Ovulatory response of different GnRH analogues and subsequent corpus luteum lifespan in the presence of norgestomet in Holstein heifers

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Summary

This study investigated the effect of GnRH analogues on inducing ovulation of dominant follicle (DF) and subsequent corpus luteum (CL) lifespan in the presence of norgestomet implant in Holstein heifers. On day 6 to 8 of the estrous cycle (day 0 of the experiment), all heifers (n=15) received norgestomet implant followed by prostaglandin injection on days 0 and 1. On day 4, group 1 (n=4) and 2 (n=4) heifers received 12.5 and 25 μg Alarelin, respectively. Heifers in group 3 (n=3) received 10 μg Buserelin. Heifers in control group (n=4) did not receive any further treatment. Norgestomet was removed on day 8 in the treatment group and kept throughout the experiment in control group. From day 0, blood sampling and ultrasonography were performed. Heifers were monitored after inducing ovulation for 2 consecutive estrous cycles. DF ovulated within 33 h after GnRH injection. Progesterone started to rise on day 3 and declined on day 7.45 ± 0.28 after GnRH injection. Heifers in treatment groups displayed estrus 7.73 ± 0.24 days after GnRH injection followed by spontaneous ovulation. The lifespan of CLs were 5.36 ± 0.28 and 16.9 ± 0.37 days after inducing ovulation of DF in the presence of norgestomet and following spontaneous ovulation, respectively ($P<0.05$). In conclusion, the half dose of Alarelin induced ovulation of DF and the presence of single norgestomet implant after ovulation may be associated with short luteal lifespan.

Key words: Dominant follicle, GnRH, Norgestomet, Luteal lifespan, Progesterone

Introduction

GnRH, a decapeptide hormone that triggers a cascade of events leading to ovulation in mammals (Amoss *et al*., 1971; Matsuo *et al*., 1971), is being used extensively in breeding programs to control ovarian follicle development in cattle (Schmitt *et al*., 1996; Pursley *et al*., 1998). Because of the short half-life of endogenous GnRH (about 5 min), the development has been centered on producing agonists with a high affinity for the GnRH receptors and increased resistance to degradation or elimination resulting in the production of over 2000 different GnRH analogues (Karten and River, 1986).

The ovulatory response to GnRH analogues is variable depending on the stage of estrous cycle (Price and Webb, 1989), age (Pursley *et al*., 1995; Schmitt *et al*., 1996), nutrition (Murphy *et al*., 1991), heat stress (Ullah *et al*., 1996) and stage of the ovarian follicle growth (Pursley *et al*., 1995).

Maintaining dominant follicle for several days prior to induced ovulation is a suitable approach to reduce the variability of response to GnRH (Niasari-Naslaji *et al*., 1996; Rastegarnia *et al*., 2004). Maintaining dominant follicle could be achieved by replacing the CL with a single norgestomet implant (Kojima *et al*., 1992; Kinder *et al*., 1996). Under this circumstance, pulsatile secretion of LH is enhanced, resulting in the maintenance of dominant follicle. The objectives of this study were to investigate the ovulatory response of dominant follicles to different GnRH analogues and to evaluate the lifespan of CL after either ovulation of a dominant follicle (DF) in the presence of norgestomet or ovulation of a newly recruited DF without norgestomet in

Holstein heifers.

Materials and Methods

Experimental location and animals

The experiment was conducted at Veterinary Research Institute, Faculty of Veterinary Medicine, University of Tehran, Iran (Latitude: 35° 39' 8" N; Longitude: 51° 26' 38" E; Altitude: 1029 m) during October to December 2009. Cyclic and healthy Holstein heifers (n=15; 18.6 ± 0.91 months of age; 364 ± 11.95 kg weight) with regular estrous cycles were selected for this study. Heifers received a total mixed ration (TMR) of alfalfa hay (35%) and corn silage (15%) and concentrate including 25% barley, 5% cotton seed meal, 18% bran, 1% salt and 1% DCP.

Experimental design

Estrous cycle of heifers was synchronized using two consecutive injections of PG (500 µg Cloprostenol; Vetaprost®, Aburaihan, Iran), 14 days apart. On day 6-8 of the ensuing cycle (day 0 of the experiment; depending on estrus manifestation), heifers received norgestomet implant (3 mg, 17α-acetoxy-11β-metyl-19-norpreg-4-en-3, 20-dione, Crestar®, Intervet, Holland) in association with PG on days 0 and 1. On day 4 of the experiment, heifers in group 1 (n=4) and 2 (n=4) received 12.5 µg (2.5 ml) and 25 µg (5 ml) GnRH analogue (Alarelin, Vetaroline®, Aburaihan, Iran), respectively. Heifers in group 3 (n=3) received 10 µg (2.5 ml) GnRH analogue (Buserelin, Receptal®, Intervet, Holland). Norgestomet was removed on day 8 in all treatment groups but remained intact in the control group (n=4) throughout the experiment. From day 0, blood samples were collected daily to measure serum concentration of progesterone and ultrasound examinations were carried out every 4 days to follow the maintenance of DF. Ultrasonography was also conducted at 24, 36 and 48 h after GnRH injection to confirm ovulation. Heifers were monitored for 2 consecutive estrous cycles after inducing ovulation.

Ultrasound examination

Ovarian ultrasonography was performed as previously described (Pierson and Ginther, 1984) using a real time, B-mode scanner (SonoVet 2000, Madison, South Korea) equipped with a 7.5 MHz linear-array trans-rectal transducer. During each examination, a sketch of the ovary was made, recording the location and the diameter of the individually identified follicles ≥4 mm in diameter (Knopf *et al*., 1989) and CL. An ovarian follicle ≥10 mm in diameter was considered as morphologically dominant follicle (Knopf *et al*., 1989). The time that the dominant follicle disappeared was considered as the day of ovulation.

Blood sampling and plasma progesterone assay

Blood samples were collected by coccygeal venipuncture into 10 ml vacutainer tubes. Collected blood samples were centrifuged within 2 h at 1200 g for 15 min and serum samples were stored at -20°C until hormone analyses. Plasma concentrations of progesterone were determined according to single antibody radioimmunoassay (D'Occhio *et al*., 1988). The sensitivity of assay was 0.2 ng/ml and the inter and intra-assay coefficients of variation were 10.2% and 8.3%, respectively.

Statistical analyses

Changes in the serum concentrations of progesterone over time following induced and spontaneous ovulation were analysed using procedure GLM in SAS (SAS, 2001) with repeated measures analysis included in the model. Single-points measurements for follicle diameter and the lifespan of CL were compared using ANOVA. In a situation in which the assumptions of parametric tests were not achieved, Kruscal-Wallis one-way ANOVA of SAS was used. Data were presented as mean ± SEM.

Results

On day 0, there was a functional CL (diameter: 18.86 ± 1.22 mm; progesterone: 3.53 ± 0.43 ng/ml) and growing follicle (10.78 ± 0.55 mm in diameter) in all heifers. Progesterone declined to basal

concentrations (0.2 ng/ml) by day 2 after the first PG injection. On day 4, when heifers received GnRH analogue, there was a growing DF (14.75 ± 0.47 mm) in the ovary. This follicle ovulated within 33 h after GnRH analogue injection. There were no significant differences among the progesterone profile of the heifers treated with different kinds or doses of GnRH analogues (P>0.05); therefore, the data were pooled and reported. Progesterone started to rise on day 3 (0.51 ± 0.08 ng/ml), remained elevated for 4.09 ± 0.34 days and declined to basal level on day 7.45 ± 0.28 after GnRH analogue injection. Maximum concentration of progesterone (1.25 ± 0.18 ng/ml) was detected on day 4 after GnRH injection. Heifers in the treated groups displayed estrus 7.73 ± 0.24 days after GnRH analogue injection followed by spontaneous ovulation of the newly recruited dominant follicle (Fig. 1). Ovarian follicle of heifers in the control group became persistent throughout the study, while progesterone remained at basal concentration till day 20 of the experiment (Fig. 1), when one heifer displayed estrus followed by ovulation.

The length of subsequent estrous cycle was 19.73 ± 0.34 days with the maximum progesterone concentration of 6.27 ± 0.54 ng/ml occurring 14.27 ± 0.24 days after estrus. The lifespans of CL (from the day of ovulation to the day of CL regression) in the first estrous cycle, in the presence of norgestomet, was significantly shorter (5.36 ± 0.28 days) than the second estrous cycle (16.9 ± 0.37 days; P<0.05).

Discussion

This experiment demonstrates that a) two GnRH analogues, including Alarelin (12.5 or 25 μg) and Buserelin (10 μg), are effective in inducing ovulation of DFs in Holstein heifers; and b) the duration of luteal phase following formation of CL in the presence of norgestomet (for 4 days after ovulation) is shorter than that of CL formed in the absence of norgestomet implant.

The model used in the present study, including the replacement of CL with norgestoemt implant (Kojima *et al*., 1992; Savio *et al*., 1993; Niasari-Naslaji *et al*., 1996) was effective in maintaining DF responsive consistently to GnRH injection. This particular model removed variation in response to GnRH injection, consistent with our previous reports in cattle (Niasari-Naslaji *et al*., 1996) and buffalo (Rastegarnia *et al*., 2004). The ovarian

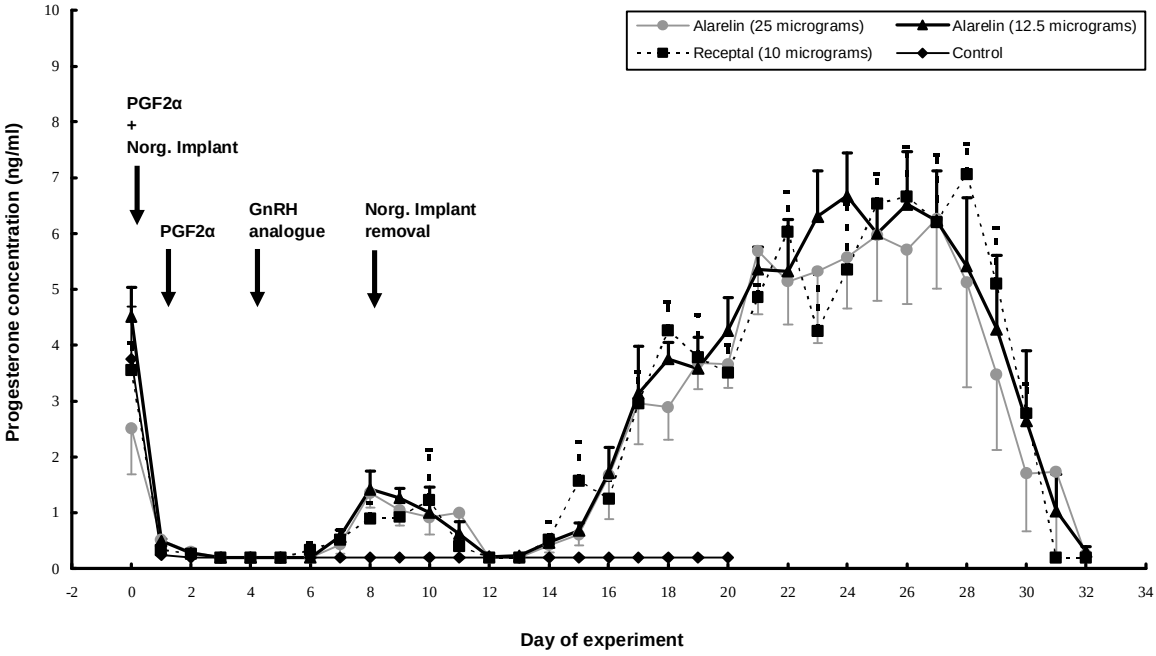

Fig. 1: Concentration of progesterone (ng/ml) following inducing ovulation of dominant follicle with different GnRH analogues in the presence of norgestomet implant and that of spontaneous ovulation of newly recruited dominant follicle

dominant follicle in the females of control group persisted throughout the experiment. This confirms the previous findings in cattle in which the replacement of corpus luteum with the low plasma concentration of progestagens, similar to pro-estrous, increased the pulse frequency of LH followed by the persistence of dominant follicle (Kojima *et al*., 1992; Savio *et al*., 1993; Niasari-Naslaji *et al*., 1996).

The largest ovarian follicles, present at the time of norgestomet insertion, were maintained and displayed progressive growth and attained the diameter of 14.75 ± 0.47 mm at the time of GnRH injection (4 days after norgestomet insertion). These DFs ovulated in response to different treatments of GnRH in all heifers, indicating that Alarelin has the potency to induce ovulation at half the recommended dose (12.5 µg). Treatment of lactating dairy cows with 100 µg of GnRH on day 3 to 8 of the cycle resulted in ovulation in 8/8 cows; while, treatment on days 0 to 2 or 9 to 14 of the cycle resulted in ovulation in 0/2 and 7/10 cows, respectively (Wiltbank, 1997). In another study, ovulation occurred in 93% (13/14) of Holstein heifers treated with Buserelin 5 days after estrus (Schmitt *et al*., 1996). The percentage of ovulation was 55.3, 76.7, 73.6 and 85.0% following injection of different GnRH analogues including: factrel, cystorelin, fertagyl, ovacyst, respectively, in lactating dairy cows (Souza *et al*., 2009). Administration of 100 µg cystorelin at the growing, static and regressing phases of the dominant follicle induced ovulation in 100, 33 and 1 percent of cows, respectively (Silcox *et al*., 1993). Although it is supposed that GnRH administration induce ovulation in growing dominant follicles (Pursley *et al*., 1995; Bergfeld *et al*., 1996; Moghiseh *et al*., 2008), ovulation occurred only in 50% of dominant follicles when fertagyl was injected during the growing phase in Holstein heifers (Moghaddam *et al*., 2002).

Short lived CLs have been detected at puberty in cattle (Gonzalez-Padilla *et al*., 1975) and sheep (Berardinelli *et al*., 1980), following the first postpartum ovulation in cattle (Odde *et al*., 1980), following induction of ovulation in anestrous ewes (Haresign *et al*., 1975) and also subsequent to inducing ovulation of small size ovulating follicles in cyclic cows (Peters and Pursly, 2003; Taponen *et al*., 2003). During the first days of the estrous cycle, down regulation of endometrial oxytocin receptors appears to be positively correlated to pre-ovulatory estradiol concentrations (Mann and Lamming, 2000). As a result, luteolytic episodes of PG do not occur early in the luteal phase (Mann and Lamming, 1995). Several reports showed that cows which developed short lived CL had less pre-ovulatory estradiol concentrations (Garverick *et al*., 1988). Therefore, cyclic cows that were induced to ovulate small follicles (with lower estradiol synthesis capacity) had premature PG secretion (Taponen *et al*., 2003) leading to short estrous cycles (Peters and Pursley, 2003). Accordingly, GnRH administered 24 h after PG could induce ovulation but resulted in the formation of short lived CL and ultimately reduced fertility (Taponen *et al*., 1999; Taponen *et al*., 2002).

Earlier studies in cow (Woody *et al*., 1967) and sheep (Ottobre *et al*., 1980) have shown that serial injections of progesterone, initiated at the end of estrous phase, decreased subsequent luteal lifespan. In other words, formation of CL in the presence of progesterone may provide the condition for premature luteolysis. In our experiment, norgestomet implant was inserted to prevent ovulation of dominant follicle, 4 days before inducing ovulation with GnRH analogues, and was maintained until 4 days after GnRH injection to evaluate the CL lifespan. One reason for decreased corpus luteum lifespan in our experiment might be due to the presence of norgestomet implant in the formative stage of corpus luteum during the first 4 days after GnRH injection. In conclusion, alarelin, at half the recommended dose, is capable of ovulating DF. Ovulation of DF in the presence of low concentration of progestogen (replacing CL with single norgestomet implant) may not be associated with the normal lifespan of CL, which is necessary for supporting early pregnancy in cattle.

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