Cattle physiology and the CO-Synch + CIDR synchronization program
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Introduction
The most commonly used approaches to timed-artificial insemination (AI) in beef cattle in the USA are based upon the CO-Synch program.1 In the USA, three hormones are available to synchronize cows; progesterone (usually a vaginal insert; CIDR), prostaglandin F2α (PGF; or its analog) and gonadotropin releasing hormone (GnRH). The original CO-Synch program consisted of an initial GnRH treatment, PGF seven 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 luteinizing hormone (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 one to two days after GnRH a new follicular wave should be initiated in a majority of cows.2-5 The efficacy of the initial GnRH, however, varies among animal class and stage of the estrous cycle.6-10 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 54 and 72 hours 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.11,12

The influence of ovulatory follicle maturity on fertility in beef cattle has been investigated.12-15 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 113,14) 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;15) 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.12,16 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.

Keywords: CO-Synch + CIDR, estrus synchronization, follicle growth, follicle age, proestrus
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.

<table>
<thead>
<tr>
<th>Conception Rate (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Follicle diameter at Ovulation (mm)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Duration of Proestrus (days)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>n</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>11.1 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>45</td>
<td>Mussard et al., 2003a&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>11.1 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>12</td>
<td>Mussard et al., 2003b&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>12.6 ± 0.2</td>
<td>1.25</td>
<td>10</td>
<td>Bridges et al., 2010&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>57</td>
<td>13.6 ± 0.2</td>
<td>2.2 ± 0.1</td>
<td>54</td>
<td>Mussard et al., 2003a&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>67</td>
<td>13.7 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>12</td>
<td>Mussard et al., 2003b&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>71</td>
<td>12.9 ± 0.2</td>
<td>2.25</td>
<td>28</td>
<td>Bridges et al., 2010&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>76</td>
<td>10.7 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>29</td>
<td>Mussard et al., 2007&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>12.0 ± 0.3</td>
<td>4.7 ± 0.2</td>
<td>24</td>
<td>Mussard et al., 2007&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of animals determined to be pregnant following insemination. Pregnancy determination was conducted via ultrasonography at approximately 30 days post-insemination.

<sup>b</sup> Diameter of the largest ovulatory follicle as determined by ultrasonography conducted either at GnRH administration or estrus.

<sup>c</sup> Interval from PGF<sub>2α</sub> until GnRH administration.

<sup>d</sup>Cows were either induced with GnRH to ovulate a small (~11 mm) follicle or allowed to spontaneously exhibit estrus. Cows were inseminated 12 hours following estrus or GnRH.

<sup>e</sup>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.

<sup>f</sup>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.

<sup>g</sup>Cows were induced to ovulate an ovarian follicle of similar diameter with GnRH either 1.25 or 2.25 days following PGF<sub>2α</sub> 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 hours 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 hours. Others have reported that timed-AI pregnancy rates were greater when proestrus was 66 than 54 hours. In practice, the second GnRH is given and timed-AI is performed in most herds between 54 and 66 hours 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-d CO-Synch + CIDR program and is the focus of the companion paper (Bridges and Day, 2013) to this review. The remainder of this review focuses 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. Proestrus is characterized by LH pulses at an increasing frequency as proestrus progresses and the LH surge approaches. 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 hr; LPE) or short (30 hr; SPE) proestrus. 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 hours preceding GnRH (Figure 1). Consistent with this observation, cows that received the 5-d vs. the 7-d CO-Synch + CIDR program ovulated follicles of similar diameter that tended to produce greater peak estradiol concentrations. 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-d vs. 7-d program is that removal of progesterone restraint of LH secretion occurs earlier relative to follicular wave emergence. With a 5-d, as compared to a 7-d program, follicles resulting from the new wave initiated after the first GnRH injection would be approximately three to four days post-emergence, vs. five to six days from emergence, respectively, at PGF and CIDR removal. It has been reported that growing dominant follicles, four 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. Furthermore, it has been demonstrated that concentrations of estradiol in the caudal vena cava were greater at approximately three 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. 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-d as compared to the 7-d CO-Synch + CIDR program.
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.11,12 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 contribute to the variation in diameter of follicles at the synchronized ovulation.8,9 Reducing the length of CIDR treatment from seven to five 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-d but not in the 5-d CO-Synch + CIDR program.22 In a recent preliminary experiment (Bridges GA, unpublished) pregnancy rate to timed-AI in cows not responding to the first GnRH was similar to, or greater than that of cows that did respond to the initial GnRH. Further research regarding this preliminary 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-d CO-Synch + CIDR program, the interval from follicle wave emergence at one to two days after the first GnRH, to induction of ovulation with the second GnRH (day 8) is six to seven days. In the 7-d program, this interval is eight to nine days. We refer to this interval as follicle age, and due to the design of the 5-d program, younger follicles are induced to ovulate with the second GnRH as compared to the 7-d program. Variation in follicle age at ovulation does exist in spontaneously ovulating cows. For example, in spontaneously ovulating dairy cows that have either two or three waves of follicular growth during their estrous cycle, the interval from follicle emergence to estrus (age of the follicle) is greater by approximately three days in cows with two follicular waves30 and pregnancy rate to AI is lower when compared to cows with three follicular waves during the estrous cycle.31 Use of a five day interval between GnRH and PGF increased pregnancy rate in lactating dairy cows32 and Cerri et al33 demonstrated

Figure 1. Concentrations of estradiol in cows that experienced either a long (LPE) or short (SPE) proestrus. Adapted from Bridges et al., 2010.
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 (n = 280, Montana and Ohio heifers), luteal regression was induced with PGF either two (young follicle = YF) or six (mature follicle = MF) d after emergence of a new follicular wave and heifers were AI 12 hours after expression of estrus. 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 three 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.

Table 2. Estrous response, proestrus interval, follicle age and size at AI (Mean ± SE), and conception rate in heifers ovulating a mature (MF) or young (YF) follicle.

<table>
<thead>
<tr>
<th>Location</th>
<th>Group</th>
<th>Estrous response, %</th>
<th>Proestrus interval, h</th>
<th>Follicle age at AI, d</th>
<th>Follicle size at AI, mm</th>
<th>Conception rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montana</td>
<td>MF</td>
<td>53 92.5</td>
<td>55.8 ± 2.7a</td>
<td>8.3 ± 0.11a</td>
<td>11.0 ± 0.18a</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>YF</td>
<td>75 90.7</td>
<td>67.4 ± 1.6b</td>
<td>4.8 ± 0.06b</td>
<td>10.4 ± 0.15b</td>
<td>64.7</td>
</tr>
<tr>
<td>Ohio</td>
<td>MF</td>
<td>77 87.0</td>
<td>53.7 ± 2.2a</td>
<td>8.2 ± 0.10a</td>
<td>-</td>
<td>64.2</td>
</tr>
<tr>
<td></td>
<td>YF</td>
<td>75 90.7</td>
<td>78.5 ± 1.4c</td>
<td>5.3 ± 0.06c</td>
<td>-</td>
<td>69.1</td>
</tr>
</tbody>
</table>

Values with different superscripts in the same column differ (P < 0.01).
rt x loc (P < 0.01) for proestrus interval and follicle age at AI.

In postpartum cows (n = 243), luteal regression was induced with PGF either 2.5 (young follicle = YF) or 6.5 (mature follicle = MF) d after emergence of a new follicular wave. Based upon the intervals to estrus in heifers, cows in the MF group were AI based upon estrus detection until 72 hours 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 three 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).
Table 3. Reproductive variables (Mean ± SE) in postpartum beef cow ovulating either a mature (MF) or young (YF) follicle.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MF</th>
<th>YF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrous response within 72 h, %</td>
<td>76.6</td>
<td>48.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Estrous response, PGF to TAI (72 vs. 96 h), %</td>
<td>76.6</td>
<td>88.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Interval from PGF to estrus, h</td>
<td>56.7 ± 1.7</td>
<td>79.0 ± 0.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Follicle age at AI, d</td>
<td>9.01 ± 0.06</td>
<td>5.87 ± 0.03</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Follicle diameter at AI, mm</td>
<td>13.1 ± 0.2</td>
<td>13.0 ± 0.1</td>
<td>&gt; 0.10</td>
</tr>
<tr>
<td>Follicle growth rate (PGF to AI), mm/d</td>
<td>1.15 ± 0.08</td>
<td>1.29 ± 0.04</td>
<td>= 0.09</td>
</tr>
<tr>
<td>Pregnancy rate, %</td>
<td>72.3</td>
<td>67.1</td>
<td>&gt; 0.10</td>
</tr>
</tbody>
</table>

As previously described, in cattle that initiate a new follicle wave after the first GnRH, age of the ovulatory follicle for a 5-d program, by design, is approximately two days less than with a 7-d CO-Synch + CIDR program. Results of experiments by Abreu et al\textsuperscript{34,35} 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.

Summary

Based upon observations across a series of experiments that pregnancy rate to timed-AI was positively related to length of proestrus, the traditional 7-d CO-Synch + CIDR program was modified to allow an increased interval from PGF/CIDR removal to GnRH/timed-AI; resulting in the 5-d 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.

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References