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Summary

In postpartum cows, ovulatory follicle size at time of insemination (GnRH/TAI) influenced pregnancy rates following TAI, but had no effect on pregnancy rates when cows spontaneously ovulated. Furthermore, cows that exhibited estrus (± 24 h of GnRH/TAI) had higher pregnancy rates compared to cows not in estrus. The objective was to assess the relationship between ovulatory follicle size and estradiol concentrations, timing of the LH surge, timing of ovulation, and subsequent progesterone concentrations. Cows were synchronized with the CO-Synch (n = 64; induced ovulation) or the Select Synch (n = 20; spontaneous ovulation) protocol. Cows that exhibited estrus and were induced to ovulate medium (11.5-14 mm) or large (>14) follicles had preovulatory concentrations of estradiol similar (*P > 0.05*) to cows that spontaneously ovulated and higher (*P* < 0.05) than cows not exhibiting estrus. Cows not exhibiting estrus had lower (*P < 0.05*) preovulatory concentrations of estradiol compared to cows that spontaneously ovulated. There was no effect (*P > 0.36*) of follicle size or estrus on concentrations of LH. Among cows induced to ovulate, cows detected in estrus had a shorter (*P < 0.01*) interval from GnRH to the LH surge and ovulation compared to cows not exhibiting estrus. Cows that spontaneously ovulated had an intermediate interval from onset of estrus to the LH surge, but a shorter (*P = 0.02*) interval to ovulation compared to cows not exhibiting estrus. Cows that ovulated medium follicles had a longer (*P = 0.03*) interval to ovulation compared to cows that ovulated large follicles, with cows that ovulated small follicles (≤ 11 mm) intermediate. The rate at which concentrations of progesterone increased following ovulation was similar (*P* > 0.30) among cows that spontaneously ovulated, cows detected in estrus and cows not detected in estrus and induced to ovulate medium and large follicles. Concentrations of progesterone were lower in cows not detected in estrus and induced to ovulate small follicle compared to cows not detected in estrus and induced to ovulate medium or large follicles (*P < 0.08*), cows that spontaneously ovulated (*P < 0.07*), and cows detected in estrus and induced to ovulate medium follicles (*P < 0.01*). In summary, concentrations of estradiol, timing of the LH surge, timing of ovulation, and rate subsequent progesterone rose could explain the increased pregnancy rates in cows that exhibit estrus and are induced to ovulate compared to cows that do not exhibit estrus.

Introduction

Most fixed-time insemination protocols utilize gonadotropin-releasing hormone (**GnRH**) to induce ovulation. Gonadotropin-releasing hormone administered nine days before insemination (day -9) induces ovulation, corpus luteum (**CL**) formation, and initiates a new follicular wave. Two days before insemination (day -2) prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) is administered to induce luteolysis, and GnRH is administered to induce ovulation of the preovulatory follicle around the time of insemination (day 0). Insemination is performed at the time of the second GnRH injection (Geary and Whittier, 1998) or 16 to 24 hours after the second GnRH injection (Pursley et al., 1998).

Bovine follicles achieve ovulatory capacity at approximately 10 mm, however a larger dose of LH was required to induce ovulation of a 10 mm follicle compared to larger follicles (Sartori et al., 2001). Furthermore, a decrease in pregnancy rates (Lamb et al., 2001; Perry et al., 2005) and an increase in embryonic mortality (Perry et al.,

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2005) occurred in postpartum cows when small follicles were induced to ovulate following a fixed-time AI protocol. However, in postpartum beef cows ovulatory follicle size had no effect on fertility when ovulation occurred spontaneously following detection in standing estrus (Perry et al., 2005). Therefore, the objectives of this study were to assess the relationships between ovulatory follicle size and circulating concentrations of estradiol and LH and timing of ovulation.

Materials and Methods

Experimental Design

Postpartum multiparous (3 to 13 years old) Angus-crossed beef cows at the South Dakota State Uniersity Beef Breeding Unit were synchronized with the CO-Synch (Induced ovulation; n = 64) or the Select Synch (Spontaneous ovulation; n = 20) synchronization protocol in 4 replicates. Cows were injected with GnRH (100 μg as 2 mL of Ovasynch i.m.; Pheniox Scientific, St. Joseph Missouri) on day -9, and $PGF_{2\alpha}$ (25 mg as 5 mL of Prostamate i.m., Pheniox Scientific, St. Joseph Missouri) on day -2 (Select-Synch). Forty-eight hours after $PGF_{2\alpha}$ (day 0) cows in the CO-Synch group received GnRH (Ovasynch; 100 μg i.m.). Cows in each replicate were maintained as a single group and calves were allowed to suckle without restriction throughout the experiment.

Blood samples were collected by venipuncture into 10 mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA) every three hours from day -2 through day 2 and daily from day 2 through day 21. Blood was allowed to coagulate at room temperature, stored at 4°C for 24 hours, and centrifuged at 1,200 x *g* for 30 minutes. Serum was harvested and stored at -20°C until analysis was performed. Serum concentrations of progesterone, estradiol, and LH were analyzed in all samples by radioimmunoassay (**RIA**). Intra- and interassay coefficients of variation were 3.2% and 7.0%, 4.0% and 15.4%, 5.0% and 6.8%, for progesterone, estradiol and LH respectively.

Ovaries of all cows were examined by transrectal ultrasonography to characterize follicular development and to determine time of ovulation (day -2, day 0, and every four hour from 20 hours after GnRH or standing estrus through ovulation) using an Aloka 500V ultrasound with a 7.5 MHz linear probe (Aloka, Wallingford, CT). All follicles ≥ 8 mm in diameter were recorded. Follicle size was determined by averaging follicular diameter at the widest point and at a right angle to the first measurement using the internal calipers on the Aloka 500V. Ovulation was defined as the disappearance of a large follicle from an ovary.

Differences between follicle size groups in timing of ovulation and timing of LH surge were determined by analysis of variance in SAS (SAS Inst. Inc., Cary, NC). When the F statistic was significant ($P < 0.05$), mean separation was performed using least significant differences (Means ± SEM, Snedecor and Cochran, 1989). Circulating concentrations of progesterone, estradiol-17□, and LH were analyzed by analysis of variance for repeated measures in SAS (proc mixed, Littell et al., 1998). The statistical model consisted of follicle size and standing estrus, time, and their interactions. The effect of follicle size or standing estrus was analyzed using animal within treatment as the error term, and effects of time and any interaction were analyzed using the residual as the error term.

Results

There was an affect of treatment ($P < 0.01$), time ($P < 0.01$), and a treatment by time interaction ($P < 0.01$) on preovulatory concentrations of estradiol (Figure 1). More specifically, cows that did not exhibit estrus and were induced to ovulate small (≤11 mm) or medium (11.5-14 mm) follicles had lower ($P < 0.05$) preovulatory concentrations of estradiol compared cows that spontaneously ovulated. Cows not detected in standing estrus and induced to ovulate small or medium follicles also had lower ($P < 0.05$) preovulatory concentrations of estradiol compared to cows that exhibited estrus and were induced to ovulate small, medium, or large (> 14 mm) follicles. Cows not detected in standing estrus and induced to ovulate large follicles had lower ($P < 0.05$) preovulatory concentrations of estradiol compared to cows detected in standing estrus and induced to ovulate medium or large follicles and similar ($P > 0.05$) preovulatory concentrations of estradiol to cows detected in standing estrus and induced to ovulate small follicles. Preovulatory concentrations of estradiol did not differ ($P > 0.05$) among cows that exhibited estrus and were induced to ovulate and cows that spontaneously ovulated.

There were no detectable differences (*P* > *0.36*) among groups on circulating concentrations of LH (Figure 2). However, among cows induced to ovulate, cows that exhibited estrus had a shorter (*P* < *0.01*) interval from GnRH to the LH surge compared to cows not exhibiting estrus (Table 1). The interval from the onset of estrus until the LH surge was intermediate for cows that spontaneously ovulated. Estrus and follicle size also affected the interval from GnRH or onset of estrus to ovulation. Cows that did not exhibit estrus and were induced to ovulate had a longer interval to ovulation compared to cows that exhibited estrus and were induced to ovulate (*P* < *0.01*) and cows that spontaneously ovulated (*P* = *0.02*; Table 1). Furthermore, cows that ovulated medium follicles had a longer (*P* = 0.03) interval to ovulation compared to cows that ovulated large follicles (Table 1). Interval to ovulation was intermediate for cows that ovulated small follicles (≤ 11 mm).

There was a tendency (*P = 0.10*) for a treatment by time interaction of subsequent concentrations of progesterone (Figure 3). However, cows not detected in standing estrus and induced to ovulate small follicles had a slower rise in progesterone compared to cows not detected in standing estrus and induced to ovulate medium (*P = 0.05*) or large (P = *0.08*) follicles , cows that spontaneously ovulated (*P = 0.06*), and cows detected in standing estrus and induced to ovulate medium follicles (*P < 0.01*). There were no differences detected (*P > 0.30*) in the rate at which progesterone increased among cows not detected in standing estrus and induced to ovulate medium and large follicles, cows that spontaneously ovulated, and cows that were detected in standing estrus and induced to ovulate.

Discussion

The efficiency of timed-insemination protocols is dependent on precisely controlling the timing of ovulation, and for pregnancy to be maintained a proper uterine environment and adequate progesterone production by the subsequent CL must occur. In the present study, ovulation was induced by an injection of GnRH, however the interval from the GnRH injection until ovulation was influenced by both the ovulatory follicle size and if the animal had exhibited standing estrus. A longer interval from the GnRH injection (insemination) until ovulation could lead to decreased fertility. Sacke et al., (2000) reported that when insemination occurs before the onset of standing estrus (>30 hrs before ovulation), fertilization rates are low, but when insemination occurs >12 hours after the initiation of estrus (<18 hours before ovulation), fertilization rates are high. Furthermore, the timing of insemination after the onset of standing estrus can not only influenced fertilization rates but also influenced embryo quality (Dalton et al., 2001).

Preovulatory concentrations of estradiol may also play an important role in both preparing the uterus for pregnancy and in preparing follicular cells for luteal formation and function. Previous reports have shown cows that exhibit standing estrus around (± 24 hours) of fixed-time insemination had significantly higher pregnancy rates compared to cows that did not exhibit standing estrus (Perry et al., 2005). In the present study cows that were induced to ovulate and were detected in standing estrus had higher preovulatory concentrations of estradiol compared to cows not detected in standing estrus and induced to ovulate. In postpartum beef cows, preovulatory concentrations of estradiol-17β were lower preceding a short compared to a normal length luteal phase (Sheffel et al., 1982; Garcia-Winder et al., 1986; Garverick et al., 1988; Braden et al., 1989). Furthermore, reduced concentrations of estradiol-17β during the preovulatory period have been associated with decreased numbers of endometrial progesterone receptors during the early luteal phase (Zollers et al., 1993). Ovulation of follicles producing suboptimal preovulatory concentrations of estradiol may result in reduced numbers of endometrial progesterone receptors. Consequently, pregnancy maintenance may be decreased due to inadequate response of the endometrium to progesterone. Furthermore, ovariectomized ewes that did not receive an injection of estrogen corresponding with initiation of estrus before embryo transfer had decreased embryo survival (Miller and Moore, 1976), uterine weight, uterine protein, RNA to DNA ratio, and the rate of protein synthesis (Miller et al., 1977).

In addition to playing a role in preparing the uterus for pregnancy, increased preovulatory concentrations of estradiol likely plays a role in luteal progesterone production. Luteinized human granulosa cells secreted more progesterone when they were collected from follicles having increased follicular fluid

concentrations of estradiol compared to granulosa collected from follicles that had lower concentrations of estradiol (McNatty et al., 1979). In dairy cows, ovulation of small follicles resulted in the development of a smaller CL and lower serum progesterone concentrations (Vasconcelos et al., 2001). Furthermore, premature stimulation of ovulation by intrafollicular injections of LH or FSH reduced subsequent luteal progesterone secretion in ewes (Murdoch et al., 1983), and induced ovulation of small ovine follicles resulted in formation of small CL that had fewer large luteal-granulosa cells (Murdoch and Van Kirk, 1998). A decrease in CL size and progesterone production is believed to be related to a reduction in the number of granulosa cells present at the time of ovulation. Granulosa cells are generally believed to differentiate into large luteal cells (Smith et al., 1994) and approximately 80% of progesterone secreted by ovine corpora lutea is believed to be secreted by large luteal cells (Niswender et al., 1985). Therefore, a decrease in the number of granulosa/large luteal cells could influence circulating concentrations of progesterone.

Previous studies have reported cows treated with GnRH following detection in standing estrus had an LH surge of greater amplitude than cows that had a spontaneously induced LH surge (Kaim et al., 2003), and a GnRH-induced LH surge is of shorter duration when cows were not detected in standing estrus compared to cows that spontaneously initiated surge (Chenault et al., 1990). In the present study no differences were detected among treatments in serum concentrations of LH during the LH surge. This is likely due to a sample being collected only once every three hours in the present study.

Luteal progesterone secretion is required for maintenance of pregnancy (McDonald et al., 1952) and stimulates endometrial secretions (Geisert et al., 1992) as well as embryonic growth/development (Garrett et al., 1988; Mann et al., 1996). In previous studies cows induced to ovulate smaller follicles had significantly lower concentrations of progesterone beginning on day 9 after insemination and a slower rise in progesterone following insemination (Perry et al., 2005), and cows with normal developing embryos had higher concentrations of progesterone on days 3 and 6 after insemination compared to cows with degenerating embryos (Maurer and Echternkemp, 1982). In the present study there was a tendency for treatment to influence concentrations of progesterone, and cows not detected in standing estrus and induced to ovulate small follicle had a slower rise in progesterone following ovulation compared to cows not detected in standing estrus and induced to ovulate medium or large, cows that spontaneously ovulated, and cows detected in standing estrus and ovulated medium follicles. The rate at which concentrations of progesterone increase following ovulation can likely influence pregnancy rates. Cows that had an earlier rise in progesterone had embryos that were more advanced developmentally, produced more interferon τ (INF-τ) and were capable of inhibiting the $PGF_{2\alpha}$ release on day 16 after breeding, but cows that had a delayed rise in progesterone had less developed embryos, produced less IFN-τ, and were not capable of inhibiting the $PGF_{2\alpha}$ release on day 16 (Kerbler et al., 1997; Mann et al., 1999; Mann and Lamming, 2001). Furthermore, luteal progesterone secretion has been associated with fertility in cattle by stimulating endometrial secretions (Geisert et al., 1992). Endometrial secretions include nutrients, growth factors, immunosuppressive agents, enzymes, ions, and steroids contribute to early conceptus growth/survival (Geisert et al., 1992; Gray et al., 2001).

Implications

The most efficient and economical method for genetic improvement of economically important traits in the beef industry is artificial insemination with semen from genetically superior sires. However, cows induced to ovulate that have not been detected in standing estrus have decreased pregnancy rates compared to cows induced to ovulate that have exhibited standing estrus. This decrease in pregnancy rates might result from decreased preovulatory concentrations of estradiol, increased interval from GnRH until ovulation, or decrease rate of increase in subsequent concentrations of progesterone when cows that have not exhibited standing estrus are induced to ovulate.

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Tables

Table 1. Effect of standing estrus and follicle size on interval to LH surge and ovulation

	Estrus Status			Follicle Size (mm)		
	Standing Induced[a]	Not Standing Induced[b]	Spontaneous[c]	≤ 11[d]	11.5 to 14[e]	> 14[f]
Interval to LH surge (h)[g]	0.0 ± 0.79[x]	2.65 ± 0.71[y]	0.81 ± 1.76[xy]	1.6 ± 1.15	1.9 ± 0.74	0.14 ± 1.23
Interval to ovulation (h)[h]	25.75 ± 0.85[x]	28.97 ± 0.82[y]	25.83 ± 1.11[x]	26.5 ± 1.2[xy]	28.2 ± 0.6[x]	25.8 ± 0.9[y]

[a]Cow detected in standing estrus and inducted to ovulate
[b]Cow not detected in standing estrus and induced to ovulate
[c]Cow detected in standing estrus and spontaneously ovulated
[d]Cows that ovulated (induced or spontaneous) follicles ≤ 11 mm in diameter
[e]Cows that ovulated (induced or spontaneous) follicles 11.5 to 14 mm in diameter
[f]Cows that ovulated (induced or spontaneous) follicles > 14 mm in diameter
[g]Interval from GnRH injection (induced ovulation) or onset of standing estrus (spontaneous ovulation) to the onset of the LH surge
[h]Interval from GnRH injection (induced ovulation) or onset of standing estrus (spontaneous ovulation) to ovulation
[xy]Means within a row and category (estrus status or Follicle size) having different superscripts are different ($P \leq 0.03$)

Figures

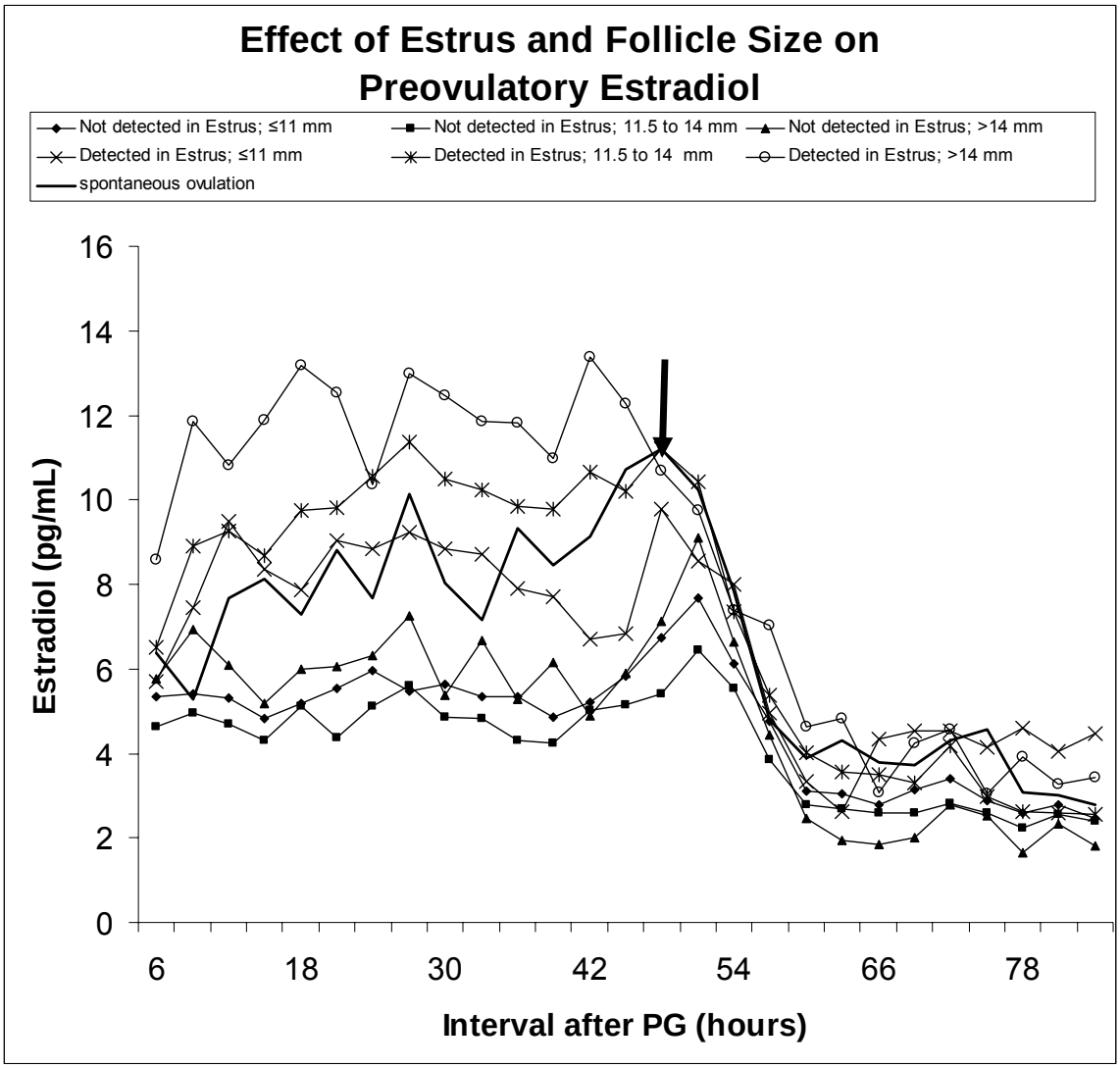

Figure 1. Influence of standing estrus and ovulatory follicle size on preovulatory concentrations of estradiol. Timing of the second GnRH injection for cows treated with the CO-Synch protocol is indicated by the arrow. (Treatment $P < 0.01$; Day $P < 0.01$; Treatment x Day $P < 0.01$).

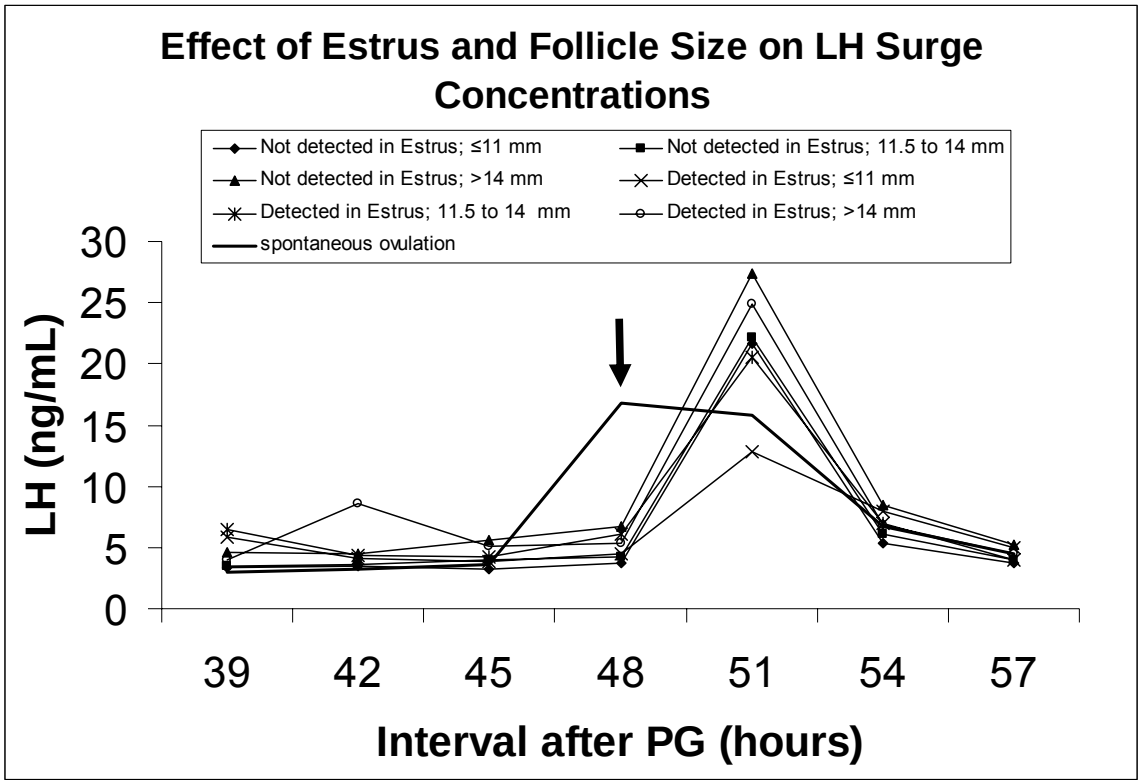

Figure 2. Influence of standing estrus and ovulatory follicle size on concentrations of LH. Timing of the second GnRH injection for cows treated with the CO-Synch protocol is indicated by the arrow. (Treatment $P = 0.74$; Day $P < 0.01$; Treatment x Day $P = 0.77$)

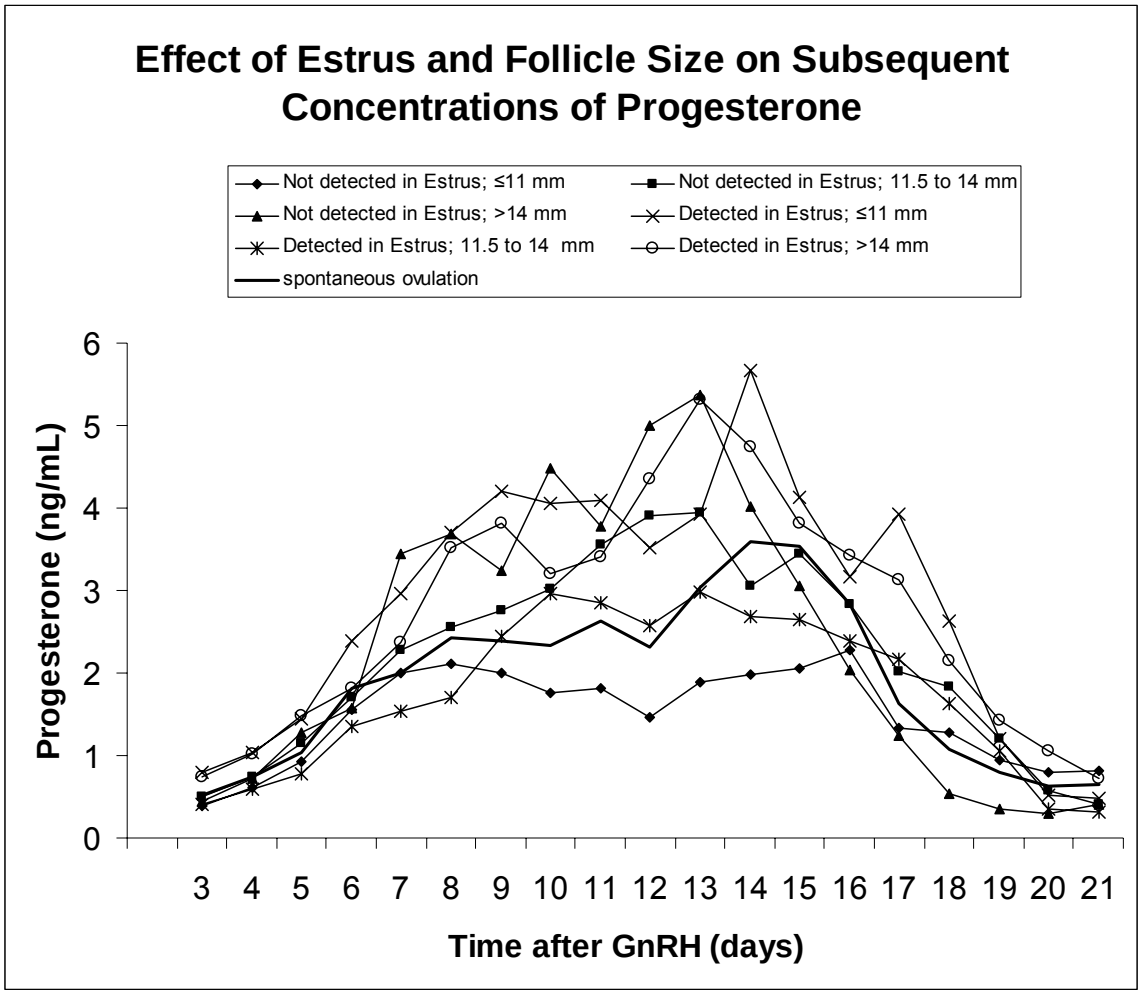

Figure 3. Influence of standing estrus and ovulatory follicle size on postovulatory concentrations of progesterone. (Treatment $P = 0.59$; Day $P < 0.01$; Treatment x Day $P = 0.10$)