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State of the art of GnRH - based timed AI in beef cattle

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Abstract

Based upon observations across a series of experiments that pregnancy rate to timed-AI was positively related to length of proestrus, the traditional 7-day CO-Synch + CIDR program was modified to allow an increased interval from PGF/CIDR removal to GnRH/timed-AI; resulting in the 5-day CO-Synch + CIDR program. This modification has been demonstrated to increase timed-AI pregnancy rates relative to the traditional approach. The impact of this modification on preovulatory estradiol concentrations, as a result of extending the period of gonadotropic stimulus provided to the follicle, initiation of proestrus at a time when ovulatory follicles are highly estrogenic and/or through reduction in the incidence of ovulation of very young follicles, are potential mechanisms for increased estradiol concentrations and enhanced fertility. Conversely, for females in these estrous control programs in which follicular growth is adequately controlled, differences in age of the ovulatory follicle may not be a significant contributor to variation in timed-AI pregnancy rate.

Keywords: bovine, estradiol, fixed-time artificial insemination.

Introduction

The most commonly used approaches to timed-AI in beef cattle in the USA are based upon the CO-Synch program (Geary and Whittier, 1998). In the USA, three hormones are available to synchronize cows; progesterone (usually a vaginal insert; CIDR), prostaglandin F2α (PGF; or it's analog) and GnRH. The original CO-Synch program consisted of an initial GnRH treatment, PGF 7 days later to induce luteolysis, and a second GnRH treatment 48 h after PGF to induce ovulation for timed-AI. The timing of the second injection of GnRH determines the length of "proestrus", or the interval between the initiation of regression of the corpus luteum (CL) and the LH surge. A CIDR is usually inserted into beef females between the initial GnRH and the PGF treatment, resulting in a CO-Synch + CIDR program. Each exogenous hormone used in this program has specific actions and the efficacy and accuracy of these actions are crucial for synchronization. The first GnRH treatment is used to induce ovulation and reset follicular growth. In other words, approximately 1 to 2 days after GnRH a new follicular wave should be initiated in a majority of cows (Thatcher *et al*., 1989; Macmillan and Thatcher, 1991; Twagiramungu *et al*., 1994, 1995). The efficacy of the initial GnRH, however, varies among animal class and stage of the estrous cycle (Pursley *et al*., 1995; Geary *et al*., 2000; Atkins *et al*., 2008, 2010; Souza *et al*., 2009). The second GnRH will induce an LH surge and subsequent ovulation of the dominant follicle that results from the new wave induced by the first GnRH. Luteolysis is induced with PGF between 48 and 72 h before the second GnRH treatment. Timed-AI is performed coincident with the second administration of GnRH. One important concern is the proportion of cows that are induced to ovulate follicles that are smaller than typical diameter with the second GnRH administration and the fact these animals are less likely to become pregnant to timed-AI (Lamb *et al*., 2001; Perry *et al*., 2005).

The influence of ovulatory follicle maturity on fertility in beef cattle has been investigated (Perry *et al*., 2005; Mussard *et al*., 2003, 2007; Bridges *et al*., 2010). One hypothesis was that diameter of ovulatory follicles was the most appropriate indicator of follicle "maturity" and that cows induced to ovulate small follicles would have lesser fertility compared to those induced to ovulate larger follicles. Within each of three experiments (Table 1; Mussard *et al*., 2003, 2007) this hypothesis was supported, but as data from multiple experiments accumulated, the relationship of follicle diameter to pregnancy rate appeared inconsistent. Across experiments, the more consistent predictor of pregnancy rate appeared to be duration of proestrus (interval from initiation of CL regression with PGF to the LH surge; Table 1). Based on the relationship between length of proestrus and conception rate, an additional experiment (Table 1; Bridges *et al*., 2010) was performed to hold follicle diameter constant and only vary length of proestrus. It was demonstrated that at a constant ovulatory follicle diameter, length of proestrus had a substantial influence on conception rate. Taken together, data from this series of studies suggested a strong positive relationship of duration of proestrus with follicle maturity and fertility and suggested that diameter of the ovulatory follicle, in itself, was not a consistent predictor of follicle maturity. The effect of ovulatory follicle diameter at GnRH-induced ovulation or at spontaneous ovulation on conception rate has also been evaluated (Perry *et al*.,

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2005, 2007). It was reported that diameter of the ovulatory follicle influenced conception rate after detection of estrus in heifers, but not in postpartum cows. In postpartum cows that did not exhibit estrus, diameter of the ovulatory follicle was positively associated with conception rate when ovulation was induced with GnRH. Thus, if a ‘complete’ spontaneous proestrus occurred in cows (confirmed by exhibition of estrus), diameter did not impact fertility, but, diameter of the ovulatory follicle when ovulation was induced with GnRH did influence conception rate; at a constant duration of proestrus. Since findings suggested that maturity of the ovulatory follicle and probability of conception was perhaps defined by length of proestrus, we applied this knowledge towards optimizing the existing CO-Synch + CIDR program.

Table 1. Conception rate, diameter and age of the ovulatory follicle, length of proestrus, and number of cows in a series of experiments investigating the effect of follicle maturity on fertility.

Conception rate (%)[a]	Follicle diameter at ovulation (mm)[b]	Duration of proestrus (days)[c]	n	Experiment
4	11.1 ± 0.2	1.0 ± 0.1	45	Mussard *et al*., 2003a[e]
8	11.1 ± 0.2	1.0 ± 0.1	12	Mussard *et al*., 2003b[f]
10	12.6 ± 0.2	1.25	10	Bridges *et al*., 2010[g]
57	13.6 ± 0.2	2.2 ± 0.1	54	Mussard *et al*., 2003a[e]
67	13.7 ± 0.2	2.0 ± 0.1	12	Mussard *et al*., 2003b[f]
71	12.9 ± 0.2	2.25	28	Bridges *et al*., 2010[g]
76	10.7 ± 0.1	3.3 ± 0.1	29	Mussard *et al*., 2007[d]
100	12.0 ± 0.3	4.7 ± 0.2	24	Mussard *et al*., 2007[d]

[a]Percentage of animals determined to be pregnant following insemination. Pregnancy determination was conducted via ultrasonography at approximately 30 days post-insemination. [b]Diameter of the largest ovulatory follicle as determined by ultrasonography conducted either at GnRH administration or estrus. [c]Interval from PGF2α until GnRH administration. [d]Cows were either induced with GnRH to ovulate a small (~11 mm) follicle or allowed to spontaneously exhibit estrus. Cows were inseminated 12 h following estrus or GnRH.[e]Cows were induced to ovulate either a small (~11 mm) or large (~13 mm) ovarian follicle with GnRH. Animals were inseminated 12 h following GnRH administration. [f]Cows were induced to ovulate either a small (~11 mm) or large (~13 mm) ovarian follicle with GnRH. Embryo from non-treated cows were then transferred 7 days after GnRH. [g]Cows were induced to ovulate an ovarian follicle of similar diameter with GnRH either 1.25 or 2.25 days following PGF2α administration. Animals were inseminated 12 h following GnRH administration. Includes only cows with a luteal phase of normal length.

Lengthening proestrus in the CO-Synch + CIDR program

The length of proestrus with the traditional 7-day CO-Synch + CIDR program was varied from 50 to 66 h in mature cows without influencing timed-AI pregnancy rate, but in younger cows (≤3 years of age), greatest pregnancy rates were achieved with timed-AI at 56 h as compared to longer intervals (Dobbins *et al*., 2009). Others (Busch *et al*., 2008) have reported that timed-AI pregnancy rates were greater when proestrus was 66 than 54 h. In practice, the second GnRH is given and timed-AI is performed in most herds between 54 and 66 h after PGF. We hypothesized that if the CO-Synch + CIDR synchronization approach could be modified in a manner in which we could increase the interval from PGF and CIDR removal to the second GnRH and timed-AI, that timed-AI pregnancy rate would increase. This end was achieved through development of the 5-day CO-Synch + CIDR program (Bridges *et al*., 2008). This paper will focus on the physiological effects of this change in the program and potential mechanisms for the increase in timed-AI pregnancy rate that is achieved.

Hormonal changes with a lengthened proestrus

Proestrus starts with removal of progesterone sources (a CL, a CIDR or both) and ends with either a spontaneous or GnRH- induced LH surge. Concentrations of progesterone decline rapidly and are sustained at basal concentrations throughout proestrus, setting off a series of crucial hormonal changes that precede ovulation. An almost immediate response to declining progesterone concentrations is an increase in the frequency of LH pulses. Frequency of release of LH from the anterior pituitary is primarily regulated by progesterone and the negative association of progesterone concentration and frequency of LH pulses has been well established (Kinder *et al*., 1996). Proestrus is characterized by LH pulses at an increasing frequency as proestrus progresses and the LH surge approaches (Imakawa *et al*., 1986). Pulsatile LH secretion is the primary factor that drives the final development of preovulatory follicles. During a spontaneous proestrus, growth of the preovulatory follicle and production of estradiol by granulosa cells in the follicle increases as proestrus progresses. We have compared preovulatory estradiol and post-ovulatory progesterone concentrations, and the magnitude of the

LH surge between female cattle experiencing either a long (54 h; LPE) or short (30 h; SPE) proestrus after synchronizing follicular growth with ultrasound-guided aspiration of the dominant follicle and altering timing of luteal regression (Bridges *et al*., 2010). Ovulatory follicle size and magnitude of the GnRH-induced LH surge did not differ, but there tended to be a greater incidence of short estrous cycles and lesser progesterone concentrations during the subsequent estrous cycle in the SPE than LPE treatment. The most striking difference between treatments was that concentrations of estradiol were greater in the LPE than SPE treatment during the 38 h preceding GnRH (Fig. 1). Consistent with this observation, cows that received the 5-day *vs*. the 7-day CO-Synch + CIDR program ovulated follicles of similar diameter that tended to produce greater peak estradiol concentrations (Bridges *et al*., 2014). A logical explanation for this difference in estradiol concentrations is the extended period of stimulation by high frequency LH pulses. However, an additional factor that we think may also contribute to enhanced systemic estradiol with a 5-day *vs*. 7-day program is that removal of progesterone restraint of LH secretion occurs earlier relative to follicular wave emergence. With a 5-day, as compared to a 7-day program, follicles resulting from the new wave initiated after the first GnRH injection would be approximately 3 to 4 days post-emergence, *vs*. 5 to 6 days from emergence, respectively, at PGF and CIDR removal. It has been reported that growing dominant follicles, 4 days after emergence, have increased intra-follicular estradiol concentrations and capacity to produce estradiol *in vitro* than non-atretic dominant follicles at a time later in the follicular wave (Valdez *et al*., 2005). Furthermore, it has been demonstrated that concentrations of estradiol in the caudal vena cava were greater (Rhodes *et al*., 1995) at approximately 3 days after emergence of the first wave dominant follicle as compared to later in the lifespan of this follicle. Hence, extending proestrus and removing progesterone at a time when steroidogenic capacity of dominant follicles is optimal may both contribute to greater peak concentrations and/or an extended period of elevated estradiol during proestrus. The concentrations of estradiol present during the preovulatory period in cattle is increasingly recognized as a key factor that influences fertility (Bridges *et al*., 2012, 2013; Atkins *et al*., 2013; Jinks *et al*., 2013; Geary *et al*., 2013). We have concluded that a key impact of increased length of proestrus is to escalate preovulatory concentrations of estradiol in response to a longer period of LH stimulation and have demonstrated greater estradiol concentrations during proestrus and an increased timed-AI pregnancy rate in the 5-day as compared to the 7-day CO-Synch + CIDR program.

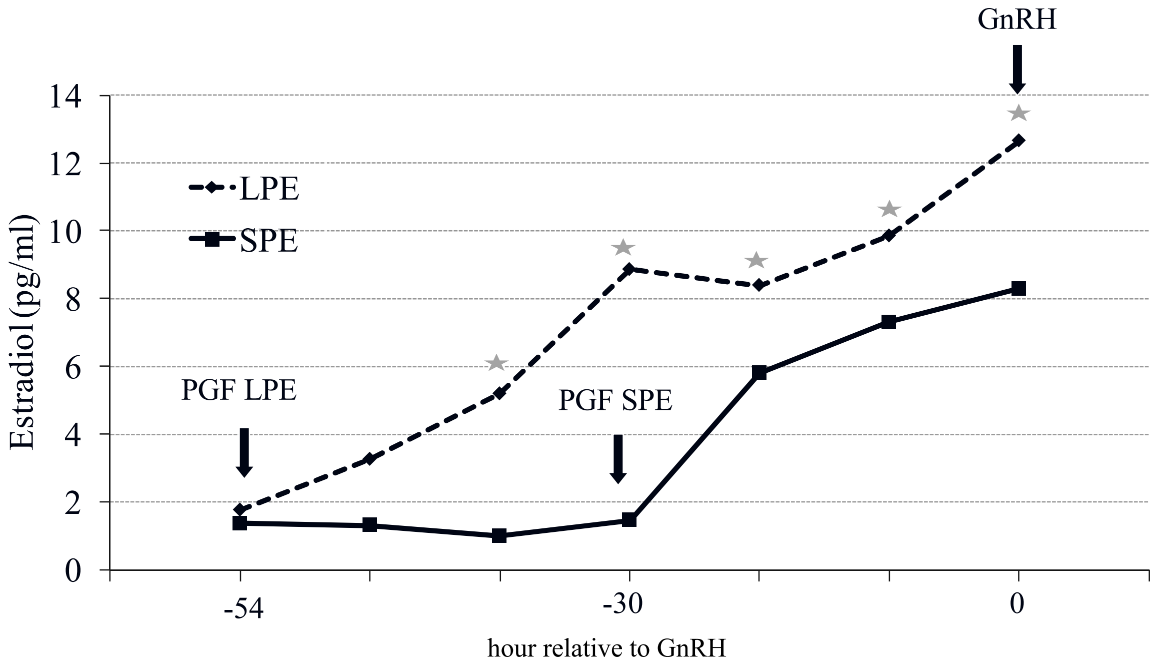

Figura 1. Concentrations of estradiol in cows that experienced either a long (LPE) or short (SPE) proestrus Adapted from Bridges *et al*., 2010.

Follicular growth and length of synchronization program

It has been demonstrated that the 7-day CO-Synch program results in a proportion of cows that ovulate follicles of smaller than typical diameter at timed-AI, which results in decreased fertility in these animals (Lamb *et al*., 2001; Perry *et al*., 2005). We and others have presumed that the smaller follicles at the time of synchronized ovulation are the result of spontaneous atresia of follicles and initiation of a new wave of follicular development during the latter stages of the interval between the initial GnRH treatment and PGF in females that do not respond to the first GnRH treatment. It has been reported that this is one of a variety of factors that contribute to the variation in diameter of follicles at the synchronized ovulation (Atkins *et al*., 2008, 2010). Reducing the length of CIDR treatment from 7 to 5 days would be expected to reduce the likelihood that this pattern of follicular growth would occur, and result in greater estradiol concentrations during proestrus in these females. Failure to ovulate to the first GnRH resulted in reduced preovulatory estradiol concentrations and progesterone concentrations during the subsequent luteal phase in the 7-day but not in the 5-d CO-Synch + CIDR program (Bridges *et al*., 2014). In a recent preliminary report (Dias *et al*., 2014), females which did not respond to the first GnRH administration in the 5-day CO-Synch + CIDR program had greater TAI pregnancy rates (65.0%; n = 163; P = 0.01) than those that did respond to GnRH-1 (51.5%). Further research regarding this finding, and whether a portion of the benefit of the 5-day program is the result of normal fertility in those females not responding to the initial GnRH, is necessary.

Age of follicles at induced ovulation

In the 5-day CO-Synch + CIDR program, the interval from follicle wave emergence at 1 to 2 days after the first GnRH, to induction of ovulation with the second GnRH (day 8) is 6 to 7 days. In the 7-d program, this interval is 8 to 9 days. We refer to this interval as follicle age, and due to the design of the 5-day program, younger follicles are induced to ovulate with the second GnRH as compared to the 7-day program. Variation in follicle age at ovulation does exist in spontaneously ovulating cows. For example, in spontaneously ovulating dairy cattle that have either 2 or 3 waves of follicular growth during their estrous cycle, the interval from follicle emergence to estrus or ovulation (age of the follicle) is greater by approximately 3 days in cows (Bleach *et al*., 2004) and 4 days in heifers (Sartori *et al*., 2004) with 2 follicular waves; and pregnancy rate to AI is lower when compared to cows with 3 follicular waves during the estrous cycle (Townson *et al*., 2002). Use of a 5 day interval between GnRH and PGF increased pregnancy rate in lactating dairy cows (Santos *et al*., 2010) and Cerri *et al*. (2009) demonstrated a greater proportion of good quality embryos collected from lactating dairy cows that were induced to ovulate younger follicles; within the range normally observed in spontaneously ovulating females. The cumulative interpretation of reports in lactating dairy cows suggests that age of the follicle is a significant source of variation in fertility. We have recently completed two experiments to directly address the effect of age of the ovulatory follicle on fertility in cattle and tested the hypothesis that conception rate to AI after ovulation of a younger follicle would be greater in beef heifers after spontaneous ovulation and in postpartum beef cows after either a spontaneous or GnRH-induced ovulation.

In the first experiment in heifers, luteal regression was induced with PGF either 2 (young follicle = YF) or 6 (mature follicle = MF) days after emergence of a new follicular wave and heifers were AI 12 h after expression of estrus (Abreu *et al*., 2014a). As expected, the interval from PGF to estrus was greater in the YF than MF group with some variation in this interval between locations (Table 2). Age of follicles at AI was greater by approximately 3 days in the MF group, and diameter of the ovulatory follicle was marginally greater in the MF than YF heifers. However, conception rate to estrus-AI did not differ between groups.

In postpartum cows (Abreu *et al*., 2014b), luteal regression was induced with PGF either 2.5 (young follicle = YF) or 6.5 (mature follicle = MF) days after emergence of a new follicular wave. Based upon the intervals to estrus in heifers (Abreu *et al*., 2014a), cows in the MF group were AI based upon estrus detection until 72 h after PGF with the cows not detected in estrus receiving GnRH and timed-AI at hour 72. In the YF group, estrus detection and AI was performed to hour 96, with timed-AI in the remaining cows at hour 96. Interval to estrus after PGF was approximately 24 h greater in the YF than MF treatment (Table 3). This resulted in a difference in follicle age at AI of approximately 3 days (MF > YF) yet diameter of the ovulatory follicle did not differ between treatments. Pregnancy rate during the synchronization period did not differ between cows in the MF and YF treatments (Table 3).

As previously described, in cattle that initiate a new follicle wave after the first GnRH, age of the ovulatory follicle for a 5-day program, by design, is approximately 2 days less than with a 7-day CO-Synch + CIDR program. Results of experiments by Abreu *et al*. (2014a, b) suggest that age of ovulatory follicles, in itself, for females that respond to the first GnRH may not be a substantial source of variation in timed-AI pregnancy rate.

Table 2. Effect of treatment (Trt) on estrous response, proestrus interval, follicle age and size at AI (Mean ± SE), and conception rate in both locations.

	Trt[1]	n	Estrous response (%)	Proestrus[2] interval (h)	Follicle age[3] at AI (day)	Follicle size at AI (mm)	Conception rate (%)
Montana	MF	53	92.5	55.8 ± 2.7^{a}	8.3 ± 0.11^{a}	11.0 ± 0.18^{a}	63.3
	YF	75	90.7	67.4 ± 1.6^{b}	4.8 ± 0.06^{b}	10.4 ± 0.15^{b}	64.7
Ohio	MF	77	87.0	53.7 ± 2.2^{a}	8.2 ± 0.10^{a}	-	64.2
	YF	75	90.7	78.5 ± 1.4^{c}	5.3 ± 0.06^{c}	-	69.1

$^{a-c}$Values with different superscripts in the same column differ ($P < 0.01$). [1]MF = mature follicle; YF = young follicle. [2]Proestrus interval was defined as the interval from prostaglandin F2α (PGF2α) administration to estrus. [3]Follicle age was defined as the interval from estradiol benzoate (EB) administration to 12 h after estrus minus 3 days for new follicle wave formation to occur for heifers that received PGF2α either 5 (YF) or 9 days (MF) after EB. Adapted from Abreu *et al.* (2014a.

Table 3. Effect of treatments on response variables (mean ± SE) in beef cows.

Variable[1]	Mature follicle	Young follicle	P- value
Estrous response within 72 h (%)	76.3	47.7	< 0.01
Estrous response from PGF to TAI - 72 *vs*. 96 h (%)	76.3	88.6	< 0.01
Interval from PGF to estrus (h)	57.5 ± 1.6	78.9 ± 0.8	< 0.01
Follicle age at AI (day)	9.32 ± 0.04	6.26 ± 0.02	< 0.01
Follicle diameter at AI (mm)	13.1 ± 0.2	12.9 ± 0.1	> 0.10
Follicle growth rate - PGF to AI (mm/day)	0.95 ± 0.07	1.14 ± 0.04	< 0.05
Follicle growth rate, 5.5 d after EB to PGF (mm/day)	0.77 ± 0.06	n/a	-
Pregnancy rate (%)	72.0	67.1	> 0.10
Progesterone concentration (~ 7 d) after AI, ng/ml (G1 only)	3.56 ± 0.21	3.85 ± 0.13	> 0.10

[1]TAI = timed AI; EB = estradiol benzoate; G1 = group 1; PGF2α = prostaglandin F2α. Adapted from Abreu *et al.* (2014b).

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References

Abreu FM, Cruppe LH, Maquivar M, Utt MD, Madsen CA, Vasconcelos JLM, Day ML, Geary TW. 2014a. The effect of follicle age on conception rate in beef heifers. *J Anim Sci*, 92:1022-1028.

Abreu FM, Geary TW, Cruppe LH, Madsen CA, Jinks EM, Pohler KG, Vasconcelos JLM, Day ML. 2014b. The effect of follicle age on pregnancy rate in beef cows. *J Anim Sci*, 92:1015-1021.

Atkins JA, Busch DC, Bader JF, Keisler DH, Patterson DJ, Lucy MC, Smith MF. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: effect of day of the cycle. *J Anim Sci*, 86:83-93.

Atkins JA, Smith MF, Wells KJ, Geary TW. 2010 Factors affecting pre-ovulatory follicle diameter and ovulation rate to GnRH in postpartum beef cows Part I: Cycling cows. *J Anim Sci*, 88:2300-2310.

Atkins JA, Smith MF, MacNeil MD, Jinks EM,

Abreu FM, Alexander LJ, Geary TW. 2013. Pregnancy establishment and maintenance in cattle. *J Anim Sci*, 91:722-733.

Bleach, ECL, Glencross RG, Knight PG. 2004. Association between ovarian follicle development and pregnancy rates in dairy cows undergoing a spontaneous oestrus cycle. *Reproduction*, 127:621-629.

Bridges GA, Helser LA, Grum DE, Mussard ML, Gasser CL, Day ML. 2008. Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. *Theriogenology*, 69:843-851.

Bridges GA, Mussard ML, Burke CR, Day ML. 2010. Influence of the length of proestrus on fertility and endocrine function in female cattle. *Anim Reprod Sci*, 117:208-215.

Bridges GA, Mussard ML, Pate JL, Ott TL, Hansen TR, Day ML. 2012. Impact of preovulatory estradiol concentrations on conceptus development and uterine gene expression. *Anim Reprod Sci*, 133:16-26.

Bridges GA, Day ML, Geary TW, Cruppe LH. 2013. Triennial Reproduction Symposium: Deficiencies in the uterine environment and failure to support embryonic development. *J Anim Sci*, 91:3002-3013.

Bridges GA, Mussard ML, Helser LA, Day ML. 2014. Comparison of follicular dynamics and hormone concentrations between the 7-day and 5-day CO-Synch + CIDR program in primiparous beef cows. *Theriogenology*, 81:632-638.

Busch DC, Schafer DJ, Wilson DJ, Mallory DA,

Leitman NR, Haden JK, Ellersieck MR, Smith MF, Patterson DJ. 2008. Timing of artificial insemination in postpartum beef cows following administration of the CO-Synch + controlled internal drug-release protocol. *J Anim Sci*, 86:1519-1525.

Cerri, R L, Rutigliano HM, Chebel RC, Santos JE. 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. *Reproduction*, 137:813-823.

Dias, HP, Kruse SG, Bird SL, Funnell BJ, Geppert T, Lundy E, Gunn PJ, Bridges GA. 2014. Incidence of ovulation to GnRH at onset of 5-d CO-Synch + CIDR protocol and impact on reproductive responses. *J Anim Sci*, 91(E-suppl. 1):698. (abstract).

Dobbins CA, Eborn DR, Tenhouse DE, Breiner RM, Johnson SK, Marston TT, Stevenson JS. 2009. Insemination timing affects pregnancy rates in beef cows treated with CO-Synch protocol including an intravaginal progesterone insert. *Theriogenology*, 72:1009-1016.

Geary TW, Whittier JC. 1998. Effects of a timed insemination following synchronization of ovulation using the Ovsynch or COSynch protocol in beef cows. *Prof Anim Sci,* 14:217-220.

Geary TW, Downing ER, Bruemmer JE, Whittier JC. 2000. Ovarian and estrous response of suckled beef cows to the select synch estrous synchronization protocol. *Prof Anim Sci*, 16:1-5.

Geary TW, Smith MF, MacNeil MD, Day ML, Bridges GA, Perry GA, Abreu FM, Atkins JA, Pohler KG, Jinks EM, Madsen CA. 2013. Triennial Reproduction Symposium: Influence of follicular characteristics at ovulation on early embryonic survival. *J Anim Sci*, 91:3014-3021.

Imakawa K, Day ML, Zalesky DD, Garcia-Winder M, Kittok RJ, Kinder JE. 1986. Regulation of pulsatile LH secretion by ovarian steroids in the heifer. *J Anim Sci*, 63:162-168.

Jinks EM, Smith MF, Atkins JA, Pohler KG, Perry GA, MacNeil MD, Roberts AJ, Waterman RC, Alexander LJ, Geary TW. 2013. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. *J Anim Sci*, 91:1176-1185.

Kinder JE, Kojima FN, Bergfeld EGM, Wehrman ME, Fike KE. 1996. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *J Anim Sci*, 74:1424-1440.

Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. *J Anim Sci*, 79:2253-2259.

Macmillan KL, Thatcher WW. 1991. Effects of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. *Biol Reprod*, 45:883-889.

Mussard ML, Burke CR, Day ML. 2003. Ovarian follicle maturity at induced ovulation influences fertility in cattle. *Proc Annu Conf Soc Theriogenol*, 79:185-197.

Mussard ML, Burke CR, Behlke EJ, Gasser CL, Day ML. 2007. Influence of premature induction of an LH surge with GnRH on ovulation, luteal function and fertility in cattle. *J Anim Sci*, 85:937-943.

Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, Roberts AJ, Geary TW. 2005. Relationship between follicle size at insemination and pregnancy success. *Proc Nat Acad Sci USA*, 102:5268-5273.

Perry GA, Smith MF, Roberts AJ, MacNeil MD, Geary TW. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J Anim Sci*, 85:684-689.

Pursley JR, Mee MO, Wiltbank MC. 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology*, 44:915-923.

Rhodes FM, Fitzpatrick LA, Entwistle KW, Kinder JE. 1995. Hormone concentrations in the caudal vena cava during the first ovarian follicular wave of the oestrous cycle in heifers. *J Reprod Fertil*, 104:33-39.

Santos, J E, Narciso CD, Rivera F, Thatcher WW, Chebel RC. 2010. Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. *J Dairy Sci*, 93:2976-2988.

Sartori R, Haughian JM, Shaver RD, Rosa GJ, Wiltbank MC. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J Dairy Sci*, 87:905-920.

Souza AH, Cunha AP, Silva EPB, Gumen A, Ayres H, Guenther JN, Wiltbank MC. 2009. Comparison of gonadorelin products in lactating dairy cows: efficacy based on induction of ovulation of an accessory follicle and circulating luteinizing hormone profiles. *Theriogenology*, 72:271-279.

Thatcher WW, Macmillan KL, Hansen PJ, Drost M. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology*, 31:149-164.

Townson DH, Tsang PC, Butler WR, Frajblat M, Griel Jr LC, Johnson CJ, Milvae RA, Niksic GM, Pate JL. 2002. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. *J Anim Sci*, 80:1053-1058.

Twagiramungu H, Guilbault LA, Proulx JG, Dufour JJ. 1994. Influence of corpus luteum and induced ovulation on ovarian follicular dynamics in postpartum cyclic cows treated with buserelin and cloprostenol. *J Anim Sci*, 72:1796-1805.

Twagiramungu H, Guilbault LA, Dufour JJ. 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review. *J Anim Sci*, 73:3141-3151.

Valdez KE, Cuneo SP, Gorden PJ, Turzillo AM. 2005. The role of thecal androgen production in the regulation of estradiol biosynthesis by dominant bovine follicles during the first follicular wave. *J Anim Sci*, 83:597-603.