Effect of Ovulatory Follicle Size on the Establishment and Maintenance of Pregnancy in Beef Cattle.

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Points to Remember

It is the physiological maturity of a dominant follicle and not dominant follicle size that affects pregnancy rates in postpartum beef cows following fixed-time artificial insemination.

Gonadotropin releasing hormone (GnRH)-induced ovulation of a physiologically immature dominant follicle decreases pregnancy rate and decreases late embryonic/fetal survival.

Decreased fertility following GnRH-induced ovulation of a physiologically immature dominant follicle was associated with reduced concentrations of estradiol at insemination and a lower rate of luteal progesterone secretion after insemination.

In postpartum beef cows, small dominant follicles generally arise from failure to synchronize a follicular wave at the start of an estrus synchronization protocol.

Strategies to increase the physiological maturity of a dominant follicle have focused on increasing gonadotropin stimulation to the ovulatory follicle during the preovulatory period by increasing the duration of proestrus, short term calf removal, and exogenous gonadotropin stimulation (PMSG/eCG).

Introduction

Although artificial insemination (AI) is the most powerful tool available for genetic improvement, cow calf producers in the USA have been slow to adopt this technology due to the time and labor associated with estrous detection and a market structure that until recently has not provided an incentive to cow calf producers to emphasize genetic improvement of their herds. However, development of estrus synchronization protocols that precisely synchronize the time of ovulation have increased the adoption of fixed-time artificial insemination (FTAI). In addition, a changing market structure in the USA that recognizes and provides an economic incentive for genetic improvement (e.g. premiums for high quality carcasses) has also contributed to the increased use of FTAI by commercial beef producers.

Current estrus synchronization protocols do a good job of synchronizing the time of ovulation; therefore, future research emphasis should focus on understanding the mechanisms that increase the physiological maturity of a dominant follicle prior to GnRH-induced ovulation, identifying factors that affect late embryonic/fetal mortality following FTAI, and identifying bulls that work well in FTAI protocols. The purpose of this paper is to review the effect of ovulatory follicle size in FTAI programs on pregnancy rates and late embryonic/fetal survival, discuss why physiologically immature follicles are present at FTAI, and to discuss some strategies to increase the physiological maturity of the ovulatory follicle.

Overview of synchronization of ovulation

Exogenous hormone regimes that precisely control timing of ovulation have been implemented in domestic livestock species and humans. In cattle, synchronization of estrus/ovulation and artificial insemination (AI) remain the most powerful technologies available to cattle producers for genetic improvement and reproductive management (Seidel, 1995). However, adoption of these technologies by beef producers has been relatively low due to the time and labor associated with estrous detection. Therefore, fixed-time AI (FTAI) protocols that eliminate estrous detection and permit insemination of heifers and cows at a predetermined time were developed and result in pregnancy rates that are similar to

insemination following detection of estrus. Furthermore, FTAI protocols increase the proportion of heifers and cows that conceive early in the breeding season which has important benefits for reproductive management and beef production.

Development of effective FTAI protocols in cattle requires control of the following physiological processes: 1.) Synchronization of a follicular wave following an ovulatory stimulus (e.g. gonadotropin releasing hormone [GnRH] injection) or induction of dominant follicle turnover (e.g. administration of estradiol or progesterone) culminating in development of a physiologically mature dominant follicle at insemination, 2.) Control of luteal lifespan via prostaglandin $F_{2\alpha}$ (PGF)-induced luteolysis, 3.) GnRH-induced ovulation of a physiologically mature dominant follicle, and 4) deposition of semen at the appropriate time relative to induction of ovulation. The preceding GnRH-PGF-GnRH injection sequence (Figure 1; Panel A) is based on the premise that the initial injection of GnRH will induce ovulation of a dominant follicle resulting in synchrony of a new follicular wave, followed by an injection of PGF seven days later to induce luteolysis. Approximately 60-72 hours following PGF injection a second injection of GnRH is administered to induce ovulation of a physiologically mature dominant follicle and insemination normally occurs at the second GnRH injection (Figure 1; Panel A). Essentially all FTAI protocols in the USA are variations of the GnRH-PGF-GnRH injection sequence with some differences in timing of insemination.

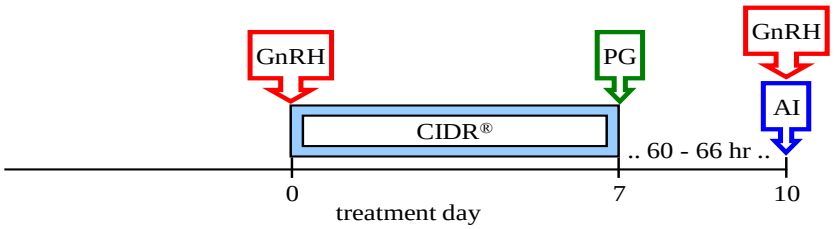
What is the effect of ovulatory follicle size on pregnancy in beef heifers and cows?

In *Bos tarus* and *Bos indicus* cattle, ovulatory capacity of a follicle is obtained between 7 and 10 mm in diameter (Sartori et al., 2001; Gimenes et al., 2008) and is associated with acquisition of LH receptors in granulosa cells. However, a larger dose of LH was required to induce ovulation in a 10 mm follicle verses larger sized follicles (Sartori et. al., 2001) suggesting a difference in the physiological maturity of small verses large dominant follicles.

A

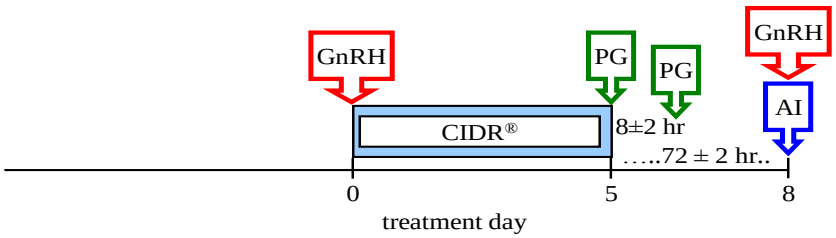
7-day CO-Synch + CIDR®

Perform TAI at 60 to 66 hr after PG with GnRH at TAI.



5-day CO-Synch + CIDR®

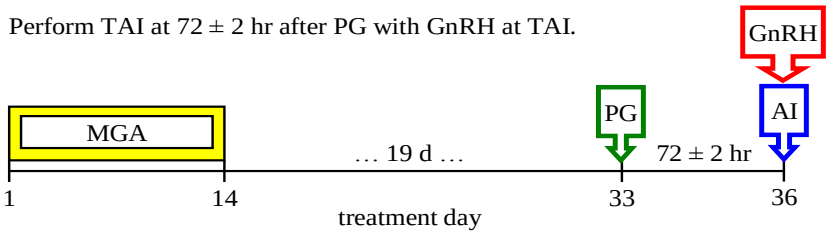
Perform TAI at 72 ± 2 hr after 1st PG with GnRH at TAI.
Two injections of PG 8 ± 2 hr apart are required for this protocol.



B

MGA®-PG

Perform TAI at 72 ± 2 hr after PG with GnRH at TAI.



14-day CIDR®-PG

Perform TAI at 66 ± 2 hr after PG with GnRH at TAI.

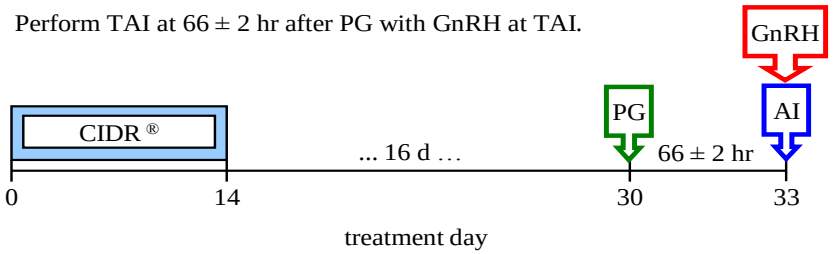


Figure 1. Current recommended methods for fixed-timed insemination (FTAI) in beef cows (panel A) and beef heifers (panel B). GnRH – gonadotropin releasing hormone; PG – prostaglandin $F_{2\alpha}$; CIDR – controlled internal drug release, MGA – melengestrol acetate (orally active progestin).

Ovulatory follicle size and pregnancy rates:

Ovulatory follicle size at GnRH-induced or spontaneous ovulation is variable in cattle (Table 1). In postpartum beef cows, ovulatory follicle size was 15.0 ± 0.3 mm (mean ± std) with a range of ≤ 12mm to ≥ 18mm (Lamb et al., 2001). In the preceding study, there was a significant decrease in pregnancy rate following GnRH-induced ovulation of follicles ≤ 12.0 mm regardless of treatment. Perry et al., (2005) also reported a decrease in pregnancy rates following GnRH-induced ovulation of small ovulatory sized follicles; however, there was no effect on pregnancy rate when follicles within the same size range ovulated spontaneously (Figure 2). In dairy cows, GnRH-induced ovulation of dominant follicles resulted in a quadratic relationship between follicle size and pregnancy establishment in which pregnancy rate increased with dominant follicle size to a certain follicle size and then decreased (Bello et al., 2006). Other investigators have also reported that induced ovulation of small physiological immature follicles reduced pregnancy rates in both beef and dairy cattle (Vasconcelos et al., 2001; Waldmann et al., 2006; Perry et al., 2007; Dias et al., 2009; Meneghetti et al., 2009; Peres et al., 2009; Sa Filho et al., 2009; Sa Filho et al., 2010; see Table 1).

Ovulatory follicle size and late embryonic/fetal mortality:

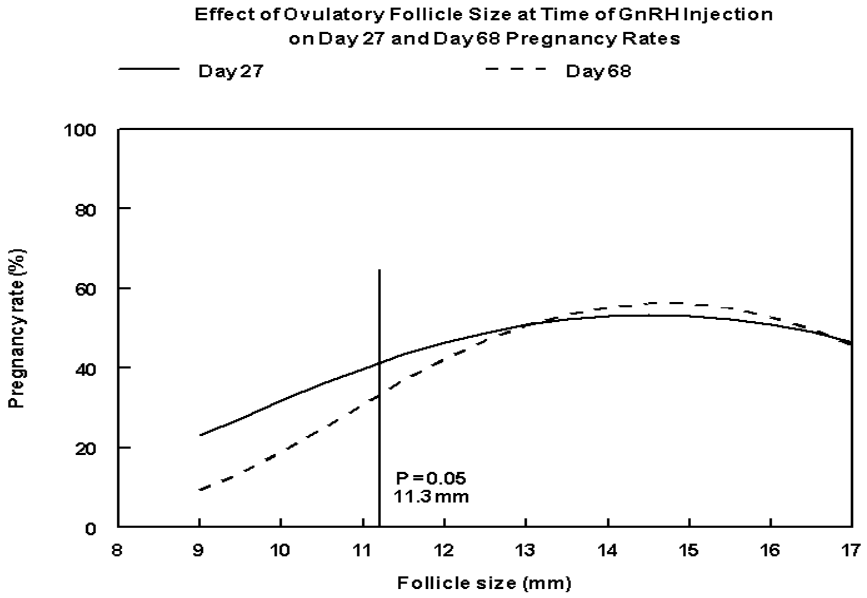
There was an increase in late embryonic/early fetal mortality (i.e. pregnancy loss between d 28 to 60) in cows in which ovulatory follicles < 11.3 mm were induced to ovulate; however, late embryonic/early fetal mortality was not related to ovulatory follicle size in cows that spontaneously ovulated (Figure 2; Perry et al., 2005). When lactating dairy cows were administered a second injection of GnRH and subsequent AI there was a 20.2% incidence of late embryonic/early fetal loss following confirmation of pregnancy on day 28, with a majority of the loss occurring between days 28 to 42 (Vasconcelos et al., 1997). This suggests that not only is there an effect of ovulatory follicle size on pregnancy establishment, but also on pregnancy maintenance. However, there was no effect of ovulatory follicle size on pregnancy rates or late embryonic/fetal survival in cows that spontaneously ovulated (Perry et al., 2005). Collectively these observations suggest that the decrease in pregnancy establishment and maintenance is

due to the physiological immaturity of the ovulatory follicle rather than ovulatory follicle size alone.

Why do heifers and cows have small dominant follicles at fixed-time insemination?

Since GnRH-induced ovulation of small dominant follicles decreased pregnancy rates and late embryonic/fetal survival a common question is what causes small dominant follicles to be present at FTAI and how can I reduce the proportion of small dominant follicles at FTAI. These are questions that are addressed below. Administration of the first injection of GnRH (GnRH-1) 7 days before PGF and a second GnRH injection (GnRH-2) with FTAI 48 hr after PGF has been used to inseminate beef cows by appointment (CO-Synch; Geary et al., 1998). The GnRH-1 injection is expected to ovulate a dominant follicle and initiate a new follicular wave so that a viable pre-ovulatory follicle is present at FTAI; however, approximately 50% of heifers and 66% of postpartum cows have a dominant follicle capable of responding a to a single GnRH injection. It is logical that small dominant follicles present at the time of GnRH-2 (FTAI)

A



B

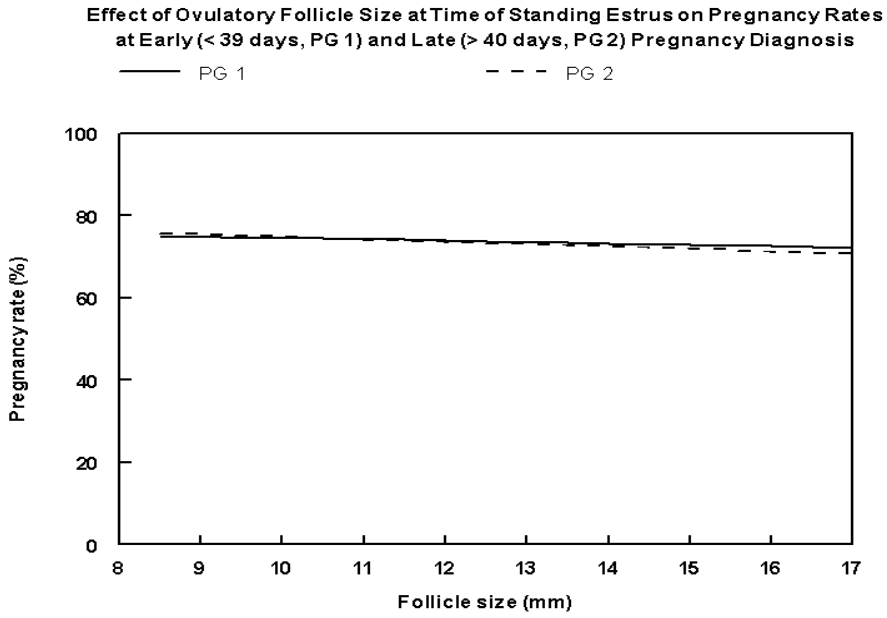


Figure 2. Regression analysis of the effect of ovulatory follicle size on pregnancy rates. Follicle sizes at which pregnancy rates were decreased ($P < 0.05$) below the maximal pregnancy rates are indicated with vertical lines (Panel A). The size of follicles that ovulated spontaneously had no effect on subsequent pregnancy rates or late embryonic/fetal mortality (Panel B).

Table 1- Ovulatory follicle size and reproductive success (embryo development, conception, and pregnancy)

Species	[1]Follicle Size at which Embryo Development/Conception/Pregnancy Decreased	Range in Follicle Size	Source
Beef cows	≤ 12.0 mm	< 12 mm to > 18 mm	Lamb et al., 2001
Beef cows	≤ 11.3 mm	10 mm to 17 mm	Perry et al., 2005
Beef heifers	< 10.7 mm >15.7 mm	<10 mm to > 16 mm	Perry et al., 2007
Beef cows and heifers	Linear	7.5 mm to 18.0 mm	Peres et al., 2009
Beef heifers	Linear	6 mm to 16 mm	Dias et al., 2009
Beef cows	Linear	< 9 mm to > 17 mm	Sa Filho et al., 2009
Beef cows	Linear	< 9 mm to > 16 mm	Meneghetti et al., 2009
Dairy cows	Quadratic	10 mm to 23 mm	Bello et al., 2006
Dairy cows	15 mm and 14.5 mm	8 mm to 17 mm	Lopes et al., 2007

[1]Follicle size at which reproductive success was significantly decreased. Linear and quadratic refer to the significant line, which was fit to these data. Linear: As ovulatory follicle size increased there was an increase in pregnancy rates. Quadratic: As ovulatory follicle size increased there was an increase in pregnancy rates until a follicle diameter of ≅ 15.0 mm was reached in which time an increase an ovulatory follicle size decreased pregnancy rate.

could result from failure to ovulate a dominant follicle and initiate a new follicular wave with GnRH-1. Consequently, at GnRH-2 there will be cows in which a follicular wave has been correctly synchronized and there will be cows which do not have a synchronized follicular wave. We hypothesize that cows that do not have a synchronized wave may be the ones with a small dominant follicle at GnRH-2. Alternatively, a slower growth rate of the dominant follicle could result in a small dominant follicle at GnRH-2. Our hypothesis was that day of the estrous cycle

at GnRH-1 would affect ovulatory response to GnRH-1 and that ovulatory response to GnRH-1 would affect growth rate, diameter, and physiological maturity of the ovulatory follicle at GnRH-2. Therefore, we tested this hypothesis in beef heifers and both cycling and anestrus postpartum beef cows. In each of the following experiments we used the Co-Synch protocol (GnRH-1 on day -9, $PGF_{2\alpha}$ on day -2, and GnRH-2 on day 0). The results of these studies are described below.

Beef heifers:

Induced ovulation of small dominant follicles in beef heifers decreased pregnancy rate following FTAI (Perry et. al., 2007); however, the reason for the presence of small dominant follicles at GnRH-2 was not clear. The objectives of this study were to: 1) Determine the effect of ovulatory response at GnRH-1 on diameter and variation in diameter of the largest follicle at GnRH-2, 2) Determine the effect of day of the cycle (stage of a follicular wave) on GnRH-induced luteinizing hormone (LH) release, and the resulting ovulatory response after GnRH-1 and 2. Two experiments were conducted using cycling Bos tarus beef heifers synchronized to be on different days of the estrous cycle (days 2, 5, 10, 15, and 18 after estrus) in which a dominant follicle would or would not respond to GnRH-1. In Exp. 1, ovulatory response after GnRH-1 and GnRH-2 was affected by day of the cycle when GnRH-1 was administered (Table 2). Ovulatory response to GnRH-1 did not affect size or variation in diameter of the largest follicle at GnRH-2 in Exp.1 or 2 (Table 3). In Exp. 2, the GnRH-induced LH surge was of greatest magnitude in heifers receiving GnRH-1 on d 18 of the cycle followed by days 5, 15, 10, and 2 ($9,054^{b}$, $5,774^{bc}$, $4,672^{c}$, $2,548^{c}$, and 915^{d} arbitrary units; respectively; $^{abcd}P < 0.05$). In summary, presence of small dominant follicles at GnRH-2 was not affected by ovulatory response to GnRH-1 (Table 3). The day of the cycle at GnRH-1 affected dominant follicle size and ovulatory response at GnRH-2. Heifers receiving GnRH-1 in the latter part of the cycle had a greater incidence of luteolysis before PGF and earlier onset of estrus regardless of the presence of an accessory CL after GnRH-1 which resulted in smaller follicles at GnRH-2. Therefore, presynchronizing heifers to be in the early stage of the estrous cycle (≤ d 10) at GnRH-1 would increase the proportion of heifers having a follicle large enough to respond to GnRH-2 and increase the ovulatory response after GnRH-2.

Postpartum beef cows:

Cycling postpartum beef cows: Estrous cycles of beef cows were manipulated so that cows were on specific days of the estrous cycle (days 2, 5, 9, 13, and 18, day 0 = estrus; n = 12 per treatment group) at the start of the CO-Synch protocol. As described above for the experiment with heifers, the days were selected based on prediction of the presence of a dominant follicle that either would or would not respond to GnRH-1 and ovulate a 1st, 2nd, or 3rd, wave dominant follicle in response to GnRH-2. Day of the estrous cycle at GnRH-1 did not affect the size of the preovulatory follicle or the proportion of cows ovulating at GnRH-2 (*P = 0.65* and *0.21*, respectively). When all cows were included in the analysis, cows that ovulated after GnRH-1 had similar follicle size at GnRH-2 compared to cows that did not ovulate after

Table 2 Mean diameter (mm) of the largest follicle (± SEM) at GnRH-1 and GnRH-2, number (%) of heifers ovulating to GnRH-1 and 2, undergoing luteolysis before prostaglandin $F_{2\alpha}$ (PGF), in estrus from GnRH-1 to PGF.

	GnRH 1[b]		GnRH 2[c]			
Treatment Group[a]	Follicular Diameter	Ovulation	Follicular Diameter	Ovulation	Luteolysis Before PG	Proportion in Estrus from GnRH 1 to PG
d 2	5.3^{d} ± 0.4	0/14^{g}	12.4^{g} ± 0.7	13/14^{g}	0/14^{d}	0/14^{d}
d 5	9.4^{e} ± 0.5	12/13^{h}	11.4^{g} ± 0.8	12/13^{g}	0/13^{d}	0/13^{d}
d10	11.8^{f} ± 0.5	4/13^{gi}	12.3^{g} ± 0.8	12/13^{g}	2/13^{d}	1/13^{d}
d 15	10.2^{ef} ± 0.5	9/13^{hi}	7.2^{h} ± 0.8	2/13^{h}	12/13^{e}	6/13^{e}
d 18	10.7^{ef} ± 0.5	2/10^{g}	9.6^{gh} ± 0.9	2/10^{h}	10/10^{e}	7/10^{e}

[a]Treatment groups were based on the day of the estrous cycle at the start (GnRH -1) of the CO-synch protocol.

[b]GnRH-1- Injection of GnRH on the first day of the CO-Synch protocol (d -9).

[c]GnRH-2- Injection of GnRH and TAI (d 0) in the CO-Synch protocol.

Means or proportions having different superscripts within a column were different ($^{def}P < 0.01$, $^{ghi}P < 0.05$).

Experiment	Ovulation to GnRH 1	n	Follicle diameter (mm) at GnRH 1	Variance follicle diameter (mm) at GnRH 1	Follicle diameter (mm) at GnRH 2	Variance follicle diameter (mm) at GnRH 2	Ovulating to GnRH 2	Luteolysis before PG
1	Yes	27	10.61[a]	1.89[a]	10.06	12.78	17 (63)	11 (41)
	No	35	8.2[b]	9.55[b]	11.1	7.84	24 (69)	14 (39)
2	Yes	18	11.0[a]	2.6[a]	10.8	17.6	11 (61)	8 (44)
	No	19	9.0[b]	8.4[b]	11.8	9.0	8 (42)	7 (37)

Table 3 Effect of ovulatory response at gonadotropin releasing hormone (GnRH-1) of the Co-Synch protocol on mean follicular diameter (mm) at GnRH-1 and 2, variance in diameter of the largest follicle at GnRH 1 and 2, number and percent (in parentheses) ovulating at GnRH-2, undergoing luteolysis before prostaglandin $F_{2\alpha}$ (PGF).

Means having different superscripts within experiments and across columns were different ($^{ab}P<0.01$, $^{cd}P<0.05$).

GnRH-1 (11.4 and 10.4 mm, respectively; *P = 0.23*). When only cows that could ovulate after GnRH-1 (excluding Day 2 cows) were included in the analysis, cows that ovulated to GnRH-1 had a larger follicle at GnRH-2 than cows that did not ovulate after GnRH-1 (11.4 and 9.5 mm, respectively; *P = 0.04*). Follicle growth from d -5 to d 0 (d 0 = GnRH-2) was similar between cows that ovulated after GnRH-1 and cows that did not ovulate (1.01 vs. 0.89 mm/d, respectively; *P = 0.75)*. There was a tendency for increased follicle growth rate in cows that ovulated a large follicle (> 11 mm) compared to cows that ovulated a small follicle (≤ 11 mm; 1.01 vs. 0.86 mm/d, respectively; *P = 0.07*). Serum concentrations of estradiol at GnRH-2 and progesterone following ovulation were reduced in cows that ovulated small follicles compared to cows that ovulated large follicles (*P = 0.006* and *< 0.05*, respectively). In summary, day of the estrous cycle at initiation of synchronization did not affect ovulatory follicle size, but both synchronization of the follicular wave and follicle growth rates affected the size of the follicle at GnRH-2.

Anestrus postpartum beef cows: At the beginning of the breeding season there are a mixture of cycling and anestrus postpartum cows which may contribute to the large variation in

dominant follicle diameter at the time of GnRH-induced ovulation in the CO-Synch protocol (Table 1). Our hypothesis was that ovulatory response to GnRH-1 and progesterone exposure (controlled intravaginal drug releasing insert [CIDR]) would affect ovulatory follicle size at GnRH-2 in anestrous cows. This study used a two by two factorial design in which anestrous suckled beef cows (n = 55) either ovulated (Ov1+) or failed to ovulate (Ov1-) after GnRH1 and either received (CIDR+) or did not receive (CIDR-) a 7 d CIDR treatment (from GnRH-1 to PGF) resulting in the following treatment groups: Ov1+CIDR+, Ov1-CIDR+, Ov1+CIDR-, and Ov1-CIDR- (n = 9, 17, 11, and 18, respectively). The Ov1+ had larger follicles at GnRH-2, a decreased proportion of small follicles within cows that ovulated to GnRH-2, and a similar growth rate of the ovulatory follicle from d -5 to d 0 (d 0 = GnRH-2) compared to Ov1- cows (12.3 vs. 11.0 mm, 2/16 vs. 14/23, and 1.1 vs. 1.1 mm/d, respectively; *P = 0.04, 0.003, and 0.99*, respectively). Administration of a CIDR had no effect on follicle diameter at GnRH-2, proportion of small ovulatory follicles at GnRH-2, and follicular growth rate from d -5 to d 0 (d 0 = GnRH-2; 11.8 vs. 11.2 mm, 7/19 vs. 9/20, and 1.2 vs. 1.1 mm/d for the CIDR+ vs. CIDR- cows, respectively; *P = 0.3, 0.6, and 0.76*, respectively). Administration of a CIDR, but not ovulation to GnRH-1, increased follicle growth from d -2 to d 0 (d 0 = GnRH-2; *P = 0.03 and 0.9*, respectively). Large follicles (> 11 mm) had a similar growth rate from d -5 to d 0 (d 0 = GnRH-2; *P = 0.44*) compared to small follicles (1.1 vs. 1.2 mm/d) but the large ovulatory follicles were larger at d-5 compared to small ovulatory follicles (*P < 0.001).* Follicle diameter was positively correlated with serum concentrations of estradiol at GnRH-2 (r = 0.622; *P < 0.0001*). In summary, ovulation to GnRH-1 but not CIDR administration resulted in increased dominant follicle diameter at GnRH-2 in anestrous suckled beef cows. Large follicles were already larger five days before GnRH-2 but grew at a similar rate to small follicles and follicle size was positively correlated with serum concentrations of estradiol at the time of GnRH-induced ovulation.

How do you enhance the physiological maturity of dominant follicles?

As previously mentioned, it is the physiological maturity of a dominant follicle and not diameter that affects the establishment and maintenance of pregnancy in beef cattle. A dominant follicle that is physiologically mature may be defined as follows: 1) contains a

competent oocyte, 2) secretes adequate amounts of estradiol during the preovulatory period, and 3) has the ability to form a corpus luteum capable of secreting adequate amounts of progesterone for establishment and maintenance of pregnancy. Gonadotropin secretion during the preovulatory period has been shown to be important for acquisition of oocyte competence as well as estradiol secretion. Following the decrease in progesterone at luteolysis or removal of a CIDR there is an increase in the pulse frequency of luteinizing hormone (LH) in circulation that stimulates a dominant follicle to synthesize and secrete estradiol during the preovulatory period. Estradiol has an important effect on expression of estrus, preparation of the maternal environment for pregnancy, and perhaps the acquisition of oocyte competence. Heifers or cows that express estrus at FTAI consistently have higher pregnancy rates than heifers or cows that do not express estrus. Furthermore, estradiol supplementation during the preovulatory period increased pregnancy rates in postpartum beef cows induced to ovulate smaller dominant follicles. Below is a description of three potential strategies for increasing the amount of gonadotropin stimulation to the dominant follicle resulting in increased estradiol secretion during the preovulatory period.

Increase the length of proestrus:

Proestrus is generally defined as the period from initiation of luteolysis to the onset of estrus during which a dominant follicle and oocyte continues the maturation process. There is accumulating evidence that the length of proestrus can affect the establishment of pregnancy in cattle. Regardless of follicular diameter, luteal function and embryo development were reduced when bovine follicles ovulated following a short vs. long proestrus period (Burke et al., 2001; Mussard et al., 2003, 2007; Bridges et al., 2006). Reducing the length of proestrus resulted in inadequate luteal function following ovulation independent of follicle diameter (Mussard et al., 2003). In the same study, pregnancy rates following embryo transfer were lower in cows with a shorter proestrus compared to cows with a longer proestrus (Mussard et al., 2003). The preceding data provide further support that it is the physiological maturity of the follicle and not simply size that contributes to establishment and maintenance of pregnancy. In the CO-Synch + CIDR protocol removing a CIDR after 5 days instead of 7 days will increase the length of proestrus and increased pregnancy rates in beef cows (Bridges et. al., 2008).

Temporary calf removal

Postpartum beef cows that are suckled ad libitum have a longer postpartum anestrous period than cows that are suckled once daily, or not suckled at all (see review by Williams, 1990). This extended anestrous period is a direct function of suckling intensity and presence of a cow's own calf on LH pulse frequency. Short term calf removal is reported to increase the proportion of anestrous postpartum beef cows that begin cycling and has been combined with progestin-based estrus synchronization protocols to increase pregnancy rates. Removal of a calf from an anestrous cow will increase circulating concentrations of LH within 24 hr of temporary weaning and LH concentrations decrease following calf return. There is variation in the response of anestrous cows to temporary weaning and the source of this variation is not clear. We hypothesized that using methods to initiate a new follicular wave combined with short term calf removal to induce ovulation may be an effective strategy to increase the proportion of cows that ovulate in response to calf removal. Calves were removed for 48 hr on days 2, 4, or 8 of a follicular wave and the ovulatory response to calf removal was maintained through day 8; however, stage of a follicular wave did not affect response to calf removal (Salfen et. al., 2001). In summary, increased LH pulse frequency following calf removal will likely stimulate estradiol secretion and promote physiological maturity of a dominant follicle in a proportion of postpartum cows.

Administration of PMSG/eCG

While temporary calf removal provides increased endogenous gonadotropin (LH) secretion that may enhance the physiological maturity of a dominant follicle, administration of equine chorionic gonadotropin (eCG) provides an exogenous method of providing gonadotropin stimulation to a dominant follicle. Equine chorionic gonadotropin will bind to both FSH and LH receptors in the follicle wall and eCG has a relatively long half life. Therefore, eCG administration can be used to enhance follicular maturity and estradiol secretion. Since temporary calf removal and eCG both provide gonadotropin stimulation to the ovary, it is not surprising that there is no benefit of combining eCG injection with calf removal (Sa Filho et al., 2009).

Summary:

In summary, GnRH-induced ovulation of follicles ≤11 mm resulted in decreased pregnancy rates and increased late embryonic/fetal mortality. This observation is important due to the extensive use of GnRH in many synchronization regimens. The decrease in fertility was associated with decreased circulating concentrations of estradiol on the day of insemination, a lower rate of increase in progesterone following insemination, and decreased circulating concentrations of progesterone. However, ovulatory follicle size had no apparent effect on fertility when ovulation occurred spontaneously. Thus, follicles undergoing spontaneous ovulation do so at a wide range of sizes when they are physiologically mature. Therefore, administration of GnRH to induce ovulation likely initiates a preovulatory gonadotropin surge before a dominant follicle has attained physiological maturity, and GnRH-induced ovulation of follicles that are physiologically immature has a negative impact on pregnancy rates and late embryonic/fetal survival. In heifers, small dominant follicles arise from decreased growth rate or the day of the cycle when GnRH is administered to synchronize a follicular wave; whereas, in postpartum beef cows, small dominant follicles arise from failure to synchronize a follicular wave at the start of an estrus synchronization protocol. Strategies to increase the physiological maturity of a dominant follicle have focused on increasing gonadotropin stimulation by increasing the duration of proestrus, short term calf removal, and exogenous gonadotropin stimulation (eCG/PMSG).

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