Embryos with a small blastocoel and thick zona pellucida were classified as early blastocysts. Embryos with many blastomeres, a large blastocoel, but a thick zona pellucida, were classified as non-expanded blastocysts. Embryos with a very large blastocoel that made the embryo transparent in appearance and a very thin zona pellucida were classified as expanded blastocysts. Embryos that had a visible blastocoel and had clearly stretched the zona pellucida, but had since compacted, were classified as de-expanded blastocysts. Embryos that were no longer encased in a zona pellucida, regardless of the degree of expansion, were classified as hatched blastocysts.

2.3. Experiment 2

2.3.1. Hormonal treatment

Starting between Days 3 and 16 of their second or third estrous cycle, 15 sets of three littermate gilts were given oral altrenogest treatment (Regu-mate™, 15 mg/d) for 14–18 d to synchronize their subsequent estrus. Littermates completed altrenogest treatment at the same time, were treated with 600 IU eCG im (Folligon) on the following day, and then randomly assigned to receive either 5 mg pLH im (Lutropin-V), 750 IU hCG im (Chorulon, Intervet Canada Inc., Whitby, ON, Canada), or saline im, 80 h after eCG treatment.

2.3.2. Determination of ovulation timing and breeding

Onset of estrus and ovulation following hormonal stimulation were determined as for Experiment 1. The pLH- and hCG-treated gilts were inseminated 32 h after the pLH/hCG treatment, and if ovulation was not yet confirmed, again at 40 h. Control gilts were inseminated with the same source and dose of semen as treated gilts, 16 h following the initial detection of standing estrus, and then every 24 h until ovulation was confirmed. Source and processing of semen for AI were as described for Experiment 1.

2.3.3. Embryo collection, classification and staining

At Day 6 of gestation (mean Day 5.9 ± 0.03, with ovulation considered to be Day 1), gilts were euthanized on site. Reproductive tracts were recovered, ovulation rate was determined, and embryos were harvested from the uterus and classified as described for Experiment 1. Prior to the uterine flush, each oviduct was flushed to determine if any embryos or unfertilized oocytes were retained in this portion of the tract. This involved a retrograde flush of the oviducts using two 10 mL volumes of warmed PBS, as previously described [24].

All blastocyst stage embryos were viewed under conventional bright-field microscopy using an inverted microscope (Olympus IX81-UCB-2, Tokyo, Japan) at 200× magnification and three-dimensional images were obtained using Metamorph software (Version 7.1.0.0, Molecular Devices, Sunnyvale, CA, USA) to determine the degree of expansion. If expanded blastocysts were present, a subset of these blastocysts from each gilt was stained using a previously described differential staining procedure utilizing the two fluorochromes, propidium iodide and bisbenzimide, to visualize trophectoderm (TE) and inner cell mass (ICM) cells [25]. Once again, Metamorph software was used to obtain three-dimensional images of each stained embryo and for blastomere counting procedures.

2.3.4. Embryo scoring

To evaluate embryonic development within a litter, embryos were subjectively scored using the method described by Vinsky et al. [26], similar to the method used by Machaty et al. [23]. Briefly, non-fertilized and degenerated embryos were given a score of 2, early stage embryos scored 4, morula stage embryos scored 6, non-expanded blastocysts scored 8, and expanded, de-expanded, and hatched blastocysts scored 10. The sum of the scores for individual embryos within a litter was calculated, generating an overall litter gilt score. Higher scores indicated that a large number of well-developed embryos were recovered. Lower scores indicated poor fertilization, less developed embryos, and fewer embryos recovered.

2.3.5. Blood sample collection and progesterone assay

At euthanasia, 2–5 mL blood samples were collected by jugular venipuncture from a subset of gilts ($n = 30$; 10 littermate sets) into heparinized tubes (1000 IU heparin/mL solution, one or two drops per tube). Samples were centrifuged for 15 min at $2500 \times g$, and the plasma was transferred into separate tubes and stored at approximately −30 °C until analysis. Samples were assayed using the Coat-A-Count® Progesterone radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA, USA,). Plasma samples were diluted 20-fold in assay buffer, and a 100 μL aliquot of diluted sample was taken to assay in triplicate on the basis of preliminary assays with undiluted plasma. Assay sensitivity defined as 89.6% to total bound was 0.01 ng per tube and all samples were within the sensitivity of the assay. The intra-assay coefficient of variation was 3% and a serially diluted plasma pool showed parallelism to the standard curve.

2.4. Statistical analysis

All data were verified for normality and equality of variance using the PROC UNIVARIATE and PROC GLM (Bartlett and Levene tests) procedures in SAS (SAS Institute, Cary, NC, USA, 1990). Data that met the assumptions of typical statistical tests were analyzed using the PROC MIXED procedure. Other data were transformed to a normal distribution and then tested using the PROC MIXED procedure. All proportional data were arcsin transformed before analysis. The data were analyzed as a randomized complete block design with no replication within the blocks. The fixed effect was considered to be the treatment (pLH, hCG or saline), the random effect in the model was the pairs of littermates, and the experimental unit was the gilt. For Experiment 2, length of altrenogest treatment was tested as a covariate and included when significant. Given that the experimental unit was the gilt, individual embryo parameters (diameter and total cell count) were averaged within each litter, and the averages were used to evaluate the effect of treatment. Correlations between embryonic characteristics were evaluated using the CORR procedure. All results given are the least square of the means and standard errors calculated by SAS

Table 1
Least square means (±S.E.M.) for plasma LH surge characteristics, and for the relationship between the surge and estrus and ovulation in gilts treated with saline (Control) or pLH (Experiment 1)

	Control ($n = 4$)	pLH ($n = 4$)
LH baseline (ng/mL)	0.5 ± 0.1	0.7 ± 0.1
Duration of LH surge (h)	29.0 ± 3.1	32.0 ± 3.1
Peak of LH surge (ng/mL)	6.3 ± 1.7a	11.8 ± 1.7b
Amplitude of LH surge (ng/mL)	5.9 ± 1.2a	11.0 ± 1.2b
Onset of LH surge to estrus (h)[a]	−7.4 ± 3.9	−5.2 ± 3.9
Onset of LH surge to ovulation (h)[b]	−45.4 ± 2.3	−39.4 ± 2.3
Peak of LH surge to ovulation (h)[b]	−33.4 ± 2.7	−33.4 ± 2.7
Treatment injection to onset of LH surge (h)[c]	−1.0 ± 2.8	1.0 ± 2.8
Treatment injection to peak of LH surge (h)[d]	−13.0 ± 4.6	−5.0 ± 4.6

LH potencies were expressed as nanogram equivalents of the purified pLH preparation AFP12389A, with a stated potency of 2.2 × NIH S1. Values without a common superscript (a and b) differed ($P = 0.01$).
[a] Onset of estrus as time 0.
[b] Time of ovulation as time 0.
[c] Onset of the LH surge as time 0.
[d] LH surge as time 0.

following PROC MIXED analysis and were considered statistically significant at $P < 0.05$.

3. Results

3.1. Experiment 1

3.1.1. Plasma LH concentrations

Characteristics of the endogenous or pLH-enhanced plasma LH surge are shown (Table 1). The onset of the endogenous LH surge consistently preceded the onset of standing estrus; ovulation occurred 39.4–45.4 h after the defined onset of the natural or pLH-enhanced LH surge, and 33.4 ± 2.7 h after the peak of the natural or pLH-enhanced LH surge. Interestingly, exogenous pLH was consistently given before the start of the endogenous LH surge. There was no difference between treatments in the duration of the LH surge. The maximum plasma pLH concentration reached at the peak of the LH surge, as well as the amplitude of the LH surge, were greater in pLH-treated gilts than in the littermate controls ($P = 0.01$).

Evaluation of individual pLH profiles from littermate pairs (Fig. 1A and B) revealed that the exogenous pLH treatment consistently increased both peak plasma LH concentrations and the amplitude of the LH surge. Although pLH treatment did not affect onset of the LH surge, variation in the interval between treatment and the time peak LH was greater in saline-treated controls than the pLH-treated gilts ($P = 0.01$).

3.1.2. Estrus, ovulation timing and embryo data

All gilts in the study were of similar metabolic state and day of gestation at the time of euthanasia,

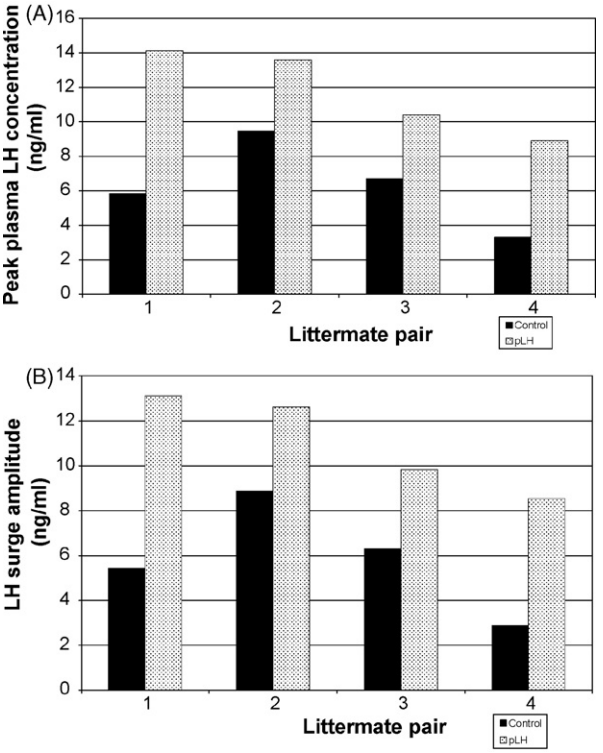

Fig. 1. Plasma LH concentrations in gilts treated with saline (Control) or pLH (Experiment 1). (A) Peak plasma LH concentrations during the endogenous or pLH-enhanced LH surge for each gilt; (B) amplitude of the endogenous or pLH-enhanced LH surge for each gilt.
Plasma LH concentrations are expressed as nanogram equivalents of the purified pLH preparation AFP12389A, with a stated potency of 2.2 × NIH S1.

Table 2
Least square means (±S.E.M.) for characteristics of estrus and ovulation in gilts treated with saline (Control), pLH, or hCG

	Experiment 1		Experiment 2		
	Control ($n = 4$)	pLH ($n = 4$)	Control ($n = 15$)	pLH ($n = 15$)	hCG ($n = 15$)
Weight at euthanasia (kg)	155.1 ± 4.2	155.9 ± 4.2	143.1 ± 2.1	139.2 ± 2.1	145.1 ± 2.1
Duration of estrus (h)	51.6 ± 5.6	49.9 ± 5.6	48.9 ± 4.1	43.4 ± 4.2	41.5 ± 4.1
Onset of estrus to ovulation (h)	38.0 ± 3.9	34.2 ± 3.9	38.8 ± 3.5[a]	32.7 ± 3.6[b]	32.8 ± 3.5[b]
% Onset of estrus to ovulation[*]	73.9 ± 7.1	70.3 ± 7.1	80.6 ± 5.7	73.9 ± 5.9	81.8 ± 5.7
First insemination to ovulation (h)	17.5 ± 2.8[a]	6.0 ± 2.8[b]	17.6 ± 2.3	11.0 ± 2.3	15.2 ± 2.3
Second insemination to ovulation (h)	−3.8 ± 3.3	−2.1 ± 2.9	7.1 ± 2.4	3.5 ± 1.6	8.0 ± 1.6
Insemination closest to ovulation (h)	11.9 ± 2.2[a]	4.0 ± 2.2[b]	7.9 ± 1.5	3.5 ± 1.5	7.7 ± 1.5
Saline/pLH/hCG to ovulation (h)	46.4 ± 3.7[a]	38.4 ± 3.7[b]	59.5 ± 2.5[c]	43.2 ± 2.5[d]	47.6 ± 2.5[d]
Largest measured follicle (mm)	N/A	N/A	8.6 ± 0.2[e]	8.1 ± 0.2[f]	8.1 ± 0.2[f]

Within an experiment and row, values without a common superscript (a and b) tended to differ ($P = 0.06$). Within an experiment and row, values without a common superscript (c and d) values differed ($P = 0.001$). Within an experiment and row, values without a common superscript (e and f) values differed ($P < 0.05$).

[*] % Estrus was calculated by expressing the interval between onset of estrus and ovulation as a proportion of the total duration of estrus.

as manifested by their weights. There were differences between treatments in the duration of estrus and timing of ovulation within the estrous period (Table 2).

Duration of standing estrus following hormonal stimulation treatments was similar to control gilts, with an overall mean of 2.1 ± 0.2 d (50.7 ± 3.7 h), with no difference in the timing of ovulation in relation to the duration of estrus. The interval between pLH treatment and ovulation averaged 38.4 ± 3.7 h following treatment and tended to occur later in the control gilts (46.4 ± 3.7 h; $P = 0.06$). Consequently, the interval from AI to ovulation interval tended ($P = 0.06$) to be shorter for pLH-treated gilts (4.0 ± 2.1 h) than controls (11.9 ± 2.2 h). There were no significant differences between groups in any reproductive end points assessed after euthanasia (Table 3).

3.2. Experiment 2

3.2.1. Estrus and ovulation timing

The duration of estrus following hormonal stimulation did not differ among the groups, nor did the timing of ovulation in relation to onset of estrus. However, there was a littermate effect ($P = 0.01$), for duration of standing estrus, which then tended to influence the interval between onset of estrus and ovulation ($P = 0.06$). Ovulation occurred earlier ($P < 0.001$) in the pLH and hCG groups (43.2 ± 2.5 and 47.6 ± 2.5 h after treatment, respectively) when compared to control gilts (59.5 ± 2.5 h), although the duration of standing estrus did not differ (Table 2). The ranges in ovulation timing following treatment were 35.6–52.1 h for the pLH group, 36.2–67.3 h in the hCG group, and

Table 3
Least square means (±S.E.M.) for reproductive performance and characteristics of recovered embryos in gilts treated with saline (Control), pLH, or hCG

	Experiment 1		Experiment 2		
	Control ($n = 4$)	pLH ($n = 4$)	Control ($n = 15$)	pLH ($n = 15$)	hCG ($n = 15$)
Day of gestation	6.1 ± 0.2	6.6 ± 0.2	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
Ovulation rate	18.5 ± 3.2	23.5 ± 3.2	19.3 ± 1.5	20.3 ± 1.5	17.5 ± 1.5
Ovarian cysts	0.8 ± 0.6	2.0 ± 0.6	0.7 ± 0.4	0.5 ± 0.4	0.8 ± 0.4
Total embryos	15.3 ± 3.7	21.3 ± 3.7	15.3 ± 1.2	16.1 ± 1.2	13.7 ± 1.2
% Embryo recovery	83.7 ± 6.5	88.4 ± 6.5	80.7 ± 3.9	78.8 ± 3.8	78.9 ± 3.4
Non-fertilized and degenerated	1.0 ± 2.2	3.8 ± 2.2	3.7 ± 1.5	2.1 ± 1.5	2.9 ± 1.5
Early embryos (2–4 cell to morula)	3.0 ± 2.1	0.3 ± 2.1	0.9 ± 0.4	0.3 ± 0.4	0.3 ± 0.4
Early blastocysts	2.0 ± 1.9	2.0 ± 1.9	0.5 ± 0.2	0.3 ± 0.2	0.5 ± 0.2
Non-expanded blastocysts	2.8 ± 1.1	2.5 ± 1.1	1.3 ± 0.6	1.9 ± 0.6	1.1 ± 0.6
Expanded blastocysts	6.3 ± 2.8	10.5 ± 2.8	6.9 ± 1.4	9.4 ± 1.4	6.6 ± 1.4
De-expanded blastocysts	0.0 ± 0.4	0.5 ± 0.4	0.1 ± 0.2	0.9 ± 0.2	0.4 ± 0.2
Hatched blastocysts	0.3 ± 1.0	1.8 ± 1.0	1.9 ± 0.9	0.7 ± 0.9	1.6 ± 0.9
Progesterone (ng/mL; $n = 30$)[*]	–	–	20.4 ± 1.6	17.9 ± 1.6	20.0 ± 1.6

[*] Significant covariate (ovulation rate) $P < 0.001$.

36.1–83.8 h in the control group. Furthermore, overall variance of the treatment–ovulation interval differed among treatments ($P < 0.01$; Fig. 2), with the least variation in the pLH-treated group. The average diameter of the largest follicles prior to ovulation was greater ($P = 0.05$) in the control gilts (8.6 ± 0.2 mm) than in the pLH- and hCG-treated gilts (8.1 ± 0.2 and 8.1 ± 0.2 mm, respectively).

The majority of the hormonally stimulated gilts received two inseminations (28 out of 30), with the second insemination being very close to the time of ovulation (Table 2). However, with the treatments and AI protocols used, there was no difference in the timing of the last insemination prior to ovulation among control (7.9 ± 1.5 h) and treated gilts (3.5 ± 1.5 and 7.7 ± 1.5 h in pLH- and hCG-treated gilts, respectively).

3.2.2. Progesterone concentrations

There were no significant differences among treatments in progesterone concentrations on the day of euthanasia (Day 6 of gestation; Table 3). There was variation in plasma progesterone concentrations among individuals (data not shown); however there were no characteristic differences among littermate sets. Plasma progesterone concentrations at Day 6 of gestation were positively correlated with ovulation rate ($r = 0.75$, $P < 0.001$), with the strongest correlation in hCG-treated gilts.

3.2.3. Embryo results

After euthanasia at Day 5.9 (± 0.03) of gestation, no unfertilized oocytes or embryos were recovered from oviductal flushings. Number of ovulations, occurrence of ovarian cysts, total number of embryos recovered, and recovery rate of embryos did not differ among treatments (Table 3). The number of ovulations ranged from 13 to 32 in control gilts, 13 to 40 in pLH-treated gilts, and 11 to 32 in hCG-treated gilts. Total embryos recovered ranged from 8 to 23 in control gilts, 9 to 33 in pLH-treated gilts, and 7 to 20 in hCG-treated gilts.

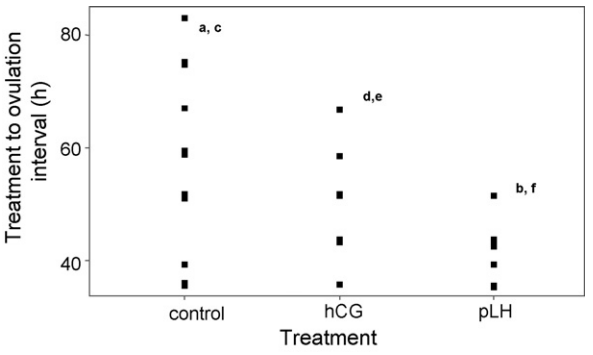

Fig. 2. Variation in timing of ovulation in gilts following treatment with saline (Control), hCG, or pLH (Experiment 2).
[a,b]Values without a common superscript differed ($P = 0.001$); [c,d]values without a common superscript differed ($P < 0.05$); [e,f]values without a common superscript tended to differ ($P = 0.06$).

There were no differences among treatments in the developmental stages of embryos collected at Day 5.9 (± 0.03) of gestation (Table 3). However, an effect of litter was observed on the number of expanded blastocysts recovered ($P = 0.05$). The total numbers of blastocysts evaluated by developmental stage were as follows: 16 early blastocysts, 64 non-expanded blastocysts, 321 expanded blastocysts (194 of which were used for total cell counts), 21 de-expanded blastocysts, and 60 hatched blastocysts. Effective embryonic staining was obtained; however, consistent differential staining of the ICM and TE was difficult to achieve, so total cell counts were used. Embryo diameter of unhatched blastocysts and total cell number of expanded blastocysts was not affected by treatment (Table 4). There was a strong correlation between cell number and diameter of expanded blastocysts (Fig. 3; $r = 0.88$, $P < 0.0001$); this relationship was strongest for embryos from control gilts ($r = 0.90$).

Length of altrenogest treatment was evaluated as a covariate in the statistical model, and was a significant correlation for the number of expanded blastocysts recovered ($P = 0.02$). However, when evaluating the length of altrenogest treatment following luteolysis, assuming luteolysis at Day 15 of the estrous cycle [27], the effect of the length of residual altrenogest treatment was no longer a significant covariate.

There was little overall heterogeneity in embryonic development and no difference in embryonic heterogeneity scores among treatments (Table 4; Fig. 4). Most embryos recovered were at the blastocyst stage of development, and no relationship could be established between the number of expanded and hatched blastocysts recovered and ovulation rate, or embryo recovery rate. There was no difference among treatment groups in the number of non-fertilized, degenerated, very early stage, or advanced stage embryos collected (Table 4). As well, there was a range embryonic development within litters from all treatments.

4. Discussion

The studies described here are, to our knowledge, the first to demonstrate the efficiency of exogenous pLH treatment for inducing ovulation in cyclic gilts. Based on endocrine data from Experiment 1, even though the onset of the LH surge was not affected by the pLH

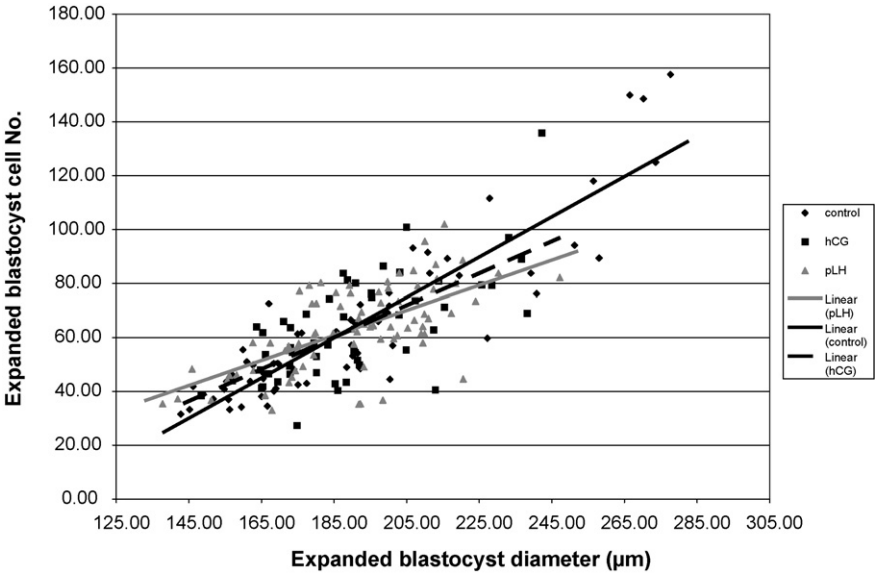

Fig. 3. Relationship between individual expanded blastocyst diameter and total cell number (separated by treatment) in gilts treated with saline (Control), pLH, or hCG (Experiment 2).

treatment, the surge was most synchronous in pLH-treated gilts. It has been suggested that follicles only require a specific threshold of LH stimulation to initiate ovulation [28]. Although the peak concentration of LH during the surge may not be critical, the timing of ovulation is closely related to the timing of peak LH [28]. Therefore, pLH treatment should initiate an earlier and more synchronized ovulation. Although there appeared to be a reduction in variation in the interval between treatment and peak LH concentrations for pLH-treated gilts in this experiment, due to the limited number of gilts used, further studies are needed before firm conclusions can be made.

The average treatment to ovulation interval for pLH-treated gilts in Experiment 1 was similar to that observed for weaned sows [6–8,17], but was somewhat longer for the gilts in Experiment 2, regardless of treatment. The variation between these groups may be a consequence of the surgical cannulation done in Experiment 1. As well, Experiment 2 was carried out during an altrenogest-synchronized estrous cycle, whereas Experiment 1 was conducted during the subsequent cycle after receiving cloprostenol treatment. In previous studies, reproductive characteristics have not been affected by altrenogest treatment [14]. However, when this parameter was included as a covariate in the analysis, it influenced the number of expanded blastocysts recovered, and the overall number of expanded blastocyst was significantly lower after 18 d of altrenogest treatment when compared to 14 d ($P = 0.02$; data not shown). Altrenogest treatment prevented preovulatory follicular growth [29], resulting in an extended interval to ovulation and possibly producing 'aged' oocytes at the time of ovulation. Therefore, the length of altrenogest treatment may need to be considered when optimizing future synchronization protocols.

There was no significant difference between pLH- and hCG-treated gilts for the average interval between

Table 4
Least square means (±S.E.M.) for embryo characteristics, as measured using Metamorph, in gilts treated with saline (Control), pLH, or hCG (Experiment 2)

	Control	pLH	hCG
Early blastocyst diameter (μm; $n = 10$)	116.8 ± 8.0	114.7 ± 8.0	119.5 ± 6.9
Non-expanded blastocyst diameter (μm; $n = 17$)	159.3 ± 6.0	151.2 ± 6.0	155.8 ± 6.6
Expanded blastocyst diameter (μm; $n = 36$)	190.0 ± 7.7	189.2 ± 6.8	193.3 ± 7.7
Total expanded blastocyst cell number ($n = 35$)	64.3 ± 6.7	62.9 ± 5.7	67.9 ± 6.4
Litter score[a]	114.4 ± 14.0	136.8 ± 14.0	106.7 ± 14.0

n Value in brackets indicates the total number of gilts that contributed the embryos used in the analysis.
[a] Litter score is the total of individual embryo scores within a litter; scoring was based on the method of Vinsky et al. [26].

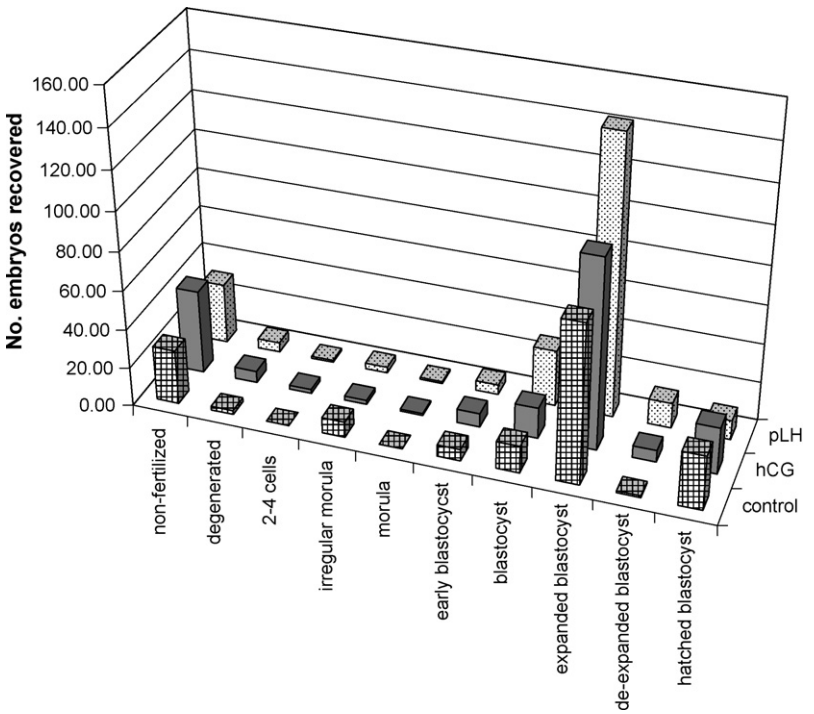

Fig. 4. The stage of the embryos recovered for each treatment at Day 5.9 (±0.03) of gestation in gilts treated with saline (Control), pLH, or hCG (Experiment 2).

hormone treatment and ovulation. However, the reduced variation in the treatment–ovulation interval for pLH-treated gilts compared to either hCG-treated or control gilts, was of particular interest. This is a major consideration for fixed-time AI protocols, as pLH-treated gilts would be more likely to receive a properly timed insemination. Despite some variation among gilts in response to treatment, it was small enough to allow for one insemination to be done very close to the time of ovulation, which will optimize fertilization. For gilts with a known stage of the estrous cycle, pLH could be given late into the follicular phase (Day 18) to induce ovulation. However, when the status of the gilt is unknown, pLH should be combined with other synchronization techniques, to ensure that pLH is given at an appropriate time.

In Experiment 2, treatment had no effect on plasma progesterone concentrations at Day 6 of gestation; although progesterone concentrations were influenced by ovulation rate, ovulation rate did not differ among treatments. The relationship between the number of CL and progesterone concentrations was similar to that observed by Knox et al. [30]. Therefore, pLH had no apparent effect on the capacity of the resulting CL to produce progesterone at this stage of gestation. However, the time of sampling in this study may not have been ideal to allow for detection of physiologically critical differences in progesterone concentrations. Based on evaluation of the diameter of the largest follicle present prior to ovulation in this study, hormonal stimulation induced smaller follicles to ovulate when compared to control gilts. This brings into question the potential quality of the oocytes and embryos resulting from this protocol. Therefore, the effect of hormone treatment on resulting embryo quality was also evaluated. Various approaches have been used to evaluate embryonic quality, including morphology, rate of development, cell counting, and rates of apoptosis [17,22,31,32]. Differential staining of the ICM and TE cells has been established for bovine and mouse embryos [25,28,31] and has also been applied to porcine embryos [19,23,33]. In our laboratory, effective embryonic staining was obtained; however, consistent differential staining of the ICM and TE was difficult to achieve. Regardless, total cell counts have been used on numerous occasions to evaluate embryo quality [23,32–34]. In the present study, total cell counts for expanded blastocysts did not differ among treatments, suggesting that embryo quality was not compromised by hormonal stimulation, even though the size of the follicles being ovulated was smaller. This is encouraging for those who employ embryo transfer procedures, as hormonal

control of estrus and ovulation are inherent components of these protocols. It was also determined that expanded blastocyst diameter correlates well with total cell number. However, this relationship may not extend as effectively to de-expanded or hatched blastocysts. During the present experiments, the degree of expansion among de-expanded and hatched blastocysts varied greatly and could change within a matter of hours.

A recent study by Vinsky et al. [26], identified a subpopulation of sows which produced very heterogeneous litters with regard to embryo development. This finding was not supported by the present study in which there was general homogeneity within the litters, with most gilts producing embryos at an advanced stage of development. Vinsky et al. [26] also found a relationship between the number of advanced blastocysts recovered and the overall embryo recovery rate. It was unclear if this effect was due to the possibility these unrecovered embryos remained in the oviduct and could not be collected by uterine flush, or whether it was a true loss or destruction of the embryos that were not recovered. In the present study, retrograde flushing of the oviduct did not reveal any additional oocytes or embryos, indicating that embryos not recovered by uterine flushing were not missed because they were in the oviduct. Therefore, although the interval to ovulation in the pLH-treated gilts was less variable in this study, this did not translate into differences among treatments in embryo heterogeneity.

Overall, we concluded that pLH was an appropriate product to use in synchronization protocols for cyclic gilts, resulting in consistent and reliable timing of ovulation following treatment. Reduced variability in the treatment to ovulation interval represented an important benefit of pLH treatment compared with hCG. These results also illustrated the potential of this product for use in fixed-time AI protocols in which only one insemination, 30–40 h after pLH treatment, would be needed. A lack of any detectable effects on embryo quality was also encouraging for embryo transfer procedures, in which large numbers of well developed, viable embryos are required. The use of pLH in these situations should be explored further, in order to establish reliable techniques that can be used in a production setting.

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