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Induction and synchronization of ovulation in sows using a Gonadotropin-releasing Hormone Analog (Lecirelin)

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Abstract

The GnRH agonist, Lecirelin (Gestran Plus®; ARSA S.R.L., Buenos Aires, Argentina), was tested for the ability to induce and synchronize ovulation in sows. Sows were uniformly allocated in two groups (n = 56, each) according to parity (2-6), weaning to estrus interval (WEI) and body condition score (BCS). The GnRH analog (25 µg, 1 ml) and saline solution (control group) were injected at estrus onset. Estrus detection and transcutaneous real-time ultrasonography were performed every 8 h. Duration of estrus for the control and treated groups was 66.3 ± 1.3 h and 61.3 ± 1.3 h ($P < 0.007$), respectively. Interval from estrus onset to ovulation (IEO) was 44.3 ± 1.2 h and 39.9 ± 1.2 h ($P < 0.012$) for the control and treated groups, respectively. Up to 40 h after treatment administration, 70.9% of Lecirelin sows had ovulated whereas 48.2% of control sows ovulated in the same period ($P < 0.01$). There was a tendency for a greater proportion of Lecirelin sows (92.7%) to ovulate during the 48-h period post treatment compared to control sows (82.4%). Reproductive performance (farrowing rate, number of total piglets born, piglets born alive, stillborn piglets and mummified fetuses) was not affected ($P > 0.05$) by induction of ovulation with Lecirelin. Based on the results found, it can be concluded that Lecirelin injection at estrus onset reduces estrus length and interval between estrus onset and ovulation with no influence on the subsequent reproductive performance.

Keywords: GnRH analog, Lecirelin, ovulation, reproductive performance, sows.

Introduction

On average, ovulation takes place at 35 to 45 h after onset of estrus (standing response in the presence of a boar; Soede *et al*., 2003). However, the variability in time of ovulation among sows is large, between 10 and 85 h (Weitze *et al*., 1994) or 10 and 58 h (Soede *et al*., 1995). Therefore, this variability represents a challenge in determining a reliable AI schedule. Unfortunately, no physical changes or behavioral parameters allow for a practical way to predict the moment of ovulation (Soede and Kemp, 1997).

A pharmacological induction of ovulation with GnRH agonists or pLH seems to be the most effective way to synchronize ovulation allowing the use of fixed time AI (Kirkwood, 2008; Brüssow *et al*., 2009). Synthetic gonadotropin-releasing hormone (GnRH) and their derivatives are used for stimulation and synchronization of ovulation in pigs (Webel and Rippel, 1975; Von Kaufmann and Holtz, 1982; Brüssow *et al*., 1990; Baer and Bilkei, 2004; Taibl *et al*., 2007) and minimize the variability in the time interval between onset of estrus and ovulation (IEO). Several protocols using GnRH agonists were proposed for swine (Von Kaufmann and Holtz, 1982; Brüssow *et al*., 1990; Knox *et al*., 2003). Lecirelin is a synthetic hypothalamic hormone, a superanalog of GnRH of prolonged action, which is obtained through the modification of gonadorelin's structure (Baruselli *et al*., 2001). However, its efficacy on synchronization of ovulation in weaned sows has not been reported yet. The objective of the present study was to determine the effect of GnRH analog (Lecirelin) on synchronizing and advancing the time of ovulation in weaned sows and the consequences on the reproductive performance.

Materials and Methods

The experiment was conducted on a breeding farm, in the west of Santa Catarina state, Brazil, from January to March 2009. A total of 112 weaned sows (Camborough®) of parity 2-6 were selected for the experiment. After weaning, sows were placed in individual crates and checked for estrus every 8 h (1:00 AM, 9:00 AM and 5:00 PM). Estrus detection was performed in the presence of a sexually mature boar, using the standing reflex in response to back pressure. Duration of estrus (DE) corresponded to the period between the first observed standing reflex minus 4 h and the moment where standing reflex was no longer observed minus 4 h. Based on parity, body condition score (BCS), weaning-to-estrus interval (WEI) and lactation length, sows were paired and then randomly assigned to

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one of the two groups. Sows in group A (n = 56) were treated with 25 µg (1 ml; i.m.) of Lecirelin (Gestran Plus®; ARSA S.R.L., Buenos Aires, Argentina). Control group (B, n = 56) received an i.m. saline (1 ml) injection. Lecirelin and saline were injected at estrus onset (at the first observed standing reflex).

In order to determine the ovarian status, transcutaneous real-time ultrasonography (Weitze *et al*., 1994) was performed using a 5 MHz Aloka (Aloka Co., Ltd., Mure, Mitaka-shi, Tokyo 181-8622, Japan) convex linear transducer. Ultrasonographic evaluations started at estrus onset and were performed every 8 h (2:00 AM, 10:00 AM, and 6:00 PM), always by the same technician. Ovulation time was defined as the first examination where no pre-ovulatory follicles were observed minus 4 h. One additional ultrasound examination was performed at the next estrus detection to confirm that ovulation had occurred. The IEO was measured from the time when the female was first detected in estrus, minus 4 h, to the time when no follicles were observed, minus 4 h.

The first insemination was performed 12 h after estrus onset. Sows were subsequently inseminated at 12 h intervals as long as they exhibited standing estrus (for the three first inseminations and at 24 h intervals thereafter if estrus persisted). Sows were inseminated with semen doses containing 3.0 x 10^9 sperm cells diluted in Beltsville Thawing Solution (total volume of 90 ml), which were stored up to a maximum of 48 h after collection.

Farrowing rate (FR) was calculated based on the number of females that farrowed as a proportion of those inseminated. Number of total piglets born (TB), including piglets born alive (BA), stillborn piglets and mummified fetuses were recorded at farrowing.

All statistical analyses were performed with SAS version 9.1.3 (SAS, 2005). Variables such as parity, lactation length, WEI, DE, and ovulation time were submitted to analysis of variance by GLM procedure. GLM procedure was also used for the analyses of TB and BA with number of total born piglets and piglets born alive at previous farrowing included, respectively, as covariates in the model of analysis. Stillborn and mummified piglets were analyzed with NPAR1WAY procedure and groups were compared by Wilcoxon test. Percentages of sows ovulating at different intervals after the estrus onset were calculated by FREQ procedure and compared by chi-square test. Percentages of sows that returned to estrus after insemination and of sows that farrowed were compared by chi-square test.

Results

Sows were inseminated with a mean of 3.4 ± 0.05 doses of semen, and all of them received at least one insemination within 24 h before ovulation. Parity, BCS, WEI and lactation length were similar for the two groups (Table 1). The DE was 5.0 h shorter and ovulation was advanced in 4.4 h in the Lecirelin group. Up to 32 h after onset of estrus no differences were found (P = 0.22) between control and Lecirelin groups regarding percentage of sows that ovulated (16.1 and 25.4%, respectively; Fig. 1). However, up to 40 h after estrus onset more sows (70.9%) of Lecirelin group ovulated (P < 0.01) than in control group (48.2%). A trend of more sows ovulating in Lecirelin than in control group (92.7 vs. 82.4%) was also seen at 48 h after estrus onset (P > 0.09).

From 112 inseminated sows none were removed due to non-reproductive reasons. Return to estrus rate, abortion rate, farrowing rate, TB, BA, stillborn piglets and mummified fetuses were not different (P > 0.05) between groups (Table 2).

Table 1. Least square means (±SEM) for characteristics of weaned sows treated with saline (control) or GnRH agonist (Lecirelin) at the estrus onset.

	Control	Lecirelin*	P-value
Number of sows	56	56	-
Parity	4.3 ± 0.2 (2-6)	4.3 ± 0.2 (2-6)	1.0
Body condition score	2.9 ± 0.04 (2.5-3.5)	3.0 ± 0.05 (2-4)	-
Lactation length (days)	22.1 ± 0.2 (19-27)	22.6 ± 0.3 (20-30)	0.2
Weaning-to-estrus interval (h)	90.4 ± 1.6 (76-132)	90.3 ± 1.6 (76-132)	0.9
Duration of estrus (h)	66.3 ± 1.3 (48-88)	61.3 ± 1.3 (16-80)	0.007
IEO (h)	44.3 ± 1.2 (24-64)	39.9 ± 1.2 (16-64)**	0.01

IEO = interval from estrus onset to ovulation. Range values are presented within parentheses.
*25 µg of Lecirelin (Gestran Plus®) i.m. (1ml);
**One sow was not examined by ultrasound.

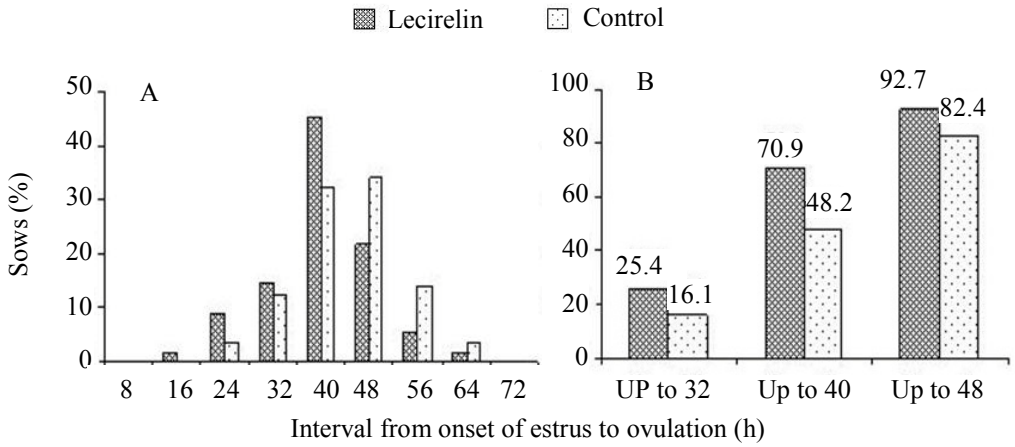

Figure 1. (A) Percentage of sows ovulating in different intervals after estrus onset ($P > 0.05$). (B) Cumulative percentage of females ovulating up to 32 h ($P = 0.22$), up to 40 h ($P < 0.01$) and up to 48 h ($P > 0.09$).

Table 2. Reproductive performance of weaned sows submitted to a GnRH agonist (Lecirelin) at estrus onset.

	Control	Lecirelin***	P-value
Number of sows	56	56	
Farrowing rate, %	92.9	92.9	1.0
Return to estrus rate, %	1.8	5.4	0.3
Abortion rate, %	5.4	1.8	0.3
Total born*	13.7 ± 0.4	13.4 ± 0.4	0.4
Born alive*	11.8 ± 0.4	11.9 ± 0.4	0.6
Stillborn, %**	8.6 ± 1.0	8.2 ± 1.2	0.7
Mummified, %**	4.8 ± 0.9	4.0 ± 1.3	0.2

*LSMeans ± SEM.
**Means ± SEM.
***25 µg of Lecirelin (Gestran Plus®) i.m. (1ml).

Discussion

As the optimum breeding time is difficult to predict, controlling time of ovulation is the only approach whereby an optimal interval between AI and ovulation may be achieved (Martinat-Botté e*t al*., 2009), allowing a fixed time insemination (Taibl *et al*., 2007). Several GnRH analogues have been evaluated for ovulation induction in swine (Knox *et al*., 2003; Brüssow *et al*., 1990) as buserelin (Möller-Holtkamp *et al*., 1995), goserelin (Brüssow *et al*., 2007) and triptorelin (Taibl *et al*., 2007). All of them are effective at stimulating preovulatory luteinizing hormone (LH) secretion in both gilts and sows (Brüssow *et al*., 2009). In the present study, the average estrus length (66.3 h) and IEO (44.3 h) in the control group were similar to those observed in other studies performed with not hormonally treated multiparous sows (Weitze *et al*., 1994; Soede *et al*., 1995; Kemp and Soede, 1996; Knox and Rodriguez-Zas, 2001). Sows of the GnRH agonist group showed a decrease of 5 h for DE compared to control group. Although DE was not affected in another study in which ovulation was synchronized with pLH (Degenstein *et al*., 2008), it was decreased when a GnRH-agonist was used (Knox *et al*., 2003; Baer and Bilkei, 2004).

GnRH agonist-induced LH surge can be of short duration (180-240 min) compared to the natural surge, which induces a serum LH elevation for longer than 12 h (Gooneratne *et al*., 1989). A positive aspect is that induced LH surge does not interfere with the natural preovulatory surge, and may even act in conjunction enhancing its effects by prolonging the duration of elevated LH, as well as by increasing the total amount of LH released by the anterior pituitary prior to ovulation (Gooneratne *et al*., 1989). The decrease of 4.4 h for IEO observed in Lecirelin sows confirms the effect of GnRH agonists on the anticipation of ovulation previously reported (Brüssow *et al*., 1996, 2007, 2009; Baer and Bilkei, 2004; Kauffold *et al*., 2007), showing that they are able to stimulate adequate surge of LH from the pituitary gland to induce ovulation (Brussow *et al*., 2007). Advancement of ovulation to an IEO of approximately 40 h is similar to intervals observed in other studies, in which an intravaginal GnRH agonist (100 µg of triptorelin) was administered at the onset of estrus (Knox *et al*., 2003) or 50 µg of gonadorelin was used

80 h after eCG injection (Brüssow *et al*., 1990). The degree of synchronization of ovulation with the use of GnRH agonists can even be higher as it was shown by Martinat-Botté *et al*. (2009), who administered 10 µg of buserelin 94 h post-weaning and observed 100% of the sows ovulating within 24 h compared to 69% of controls.

Our results are in accordance with those of other experiments in which GnRH has been shown to increase the percentage of sows ovulating in a specific time interval (Brüssow *et al*., 1996; Taibl *et al*., 2007; Martinat-Botté *et al*., 2009). Although Lecirelin anticipated time of ovulation, there is a major consideration for fixed-time AI protocols, which is the degree of ovulation synchronization. Then, it remains to be established if the synchrony obtained is suitable for the use of fixed-AI protocols. It is generally recommended to inseminate swine females whose ovulation has been induced with GnRH analogues or hCG twice, i.e. 24 and 40 h after ovulation induction (Brüssow *et al*., 2009). Nevertheless, the use of a single insemination at a predetermined time seems to be more risky since the optimal time for insemination, to ensure good fertility in sows, is the interval 28 h before to 4 h after ovulation (Nissen *et al*., 1997). The use of a fixed-AI protocol was not tested in the present study but it seems that the use of a single insemination within 24-30 h after Lecirelin injection would allow for most of the induced females to be inseminated within an optimal interval insemination-ovulation.

Besides GnRH analogues, human chorionic gonadotropin (hCG; Hunter, 1967; Degenstein *et al*., 2008) and porcine LH (pLH; Candini *et al*., 1999; Cassar *et al*., 2005; Degenstein *et al*., 2008) can also induce ovulation. Compared to pLH, hCG injection does not provide adequate synchronization of ovulation (Cassar *et al*., 2005; Degenstein *et al*., 2008). Reduced variability in the treatment to ovulation interval represents an important benefit of pLH compared with GnRH (Cassar *et al*., 2005; Degenstein *et al*., 2008). However, pLH is a biological product that could have some restrictions for uses and cost is higher compared to GnRH.

According to De Rensis *et al*. (2003), if GnRH or hCG is employed to induce a predictable time of ovulation, the potential to improve the timing of insemination relative to the time of ovulation may enhance sow fertility. In the present study, however, the subsequent reproductive performance of sows was not affected by Lecirelin administration. It must be pointed out that multiple inseminations with 3 billion sperm doses were performed (overall average of 3.4 doses/sow) and all females received at least one insemination within 24 h before ovulation. Moreover, no sows with previous reproductive disorders, locomotor problems and low BCS were used in the experiment. The lack of a GnRH agonist treatment effect on conception rate (Gooneratne *et al*., 1989), farrowing rate (Martinat-Botté *et al*., 2009) or litter size (Gooneratne *et al*., 1989; Martins *et al*., 1996; Knox *et al*., 2003; Martinat-Botté *et al*., 2009) has been previously reported.

From these data, it could be concluded that Lecirelin injection at estrus onset reduces estrus length and interval between estrus onset and ovulation. Almost 93% of the females ovulate up to 48 h after Lecirelin injection. Subsequent reproductive performance, regarding farrowing rate and litter size, is not affected by Lecirelin use.

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