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2012 Sponsors & Exhibitors: Inside front cover
Optimizing Timed-Al Pregnancy Rates with the 5 d CO-Synch + CIDR Program

L. H. Cruppe and M. L. Day
The Ohio State University, Department of Animal Sciences, Columbus, OH 43210

Introduction
Increasing the use of artificial insemination through timed-Al programs maximizes the incorporation of superior genetics in a herd, very often shortens the length of the breeding season and avoids the challenges associated with estrus detection. The most commonly used approaches to timed-Al in beef cattle in the USA are based upon the CO-Synch program (Geary et al., 1998). Five different variations of this program were tested and achieved an average timed AI pregnancy rate of 54% in a multistate study performed with over 2500 postpartum cows (Larson et al., 2006). Having approximately half of all the cows pregnant on the first day of the breeding season is attractive to many producers. However, this proportion, in relation to conception rates with estrus AI, suggests that there may be potential to further improve the efficacy of timed-Al in beef cattle.

In the USA, three hormones are available to synchronize cows; progesterone (usually a vaginal insert; CIDR), prostaglandin F$_{2\alpha}$ (PGF; or it’s analog) and GnRH. The original CO-Synch program consisted of an initial GnRH treatment followed by PGF 7 days later and a second GnRH treatment 48 h after PGF that was accompanied by timed AI. The timing of the second injection of GnRH determines the length of “proestrus”, or the interval between the initiation of the luteal regression and the LH surge. A CIDR is usually inserted into beef females during the period between the initial GnRH and the PGF treatment, resulting in a CO-Synch + CIDR program. Every exogenous hormone used in this program has specific actions and the efficacy and accuracy of these actions are crucial for the synchronization process. The first GnRH treatment is used to induce ovulation and reset follicular growth. In other words, 1 to 2 days after GnRH, a new follicular wave will 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 varies among animal class and stage of the estrous cycle (Pursley et al., 1995; Atkins et al., 2008, 2009; Souza et al., 2009), and is around 60% (Geary et al., 2000). The second GnRH will induce a LH surge and subsequent ovulation of the dominant follicle resulting from the new wave induced by the initial GnRH treatment. Luteolysis is induced by PGF administration from 48 to 66 hours before to the second GnRH treatment. At this moment, cycling cows may present 2 corpora lutea due to the presence of the corpus luteum induced by the initial GnRH and a spontaneously formed corpus luteum from an earlier ovulation. Timed-Al is performed coincident with the second GnRH treatment. One important concern with this sequence is the proportion of cows that are induced to ovulate small dominant follicles following the second GnRH treatment and, in fact, these animals are less likely to become pregnant to timed-Al (Lamb et al., 2001; Perry et al., 2005).

We and many others have investigated the influence of ovulatory follicle maturity on fertility in beef cattle (Mussard et al., 2003a, b; 2007; Bridges et al., 2010; Perry et al., 2005). The logical postulate was that diameter of ovulatory follicles was the most appropriate indicator of follicle “maturity” and that cows induced to ovulate small follicles would have decreased fertility compared to cows which are induced to ovulate large dominant follicles. Within each of three experiments (Table 1; Mussard et al., 2003a, b; 2007) this hypothesis was supported, but as data accumulated, from multiple experiments, the relationship of follicle diameter to pregnancy rate appeared inconsistent. In fact, across experiments, the more consistent predictor of pregnancy rate appeared to be the interval from initiation of luteal regression with PGF to either a spontaneous or GnRH-induced LH surge (Table 1). Since proestrus is defined as the interval between luteal regression and initiation of estrus/LH surge in a
spontaneous estrous cycle, we refer to the interval from PGF (and progestin withdrawal when applicable) to induction of the LH surge with GnRH as ‘proestrus’ in our research. The emerging relationship between length of proestrus and conception rate in three previous studies (Mussard et al., 2003a, b; 2007) led to an additional experiment (Table 1; Bridges et al., 2010) designed to standardize follicle diameter and age, and only vary length of proestrus. In this experiment, 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 furthermore, suggested that diameter of the ovulatory follicle, in itself, was not as consistent of a predictor of follicle maturity. The effect of ovulatory follicle size at GnRH-induced ovulation or at spontaneous ovulation on conception rate has also been evaluated by Perry et al. (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 of the ovulatory follicle did not impact fertility. The impact of follicle diameter on conception rate was evident when ovulation was induced with GnRH; at a constant duration of proestrus. Since our findings suggested that maturity of the ovulatory follicle and probability of conception is perhaps best defined by length of proestrus, especially when ovulation is timed with GnRH, we applied this knowledge towards optimizing the existing 7 day CO-Synch + CIDR program.

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

<table>
<thead>
<tr>
<th>Conception Rate (%)</th>
<th>Follicle diameter at Ovulation (mm)</th>
<th>Duration of Proestrus (days)</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</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</td>
</tr>
<tr>
<td>10</td>
<td>12.6 ± 0.2</td>
<td>1.25</td>
<td>10</td>
<td>Bridges et al., 2010</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</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</td>
</tr>
<tr>
<td>71</td>
<td>12.9 ± 0.2</td>
<td>2.25</td>
<td>28</td>
<td>Bridges et al., 2010</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</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</td>
</tr>
</tbody>
</table>

*Percentage of animals determined to be pregnant following insemination. Pregnancy determination was conducted via ultrasonography at approximately 30 days post-insemination.

*Diameter of the largest ovulatory follicle as determined by ultrasonography conducted either at GnRH administration or estrus.

*Interval from PGF$_{2\alpha}$ until GnRH administration.
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.

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.

Cows were induced to ovulate either a small (~11 mm) or large (~13 mm) ovarian follicle with GnRH. Embryos from non-treated cows were then transferred 7 days after GnRH.

Cows were induced to ovulate an ovarian follicle of similar diameter with GnRH either 1.25 or 2.25 days following PGF$_{2\alpha}$ 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 (PGF to second GnRH and timed AI) with the traditional 7-day CO-Synch + CIDR program was varied from 54 to 66 hours in mature cows without influencing timed AI pregnancy rate, but in younger cows (≤ 3 years of age), greater pregnancy rate was achieved with timed AI at 56 hours (Dobbins et al., 2009). Others (Busch et al., 2008) 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 approach could be modified in a manner that would extend proestrus, that this would increase secretion of estradiol by the ovulatory follicle, decrease the incidence of induced ovulation of follicles with lesser estrogenic capacity, and result in increased timed AI pregnancy rates. To achieve this end, we decided to shorten the interval from the initial GnRH treatment and insertion of the CIDR to CIDR withdrawal and PGF from 7 to 5 days to attempt to increase the length of proestrus in this timed AI program.

**The 5-d CO-Synch + CIDR Program in Postpartum Beef Cows**

The 5-d CO-Synch + CIDR protocol was developed at The Ohio State University over the past 7 years and is now recommended as a timed AI option for beef cows. In postpartum beef cows, this approach increased timed AI pregnancy rate by 10.5 percentage points compared with the traditional 7-d CO-Synch + CIDR program (Bridges et al., 2008). In our first 5 years of working with the 5-d program, timed AI pregnancy rates averaged 68% across 23 herds in 3 states. This paper describes how the 5-d CO-Synch + CIDR program was developed.

**2004 breeding season – Ohio**

The objectives of the first study conducted in 2004 (Bridges et al., 2008) were to compare estrous response, interval to estrus, and conception rate during the synchronization period when the interval from GnRH to PGF in a Select Synch + CIDR (estrus detection followed by AI with no second GnRH) program was reduced from 7 (7-d) to 5 (5-d) days. Postpartum beef cows (n = 156) were assigned by parity and days postpartum (68.9 ± 1.3; range 25-101) to either the 7-d (n = 79) or 5-d (n = 77) Select Synch + CIDR treatment. For all experiments discussed in this review, the day of administration of the initial injection of PGF is defined as day 0 or hour 0 of the experiment. Blood samples for progesterone analysis were collected on day -15 and -5 of the experiment to determine if cows were cyclic or anestrus. All cows received 100 µg of GnRH im (Ovacyst®, IVX Animal Health Inc.) and a CIDR on either day -7 (7-d) or day -5 (5-d). On day 0 and at hour 0, the CIDR were removed and cows received 25 mg of PGF im (Dinoprost tromethamine; Lutalyse®, Pfizer Animal Health). Due to the relatively short interval between the GnRH to PGF$_{2\alpha}$ in the 5-d treatment, it was unknown whether the accessory CL would regress in all cows with a single dose of PGF in this treatment. Therefore, a second dose of 25 mg of PGF was given in both treatments approximately 12 hours after the first PGF treatment to avoid the possible occurrence of incomplete luteal regression that would confound determination of the effect of the 5-d program on fertility. Estrus detection was performed in the AM and PM for 7 days after CIDR withdrawal and all cows detected in estrus were artificially...
inseminated approximately 12 hours after estrus detection. In this first study, 54.4% of the cows in the 7-d treatment and 57.1% of the cows in the 5-d treatment were cyclic at CIDR insertion. Estrous response was similar between treatments (Table 2). The interval from CIDR withdrawal to estrus did not differ between the 7-d (55.9 ± 1.5 h) and 5-d (58.9 ± 1.3 h) treatment. Estrus distribution between treatments is presented in Fig. 1. Conception rate and first service AI pregnancy rate also did not differ between treatments (Table 2). Breeding season pregnancy rate did not differ between treatments (Table 2).

Table 2. Reproductive performance of postpartum beef cows treated with either the 7-d or 5-d Select Synch + CIDR protocol (Adapted from Bridges et al., 2008).

<table>
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<tr>
<th>Variable</th>
<th>7-d</th>
<th>5-d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus response, %</td>
<td>91.1</td>
<td>83.1</td>
</tr>
<tr>
<td>Conception rate, %</td>
<td>51.4</td>
<td>56.3</td>
</tr>
<tr>
<td>First service pregnancy rate, %</td>
<td>46.8</td>
<td>46.8</td>
</tr>
<tr>
<td>Breeding season pregnancy rate, %</td>
<td>82.3</td>
<td>85.7</td>
</tr>
</tbody>
</table>

Figure 1. Estrus distribution for postpartum beef cows treated with either 7-d or 5-d Select Synch + CIDR program (Adapted from Bridges et al., 2008)
In a complementary study in 2004 (Bridges et al., 2008), the objective was to compare timed AI pregnancy rates with timed AI at 60 hours after the first PGF in both the 7-d and 5-d CO-Synch + CIDR program. Postpartum beef cows (n = 223) were assigned by parity and days postpartum (69.9 ± 1.4; range 21-99). Blood samples for progesterone analysis were collected on day -15 and -5 of the experiment to determine if cows were cyclic or anestrus. Approximately 59% of cows were anestrus at the beginning of synchronization program. All cows received 100 µg of GnRH (Ovacyst®) and a CIDR on either day -7 or day -5. On day 0 at hour 0, the CIDR was removed and the first 25 mg of PGF (Lutalyse®) was given. At approximately hour 12, a second 25 mg of PGF was administered to all cows. At hour 60, timed AI was performed in all cows with a second treatment of 100 µg of GnRH (Ovacyst®). Timed AI pregnancy rates did not differ between treatments and were 52.7% in the 7-d CO-Synch + CIDR and 56.8 % in the 5-d CO-Synch + CIDR when timed AI was performed at hour 60.

2005 and 2006 Breeding Season - Ohio

Our original hypothesis was that when interval from the first GnRH and CIDR insertion to PGF was shortened to 5 days, proestrus could be lengthened and timed AI pregnancy rate would increase. Therefore, during the breeding seasons of 2005 and 2006, the interval from PGF to the second GnRH and timed AI was extended to 72 hours in the 5-d CO-Synch + CIDR program and compared to the standard 7-d CO-Synch + CIDR with timed AI and GnRH at the recommended 60 hours after PGF. In 2005, postpartum beef cows (n =223) were assigned by parity and days postpartum to either the 7-d or 5-d CO-Synch + CIDR with timed AI at hour 60 or hour 72, respectively. Blood samples for progesterone analysis were collected on day -15 and -5 of the experiment to determine if cows were cyclic or anestrus. All animals received 100 µg of GnRH (Ovacyst®) and a CIDR was inserted on either day -7 or day -5. On day 0 at hour 0, the CIDR was removed from all cows and the first 25 mg of PGF (Lutalyse®) was given. At approximately hour 12, a second dose of 25 mg of PGF was administered to all cows. Timed-AI was performed at hour 60 for the 7-d CO-Synch + CIDR and at hour 72 for the 5-d CO-Synch + CIDR program, concomitant with an administration of 100 µg of GnRH. In this, and all other experiments described herein, synchronization treatments were offset such that all cows, regardless of treatment, received GnRH and timed AI in the morning, on the same date and at random relative to treatment. In 2006, we repeated the same approach described above with 400 postpartum cows in 2 locations in Ohio. The only difference between 2005 and 2006 was that instead of Lutalyse®, estroPLAN™ (cloprostenol sodium; Pfizer Animal Health) was used in 2006. Table 4 summarizes the results achieved in 2005 and 2006. Estrus detection was performed in 2006 with one herd and figure 2 illustrates the results.
Table 3. Reproductive performance of postpartum beef cows synchronized with either the 7-d or 5-d CO-Synch + CIDR* program (Adapted from Bridges et al., 2008)

<table>
<thead>
<tr>
<th>Variable</th>
<th>7-d with AI @ 60 vs. 5-d with AI @ 72 2005</th>
<th>7-d AI with @ 60 vs. 5-d AI @ 72 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7-d</td>
<td>5-d</td>
</tr>
<tr>
<td>n</td>
<td>111</td>
<td>105</td>
</tr>
<tr>
<td>Cyclic, %</td>
<td>11.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Location 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Location 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Timed AI pregnancy rates, %</td>
<td>66.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breeding Season pregnancy rates, %</td>
<td>93.7</td>
<td>96.2</td>
</tr>
</tbody>
</table>

* Cows in all experiments received 100 mg of GnRH and a CIDR on either d -7 (7-d) or d -5 (5-d). On d 0 the CIDR was withdrawn, and PGF was given. A second dose of PGF was given to all cows approximately 12 h later.

<sup>ab</sup> Means differ between treatments within experiment, P < 0.05.

Figure 2. Estrus distribution for postpartum beef cows synchronized with either a 7-d CO-Synch + CIDR with timed AI at hour 60 or 5-d CO-Synch + CIDR with timed AI at hour 72. Visual estrus detection was performed twice daily (AM and PM) following PGF until timed-AI. In both treatments, two doses of 500 mg of cloprostenol sodium were administered 12 h apart, with the initial dose given at CIDR withdrawal. Timed-AI pregnancy rates (%) for cows in each period of estrus are reported in parenthesis (Bridges et al., 2008)
2006 breeding season - Virginia

The increased timed AI pregnancy rates with the 5-d CO-Synch + CIDR with timed AI at hour 72 were achieved when the second PGF treatment was given 12 hours after CIDR withdrawal. We had previously observed that similar reproductive performance was achieved in yearling heifers with a 7-d or a 5-d Select-Synch + CIDR program with a single 25 mg PGF treatment (Helser et al., 2006). To test the necessity of the second PGF treatment in postpartum cows, Kasimanickam et al. (2009), compared the reproductive performance of 830 postpartum beef cows synchronized with the 5-d CO-Synch + CIDR program when given either 2 doses of 25 mg of PGF (2 x PGF; Lutalyse®) 7 hours apart, or a single treatment of either 25 mg of PGF (1 x PGF; Lutalyse®) or 500 µg of cloprostenol sodium (1 x CLP; estroPLAN™) at CIDR removal. On day -5, all cows received 100 µg of GnRH (Cystorelin®, Merial) and a CIDR. On day 0, all cows received their luteolytic treatment as described above and the CIDR was withdrawn. At hour 72, cows received 100 µg of GnRH concomitant with timed AI. Reproductive performance is presented in table 4.

Table 4. Effect of treatment on Timed AI pregnancy rate, breeding season pregnancy rate and estrus response in postpartum beef cows synchronized with the 5-d CO-Synch + CIDR program (Adapted from Kasimanickam et al., 2009).

<table>
<thead>
<tr>
<th>Pregnancy Rate</th>
<th>Treatment</th>
<th>n</th>
<th>Pregnancy rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timed AI</td>
<td>1 x PGF</td>
<td>277</td>
<td>52.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2 x PGF</td>
<td>282</td>
<td>69.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1 x CLP</td>
<td>271</td>
<td>54.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1 x PGF</td>
<td>277</td>
<td>87.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breeding Season</td>
<td>2 x PGF</td>
<td>282</td>
<td>92.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1 x CLP</td>
<td>271</td>
<td>87.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Estrus behavior, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus</td>
<td>1 x PGF</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>2 x PGF</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>1 x CLP</td>
<td>40</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means differ between treatments, P < 0.05.

This study demonstrated that 2 doses of PGF in postpartum beef cows resulted in greater pregnancy rate at timed AI that a single dose of PGF or CLP. Based on these results, it was concluded that in order to achieve higher timed AI pregnancy rates associated with the 5-d CO-Synch + CIDR program in postpartum beef cows, 2 doses of PGF at CIDR removal were crucial. The additional cattle handling and cost of the second injection of PGF with the 5-d program resulted in a 10.5% increase in timed AI pregnancy rates (Bridges et al., 2008) when compared to the 7-d program and a 15-17% advantage when compared to a single dose of PGF or CLP (Kasimanickam et al., 2009).
2007 breeding season - Ohio

Some of our data from previous years suggested that the reduction in pregnancy rates with a single PGF dose may be associated with a delayed estrus response and time of luteal regression in the 5-d program (Helser et al., 2006). Reproductive performance and luteal regression with the 5-d CO-Synch + CIDR program when using a single dose of 500 µg CLP (estroPLAN™) was evaluated in 3 studies (Souto et al., 2009). In all experiments, cows received 100 µg GnRH (Ovacyst®) and a CIDR on day -5. The CIDR was withdrawn on day 0 at hour 0, and cows received either 2 doses of 25 mg of PGF (Lutalyse®) 12 hours apart, or a single dose of CLP. In experiment 1, cows (n = 195) received no further treatment and estrous detection with AI was performed for 7 d. Cows that received 2 doses of PGF had a greater (P < 0.05) estrus response (94.0%) than with a single CLP (77.9%), but the interval to estrus (66.4 ± 1.2 h) did not differ between treatments. In experiment 2, cows (n = 254) received 100 µg of GnRH and timed AI at hour 72 when 2 PGF were administered or at hour 84 when a single dose of CLP was given. Timed AI pregnancy rate tended to be greater in cows that received the 2 PGF doses and timed AI at hour 72 (in conjunction with GnRH) than when a single dose of CLP was given and timed AI/GnRH occurred at hour 84 (68.8% and 57.9%, respectively; P = 0.08). In experiment 3 (n = 48), blood samples for progesterone analysis were collected at hour 0, 4, 8, 12, 16, 24, 48, 72 and 96 and estrous detection was performed following treatment with either 2 doses of PGF at a 12 hour interval or a single CLP at hour 0. Progesterone concentrations did not differ between treatments from hour 0 to 12 (Figure 3), but were greater with a single dose of CLP than 2 doses of PGF from hour 24 to 96 (treatment x hour, P = 0.05). When a single dose of CLP was used, fewer cows were detected in estrus with the 5-d Select Synch + CIDR, timed AI pregnancy rate tended to be lower, even when timed AI was delayed by 12 hours in the 5-d CO-Synch + CIDR, and the incidence of a delay, or failure of luteal regression was increased. These findings combined with the results achieved in Virginia by Kasimanickam et al. (2009) confirmed that two doses of PGF were necessary to maximize timed AI pregnancy rate with the 5-d CO-Synch + CIDR program.

Figure 3. Progesterone concentrations after PGF in postpartum beef cows synchronized with the 5-d Select Synch + CIDR protocol receiving either 2 doses of 25 mg of PGF (Lutalyse®) or a single dose of 500 µg of cloprostenol sodium (estroPLAN™, CLP). Figure adapted from Souto et al., 2009.
2008 breeding season - Ohio and Indiana

Obviously, the second PGF dose creates some management challenges regarding holding the animals for the second dose, labor requirements, handling of calves, etc. These challenges would probably be exacerbated in situations in which synchronization and AI was being managed by a technician that would have responsibilities and commitments for multiple herds and locations.

Therefore, the question addressed in 2008 was if the interval between PGF doses could be shortened to an interval that would lessen some of the management challenges. In most previous experiments in Ohio, the interval between PGF doses was approximately 12 hours whereas in Virginia (Kasimanickam et al., 2009) a 7 hour interval was used. In both locations, timed AI pregnancy rates approached 70% with the 5-d program. Although not directly compared, our interpretation was that this interval could be shortened to at least 7 hours with no detrimental influence on timed AI pregnancy rate. In Figure 3, no acute response to the second PGF in terms of progesterone concentrations was evident as compared to a single CLP dose. Rather, it appeared that the second PGF simply ensured that regression of the CL continued in all animals. Conversely, with a single dose of CLP, “recovery” of function of the CL occurred in some cows. We hypothesize that the luteolytic effect of the second PGF and timed AI pregnancy rate would be similar whether the interval between doses was 2 or 12 hours in a 5-d program.

The objectives of the two experiments were to assess the impact of the interval between two PGF doses in the 5-d CO-Synch + CIDR on reproductive performance (experiment 1) and luteal regression (experiment 2) in beef cows. Cows were assigned, within parity, by days postpartum to treatments. Blood samples for progesterone analysis were collected on d -15 and -5 to classify cows as cyclic or anestrus. All cows received 100 µg of GnRH (Cystorelin®) at the time of CIDR® insertion on d -5. In experiment 1, all cows (n = 254) received their first 25 mg dose of PGF (Lutalyse®) on d 0 at h 0, at the time of CIDR withdrawal. A second dose of PGF was given at either hour 2 (2hPGF) or hour 12 (12hPGF). At hour 72, cows received 100 µg GnRH and timed AI. Blood samples were collected at hour 72 and analyzed for progesterone. In experiment 2, cows (n = 31) received the 2hPGF and 12hPGF treatments as described, with the exceptions that the CIDR was withdrawn at hour -2 and the second GnRH/Timed AI was not performed. Rather, estrous detection was performed from days 0 to days 7 and blood samples for progesterone collected at hour 0, 2, 4, 12, 14, 24, 48, 72 and 96 (Figure 4). Ultrasonography performed on d -5, -1, 3 and 10 was used to detect existing CL, induction of ovulation with the initial GnRH, regression of CL with PGF and formation of a new CL, respectively. In experiment 1, timed AI pregnancy rate did not differ between 2hPGF and 12hPGF treatments (60.8% and 58%, respectively). Concentrations of progesterone at hour 72 did not differ between treatments. In experiment 2, progesterone concentrations, incidence and timing of estrus, luteal regression and other ovarian characteristics determined by ultrasonography did not differ between treatments (Figure 4). In conclusion, in our research, reduction of the interval to the second PGF treatment from 12 to 2 hours in the 5-d CO-Synch + CIDR program did not influence timed AI pregnancy rate or the occurrence of luteal regression. However, in a subsequent study performed by Whittier et al. (2010), cows were given the second PGF dose at various times ranging from 0.5 to 8.15 hours after the first PGF. When cows were then grouped as receiving PGF from 0.5 – 3.9 hours after the initial PGF, or 4.5 to 8.15 hours after the initial PGF, a reduction in timed AI pregnancy rate was detected (52.4 vs. 57.1%, respectively).
Figure 4. Progesterone concentrations after PGF in postpartum beef cows synchronized with the 5-d Select Synch + CIDR protocol receiving either 2 doses of 25 mg of PGF (Lutalyse®) in a 2 hour interval or 2 doses of 25 mg of PGF (Lutalyse®) given 12 hour apart (Cruppe et al., 2010).

2009 breeding season - Ohio and Indiana

To this point it was clear that flexibility existed regarding the timing of the 2nd PGF. The next obvious question was whether doubling the dosage of PGF would induce luteal regression as effectively as two doses spaced between 2 to 12 h? If effective, the convenience of the 5-d program would be enhanced. While there is not data that indicates that currently approved doses of PGF are limiting, they have not been tested on groups of cows in which a majority have a CL that was induced with GnRH five days earlier. Our data in beef heifers (Helser et al., 2006; Souto et al., 2009) and in dairy heifers from Florida (Rabaglino et al., 2010) have demonstrated that a single PGF is as effective two PGF doses with the 5-d program in heifers. Obviously, on a per body weight basis, the dose of PGF being delivered to heifers is considerably greater than in postpartum beef cows. If the capacity of a single PGF to effectively induce luteal regression in heifers but not cows with the 5-d program is due to a dose/kg BW difference, the potential existed that doubling the PGF dose would be effective in postpartum cows. Alternatively, if there was some presently unknown difference in the sensitivity of the early CL to PGF between heifers and cows, then the double dose may not be as effective as spaced doses with the 5-d program in postpartum cows.

The aim of the study performed in the breeding season of 2009 was to determine the effect of timing of the second dose of PGF in the 5-d CO-Synch + CIDR program on timed-Al pregnancy rate. Postpartum beef cows (n = 662) at 5 locations were assigned, within parity (primi- or multiparous), by days postpartum to treatments. At 4 locations, blood samples for progesterone analysis were collected on d -15 and -5 of the experiment to determine if cows were cyclic or anestrous. All cows received 100 µg of GnRH (Cystorelin®) at the time of CIDR insertion on d -5. On d 0 (h 0), the CIDR was withdrawn, and all cows received their first 25 mg dose of PGF (Lutalyse®). A second dose of PGF was administered either immediately following the first injection, coincident with CIDR withdrawal (CoPGF; n = 218), hour 2 (2hPGF; n = 226) or hour 8 (8hPGF; n = 218). At hour 72, all cows received 100 µg GnRH
and timed AI. Pregnancy rates to timed AI did not differ and were 69.7% in the CoPGF, 65.5% in the 2hPGF and 66.1% in the 8hPGF treatments (Figure 5a). Timed AI pregnancy rates by location are illustrated in figure 5b. Irrespective of treatment, timed AI pregnancy rate was greater (P = 0.05) in multiparous (68.6%) than primiparous (59.8%) cows (Figure 6). In conclusion, two coincident doses of PGF at CIDR withdrawal in the 5-d program results in similar timed AI pregnancy rates as when given either 2 or 8 h apart (Cruppe et al., 2010b).

Figure 5. Timed AI pregnancy rates are shown in figure 5a when 662 cows were synchronized with the 5-d CO-Synch + CIDR program receiving a second dose of PGF either immediately following the first injection, coincident with CIDR withdrawal (CoPGF; n = 218), hour 2 (2hPGF; n = 226) or hour 8 (8hPGF; n = 218). This experiment was performed in 2 states and 5 different herds as shown in figure 5b (Adapted from Cruppe et al., 2010b).

![Figure 5a](image1.png)

![Figure 5b](image2.png)

Figure 6. Timed AI pregnancy rates by animal class and reproductive status in cows synchronized with the 5-d CO-Synch + CIDR program receiving a second dose of PGF either immediately following the first injection, coincident with CIDR withdrawal (CoPGF), hour 2 (2hPGF) or hour 8 (8hPGF).

![Figure 6](image3.png)
2010 breeding season – A Multistate Project

A large multistate project was conducted in 2010 (Bridges et al., 2011) to further test the CoPGF approach in a wider range of production conditions. Postpartum Bos taurus and Bos indicus beef cows (n = 2465; 67 ± 0.4 days postpartum) from 13 herds in 8 states were enrolled in the 5-d CO-Synch + CIDR program. All cows received 100 µg of GnRH (Factrel®, Pfizer Animal Health) and a CIDR on day -5 of the experiment. On day 0, cows were assigned to receive either 2 doses of 25 mg of PGF (Lutalyse®) 8 hours apart with the initial injection given at CIDR removal (8hPGF), 2 doses of 25 mg PGF delivered in 2 injection sites with both administered at CIDR removal (CoPGF), or a single 25 mg dose of PGF at CIDR removal (1xPGF). Cows were timed AI 72 hours after CIDR withdrawal coincident with the second GnRH. Reproductive status (54% cyclic) was determined by evaluation of progesterone in 2 blood samples taken on d -15 and -5. Determination of pregnancy was performed by transrectal ultrasonography 39 ± 0.1 d after timed AI and after the conclusion of the breeding season. Timed AI pregnancy rates were greater (P < 0.05) for the 8hPGF (55%) than the 1xPGF (48%) treatment, with the CoPGF (51%) treatment intermediate and not different from the other treatments. Contrast analysis demonstrated that cows receiving 50 mg of PGF (8hPGF and CoPGF) had greater (P < 0.05) timed AI pregnancy rates than those receiving 25 mg (1xPGF). Pregnancy rates to timed AI were greater (P < 0.05) in cyclic (55%) than non-cyclic (47%) and greater (P < 0.05) in mature (≥ 3 years of age; 54%; n = 1940) than 2 years old cows (40%; n = 525). Luteolysis following PGF treatment was assessed in a subset of cows (n = 277) and did not differ (P = 0.13) among the 8hPGF (96%), CoPGF (93%), and 1xPGF (88%) treatments. Breeding season pregnancy rates (88%) did not differ among treatments but were greater (P < 0.01) in mature (90.4%) than 2 years old cows (77.7%). In summary, 50 mg of PGF is required in the 5 d CO-Synch + CIDR protocol; however, timed AI pregnancy rates did not differ when 50 mg of PGF was administered simultaneously with CIDR removal or at 0 and 8 h following CIDR withdrawal.

The 5-d CO-Synch + CIDR in heifers

The 5-d program is also being used to synchronize beef heifers in the USA. It was reported that similar reproductive performance was achieved when heifers (n = 159) received either a 7-d or a 5-d Select Synch + CIDR program and were AI only based on estrus detection with a single 25 mg PGF treatment (Helser et al., 2006). Wilson et al. (2007) used a larger number of heifers (n = 233) that received either the 7-d or a 5-d CO-Synch + CIDR with an injection of PGF at CIDR removal and 12h later, followed by timed AI at either hour 60 (7-d) or hour 72 (5-d) accompanied by GnRH. Wilson et al. (2007) used a larger number of heifers (n = 233) that received either the 7-d or a 5-d CO-Synch + CIDR with an injection of PGF at CIDR removal and 12h later, followed by timed AI at either hour 60 (7-d) or hour 72 (5-d) accompanied by GnRH. There was a tendency (P < 0.09) for more heifers in the 5-d program to exhibit estrus than in the 7-d (70.9 % vs. 60.7%, respectively) treatment. The interval from CIDR removal to estrus was greater for 5-d (50.9 ± 1.1 h) compared to the 7-d (45.4 ± 1.2 h) program. Timed AI pregnancy rates were greater for heifers synchronized with the 5-d (57.8%) compared to the 7-d (43.6%). Lima et al. (2010) synchronized approximately 3000 dairy heifers using the 5-d CO-Synch program with a variety of modifications tested. Across these treatments timed AI pregnancy rates ranged from 50.4% to 63%. Sparks et al. (2010) assigned heifers (n = 664) to either the 7-d Select Synch + CIDR or the 5-d Select Synch + CIDR treatment. All heifers received 2 doses PGF given 8 h apart. Heifers detected in estrus by h 60 were bred by the AM/PM rule and heifers not detected in estrus were bred 72 hours after the first PGF coincident with GnRH treatment. Conception rate of heifers in estrus was greater (P < 0.05) in the 5-d (61.9%) than the 7-d (50.0%) treatment. Including heifers that were timed AI, more heifers became pregnant to a synchronized AI in the 5-d (57.1%) than 7-d (47.3%) Select Sync + CIDR treatment. Gunn et al. (2009) compared AI pregnancy rates between the 5-d CO-Synch + CIDR and the CIDR Select program in beef heifers (n = 318). The CIDR Select treatment consisted of insertion of a CIDR for 14 days, an injection of GnRH (Cystorelin®) 9 days after CIDR withdrawal, and treatment with 25 mg PGF (Lutalyse®) 7 days after GnRH. In both treatments, heifers were detected for estrus for 56 h following PGF and those exhibiting estrus were AI based on the AM/PM rule. Heifers not observed in estrus received timed AI 72 h after PGF with GnRH. Conception rate of heifers exhibiting estrus (CIDR Select; 75.2%, 5-d; 76.5%), timed AI pregnancy rate (CIDR Select; 62.1%, 5-d; 63.5%) and overall synchronized AI pregnancy rate (CIDR Select; 70.4% 5-d; 70.4%) did not differ between treatments. Luteal regression with the 5-d program when various luteolytic treatments were used.
was evaluated in 79 heifers (Souto et al., unpublished). The CIDR was withdrawn on day 0 at hour 0, and heifers received either 2 doses of 25 mg of PGF (Lutalyse®) 12 h apart, 1 dose of 25 mg of PGF or a single dose of CLP (estroPLAN™). Blood samples for progesterone analysis were collected at hour 0, 6, 12, 16, 24, 72. Progesterone concentrations after PGF injections are shown in Figure 7 and did not differ among treatments. Rabaglino et al. (2010) investigated whether the 5-d Co-Synch + CIDR program could be used in dairy heifers for timed AI with a single dose of PGF. Heifers (n = 593) were assigned to receive either 1 dose, or 2 spaced doses of PGF at CIDR withdrawal. Neither timed AI pregnancy rate (46.1 and 48.6%) nor incidence of luteolysis (86.9 and 92.8%) varied between 1 and 2 doses of PGF, respectively. In a recent study, Peterson et al. (2011) tested whether a 5- CO-Synch + CIDR program with a single dose of PGF would achieve similar timed AI pregnancy rate at hour 72 when compared to 2 doses of PGF given 6 h apart in 562 beef heifers. A significant interaction of treatment and herd was detected, however, when only main effects were considered, heifers receiving two doses of PGF tended (P = 0.06) to have greater timed AI pregnancy rates than heifers receiving a single dose of PGF.

Figure 7. Progesterone concentration in heifers synchronized with 5 d CO-Synch + CIDR receiving either 2 doses of 25 mg of PGF (Lutalyse®), 1 dose of 25 mg of PGF (Lutalyse®) or a single dose of CLP.

The 5-d CO-Synch + CIDR program is clearly an effective program for both beef and dairy heifers. Unlike in postpartum beef cows, some question still lingers as to whether heifers should receive 1 or 2 doses of PGF and whether the CoPGF approach will circumvent this challenge. Some question remains as to whether timed AI at 72 h is the ideal time for heifers, as it is in postpartum cows. Findings in this regard have been inconclusive. Finally, the necessity of the initial GnRH treatment in yearling heifers is also being investigated by a variety of groups. Our current recommendation for producers for heifers is to use the 5-d Select Synch + CIDR with a single dose of PGF, perform estrus detection and AI by the AM/PM rule for heifers in estrus by h 48, with timed AI for all remaining heifers at 72 hours in conjunction with GnRH treatment. If only timed AI is being used, this can vary from 60 to 72 h, and if possible, should be dependent upon the timing of estrus in the first heifers that are detected.
Summary

For the 5-day CO-Synch + CIDR program in postpartum beef cows (Figure 8), two rather than one dose of PGF are administered at CIDR withdrawal, which is 5 days after CIDR insertion and the first GnRH treatment. With this approach, the new follicular wave emerges 3 to 4 days before PGF is given whereas in the 7-d program, the dominant follicle emerges 5 to 6 days before PGF. Thus, with the 5-d program, the dominant follicle enters proestrus at a follicular age at which follicular fluid estradiol concentrations and capacity of granulosa cells to produce estradiol are greater than with the 7-day program (Valdez et al., 2005). Bridges et al. (2008) determined that the appropriate interval from PGF to the second GnRH and timed-AI with the 5-day program was 72 hours in postpartum beef cows. In comparison with the traditional 7-day CO-Synch + CIDR program, the 5-day CO-Synch + CIDR program increased timed-AI pregnancy rate from 59.9 to 70.4% (Bridges et al., 2008). Similarly, a 14% increase in timed-AI pregnancy rate was detected in yearling heifers with the 5-day as compared to 7-day program (Wilson et al., 2007). This substantial increase in timed-AI pregnancy rate with the 5-day program was achieved using two doses of PGF (or CLP) spaced at 12 hours apart in the initial experiments, and it is clear that pregnancy rate is decreased with a single dose of PGF in postpartum cows (Kasimanickam, et al. 2008; Souto et al., 2009) due to failure of a single dose to induce luteal regression in approximately 30% of cyclic cows (Souto et al., 2009). Cruppe et al. (2010) reported that giving two simultaneous doses of PGF at the time of CIDR withdrawal in the 5-day program resulted in pregnancy rates similar to those achieved with two injections, spaced 8 hours apart and this was confirmed by a large multistate study (Bridges et al., 2011). The 5-d program has been tested in lactating dairy cows (Bisinotto et al., 2010) and has shown promise to increased timed AI pregnancy rates in this class of cattle. In heifers, questions linger relative to the appropriate luteolytic dose, the necessity of the initial GnRH injection, and the most appropriate time of AI, but in comparisons with the 7-d approach, a similar 10+ percentage point increase in pregnancy rate relative to the 7-d program has been observed. Furthermore, comparisons of the 5-d program to longer term programs such as CIDR Select or the 14 d CIDR PG program in yearling heifers have resulted in similar synchronized pregnancy rates. The 5-d CO-Synch + CIDR program is an effective method for optimizing timed AI pregnancy rates in beef cattle.

Figure 8. The 5-d CO-Synch + CIDR program for beef cows (Adapted from Cruppe and Day, 2011).

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INTRODUCTION

Reproductive technologies have been developed in an attempt to increase the overall efficiency of bovine genetics. The first of these techniques, which has had the most predominant genetic impact on dairy herds, was artificial insemination (AI) (Foote, 2002). It is the worldwide acceptance of AI that has permitted the development and application of many commonly used technologies such as semen cryopreservation and sexing, estrus cycle synchronization and superovulation, and embryo collection, freezing, export, transfer and cloning. The transition from the originally intended use of frozen bull semen for AI to ET, IVF and sperm sexing have provided new challenges as sires can differ in their performance between the technologies.

The goal of a global bovine genetics company must include maximized in-cow fertility, semen quality and quantity combined with rapid genetic progress within the breeders herds. This paper includes information on sire development and maintenance, semen production (conventional and sexed), the evolution of quality control and bull fertility assessment.

SIRE DEVELOPMENT and MAINTENANCE

The arrival of a young dairy “test bull” at an AI production centre marks the end of the first stage of sire development. Prior to this event the genetics group has decided on the sire-dam combination the program requires, the resulting bull calf is genomically tested to determine if it is a suitable candidate and has successfully passed the required zoo-sanitary tests. About 60% of all young sires are products of this process referred to as planned mating. The reproductive technologies used to make this mating a reality include artificial insemination (AI - 24%), embryo transfer (ET – 75%) and in vitro fertilization (IVF – 1%).

In North America the young bulls enter the AI centre isolation facility between 6 and 9 months of age, will join the main herd after more zoo-sanitary screening is completed and will begin semen collection between 10 and 11 months of age. On average sufficient frozen semen will be available for use within the young sire proving program by the time the bull is 15 months old.

The zoo-sanitary demands for the production of frozen semen for global movement are extremely rigorous. The requirements outlined by the importing countries are supervised by the Canadian Food Inspection Agency whose standards are aligned with those of the World Organization for Animal Health (www.oie.int/en/).

SEMEN COLLECTION

Although semen collection from a bull with an artificial vagina (AV) is a time-tested procedure, there is a constant need to focus on the optimal pre-collection sexual preparation, a combination of false mounts and active restraint, to achieve the goal of maximized sperm harvest. Hale and Almquist, (1960), demonstrated that a single false mount increases sperm output by 50 percent and two additional mounts can double the output over that obtained with no preparation. Obviously the artificial vagina design, composition and the use of appropriate
temperatures are integral to the overall sperm harvest. Throughout the collection, evaluation and processing stages, highly reliable systems using RFID tags for bull, collector and handler combined with barcoded ejaculate ID labels provides low risk traceability throughout the production process.

PRE-FREEZE SEMEN EVALUATION AND QUALITY CONTROL

The application of technically sound and rigidly applied semen evaluation and quality standards for fresh semen pre-freeze results in the acceptance for processing of about 90% of the ejaculates collected. This section includes a review of past, present and future semen evaluation techniques.

When the fresh semen is delivered to the laboratory every ejaculate is subjected to a pre-freeze evaluation.

Ejaculate Volume

Up until the early 1990’s the ejaculate volume was estimated to the closest 0.1ml using a graduated collection tube. Most laboratories have now adapted a more precise, secure system of determining ejaculate volume by weight using an electronic balance (Kaporth, 1988). This allows a precise weight determination to 0.01g and the avoidance of information exchange errors by electronic transfer into the laboratory computer system.

Sperm Concentration

The method of sperm concentration (million sperm per ml) determination has evolved from visual estimation of fresh semen in the collection tube to the use of the haemocytometer followed by the use of a calibrated spectrometer (Foote, 1978). Recent efforts to validate techniques using fluorescent markers for sperm counting and spectrometer calibration including flow cytometry and the other instruments (example - NucleoCounter™, Chemometec, Allerod, Denmark) have demonstrated promise as new gold standards for quantifying sperm numbers. (Anzar et al. 2009, DelJarnette et al. 2008).

Motility

Motility is one of the most important characteristics believed to be associated with the fertilizing ability of spermatozoa. Several groups have reported a significant correlation between total (Wood et al., 1986; Gillan et al., 2008; Kjaestad et al., 1993; Correa et al., 1997) and progressive (Kathiravan et al., 2008) motility of bull semen and its associated field fertility. In the early days the mass wave motility of undiluted semen was used but this was replaced by a qualitative subjective motility analysis of a diluted sperm sample under a microscope which relied on technician experience and technique.

Morphology

Morphology is the second most common analysis made in AI laboratories. This analysis relies on the relationship between the incidence and type of morphological sperm abnormalities and field fertility. Traditional microscopic evaluation of fixed or stained samples, (Saacke et al., 2008) continues to be the most common method in use today although other more objective techniques including Fourier Harmonic Amplitude analysis (Ostermeier et al., 2011) and CASA-based morphometry (Aulesa et al., 2009) are under investigation.
SEMEN PROCESSING

Conventional semen

Once the ejaculate has been determined to be acceptable for processing, from the point of view of quality and quantity, an antibiotic solution (Shin S.J. and Kim S.G., 2000) is added to the raw semen. Should there be visible debris in the ejaculate, it is discarded, noted on the computer and then discussed with the collection team.

Most semen is diluted with an extender solution which includes either milk or egg yolk although there is a gradual tendency towards the use of animal protein-free extenders. Besides being a diluent, extender also functions as a protective energy source and a carrier for antibiotics and glycerol, the cryo-protectant. The volume of extender added is directly related to the number of straws to be frozen as well as the size of straw to be used. The number of straws per ejaculate is also related to the number of sperm per straw, the sperm dose. The extender is added and the diluted semen is cooled to 5°C within about 2 hours.

Since the advent of frozen semen the semen package has evolved gradually from a pellet, to glass ampoule, and then into various versions of the plastic straw. Today about 70% of the bovine semen frozen globally is packaged in the 0.25ml French straw with the remainder in the 0.5ml French straw. Numerous investigators have demonstrated the superiority of the 0.25ml straw for semen freezibility and in-cow fertility (Phatak, 2002).

Once the straws have been filled and sealed they are spread on freezing racks, counted and then prepared for freezing. There are two main methods of freezing bovine semen, under static conditions in liquid nitrogen vapour within a large nitrogen tank and within computer controlled programmable freezers. The latter system is more controlled and freezes larger numbers at the same time.

Sexed semen

Over the past decade the availability of sex-sorted semen has become a significant product line of the global bovine genetics industry. From the production perspective sex sorted semen involves at least 4 additional steps; staining, sorting, pooling and centrifugation (Figure 1) compared to conventional semen and has become a 24/7 process requiring 3 daily collection periods rather than one.

Figure 1: Comparison of conventional and sex sorted semen production
Post-Thaw Evaluation - Traditional

After freeze it cannot be assumed that the semen from all bulls froze well so it is necessary to conduct post-thaw semen evaluation on randomly selected straws of every freeze lot.

Motility

The percentage of motile sperm post-thaw is determined by light microscopy. Semen observed with a % motile exceeding the accepted minimum is retained for use. A discussion of the use of computer assisted sperm analysis (CASA) is included further on in this paper.

Sperm count per straw

Routine monitoring of the number of total sperm in frozen-thawed semen has traditionally been conducted using a haemocytometer. More recently the NucleoCounter™ (Chemometec, Allerod, Denmark) and some CASA systems which count fluorescently labeled sperm have demonstrated their potential as new gold standards.

Bacteriology

Post-thaw screening for the presence of undesirable bacteria provides a constant update on the cleanliness of the entire collection and processing system.

New Gold Standard Evaluation Systems

Computer assisted sperm analysis

Despite the power of an objective evaluation by CASA, there are some many factors known to affect CASA results including the type of specimen chamber used, the temperature at which semen is maintained, the concentration of the semen, the frame rate to analyze sperm tracks and speed and the type of extender in which semen was diluted. Some extenders contain debris of similar size to a sperm head, causing CASA software to include them in the analysis (Vincent et al., 2012). To overcome this problem, fluorescence technology allows discrimination of sperm cells from particles in the extender by staining sperm heads with a DNA binding fluorochrome. Under fluorescent light, only DNA-containing objects will be detected by the CASA software. CASA instruments collect a wide range of sperm motility parameters, allowing a more detailed, accurate and objective analysis of sperm movements and track speed. Researchers have also tried to correlate the kinetic parameters with the field fertility of semen, with some groups able to show a positive correlation between straight line velocity of spermatozoa and field fertility (Budworth et al., 1988; Gillan et al., 2008; Kathiravan et al., 2008; Farrell et al., 1998; Januskauskas et al., 1999). Another study used a combination of several motility parameters to reach a very high correlation with bull fertility (Farrell et al., 1998). Taken together, these studies show the high potential of CASA to estimate the quality of the semen, therefore becoming a powerful tool to measure sperm characteristics and predict bull fertility compared to standard semen evaluation.

Flow cytometry

Flow cytometry analyzes cells suspended in a stream of fluid passing at high velocity in front of one or several lasers. The light emitted by fluorochrome-bound cells is captured by photomultiplier tubes and converted into an electronic signal subsequently digitalized by cytometry software. Key features of flow cytometry are the acquisition and analysis of thousands of cells within seconds and the multiparametric potential of the technology.
The most modern cytometers are routinely equipped with 3 or more lasers and at least 10 photomultiplier tubes allowing cell labelling with several probes at the same time thus enabling analysis of numerous parameters simultaneously. In the last few years, the multiparametric aspect of flow cytometry allowed this technology to become a popular tool to evaluate sperm attributes (Gillan et al., 2005; Martinez-Pastor et al., 2010; Hossain et al., 2011). A wide range of fluorochromes has been developed to assess numerous characteristics of sperm cells.

Several factors influence the choice of the cytometer to use for the analysis of sperm cells. The price of the instrument remains a major factor that will influence this choice. Multiparametric analysis is usually obtained with instruments containing more than one laser and many photomultiplier tubes. An instrument with only one laser and 3 photomultiplier tubes allows detection of a maximum of 3 parameters on each cell while a multiparametric analysis including 4 and more parameters will usually require an instrument having at least 2 lasers and 4 photomultiplier tubes. The software operating the flow cytometer is another important aspect in the choice of the instrument. Most software products available are fairly easy and straightforward to operate for a novice user in flow cytometry. However, some software requires certain knowledge of flow cytometry concepts, making the instrument more difficult to operate. As an example, samples stained with a cocktail containing several probes are subjected to subpopulation gating analysis. In order to obtain representative results, gates need to be associated to the proper population in the correct hierarchy, a perspective that is difficult to handle with some software for a novice user. Moreover, some programs have gaps in export and data compilation, making it more difficult to analyze the data, and these shortcomings are time consuming for the user.

**Sperm attributes analyzed by flow cytometry**

**Viability/mortality**

Propidium iodide is the most popular dye used to identify dead cells. This membrane impermeant fluorochrome enters spermatozoa with damaged cellular membranes and binds to DNA where it can be excited with a 488nm laser present on most cytometers (Graham et al., 1990; Partyka et al., 2010; Oldenhof et al., 2011). Propidium iodide is often used in combination with SYBR-14, another DNA-labeling probe (Garner et al., 1994; Garner and Johnson, 1995). SYBR-14 is also excited by the 488nm laser and is a permeant probe staining all cells.

**Acrosome integrity**

Evaluation of acrosomal status is mainly assessed by using plant lectins recognizing acrosomal ligands. *Pivum sativum* agglutinin binds mannose and galactose moieties of the acrosomal matrix and as *pivum sativum* agglutinin cannot penetrate the intact acrosomal membrane, only spermatozoa with a reacted or damaged acrosome will be stained (Maxwell et al., 1996; Januskauskas et al., 1999; Nagy et al., 2003). However, it has been shown that *pivum sativum* agglutinin has an affinity for egg yolk and non-specific binding sites on the sperm cell surface (Purvis et al., 1990; Lybaert et al., 2009). *Arachis hypogaea* (peanut) agglutinin binds galactose moieties of the outer acrosome membrane and is the most popular lectin used to study the integrity of the acrosomal membrane with flow cytometry (Carvalho et al., 2010; Yi et al., 2011; Anzar et al., 2011). In addition, *arachis hypogaea* (peanut) agglutinin seems the most reliable probe to identify spermatozoa with a damaged acrosome as it displays less non-specific binding to other areas of spermatozoa (Carver-Ward et al., 1997). *Pivum sativum* agglutinin and *arachis hypogaea* (peanut) agglutinin are usually labeled with FITC fluorochromes, allowing them to be used by all cytometers.
Mitochondrial activity

Mitochondria are very important organelles involved primarily in the generation of the energetic substrates for the motility of the sperm cell. Rhodamine 123 was one of the first probes to monitor mitochondrial activity (Evenson et al., 1982; Garner et al., 1997). Rhodamine 123 is sequestered in active mitochondria and washed out from the cell when the membrane potential is lost. This characteristic limits its use when quantification is needed or when fixation of spermatozoa is required. To overcome the fixation problem, Mitotracker® dye could become a solution. This fixable dye accumulates and stains active mitochondria and has the advantage of availability in different ranges of excitation and emission fluorescence (Garner et al., 1997; Hallap et al., 2005; Sousa et al., 2011). The most popular probe to evaluate mitochondrial activity is JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) (Thomas et al., 1998; Gillan et al., 2005; Guthrie and Welch, 2008; Garner and Thomas, 1999). In spermatozoa with mitochondria having a high membrane potential, JC-1 enters the mitochondrial matrix where it accumulates and forms J-aggregates and become fluorescent red. In spermatozoa having mitochondria with low membrane potential, JC-1 cannot accumulate within the mitochondria and remains in the cytoplasm in a green fluorescent monomeric form. JC-1 has the advantage to quantify the mitochondrial burst of the cell compared to Rhodamine 123 and Mitotracker. A disadvantage of JC-1 probe is its dual fluorescence emission that limits its combination with other probes emitting in the green and red fluorescence.

DNA integrity

Assessment of chromatin status is important in the determination of the fertility potential of spermatozoa. In recent years, the sperm chromatin structure assay developed by Evenson and Jost (Evenson and Jost, 2000) is the main technique used to evaluate chromatin integrity in spermatozoa by flow cytometry (Januskauskas et al., 2003). The sperm chromatin structure assay uses the dual fluorescence emission of acridine orange depending whether it binds to single strand DNA (red fluorescence) or double strand DNA (green fluorescence). Following a denaturation step, the sperm sample is incubated with acridine orange then analyzed by flow cytometry. Denaturation will induce single strand DNA formation when DNA breaks are present and generate a heterogeneous population of red and green fluorescence depending on the integrity of the chromatin. The most important data derived from Sperm chromatin structure assay is the ratio red/green + red fluorescence called DNA fragmentation index where a high DNA fragmentation index correlates with high DNA damage. The DNA fragmentation index has shown some correlations with fertility in different species (Karabinus et al., 1990; Love and Kenney, 1998; Evenson et al., 1999). Another assay to assess DNA integrity developed for flow cytometry is the TUNEL assay (terminal transferase dUTP nick end labelling), which can identify DNA strand breaks (Anzar et al., 2002; Waterhouse et al., 2006; Sutovsky et al., 2002). Transferase enzyme incorporates fluorescent or modified nucleotides at the sites of DNA breakage and labelled cells can then be analyzed by flow cytometry. The TUNEL assay allows quantification of labelled nucleotides incorporated into fragmented DNA reflected by the increase of fluorescence, which gives an appreciable advantage over sperm chromatin structure assay.

Calcium influx

Calcium influx is one of the primary steps involved in the sperm capacitation process. The rise in intracellular calcium ultimately leads to the phosphorylation of tyrosine and serine residues from proteins regulating the signalling cascade. The most popular dye used to determine the intracellular calcium concentration in sperm cells is the Fluo-3/4 family probe excited by the 488nm laser line (Colás et al., 2009; Kumaresan et al., 2011; Guthrie et al., 2011). Calcium-unbound Fluo-3 is a non-fluorescent molecule but when calcium ions enter the cell and bind Fluo-3 the latter becomes fluorescent. Fluo-4 is a derivative of Fluo-3 bearing higher fluorescence intensity. Fura red is a probe also excited by the 488nm laser where its fluorescence emission decreases upon calcium binding. Dual labeling of spermatozoa with Fluo-3/Fura red allows a calculation of the ratio of unbound to bound calcium. The ratio between the two mean fluorescence intensities (Fluo-3/Fura red) is proportional to the intracellular...
calcium concentration of the spermatozoa. Penta acetoxymethyl ester Indo-1 acetoxymethyl is a membrane-
permeable calcium sensor dye used to monitor changes in intracellular calcium in the cell. Once Indo-1 enters the
cell, esterases cleave the acetoxymethyl group, yielding a membrane-permeable dye. Unbound Indo-1 has a peak
emission at 485 nm. Upon binding calcium, the peak emission shifts down to 410 nm. Measurement over time can
be represented as a ratio of the two emission wavelengths. As Indo-1 acetoxymethyl is a ratiometric probe, cell-
loading concerns (as for Fluo-3/4) are less important.

Multi-parametric analysis

Fertility is a multiparametric phenomenon that relies on the use of semen of sufficient quality and quantity,
accurate timing and method of insemination, and appropriate herd management. When using artificial
insemination, the dairy producer must manage a range of these factors, including heat detection, timing of
insemination in relation to oestrus, and correct handling of the frozen straws. However, it is the onus of the
semen production centers to supply straws containing spermatozoa of good viability that produce acceptable
conception rates if all other parameters are managed correctly.

To ensure acceptable fertility after artificial insemination, frozen-thawed spermatozoa must be present in
sufficient number in each straw (concentration), and possess a number of characteristics important for
fertilization. Accordingly, spermatozoa must survive the thawing procedure with normal morphology, an intact
acrosome, DNA integrity, active mitochondria, and maintain forward progressive motility to traverse the female
reproductive tract.

Combining CASA and cytometry will provide production centres with a powerful multiparametric approach to
evaluate the quality of the semen produced and allow the establishment of standardized procedures to make
accurate and repeatable decisions on the outcome of the semen.

The percentage of total and progressive motility and the percentage of viability, acrosome integrity and high
mitochondrial activity can be evaluated with CASA and flow cytometry and the relationship of the incidence of
these parameter to fertility in the field, we applied this analysis on semen lots of known fertility that were
released in the field after using standard quality control.

Fertility Measuring Systems

Throughout the history of bovine AI many systems have been utilized to measure the fertility of bull semen.
Obviously the percentage of cows that calve is the most precise but requires a full gestation period and pregnancy
losses can not necessarily be attributed to the semen donor. Pregnancy determination by manual palpation or
ultrasonography provides excellent early pregnancy diagnosis but are unfortunately labour and equipment
intensive. The AI industry, in an attempt to capture conception information associated with large numbers of
inseminations, developed a non-return rate (NRR) system. More sophisticated systems compared to the
traditional NRR systems, the Canadian Dairy Network (CDN) (Van Doormaal and Schaeffer, 1993) and Estimated
Relative Conception Rates (ERCR) (Clay, 2000) were developed using statistical models to adjust for factors not
attributable to the semen donor provide better estimates of bull fertility.

Sperm Dose Management

As shown in Figure 2 (Mitchell, 2012) most AI organizations have traditionally managed the extension rate or
sperm dose, total million sperm per straw, according to the amount of quality and fertility information to which
they have access.
The extension rate for a new sire begins at a higher concentration and then as the post-quality pattern and in-cow fertility information becomes known a gradual decrease in sperm per straw can result. As a sire ascends the extension rate pyramid each step requires significantly more data before a sperm dose change can be considered.

Conclusions

The contents of this paper demonstrate that the production of high quality, high fertility frozen bovine semen is a detailed process that continues to evolve as systems and technologies become available.

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BVD virus – it’s impact on female and male fertility and approaches to control

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Our understanding of Bovine Viral Diarrhea virus (BVDV) and of the diseases caused by BVDV has changed substantially in the 7 decades since BVD was first described. What was once considered to be a severe acute clinical viral disease is now recognized to also include a range of diseases affecting numerous body systems, especially the male and female reproductive systems. As our understanding of the diseases associated with BVDV infection has evolved, our understanding of the diversity of the BVD viruses themselves has also evolved. Much of our knowledge of the clinical and subclinical affects of BVDV is based on epidemiological investigations and on the results of experimental challenge studies. This reflects the diagnostic difficulties in trying to determine the exact role of BVDV as a cause of naturally-occurring disease.

Diversity of BVDV: The diversity of BVDV is often a topic of concern as people attempt to draw conclusions about BVDV behaviour by relating the virus’ laboratory growth characteristics to its ability to cause disease. BVDV are RNA viruses that exist in two biotypes, cytopathic and non-cytopathic. Biotype refers to the behaviour of the virus in tissue culture and is not indicative of virulence. Severe clinical BVD is almost always caused by non-cytopathic rather than cytopathic BVDV. The life-long, often subclinical, condition of persistent infection is always caused by BVDV of the non-cytopathic biotype.

In the 1990’s it was discovered that BVDV existed not only in two biotypes but also in a number of genotypes. The genetic differences are primarily in the untranslated region of the viral genome and are not related to ability to cause disease. This genetic diversity was discovered coincidentally with an epidemic of severe acute BVDV in eastern North America (1). BVDV isolated from these outbreaks were of the type 2 genotype. It was initially suspected that the outbreaks were related to the fact that the type 2 BVD viruses isolated during the outbreaks were newer and more virulent viruses. Further investigation actually revealed that genotype 2 BVDV were present in diagnostic submissions throughout North America for years before the outbreaks and that they were associated with a range of clinical presentations beyond severe acute disease (1, 2).

Subsequently the existence of numerous sub-genotypes within each genotype is also recognized (3). Efforts are now being made to better understand the existence of these sub-genotypes has any clinical significance (4, 5). These efforts are hampered by the fact that the most extensive collections of BVDV were developed from submissions to diagnostic laboratories. Because submissions to diagnostic laboratories are not randomly derived nor do they necessarily reflect the complete range of BVDV-related disease, it is difficult to know if they represent the true occurrence of BVDV within the entire bovine population.

Regulatory authorities recognize that it was important to have vaccines tested to demonstrate efficacy against both major genotypes of BVDV.

BVDV and female reproductive disease: It has been known for some time that exposing pregnant cows to BVDV can lead to infection of the developing fetus (6, 7, 8). Once fetal infection occurs, there can be a range of clinical outcomes depending on the virulence of the virus, the stage of fetal development when infection occurred and the immune status of the fetus.
Early Embryonic Death: Infection in the first 45 days of gestation, including infection that occurs at or before breeding, can result in apparent infertility and early embryonic death (EED) (6). EED can have a range of clinical appearances including irregular estrus cycles depending on the timing and method of pregnancy diagnosis. The mechanism of the infertility following infection in early gestation is not completely understood. BVDV may cause EED by infecting the fetus directly. BVDV may also alter the function and environment of the female reproductive tract by infecting the tissues of the tract directly. BVDV may also disrupt normal ovarian function by infecting ovarian tissues directly (9). Determining the cause of EED can be difficult because often there are no tissues available for testing. Serology on paired samples can be useful depending on the time interval between exposure to the virus and collection of serum samples.

Abortion: Once implantation occurs at approximately 45 days of gestation, transplacental infection may occur with the outcome of infection determined by the timing of infection, viral virulence and fetal immune status (6). Fetal death may be followed by fetal reabsorption, mummification or abortion. Abortion, including abortion storms, may occur during and following outbreaks of acute disease although often there are no clinical signs observed in the cow before the abortion. Abortions have been documented at all stages of gestation.

Fetal Malformation and Congenital Defects: Fetal infection may not always lead to fetal death (6, 7). The BVDV may damage developing tissues causing congenital defects without causing fetal death. The most frequently observed congenital defects affect the central nervous system and eyes. In utero exposure can also result in a range of other abnormalities including hypothyroidism, thymic hypoplasia, skeletal abnormalities and growth retardation.

Persistent Infection: The most unique outcome of fetal infection with BVDV is the development of persistent infection (PI). Persistent infection results from infection of the fetus with a non-cytopathic biotype of BVDV during (approximately) the first 125 days of gestation (6, 7). PI calves are likely to be born clinically normal but will be immunotolerant to the persistently-infecting BVDV so they are life-long carriers and life-long shedders of BVDV. It is assumed that immunotolerance is a consequence of infection occurring prior to or coincidental with the development of immunocompetence to BVDV. Once fully developed, the immune system does not recognize the antigens of the BVDV as foreign. Viral persistence is a sequel to this immunotolerance.

PI cattle are believed to be the major reservoir for BVDV in the cattle population. Programs designed to identify and remove PI cattle are capable of eradicating BVDV (10, 11). Over the past decade, numerous new technologies have become available to facilitate the identification of PI cattle. The introduction of new testing methods makes it possible to test a wide range of tissues including skin and reduce costs by testing pools of samples collected from many different animals. Skin collected from post mortem cases can even be used to determine if a dead animal had been persistently infected.

Protecting the developing fetus against BVDV: In the past ten years, vaccines marketed in Canada and the United States have been tested to assess their ability to protect the unborn calf from infection when their dam is exposed to BVDV. Vaccines that have been appropriately tested according to regulatory guidelines to demonstrate protection now carry the label statements (12). The majority of these vaccines contain type 1 and type 2 attenuated modified-live BVD viruses.

Opinion leaders have expressed concern that regulatory requirements for testing vaccines do not closely resemble the same continual or continuous exposure that occurs when pregnant cows are pastured with PI cattle. To clarify the protection that might be expected following natural exposure, several ‘field challenge’ experiments had been performed (13, 14). In these trials, pregnant cows were exposed to one or more PI cattle on pasture for weeks to months. The results of these trials suggest that fetal protection under these more natural conditions is similar to the protection observed in regulatory trials.
**BVDV and male reproductive disease:** BVDV is present in the reproductive tract secretions and the semen of PI bulls (15). The semen of PI bulls often has a very high concentration of BVDV which survives cryopreservation and processing of semen for artificial insemination. Cows can become infected with BVDV if they breed naturally by PI bulls and also if they are artificially inseminated with semen from PI bulls. Calves sired by semen from a PI bull may be born persistently infected themselves (16). It is technically difficult to test semen for BVDV, however, routine testing of blood and other tissues virtually eliminates the possibility that PI bulls would enter AI units. When the existence of PI cattle was first demonstrated, several bulls that were PI were detected AI units (17).

When non-PI but otherwise susceptible bulls become acutely infected with BVDV, the majority will develop a long-term infection in their testicles even after the virus has been cleared from the bull’s other tissues (18, 19). Their semen may contain BVDV that remains viable and capable of infecting cows even if semen is collected and frozen using technology routinely used in the artificial insemination industry. This type of long-lasting testicular infection is termed ‘prolonged testicular infection’ to differentiate it from persistent infection (19).

In a series of experiments, researchers were able to demonstrate that BVDV in semen could result in infection of cattle bred with semen from bulls with testicular BVDV infection as evidenced by seroconversion following breeding and secondary transmission to cattle in contact with the bred cattle (19, 20).

Bulls that have testicular infection following an acute BVDV infection may not be detected by routine screening as they enter AI units. At least two bulls with long-standing testicular infections have been identified in commercial artificial insemination units (20, 21, 22). In both cases, frozen semen had been exported. It has been proposed that these bulls that shed BVDV in large amounts for months after initial infection are not typical. It is more typical that bulls shed either a smaller quantity of BVDV or for shorter periods of time (18).

Several studies have demonstrated that previous vaccination of bulls with an MLV vaccine will prevent colonization of their testicles (23, 24, 25). One of the tested vaccines contained non-cytopathic BVDV. The non-cytopathic vaccine virus itself established a long-standing testicular infection following vaccination even though vaccination did protect against testicular infection by a challenge virus (24). The same situation was not observed in a vaccine containing cytopathic BVD viruses (23, 25).

**BVDV and reproductive technology:** Reproductive technologies extend and broaden the reproductive potential of bulls and cows of superior genetic merit. Many of the procedures involved in these technologies may serve as vehicles for transmission of BVDV (8, 15, 26). The industry is mindful of the potential for unwittingly transmitting BVDV.

It is technically difficult to test semen and embryos, the end products of reproductive technologies, for BVDV so control is best accomplished by implementing controls and standard practices designed to minimize the risk of transmitting contagious pathogens like BVDV. In the procedure of harvesting *in vivo* embryos for storage and for transfer, BVDV could potentially enter the system in association with the oocyte, cumulus cells, follicular fluid, oviductal cells and fluids, semen, uterine cells and fluids, serum used in flushing media and the liquid nitrogen use for storage (15, 26). The additional technical steps involved in harvesting *in vitro* embryos introduce additional potential avenues for contamination (15, 27).

The International Embry Transfer Society (IETS) publishes a manual of guidelines for the general procedures of bovine embryo transfer and minimum standards for hygienic handling of embryos. This manual is regular revised and is now in its 4th edition. Following these guidelines cannot absolutely eliminate the risk that embryos may be contaminated with BVDV (28) but it will mitigate the risk (29). The fact that there are multiple reports of the production of healthy, uninfected calves using embryos derived from PI dams demonstrates the potential for containing the risk of transmitting using the recommended procedures for embryo transfer (15).
One risk that is sometimes overlooked in the embryo transfer process is the viral and immune status of the intended recipients of the embryos. It is wise to test both donors and recipients to determine if they are PI. The potential recipients should be vaccinated prior to implantation to minimize the risk that exposure to BVDV during the subsequent gestation will result in fetal exposure to BVDV or other pathogens.

**Conclusions:** BVD remains a major cost to the cattle industry (30, 31, 32). New knowledge and technologies allow us to manage the impact of BVDV by managing the risks of transmitting the virus (10). We may not fully understand BVDV, particularly the factors that determine virulence and the importance of genotype to the ability to spread in the cattle population and cause disease. Approaches to the prevention and control of BVD will surely change as we develop better answers to these questions about the virus. However, control programs in the EU have demonstrated that it is possible to control BVD even given our incomplete understanding of BVD and BVDV.

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How DNA and Genomics Have Revolutionized the Dairy Cattle Industry

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Summary

Dairy producers in Canada and internationally now have access to various DNA genotyping services for parentage confirmation and/or discovery, genetic recessive profiling, and genomic evaluations. These advancements have revolutionized how breed associations and genetic evaluation centres offer their respective services to producers and industry organizations. Of major importance is the significant impact of genomics for increasing the accuracy of genetic evaluations for young bulls, heifers and cows, which provides new opportunities for improved genetic selection and mating strategies to boost rates of genetic progress and profitability of the dairy herd.

CDN: Canada’s National Genetic Evaluation Centre

Since 1995, Canadian Dairy Network (CDN) has been the national genetic evaluation center for dairy cattle in Canada. CDN is governed by an eight-member Board of Directors, which mainly consists of dairy producers. These directors are elected from among the voting delegates, of which there are approximately 25, who are appointed by the member organizations that include breed associations, Canadian DHI partners, A.I. organizations and Dairy Farmers of Canada. The mission of CDN is:

“To provide excellence and leadership in dairy herd improvement through efficient information infrastructures and quality genetic evaluation services ensuring Canada’s continued responsiveness and competitiveness both domestically and internationally. This mission reflects the main mandates of the company with the focus being maximum profitability for Canadian dairy producers and the industry that supports them in terms of genetic improvement.”

Industry Investment in Research

In addition to genetic evaluation services, CDN also has the mandate of coordinating industry led research and development in the area of genetic improvement. To fulfil this mandate the CDN Board of Directors appoints members to its Dairy Cattle Genetics Research and Development Council, commonly known as DairyGen. The DairyGen Council of CDN was established in 1999 and has coordinated genetic improvement research on behalf of the industry partners including breed associations, A.I. organizations, Canadian DHI partners and Dairy Farmers of Canada. As part of the CDN service fee structure, approximately $420,000 is collected from these partners for the sole purpose of research activities coordinated through the DairyGen Council with the approval of the CDN Board of Directors. Funds are used to support high priority research conducted in various universities and government research institutions across the country. During the past 5 years, DairyGen has provided over $1.7M in industry funds to support more than 25 different research initiatives. Through various federal government matching programs, an additional sum of nearly $1.9M was received for the same projects, yielding a total research investment of $3.6M, averaging $727,000 per year.
Industry Payback in Genetic Evaluations and Genomics

Over the course of its history and thanks to the industry investment in research through the DairyGen Council, CDN has implemented many major state-of-the-art systems for genetic evaluation of dairy cattle that are now worldly renowned. These include international bull (MACE) evaluations in 1995, the Canadian Test Day Model in 1999, genetic evaluations for reproductive performance in 2004, the Canadian Health Project in 2007 and national genomic evaluations for Canada in 2009.

The collection of DNA from dairy animals has become routine among many breeds in Canada, mainly by the submission of tail hairs or a nasal swab for each animal. With the aim of offering the most cost effective and efficient genotyping services to Canadian dairy producers, industry partners agreed in 2008 that Holstein Canada would act as the centralised national provider of DNA genotyping services for all dairy cattle breeds. Currently, owners of Holstein, Ayrshire, Jersey and Brown Swiss cattle in Canada submit DNA samples to Holstein Canada for genotyping. Holstein Canada negotiates the best possible arrangements and costs with various genotyping laboratories, which currently include DNA LandMarks in Québec and GeneSeek in Nebraska, USA. As of July 31, 2012, the database at CDN includes genotypes for 207,989 Holsteins (143,218 females and 64,771 males), 25,901 Jerseys (19,381 females and 6,520 males), 3,280 Brown Swiss (1,056 females and 2,224 males) and 771 Ayrshires (52 females and 719 males). Figure 1 presents the growth in total genotypes for the Holstein breed since January 2009. On a monthly basis, the number of genotypes received, processed and stored at CDN averages over 10,000 for Holsteins alone and the practise of DNA genotyping dairy cattle in North America is increasing at an increasing rate, especially for females with the low density (3K/6K) panels.

![Figure 1: Cumulative Counts of Holstein Genotypes in CDN Database for Genomic Evaluations](image)

**Value of DNA to Breed Associations**

Dairy producers in Canada have access to various DNA genotyping services through Holstein Canada. In general, they can be broken down into three categories, namely (a) parentage confirmation, (b) low density genotyping, and (c) standard genotyping. Table 1 provides an overall description of the panels commonly available in Canada for each level of genotyping and their respective application. The final panel for a SNP-based parentage verification test for cattle is expected to be approved by the International Society for Animal Genetics (ISAG) in 2012, and will include approximately 100 SNPs (markers). While this technology would provide breed associations with an effective, and likely less costly, alternative to the current microsatellite-based parentage test, some issues remain. For example, for the SNP-based parentage test to work, it requires the animal as well as the candidate
parents to be genotyped with the ISAG 100 SNP panel. In addition, for the SNP-based parentage test to be most effective all countries involved with the exchange of dairy cattle genetics (i.e.: live animals, semen, embryos) would migrate to this technology from the current microsatellite-based parentage test, which would easily take many years. For this reason, there is also an international effort being put towards finding a process to transform the microsatellite test results into the SNP-based tests, or vice versa.

| Table 1: Description of DNA Genotyping Panels Routinely Available to Producers |
|---|---|---|
| Category | No. Markers | Applications |
| Parentage | ≈100 | • Able to confirm parents as reported or from a short list of possible males and/or females |
| Low Density | 3,000 (3K) (no longer used) | • Confirmation of parents as reported or discover them from within the CDN database of genotypes • Identification of identical twins (natural or embryo splits) |
| | 6,000 (6K) (current LD norm) | • Imputation of genotypes to 50K for estimation of genomic evaluation |
| | ≈9,000 (LD+) (pending) | • Same as 6K and 3K panels • Automatically includes other diagnostics such as coat colour in Holstein, genetic recessives, haplotypes affecting fertility, and others |
| Standard | 50,000 (50K) (international standard) | • Confirmation of parents as reported or discover them from within the CDN database of genotypes • Identification of identical twins (natural or embryo splits) • Direct inclusion for calculation of genomic evaluations • Requirement for A.I. males and inclusion in reference population for estimating of marker effects by trait |

The original panel developed for genotyping animals for the purpose of genomic evaluations was the BovineSNP50 BeadChip, made commercially available in 2008. Since the original release, a second version was developed and is the current standard for genotyping dairy cattle around the world. This genotyping panel contains 54,609 highly informative SNPs that are uniformly spread across the whole bovine genome and is commonly referred to as the 50K panel.

The greatest area of technological advancement related to bovine genotyping has been the development of lower density (LD) panels for commercial use at a lower cost compared to 50K genotyping. The first LD panel, which became available in Canada in September 2010, included roughly 3,000 SNPs from a subset of those included on the 50K panel. This subset of SNPs were selected to maintain a reasonable coverage across all informative areas of the bovine genome but also to facilitate the possibility of imputing 50K genotypes from the resulting 3K ones. For this reason, the research effort placed on building accurate and efficient algorithms for carrying out such imputation calculations significantly increased in the fall of 2010. Presently, all methods developed to impute LD genotypes to 50K or higher take advantage of both pedigree-based and population-based imputation. Accuracy of pedigree-based imputation is mainly dependent upon whether the animal’s parents have been genotyped whereas population-based imputation strategies have greatest success when there is a very large set of reference genotypes available.

Nevertheless, by October 2011, Illumina made an improved LD panel with 6,000 SNPs commercially available at the same cost as the 3K panel, which effectively eliminated it from active usage within any genotyping laboratory. Again, the selection of SNPs on this improved LD panel included those from the 3K panel plus others from the 50K panel that increased the accuracy of imputation calculations even further. Table 2 presents the average
imputation error rate when genotypes from the 6K panel are used to impute to 50K genotypes compared to
genotypes from the 3K panel, which also depends of the amount of known lineage and ancestors genotyped.
Simply stated, progeny of sires that have been 50K genotyped can successfully be genotyped with the 6K panel
and have an imputation error rate that is approximately 1% or less. Imputation accuracy is lowered for animals
that have no genotyped parents and/or unknown parents.

<table>
<thead>
<tr>
<th>Sire Genotype</th>
<th>Dam Genotype</th>
<th>Average Imputation Error Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>50K</td>
<td>50K</td>
<td>0.9%</td>
</tr>
<tr>
<td>50K</td>
<td>3K</td>
<td>1.8%</td>
</tr>
<tr>
<td>50K</td>
<td>6K</td>
<td>1.4%</td>
</tr>
<tr>
<td>50K</td>
<td>Not Genotyped</td>
<td>2.9%</td>
</tr>
<tr>
<td>Not Genotyped</td>
<td>Not Genotyped</td>
<td>5.9%</td>
</tr>
<tr>
<td>Not Genotyped</td>
<td>Not Known</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

Before the end of 2012, two additional genotyping panels are expected to become commercially available. Both
are expected to add specific markers and/or gene tests (i.e.: coat colour in Holsteins, genetic recessives,
haplotypes affecting fertility, etc.) to the existing 6K and 50K panels such that they become approximately 9K and
80K panels, respectively. With an expected nominal increase in costs, these two versions of genotyping panels
will likely quickly gain popularity due to the significant increases in the value returned to breeders.

A major advantage of the SNP panels that are 3K or higher in marker density is that they provide breed
associations such as Holstein Canada with the opportunity to discover the parents of a genotyped animal from
among all genotyped males and females. This is a significant improvement over current parentage testing that
requires the submission of possible sires and/or dams only to check which qualify as parents.

In June 2012, Holstein Canada launched a new genotyping program, known as GenoID, which uses DNA genotypes
of non-registered animals to search for the exact sire and/or dam and complete the various data fields required
for official registration in the Holstein Canada herdbook at the appropriate level of purity percentage in
accordance with the discovered lineage. This program is specifically aimed at Canadian producers that wish to
start or catch up on herdbook registration activity for their herd without getting involved with the usual time-
consuming review of the on-farm documents such as breeding and calving records. In addition, GenoID is useful in
herds using natural breeding with multiple herd sires since the daughters of each sire can be determined with
certainty after genotyping each herd sire.

The Arrival of Genomic Evaluations for Genetic Selection and Improvement

The most significant benefit of DNA collection and genotyping for dairy cattle improvement has been the
development and implementation of genomic evaluations by CDN since 2009. Due to a North American
collaboration, Canada and the United States were the first two countries worldwide to publish official national
genomic evaluation for their respective industries. This initial bi-country collaboration was expanded in 2011 to
also include Italy and the United Kingdom so as to realize the greatest possible benefits from genomics in
Canadian genetic evaluations.

In Canada, the main advantages leading to significant gains in genetic evaluation accuracy due to genomics
includes (a) the very high number of genotyped progeny proven bulls used as the reference population for
estimating the effect of each of 50,000 markers on each trait of interest, and (b) the scientific expertise in Canada
involved in the research and development of high quality systems for calculating genomic evaluations for all genotyped males and females. Table 3 presents the number of progeny proven bulls included in the reference population for the estimation of marker effects for LPI within the four breeds for which CDN currently computes genomic evaluations, although those for the Ayrshire breed are not yet available on an official basis. It should be noted that the number of reference sires is specific to each trait of evaluation. For Holsteins, the total number of reference sires available exceeds 17,000 given the genotype exchange agreements involving Canada, United States, Italy and United Kingdom. For the Jersey and Brown Swiss breeds the genotypes from Canada and United States are exchanged and since USA has significantly larger populations for these breeds, the proportion of foreign sires in the reference population exceeds 85%. The opposite scenario exists for the Ayrshire breed, which is more common in Canada than the United States. Agreements for genotype exchanges at the international level are ongoing for all breeds in order to maximize the potential benefit of genomic evaluations for all dairy cattle breeds in all countries.

![Table 3: Number of Progeny Proven Sires in Reference Populations for the Estimation of Marker Effects for LPI in Canada – August 2012](image)

Possible gains in genetic selection strategies due to genomics is highly related to the size of the reference populations available in each breed, as outlined in Table 3. As of the August 2012 genetic evaluation release, genomics has increased the average Reliability of Lifetime Profit Index (LPI) values in Holsteins from 38% to 69% (gain of 31%) for young bulls being considered for purchase by A.I. organizations and heifers genotyped with the 50K panel (Figure 2).

![Figure 2: Gain in Average % Reliability for Lifetime Profit Index (LPI) with Genomics - Holstein, August 2012](image)

For heifers genotyped with a lower density panel, with either 3,000 or 6,000 markers instead of 50,000, the gain in accuracy is similar at 30%. Accuracy gains due to genomics are also achieved for genotyped cows, whether Canadian or foreign, but to a lesser degree than heifers since cows have their own performance and possibly
progeny data already included in traditional evaluations. For progeny proven bulls, accuracy gains due to genomics are less significant due to the relatively large progeny groups included in traditional proofs. Specific traits, however, such as Herd Life and Daughter Fertility, experience greater gains through genomics since they are traits with lower heritability levels and/or become available only later in the sire’s life.

Given the clear benefit that genomics provides for genetic selection opportunities, AI organizations in North America have changed the traditional young sire testing programs. Firstly, an increasing number of candidate young bulls that have interesting pedigree and related information, are genotyped by AI companies to produce a genomic evaluation estimated by CDN (Figure 3). Of the 14,615 candidate young bulls born in 2011 that were genotyped, it is estimated that approximately 1 in 12 will actually be purchased by an AI organization for semen collection and distribution. For bulls born in 2012 and beyond, this selection ratio is expected to intensify both by genotyping more candidate young bulls (i.e.: ≥25,000 per year) and by reducing the number of high quality young sires purchased.

This strategy of genotyping candidate young bulls, especially for full-sib groups, prior to selecting those for entry into AI has significantly impacted the quality of young sires purchased and made available to Canadian producers (Figure 4). The increase in genetic merit of young Holstein bulls with semen released in Canada from 2009 to the first half of 2012 averages 445 LPI points per year, which is two- to four-fold the annual rate achieved in earlier time periods.
Canadian dairy producers are also responding to the changing landscape of the dairy cattle improvement industry. A growing proportion of herd owners are genotyping females as a tool for better evaluating the genetics in the herd. Initially, breeders genotyped the cows from the milking herd they were considering as embryo transfer donor dams. Nowadays, genotyping of females is being done using a variety of strategies. Some herd owners genotype daughters and other descendants of key matriarch cows in the herd to identify superior genetic within specific cow families. Another strategy often used is genotyping all heifers in the herd not yet of breeding age and then continue for all newborn heifers, which will eventually yield whole herd of genotyped heifers and cows. Whole herd genotyping has also been carried out by some producers as a way of taking maximum advantage of genomic evaluations for genetic selection and mating decisions.

Given the fact that all young sires being offered to Canadian producers as well as essentially all AI progeny proven sires have been genotyped, all herds are using genomic evaluations when selecting AI sires to use in their herd. Figure 5 presents the trend in AI sire usage, by age category of the sire, based on Holstein inseminations from January 2007 to July 2012.

![Figure 5: Trend in Sire Usage by Age Category at Insemination](chart)

Since the arrival of official genomic evaluations by CDN in August 2009, three key trends can be observed:

- The use of semen from unproven young sires less than 2 years of age has maintained a 30% market share both before and after the arrival of genomic evaluations. It should be noted, however, that the number of young bulls contributing to this market share has decreased in recent years due to the fewer purchases made by AI companies and the higher average genetic merit of those selected genomic young bulls.

- The demand for semen from progeny proven bulls that are nine years of age or older has slowly but steadily decreased with the arrival of genomics. This represents a general trend towards the use of younger AI bulls to reduce the generation interval and increase rates of genetic gain.

- Semen from genomic young bulls between 2 and 4 years of age seems to be in direct competition with semen from younger progeny proven bulls that have their first crop of daughters included in their genetic evaluation. As the quality of progeny proven bulls increases (or decreases), producers give less (or more) consideration to semen from the genomically tested bulls expected to receive a progeny proof in the next year or two. It should be noted that these sire will already have their first crop of daughters born and CDN will have released an official genetic evaluation for calving performance traits.
Insight into the Future

Ongoing industry investments into research coordinated by the DairyGen Council of CDN have produced other interesting results for implementation in the next year or two. As an outcome of such research, CDN plans to introduce official genetic and/or genomic evaluations for Body Condition Score (BCS) in all dairy breeds in Canada, effective December 2012. In 2013, a new genetic evaluation system for Mastitis Resistance is expected to be introduced by CDN yielding official genetic and/or genomic evaluations for bulls in each dairy breed. Novel research unique to Canada has produced a patented test to identify animals that have a naturally higher or lower immune response, which therefore changes their susceptibility to various diseases common to dairy cattle such as mastitis.

In addition to using best management practices for controlling the spread of Johne’s disease and reducing its impact on dairy herd profitability, the DairyGen Council has supported research aimed at this disease from a genetic perspective. An important outcome has been the identification of specific candidate gene markers that are associated with resistance to infection of the bacteria causing Johne’s disease. In the future, these markers may be useful for incorporation into genetic selection tools to reduce the incidence of Johne’s in dairy cattle.

The fat in dairy cow milk includes various components including saturated fatty acids, commonly known as “bad fat” in terms of human health, as well as monounsaturated and polyunsaturated fatty acids (i.e.: “good fat”). Recent research has shown that several of these fatty acids are moderately heritable and therefore can be altered by genetic selection in favour of the “good fat” and against the “bad fat”. Research is continuing in this important area linking milk composition to human nutrition and health.

Specific to DNA genotyping and genomics, a major ongoing project shows promise regarding the opportunity to genotype embryos prior to embryo transfer so as to select only those of the highest genetic potential and the desired gender. The project has involved biopsying the embryos to extract small amounts of DNA, amplification of the DNA for genotyping with the 50K panel, estimation of genomic evaluations by CDN and then validating the accuracy of the genotype and genomic evaluation based on DNA collected from the resulting progeny born after embryo transfer and pregnancy.

Summary

Through Canadian Dairy Network (CDN), the partners involved in dairy cattle improvement in Canada achieve outstanding success in terms of their investment in research and development, which, in turn, yields world renowned leadership in quality genetic evaluation services provided by CDN. A major payback of this industry strategy to benefit Canadian dairy producers has been the use of DNA for improving breed association services as well as for computing national genomic evaluations for Holsteins and other dairy breeds. The accuracy gains achieved through genomics has be very significant in Canada due to large reference populations of genotyped progeny proven sires and excellence in scientific expertise of researchers involved. In the near future, Canadian dairy producers will also benefit from genetic and genomic tools for improving the health, fertility and mobility of their herd, as well as genetic selection opportunities to alter milk composition aimed at improving human health.
Estrous synchronization and embryo transfer in bison

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Introduction

Bison are the largest land mammals in North America and prior to European settlement they ranged widely over the continent in numbers estimated to be greater than 30 million (Shaw, 1995). Bison were driven to near-extinction by the beginning of the 20th century as a result of uncontrolled hunting and loss of habitat from settlement (Isenberg, 2000). Bison are divided into two variants, the southern plains bison (Bison bison bison) and the northern wood bison (Bison bison athabascae). Historically, plains bison lived on the grasslands of central North America in massive herds estimated to be in the tens of millions (Shaw, 1995). Wood bison occupied a large region of the northern boreal forest, but historically the population has been estimated to be only as high as 168,000 (Soper, 1941). Plains bison numbers have rebounded to more than 20,000 in designated conservation herds (Gates, Freese et al. 2010) and over 400,000 in commercial herds; they are no longer considered a threatened population. Wood bison numbers, however, remain low.

Why do embryo transfer with bison? The most obvious answer might be to support reproductive goals of the burgeoning ranched bison industry. However, the Canadian Bison Association (CBA) in its report titled “Bison 2000 - A Strategic Plan for Research and Development Needs of the Canadian Bison Industry” declared that the use or development of assisted reproductive technologies in bison is a low priority. Although the CBA does recognize that there is a need to establish the basic reproductive function of female bison. The Canadian bison industry does not want to “over manipulate reproduction and to apply reproduction technologies to bison” (Rutley, 2003).

Much has happened to the Canadian bison industry in the 10 years since the Bison 2000 report was issued and it remains to be seen whether or not the industry changes its mind on the use and development of assisted reproductive technologies. The price of bison meat has reached an all time high and producers cannot meet the demand for their product. To make matters even more complicated many producers have decided to take the opportunity presented by high prices to exit the industry all together. Bison slaughter numbers in Canada for 2011 were 10,961, just under half of the number slaughtered in 2008 (19,234). While many live bison were exported this drop also reflects a drop in the number of bison on farms in Canada, which has fallen more than 35%, from 195,728 in 2006 to 125,142 in 2011. In addition the number of farms raising bison in 2012 is only 64% of the number in 2006 (2011 Canadian Census of Agriculture).

Another reason to explore assisted reproductive technologies in bison is to support conservation efforts in the wild. There are 10,870 wood bison in designated conservation herds and the population remains endangered primarily because more than 50% reside in herds that are endemically infected with brucellosis and tuberculosis (Gates, Freese et al. 2010). A federal environmental assessment panel concluded that the only solution to these problems is eradication of the infected bison herds (FEARO, 1990). In addition, the panel recommended that healthy bison be reintroduced once eradication has been accomplished, and that the healthy bison be sourced from the original area through genetic salvage operations (FEARO, 1990). Interestingly, the CBA recognizes the need to support efforts to conserve bison in North America and identify this as one of only two long-term industry objectives in the Bison 2000 report.

The Wood Bison Reproductive Research Group (WBRRG) was formed to advance knowledge in the area of bison reproductive physiology and to develop reproductive technologies to enable production of disease-free gametes
and embryos that may be used to replace extirpated populations. Most of the work of the WBRRG has been conducted at the Native Hoofstock Centre (NHC) located on the University of Saskatchewan Goodale Research Farm just south of Saskatoon, Saskatchewan, Canada (52°02’N, 106°28’W).

**Bison handling facility**

The NHC is a facility designed for bison, elk and white-tailed deer. It has adequate pasture for 50 adult bison and a corral system to sort and confine bison adjacent to a handling facility that is housed in an insulated building, which provides protection from wind and precipitation. The bison are handled in a squeeze chute that was specifically designed for this species by a commercial manufacturer (Berlinic manufacturing, Quill Lake, Saskatchewan). The entry to the handling unit is composed of 4 sorting cells, which can accommodate 4 bison each, arranged to form a circle. The sorting cells are separated by sliding gates, which are moved by hydraulic motors that are controlled by an operator located on an elevated platform at the centre of the circle. The floor of one of the cells is equipped with a weigh scale.

After the bison enter the handling unit they are sorted so that only one animal enters the final cell before the hydraulic squeeze chute. The squeeze chute is equipped with a crash gate on the outside of the head catch mechanism. The crash gate is designed to stop a charging animal so that the head catch mechanism can close around the neck immediately behind the skull and prevent the animal from getting a leg or shoulder in the way. Once a bison is caught the crash gate can be lifted out of the way to allow access to the head and neck. The sides of the chute can be adjusted to apply pressure to the sides of the animal. Two doors, one on each side, located immediately behind the head catch mechanism provided safe access to the neck and jugular vein. A second set of doors at the rear of the chute allows access to the hindquarters of the animal. An operator standing to the side of the chute controls all of the moving parts of the chute hydraulically and the area is heated by an overhead natural gas radiant heater.

**Bison**

The bison at the NHC are wood bison that originated from Elk Island National Park. Bison in this park are rounded up every second year for management reasons and are otherwise left to fend for themselves. The first priority after the bison were moved to the NHC was to train these essentially wild animals so that they could safely be used in reproductive research. The bison were subjected to a program of habituation and training that involved having a single individual consistently feed them oats daily and all of the gates and alleys throughout the corral and handling system were left open to allow the animals to explore the system on their own.

Once it was apparent that the bison had become accustomed to the routine, which took about 2 months, the gates in the handling system were set to direct the bison through the hydraulic squeeze chute. To train the bison to the handling system and being caught and held in the squeeze chute the bison were worked once a day with the degree of handling increased in a series of steps over several weeks. In the first step, which lasted 1 week, the bison were allowed to move freely through the system exiting through the squeeze chute and back to their holding pen without restraint. After the handling session the bison were rewarded with whole oats. The next step, which also lasted for 1 week, repeated the same procedure of the first step but this time the bison were stopped briefly in the squeeze chute without catching their heads. In the following week the bison were caught by their heads and held in the squeeze chute for 1 minute and then released. In the final step of the process the bison underwent a short transrectal ultrasound examination before being released.
Annual reproductive pattern

Several studies involving daily transrectal ultrasound examination and blood sampling have since been completed and they have provided information about bison reproductive physiology throughout the year. Female wood bison are seasonally polyestrous with the first spontaneous ovulation occurring in all animals over a 2-week period in August. This ovulation is usually not accompanied by any evidence of estrus and is followed about 9 days later by the second ovulation, which is associated with changes to the reproductive tract and behaviour characteristic of estrus. The CL from the first ovulation is short-lived and only produces a low serum concentration of progesterone for a few days. When animals are prevented from becoming pregnant spontaneous ovulation continues at regular intervals of approximately 20 days until as late as March. The intervals between ovulation are characterized by the regular occurrence of follicular waves. There is one follicular wave between the first and second ovulation and subsequently there are two follicle waves between ovulations (McCorkell, unpublished). When an animal enters the anovulatory period regular follicular waves persist emerging about every 7 days. (McCorkell, Woodbury et al. 2008)

Synchronization of ovarian function

Once the fundamental pattern of ovarian follicle dynamics was established studies were conducted to control follicle wave emergence. Two methods to initiate a wave of ovarian follicles were compared; ultrasound-guided transvaginal ovarian follicle ablation and treatment with estradiol. Both methods caused the synchronous emergence of a follicle wave, 1.0±0.2 days after treatment for ablation and 3.3±0.3 days after treatment with estradiol (Smg; McCorkell, Paziuk et al. 2010). When estradiol treatment was used in the anovulatory period spontaneous ovulations were observed. In order to prevent this complication estradiol was combined with progesterone (100 mg). The addition of progesterone prevented spontaneous ovulation and wave emergence occurred 2.9±0.3 days after treatment. Follicle ablation, estradiol, and estradiol plus progesterone all synchronously initiated a new wave of follicle development, however follicle ablation consistently produced a quicker and more synchronous result (Palomino, McCorkell et al. 2010).

Fixed-timed artificial insemination

In order to facilitate timed breeding follicular wave emergence was synchronized with a protocol using estradiol and progesterone followed by fixed-timed artificial insemination. Ten bison were treated during the ovulatory season with estradiol 17β (2.5 mg) and progesterone (50 mg) and received a progesterone releasing intravaginal device (Cue-mate®), on Day 0. On Day 8 the Cue-mate device was removed and prostaglandin (500 mg of Estrumate®) was given. On Day 10 the bison received 5 mg of LH (Lutropin V®), and were inseminated 12 hours later with semen that had been collected and frozen from wood and plains bison previously. Ovarian follicular wave emergence occurred on Day 4.1±1.0. The interval from LH treatment to ovulation was 2.7±1.8 days. The pre-ovulatory follicle diameter averaged 15.2±1.1mm. Three of the ten cows were diagnosed pregnant. Treatment with estradiol plus progesterone prevented premature ovulation and decreased the variability in the interval to wave emergence and resulted in a more synchronous ovulatory response to LH treatment. The poor pregnancy results may have been due to problems with semen quality, timing of AI, and or quality of the ovulated oocyte (Adams, McCorkell et al. 2010).

Ovarian superstimulation and cumulus-oocyte complex collection

Several studies have been completed with the objective of superstimulating ovarian follicle development to collect oocytes by transvaginal ultrasound-guided follicle aspiration. In one study bison were divided into two groups based on age. The young group was between 2 and 4 years of age and the old group was between 10 and 13 years of age. Ovarian follicle development was synchronized by follicle ablation (Day 0). On Day 1 the bison
were treated with 200mg of FSH s.c. caudal to the shoulder. Forty-eight hours later (Day 3) the bison were given a second treatment of 200mg of FSH. This was followed on Day 5 by treatment with 25 mg of LH i.m. Ultrasound-guided transvaginal follicle aspiration to collect the cumulus-oocyte complex (COC) was conducted on Day 6. Ovarian response differed between the two groups with an average of 17.0±1.6 follicles > 5 mm per animal observed in the young group and 7.0±0.5 in the older group. The number of follicles aspirated and COC’s collected followed the same pattern with 12.8±1.3 follicles aspirated per bison in the young group and 5.7±0.7 on the older group with 8.0±1.1 and 2.3±0.3 COC’s collected respectively (McCorkell, unpublished).

In an additional study during the ovulatory season (December) the bison were synchronized by giving a luteolytic dose of prostaglandin followed 8 days later by transvaginal ultrasound-guided follicular ablation. On the day after follicular ablation (Day 0) the bison were assigned randomly to 2 groups and given either a single i.m. dose of 2500 IU of eCG, or two s.c. doses of 200 mg FSH on Days 0 and 2. Treatment with prostaglandin was repeated on Day 2. On Day 4 25 mg of LH was given i.m. COC’s were collected 24 hours after LH treatment by transvaginal ultrasound-guided follicle aspiration. The number of follicles ≥5 mm was greater in bison treated with FSH than in those treated with eCG (12.2±1.7 vs 5.8±0.5), which resulted in a greater number of follicles aspirated (11.2±1.8 vs 5.6±0.5), and more COC’s collected (7.2±1.4 vs 3.4±0.6). The number of large follicles, follicles aspirated, and COC’s collected were more than twice as high in bison treated with FSH than in those treated with eCG. (Palomino, McCorkell et al. 2011)

**Embryo Collection**

Recently two studies have been completed with the objective of collecting embryos in the anovulatory season. The first study was designed to test if progesterone priming is required for superovulation and to determine the effectiveness of LH (Lutropin®) and hCG (Chorulon®) for induction of ovulation with or without an intravaginal progesterone-releasing device (PRID®) in 32 wood bison cows. Follicular wave emergence was synchronized by transvaginal ultrasound-guided follicle ablation and the bison were assigned to 4 groups: PRID+LH (n=12), PRID+hCG (n=4), no-PRID+LH (n=12) and no-PRID+hCG (n=4). The PRID was inserted on the day of follicular ablation in the respective groups and a single s.c. dose of 400 mg FSH (Follitropin®) diluted in hyaluronan was given i.m. on Day 0. Artificial insemination was done at 24, 36, and 48 h after LH or hCG treatment. Embryos were collected non-surgically on Day 13. The number of ovulatory-sized follicles (≥10 mm) on Day 5 did not differ among groups. However the ovulation rate (number of ovulations/number of follicles ≥10 mm) was greater in bison treated with hCG and the number of CL on Day 13 was greater in bison treated with hCG without a PRID. Despite that result, no differences in number of ova/embryos and transferable embryos were found among groups. It was concluded that progesterone priming had no effect on ovarian superstimulation in wood bison in the anovulatory season and that HCG treatment produced a more satisfactory ovulatory response. (Palomino, McCorkell et al. 2012)

A second study, again in the anovulatory season was designed to repeat the previous objectives and to evaluate the effect of progesterone on embryo quality. Three groups of bison were compared: two groups had a PRID inserted from Day 1 to 3 and the third group was not treated with a PRID. In addition one of the PRID treated groups was treated with 400 IU of eCG i.m. on Day 3 as was the third group that was not treated with a PRID. All three groups were synchronized by transvaginal ultrasound guided follicle ablation on Day 0 of the study and were treated with 300mg of FSH diluted in hyaluronan i.m. on Day 1. A second treatment of 100mg of FSH diluted in hyaluronan i.m. was given to all groups on Day 3. Ovulation was induced in all animals by treating in the morning of Day 6 with 2500IU of HCG i.m. All animals were artificially inseminated on the evening of Day 6 and the morning of Day 7 using chilled semen. All three groups produced around 15 follicles > 9 mm in diameter by Day 6. However the group that was treated with a PRID and eCG had an ovulation rate of 47% which was lower than the other two groups which ranged between 69 and 79%. However, there was no difference between groups in the number of transferable embryos, which averaged around 2 per animal. The superovulatory response and
embryo collection was improved but eCG did not improve ovulation rate and progesterone had no effect on embryo quality (Palomino, Mapletoft et al. in press).

Summary

Research over the past few years has contributed much to the understanding of the reproductive physiology of bison. It is now understood that bison are seasonally polyestrous with an anestrous period from April to July and a period of spontaneous ovulation from August to March. Ovarian follicle dynamics are similar to that seen in cattle characterized by regular waves of follicle development associated with a surge in FSH. Typically, there are two waves of follicle development in one estrous cycle.

Estrous synchronization and embryo transfer in bison are areas that require more investigation and refinement before they can be applied reliably to commercial or conservation herds. However, recent trials have provided encouraging results and an effective, practical protocol is on the horizon.

References


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The Effect of Nutrition on Reproduction: Applications for ET Donors and Recipients

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Abstract

Dairy cow nutrition is moderately linked to reproduction performance. These principles can be applied to the management of ET donors and recipients. Nutritional practices that cause chronically elevated urea nitrogen (UN) concentrations are particularly detrimental to reproductive performance. Intensive monitoring of the MUN content of the milk shipped from a herd or the MUN/BUN of individual cows can be very revealing but is often overlooked. Long-term management of body condition score is crucial yet can be practically challenging. New data are emerging that suggest that higher starch diets after calving followed by higher fat diets once breeding begins could be beneficial. Specific feed additives may have beneficial effects on fertility of ET donors and recipients - particularly organic trace minerals. Generic principles of good nutritional management are especially applicable to high-value ET donors and recipients. Specific areas of nutritional management known to be related to reproductive performance include providing adequate bunk space, giving cows adequate time in the pre-fresh group, evaluating the quality of individual feed ingredients, evaluating feed mixes or the total mixed ration, evaluating the consistency and frequency of feed delivery, and evaluating the amount of feed called for each pen.

Dietary Protein and Urea Nitrogen

Perhaps the most consistent association between nutrition and reproduction is the oft-reported impairment of fertility with diets high in crude protein, and particularly ruminally degradable protein (Thatcher et al., 2011). Blood UN (BUN) or milk UN (MUN) are indirect measures of protein nutrition along with ruminally available carbohydrates. High UN may be caused by either high dietary crude protein (especially protein in the degradable fraction) and/or low dietary non-fiber carbohydrates (especially starches and sugars). High UN is a risk factor for infertility primarily due to the energy cost of detoxifying excessive ruminal ammonia into urea by the liver.

Preventing reproductive problems associated with high UN concentrations requires an effective monitoring strategy. The effect of time relative to feeding on UN concentrations is great, particularly if the protein is fed as a separate component of the diet two or three times a day. Lack of control of the time of UN sampling relative to feeding has greatly hindered the effectiveness of UN testing in the past. Sampling at about 3 hours after a major protein feeding (or the first TMR feeding of the day) should assist in determining peak daily UN concentrations. Consistent time of sampling relative to feeding is necessary when monitoring a herd or an individual cow over time.

Milk UN concentrations are closely related to BUN concentrations (Figure 1). Therefore, either BUN or MUN samples are acceptable for evaluating herd UN. Bulk tank MUN is particularly attractive because it provides a mean value for a large group of lactating cows with a single test, without concerns of getting an adequate sample size. Wet chemistry procedures for MUN are more accurate and are preferred over near infrared reflectance spectroscopy (NIRS) tests for UN. MUN tests performed by milk processors or by DHI testing centers are usually done by a NIRS method; unfortunately, these results are inconsistent compared to the gold standard test (Kohn et al., 2004).
Bulk tank MUN screening is the best place to start for herd-level monitoring of UN. A bulk tank sample accurately represents the group mean, and the extra cost of wet chemistry testing is of little consequence when the test applies to the entire herd. Individual cows (or milking strings) could then be evaluated for UN if the bulk tank MUN value falls outside the normal range (about 9 to 12 m/dL) for a group of animals.

I have been surprised at how little attention many producers, nutritionists, and veterinarians sometimes pay to bulk tank MUN values. Although MUN values from the milk plant (which are done by NIRS) are often not on the same scale as wet chemistry values, changes in these values over time are most likely real and should be monitored closely. Wet chemistry testing on bulk tank milk samples should be performed whenever the milk plant values appear to be too high.

**Control of Body Condition Score**

Cows that are either too fat or too thin are at risk for low fertility. Defining an optimal body condition score (BCS) is not as easy as it might seem, in large part because BCS is expected to be dynamic through the course of lactation. Of more practical importance than BCS by itself is negative energy balance (NEB), which is clearly associated with reduced fertility in dairy cows (Jorritsma et al., 2003). Fatter cows at calving definitely experience more profound NEB and lose more BCS after calving than thinner cows (Garnsworthy, 2007). Thus, reproductive performance is more likely to be compromised in overly fat cows compared to overly thin ones.

Visual appraisal of body condition score (BCS), while not a perfect measure, is well-correlated to actual body fat (Wright and Russel, 1984). This correlation weakens considerably at low BCS, which could be explained by body protein loss rather than body fat loss at very low scores (Macdonald et al., 1999). Accounting for both body protein and body fat would improve our assessment of BCS. Hopefully, few ET donors or recipients have very low BCS.
Preventing over-conditioning can be very challenging from a practical standpoint if donor or recipient cows have long lactations and/or long dry periods. My experience in troubleshooting nutritional management on dairies indicates that it is necessary to be very aggressive in addressing problems of over-conditioning. Minor changes in the nutrient composition of the diet (the typical approach taken by field nutritionists) are not effective. Instead, nutritionists need to aggressively add more forage (straw is particularly useful, if chopped finely enough to prevent sorting) and aggressively reduce grain feeding if cows are too fat. Limit-feeding groups of over-conditioned cows is not a good option, because individual cows may eat too much or too little and sabotage the program. Continue to feed these groups to a reasonable amount of feed refusal (typically about 2 to 5% of the feed offered).

As a practical consideration, do not attempt to reduce BCS just prior to using an animal as an ET donor or recipient. Substantial negative energy balance (i.e., enough to reduce BCS) is a strong biological signal that impairs or even halts fertility. Once ET is planned, maintain or increase the plane of nutrition for donors and recipients regardless of BCS. This dilemma highlights the importance of being proactive in managing BCS. It should be viewed as a long-term effort that plays out over several months of time, not days or weeks.

**Altering Dietary Fat or Dietary Fatty Acid Composition**

Diet supplemented with added fats may improve reproductive performance, although this effect is inconsistent and not yet fully understood (Thatcher et al., 2011). The initial appeal of including dietary fats to improve reproductive performance was that additional fat, due to its high caloric density, might blunt the NEB of early lactation. However, this potential benefit has been disappointing, probably due to the decreased dry matter intake associated with high fat feeding in early lactation (Rabiee et al., 2012).

The focus of dietary fat feeding has shifted from generic fat supplementation to adding specific fatty acids to the diet that may improve reproductive performance, independent of the caloric contributions of dietary fats. The responses have been variable (Staples et al., 1998), yet some studies offer positive and intriguing results. Certain polyunsaturated fatty acids, including some in both the n-3 and n-6 families, have shown positive physiological effects with the potential to improve reproductive performance (Thatcher et al., 2011). Flaxseed has been extensively studied, as it contains a high proportion of the n-3 fatty acids. However, the effect of feeding flaxseed on reproduction in dairy cows appears to be equivocal at best (Bork et al., 2010). The issue is complicated, and more work is needed to clarify the role of specific fatty acids on reproductive performance in dairy cattle. Whatever the final verdict is, it does not appear that the effects will be dramatic.

**High Starch (Insulin-Stimulating) Diets**

High starch diets stimulate insulin production, which has been demonstrated to increase the proportion of cows ovulating by 50 days in milk (Gong et al., 2002). Keep in mind that nutritionists generally cannot formulate diets that are both high in starch and high in fats; they have to choose one or the other. This creates a dilemma, because higher fat diets are insulin-depressing but improve oocyte quality and embryo development (Mattos et al., 2000). Garnsworthy et al. (2009) suggested a two-diet strategy for optimizing dairy cow fertility that involved a high-starch, insulin-stimulating diet until the cow resumed ovarian cyclic activity after parturition (e.g., about the first 50 days in milk), and then switching to a higher fat, insulin-depressing diet once breeding began.

It should be noted that feeding diets that are excessively high in starch could cause ruminal acidosis, although the risk is lower in the early lactation when dry matter intake is lower. High starch diets in early lactation will increase ruminal propionate production, which has been linked to increased satiety and decreased dry matter intake (Allen et al., 2005). Thus, the potential benefits of increased insulin production and more rapid return to cycling could be blunted by these practical limitations. It is clear that the amount of starch added cannot be excessive. Also, starch is becoming increasingly expensive, owing to drought conditions and increasing ethanol production.

It is also worth noting that higher fat diets are most beneficial sometime after peak milk production is reached (Staples et al., 1998). This coincides with the start of the AI period.
Organic Mineral Sources

Organic minerals may be more biologically available than their corresponding inorganic salts (Henry et al., 1992; Wedekind et al., 1992). A large meta-analysis of published studies with organic sources of Co, Cu, Mn, and Zn reported improved reproductive performance (Rabiee et al., 2010). Organic Se supplementation resulted in modest reproductive improvement in one report (Silvestre et al., 2007) but had no benefits in another (Rutigliano et al., 2008).

It is important not to provide excessive trace minerals for ET donors or recipients. The potential for toxicity is real - especially for Se and Cu. Keep in mind that supplementing more and more trace minerals cannot create a supra-physiological state with exceptional enhancement of reproductive performance. Correcting existing trace mineral deficiencies will certainly improve reproductive performance, but it is not possible to push beyond the cow’s normal physiological limits.

Trace Mineral / Vitamin Injections

Some consultants suggest giving trace mineral or vitamin injections before flushing cows. Injectable Vitamin A, vitamin E, and Se are often on the list; there are also injectable forms of Cu, Mn, and Zn.

There is very limited evidence that these injections improve fertility. If the cow was deficient prior to injection, then they would likely be beneficial. However, deficiencies of these nutrients are rare for cows consuming almost any reasonably-formulated diet. One large study (Vanegas et al., 2004) reported either no beneficial effect or even slightly reduced odds for first service conception when cows were given 2 doses of an injectable trace mineral supplement containing Cu, Mn, Se, and Zn prior to breeding. Fat-soluble vitamins (such as vitamins A and E) are stored for a very long time in body tissues, which makes deficiencies unlikely unless the dietary problem is very long term. And as mentioned above, Se can easily be toxic.

An additional concern about injectable vitamins or minerals is their potential to cause an inflammatory response at the site of injection. The amount of inflammation possible varies for different injectable nutrients and formulations; aqueous formulations are probably the least irritating. Any inflammatory response requires calories - perhaps many more than we realize. Potentially impairing energy balance before breeding seems an unnecessary risk.

Water Analysis

It seems reasonable to routinely check water samples to make sure that compounds known to impair reproduction (particularly nitrates, iron, and sulfates) are not present in high quantities. Unfortunately, there is disagreement about what constitutes ‘high’ concentrations of these minerals in water, and their direct effect on reproductive performance is not well-understood. So, some caution is in order. Mitigating the mineral content of water can be very expensive and is unlikely to yield any perceptible benefits if the mineral content was not high enough to be a true biological concern.

High Molybdenum Soils

Plants grown on high Mb soils have high Mb concentrations. When consumed, high Mb plant tissue causes a classic interference with Cu, which could impair fertility. Individuals working in areas with high Mb soils are usually well aware of the challenges there.
Monitor for Visible Molds / Test for Mycotoxins

Evaluating the potential effects of molds and mycotoxins on reproduction is very difficult. Good research data are lacking, and both producers and nutritionists are often too quick to blame molds or mycotoxins for all sorts of animal problems. But despite the misinformation and challenges, the potential still exists for real problems from molds and mycotoxins that should not be ignored.

Feed refusal should be the first clinical sign of a clinically significant mold or mycotoxin problem. This could lead to excessive NEB that spills over into fertility problems. Be careful about attributing any reproductive or health problem to mycotoxins if feed intakes are robust.

Testing for molds and mycotoxins is fraught with difficulties. Issues include the high cost of the tests, the need for proper sample handling, potential localization of molds or mycotoxins within a silo or hay mow, general lack of agreement about levels of concern for ruminant animals (who metabolize most ingested mycotoxins in the rumen), and the propensity of many herd consultants to over-react to high mold (or yeast) counts or mildly increased feed mycotoxin concentrations.

I usually start by visually inspecting the feeds for mold contamination, investigating whether or not intakes have been depressed, and then checking a TMR sample for a wide range of mycotoxins. Checking individual feed ingredients (which is what most consultants do first) is of little value without first knowing the total mycotoxin intake of the animals. Proper mold counts or mold identification is extremely difficult to accomplish under field conditions (the feed samples must be kept cool, plated the same day, and evaluated using good procedures). Most elevated mold or yeast counts are bogus and reflect nothing more than normal growth of these organisms after the sample was collected. Also, a mold or yeast count reveals nothing about mycotoxin concentrations.

Adequate Length of Time in the Pre-Fresh Pen

Allowing cows more time in the pre-fresh pen has been strongly associated with improved reproductive performance in some herds (DeGaris et al., 2010). This could be explained by better nutrient intake at this critical time, or by avoiding the stress of a pen move very close to calving.

Adequate Bunk Space

Increased bunk space in the breeding pen has been associated with improved service rates in dairy herds (Caraviello et al., 2006; Schefers et al., 2010). Many mechanisms could explain this association. Lower-rank cows may have difficulty getting access to feed when bunk space is limiting. Reduced bunk space could also limit the number of available lock ups, which could limit compliance for estrus synchronization protocols or for AI breeding. Inadequate bunk space is generally associated with inadequate resting space, which compounds the social stress imposed on over-crowded cows.

Cows need at least 30 inches of bunk space per cow. This rule seems especially applicable to ET donors and recipients.

Evaluating Individual Feed Ingredients

Sometimes nutritionists overlook the importance of evaluation of individual feed ingredients – especially the more subjective aspects such as preservation, palatability, particle length, and sortability. I find it very helpful to see all the feeds on the farm and at the minimum do a visual appraisal of each.

The dry matter content of ensiled feeds is the major determinant of the quality of the fermentation. Nothing can be practically done to alter feed dry matter content once it is harvested and ensiled. Someone needs to be alert
to check dry matter at the time the feed is harvested. Year in and year out some farms harvest forages that are either too wet or too dry, and they almost always pay the price for these mistakes. Make sure your producers get the message that ensiled feeds must be harvested at the correct dry matter – at almost any cost.

It is important to accurately know the dry matter content of a feed ingredient during feedout so that the correct amount of dry matter from that ingredient can be added to the mixer. The frequency of dry matter testing needed depends on the type of feed and the structure in which it is stored. In general, corn silage and high moisture corn have fairly consistent dry matter content. However, hay crop silages can have quite variable dry matter content at harvest. These variations are particularly important if the hay crop silage is stored in a bag or narrow vertical silo. The problem is that dry matter content can vary from load to load of chopped forage, and silage in these structures is unloaded in a manner that feed from one load to the next is not mixed. In these cases, daily dry matter monitoring may be necessary. Hay crop silages stored in bunker silos have more consistent dry matter content at feedout because feed from many different loads is fed out simultaneously. Weekly monitoring of dry matter content of the haylage coming out of a bunker silo may be sufficient. Checking forage dry matter only when feed refusals change noticeably or when the feed is visibly different is better than no testing at all. However, proactive monitoring of forage dry matter content is much better.

Adequate monitoring of forage dry matter content at feedout usually requires on-farm dry matter testing. Nutritionists will often collect and analyze forage samples once monthly, but this is often insufficient. On-farm testing methods are preferred and have the advantage of giving quick feedback so that as-fed amounts added to the mixer can be adjusted right away. The on-farm methods that appear to work the best are the Koster tester and a modified food dehydrator. Microwave ovens have lost popularity for on-farm dry matter testing because they require almost constant attention for the entire 30 to 45 minutes it takes to dry a sample.

Particle length of forages and TMR samples is both important and dynamic. Nutritionists often take on this task of running a shaker box to monitor forage and TMR particle length. Many do it well, although I often find incorrect shaking technique or the application of incorrect particle length standards. Some nutritionists may not be skilled or interested in doing this, which leaves an opportunity for the veterinarian or someone else to do it. The keys are making sure that it gets done and done correctly. Even without a shaker box, you can do informal evaluations of feed ingredient particle length or size by simply looking at a few handfuls of the feed on a clean, flat surface (e.g., the back of a clipboard) and then separating the coarse from the fine particles. Pay careful attention to the particle length of any dry, chopped hays if these are a part of the diet. Coarse, long hay or straw particles (greater than about 2 inches in length) are easily sorted away by the cows. In contrast, cows will sort toward soft, leafy hay and eat it first – even if not chopped at all. In either case, chopping the hay or straw about 1 to 2 inches long before adding it to the mixer will prevent most sorting.

Grain particle size is also important and needs to be monitored. Common errors are dry grains ground too coarsely or wet grains ground too finely. The goal is that either wet or dry grains be processed so that they have similar ruminal digestibility and rate of passage.

**Evaluating Mixes and the Total Mixed Ration**

A simple visual appraisal of custom concentrate, protein, or mineral mixes can be invaluable. Take a handful or two of the mix and separate into its different components on a flat surface. Sometimes you can catch inadvertent errors in feed mixes simply by spotting the wrong ingredients or obviously incorrect proportions of ingredients in the mix. Samples can then be submitted for wet chemistry analysis or feed microscopy for confirmation of the problem.

I find it useful to start my evaluation of TMR mixing accuracy by following the mixer as new feed is delivered and then visually inspecting the feed. Does the mix appear the same from the start to the finish of unloading? Is there more long hay in a certain part of the mix? Are there large chunks of hay that are not mixed with other ingredients? Are there more whole cottonseeds in any part of the mix? Do the whole cottonseeds appear brown
and matted (an indication of over-mixing), or are they still fluffy and white? Does the corn silage or haylage in the mix appear mashed and pulverized, or are the long forage particles still intact?

The foundation of a herd nutritional investigation is an accurate assessment of what the cows are actually eating (i.e., the “third ration” on the farm). Getting this information requires reasonably accurate analysis of all of the feed ingredients being fed, plus an in-depth understanding of how the feeds are mixed and delivered to the cows. In component-fed herds, this requires weighing all of the scoops, cans, handfuls, pitchforks, bales, and/or bunk segments of feed offered. For TMR-fed herds, the actual load sheets used to mix the feed (or even better yet the actual scale weights from TMR software programs) must be obtained and evaluated. The accuracy of the mixer scales should also be assessed. This can be done by adding 50-lb bags to the mixer with different amounts of feed already in the mixer. Alternatively, the mixer can be weighed empty and full on a platform scale. Also be alert for situations where the mixer is being loaded on a sloping surface. Most load cells are accurate only if the mixer is parked on a level surface.

A wet chemistry analysis of a carefully collected TMR bunk sample can be a useful adjunct to calculated estimates of what the cows are actually eating. Bunk sampling and testing is not a perfect representation of what the cows are eating, and several important caveats apply to interpreting these results. Nonetheless, the information often gained by appropriate TMR bunk sampling can be invaluable.

It is challenging to collect a bunk sample weighing less than a pound that is truly representative of a batch of feed that may weigh 5000 lbs or more. First, the sample should be collected at or immediately after it is put in the bunk. Otherwise, cow sorting could affect the sample. Collect about 12 handfuls of feed from the start to the end of unloading the mixer. Collect the handfuls by scooping upwards; otherwise, finer particles could be selectively lost by grabbing the sample and drawing it away from the bunk. Some suggest using pre-positioned trays in the feed bunk to collect the samples; however, the depth of feed typically placed in feed bunks makes this impractical.

After collecting the 12 or more handfuls, mix them gently on a flat table and then spread the feed out over the top of the table and separate it into quarters. Discard two of the quarters, then re-mix the remaining feed and repeat the quartering and discarding procedure. Discard different quarters each time you do the mixing. Continue mixing, quartering, and discarding until you have reduced the sample to about 1/2 pound (one pint) of feed. Then submit this entire sample to the laboratory for analysis.

Bunk samples of TMR are typically submitted for wet chemistry analyses only – NIRS analyses of TMR bunk samples are very difficult because of the challenges involved in creating a valid calibration set for a sample containing different feed ingredients in different proportions. I typically request wet chemistry analyses for dry matter, ether extract (crude fat), crude protein, bound protein, soluble protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, ash, calcium, phosphorus, magnesium, potassium, sodium, chloride, sulfur, copper, iron, manganese, and zinc. The non-fiber carbohydrate (NFC) content of the TMR can then be calculated by subtracting the ether extract, crude protein, neutral detergent fiber, and ash from 100% of the dry matter. The net energy for lactation (NEL) content of the TMR can be estimated from the ether extract, crude protein, neutral detergent fiber, and ash from 100% of the dry matter. The net energy for lactation (NEL) content of the TMR can be estimated from the ether extract, crude protein, neutral detergent fiber, and ash from 100% of the dry matter.

It is important that the laboratory not try to further sub-sample whatever TMR bunk sample you have already carefully sub-sampled. TMR samples may separate considerably during shipping and handling, especially if they are relatively dry. Therefore, it is best to submit only a relatively small quantity of feed (about 1/2 lb or one pint of sample) and to then request that the lab dry and grind the entire sample submitted. I put this request on each bag of TMR sample that I submit for analysis. Most laboratories are glad to comply with this request, as long as you submit only a small amount of feed.
Laboratory results for TMR bunk samples should be interpreted broadly, not strictly. There are numerous causes of variation between the expected and actual TMR bunk sample analysis, including poorly representative bunk samples, undetected changes in feed ingredient analyses (especially forages), undetected inconsistencies in adding feed ingredients to the mixer wagon, and laboratory error in the wet chemistry analyses. Some of these indicate an on-farm problem that requires intervention, but others are inherent errors in TMR bunk sampling. Sorting these out can be difficult. I consider the expected and laboratory results to be acceptably close if they are within about ±5% of each other (on a total nutrient basis). For instance, if the expected calcium content of a TMR was 1.00%, then any lab result between .95% and 1.05% would be considered acceptable.

Laboratory analysis of TMR bunk samples almost always results in slightly higher ADF and NDF values than estimated. I have come to expect about a 5 to 10% total over-estimation in ADF or NDF results from TMR bunk samples. For example, if the actual ADF content of a TMR was 19%, then the lab result would be likely be about 20.0 to 20.9%. Or if the actual NDF content of the TMR was 28%, then the lab result would likely be about 29.4 to 30.8%. The source of this bias is uncertain, but may involve the inclusion of some of the fat added to the TMR being retained in the ADF and NDF fractions during laboratory testing. The slight over-estimation of the NDF value lowers the NEL value calculated by the Ohio equation by about 2 to 3% of the total NEL value. So, a TMR sample with an expected NEL of 0.78 Mcal/lb would likely have an NEL value of 0.76 to 0.77 calculated by the lab.

The greatest value in TMR bunk samples is to identify gross errors in feed analysis, mixing, or delivery. For example, omitting the salt from a custom protein mix would result in a TMR bunk sample with unexpectedly low sodium and chloride content. Omitting the trace mineral/vitamin premix from the ration would result in unexpectedly low copper, iron, manganese, and zinc results. Feeding excessive dry matter from alfalfa haylage because the haylage became drier than the nutritionist’s last analysis would result in elevated dry matter, crude protein, soluble protein, ADF, and NDF values in the bunk samples.

**Evaluating the Consistency and Frequency of Feed Delivery**

It is difficult to evaluate the consistency of feed delivery on a farm, but a few simple questions and some careful observation will sometimes provide very revealing information. If the farm uses a feed mixing monitoring program (Feed Watch, etc.), then you can evaluate not only the accuracy of feed ingredient delivery, but also the consistency of the time of day that feed is delivered to each pen.

The feeding schedule on a dairy needs to be fanatic ally consistent. This is particularly in respect to the synchrony of feeding and milking times. The first feeding of the day should coincide with the cows returning from the parlor after their first milking. This will be the biggest meal of the day for most cows. It is crucial that this be done consistently. Cows apparently learn to carefully regulate their meal patterns (meal frequency and meal size) in order to self-regulate their ruminal pH. But if the feeding schedule is erratic, they will never accomplish this self-regulation. It seems particularly dangerous if cows receive their TMR later than usual – hungry cows may over-eat when feed is finally offered. Problems with an inconsistent feeding schedule are magnified by shortages in bunk space, a shortage in free stalls (cows may be more concerned about securing a place to lie down rather than eating to regulate their ruminal pH), or inadequate availability of water immediately after milking.

It is common to offer TMR once daily to most groups of cows. Many herds increase to twice daily feeding in the summer, which is an excellent decision. I prefer twice daily feeding year-round, but recognize that this usually requires extra labor. Frequent pushing up feed during the day may stimulate some additional dry matter intake, but does not appear to reduce the potential for sorting when TMR is offered infrequently. Increased feeding frequency is particularly important if the TMR is already prone to be sortable (dry TMR, excessive amount of coarse particles).
Evaluating the Amount of Feed Offered

Each day the dairy producer makes a decision as to how much feed to offer each group of cows on the farm. The goal is to keep cows from getting hungry and over-eating on a sporadic basis, and yet not waste too much feed on the farm. The ability of the farm to utilize TMR refusals often decides how much refusal they will target. If the nutritionist is not involved in monitoring the amounts of feed offered, then the herd veterinarian has an opportunity to become involved.

The decision to make “feed calls” at the start of each day should be based on the appearance of the bunk at the end of the previous feeding day. A typical goal is about a 5% daily feed refusal. More feed refusal (about 10%) is needed for pens with very dynamic populations (i.e., the transition cows in the pre- and post-fresh pens). Mid and late lactation pens can be fed to much lower feed refusal (2% or less) because pen populations are more stable. Some herds consistently run zero daily feed refusals without difficulty. However, this requires exceptionally consistent feeding management. The cows can self-regulate intakes and ruminal pH if the bunks are empty the same time each day, and if new feed is offered at the same time each day. Most dairies cannot manage their feed calls this well and need to target about 5% daily refusals.

It is very helpful if the producer records the feed offered and feed refused for each pen each day. The refusals do not have to be weighed daily; an estimation of the amount refused is usually sufficient.

The amount of feed offered to each pen each day should be an adjustment of the total batch. The producer should not lock most the ingredients and then “float” just one ingredient (usually a forage). Severe ration imbalances can occur if only one ingredient in the mix is floated. Producers should monitor forage dry matter regularly and have the confidence to adjust the entire TMR mix recipe up or down each day.

Conclusions

Dairy cow fertility is an extremely multifactorial outcome. Nutrition is one of many factors. Good nutrition starts with proper diet formulation, but also includes a number of nutritional management factors that are described above.

There are no magic strategies for diet formulation that will enhance reproductive performance. Because reproductive outcomes are so multifactorial, research results are often conflicting.

References


POSTPARTUM UTERINE DISEASES IN DAIRY COWS: WHAT’S NEW?

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1. Introduction

Reproduction is crucial in dairy production because of the necessity for a cow to calve on a regular basis in order to produce milk and provide an economically sustainable return to the farmer. Uterine health investigations in cows have been perceived as difficult to perform because of the inaccessibility of the organ. However, understanding of uterine diseases was improved in the last 20 years by the development of novel diagnostic techniques. Uterine immunology has also become an important part of the puzzle. Although great progress has been made recently, many aspects of uterine diseases remain unexplained, unexplored, or based on assumptions.

The purpose of this review is to critically evaluate the information currently available in the literature. This review aims to put this information in perspective, to raise the existing gaps in knowledge, and to highlight assumptions commonly used, but never validated. Validity of studies is assessed and criticized. Criteria used for determining the validity of studies include: appropriate design and statistical analyses, sufficient sample size, use of relevant objective outcomes, and use of randomization and controls. It is not intended to be a systematic review.

2. Normal uterine involution

After parturition, the uterus goes through a period of involution in order to regain its healthy non-pregnant size to be able to become pregnant again. Many events are considered part of this process, such as the physical shrinkage of the uterus, the regeneration of the endometrium, the elimination of bacterial contamination, and the return to ovarian activity (Sheldon et al., 2008). Although some studies have described this process, the reported normal duration of the involution period is variable, from 25 to 50 days, and is usually determined by observation over time of the size of the uterus and cervix (Morrow et al., 1966; Gier and Marion, 1968; Marion et al., 1968). Little information is available regarding microscopic involution, but it has been reported to take up to 56 days (Marion and Gier, 1959; Tennant et al., 1967; Sheldon et al., 2008). Surprisingly, the uterine involution process, as previously described, is well accepted by the scientific community. Yet, this knowledge relies on a small number of observational studies, and did not try to determine the associations of organ size or rate of shrinkage with outcomes such as risk of pregnancy (Melendez et al., 2004). For this reason, instead of referring to what is normal, the following text will describe what has been reported.

Necrosis of the endometrium and the caruncles causes lochial discharge up to 23 days after parturition (Gier and Marion, 1968; Mortimer et al., 1997). The uterus of almost every cow is contaminated by bacteria in the early postpartum period (Elliott et al., 1968; Griffin et al., 1974; Williams et al., 2005). In order to complete uterine involution and become pregnant, the uterus has to become sterile (see section “Bacteriology of the postpartum uterus”).

Return to ovarian activity, including luteal function, is an important component of uterine involution. The first dominant follicle is selected 10 to 15 days after parturition (Savio et al., 1990; Beam and Butler, 1997; Crowe,
2008). However, the fate of this follicle can be variable: atresia without ovulation, ovulation, or formation of a follicular cyst (Sheldon et al., 2008). It is not clear if early ovulation is beneficial for subsequent uterine health and fertility. It is generally accepted that return to ovulation is a positive event, when followed by normal luteal phase length and subsequent luteolysis (Darwash et al., 1997). The presence of uterine infection at first ovulation has been suggested to potentially cause pyometra (Olson et al., 1984). It is known that uterine infection in the early postpartum period can influence the side, development, and fate of first dominant follicles (Sheldon et al., 2002). Beef cows usually remain anovular for a period of at least 30 days after parturition because of suckling, whereas dairy cows may resume ovulation at or after 15 days (Crowe, 2008). It is known that anovulation in dairy cows later in lactation, between 46 and 60 days after parturition, has a detrimental effect on time to pregnancy (Walsh et al., 2007). A prolonged anovulatory period can happen in both dairy and beef cows, associated with negative energy balance or poor body condition score (Crowe, 2008). However, because beef cows generally have greater risk of pregnancy compared to dairy cows, perhaps dairy cows should not resume ovulation before 30 days after parturition or later. A recent study in dairy cows used a degradable deslorelin implant (gonadotropin releasing hormone agonist) to suppress ovulation during the first 50 days after parturition and found a reduction in the time to complete macroscopic uterine involution, but no difference in vaginal discharge as an indicator uterine health (Silvestre et al., 2009). Unfortunately, subsequent fertility was not an outcome in this study, but should be considered in the future.

Other studies investigating risk factors or postpartum treatments to accelerate uterine involution had variable success (Marion et al., 1968; Harrison et al., 1986; Sheldon et al., 2003). In the end, it seems clear that uterine infection delays uterine involution (Mateus et al., 2002a).

3. Bacteriology of the postpartum uterus

During pregnancy, the uterine lumen is sterile (Földi et al., 2006). At parturition, the cervix opens and allows bacterial contamination from the vagina and the environment into the uterus (Elliott et al., 1968; Griffin et al., 1974; Noakes et al., 1991). This situation affects over 90% of cows (Elliott et al., 1968; Griffin et al., 1974; Williams et al., 2005) and an assumption can be made that probably all cows are affected. Iatrogenic contamination, such as human intervention during a dystocia, could increase the bacterial load in the uterus (Noakes et al., 1991).

Many different species of bacteria can be found in the uterus in the early postpartum period (Elliott et al., 1968; Griffin et al., 1974; Williams et al., 2005). In the first 10 days after calving, bacteria frequently found in uterus include Escherichia coli (E. coli), Trueperella pyogenes (T. Pyogenes; formerly known as Arcanobacterium pyogenes), Streptococcus spp., Staphylococcus spp., Pseudomonas spp., Fusobacterium necrophorum (F. necrophorum), Prevotella melaninogenicus (P. melaninogenicus), and Clostridium spp. (Elliott et al., 1968; Noakes et al., 1991; Bondurant, 1999). Bacterial pathogens that contaminate the uterus have been categorized as recognized uterine pathogens, potential uterine pathogens, or opportunistic contamination bacteria (Sheldon et al., 2002; Williams et al., 2005). This classification is somewhat subjective and is based on their association with endometrial inflammation and clinical uterine disease (Griffin et al., 1974; Ruder et al., 1981; Bonnett et al., 1991). Recognized uterine pathogens include T. pyogenes, E. coli, P. melaninogenicus, F. necrophorum, and Proteus spp. (Williams et al., 2007). A study by Ruder et al. (1981) is frequently cited in the literature to support the synergy between T. pyogenes and F. necrophorum for enhancing the severity of uterine diseases.

It is known that almost all cows experience bacterial contamination after parturition. It is also known that the proportion of cows having bacterial uterine infection declines during the weeks following parturition (Elliott et al., 1968; Griffin et al., 1974; Sheldon et al., 2002). However, most studies investigating this decrease of bacterial contamination over time are weak because they are cross-sectional, have small numbers of cows, do not sample the same cows frequently enough from calving until first breeding to evaluate cows over time, use different sampling techniques, or focus only on cows with or without uterine diseases (Elliott et al., 1968; Griffin et al.,
A major gap exists in the current literature and should be addressed by performing studies that would consider these points. Such data, in addition to proper diagnosis of uterine inflammation and reproductive performance, would allow researchers to determine what really is optimal and when a sterile and/or non-inflamed uterus is desirable or achievable. According to the literature, 50% of the cows have a sterile uterus 30 days after parturition (Elliott et al., 1968; Griffin et al., 1974; Williams et al., 2005), but Sheldon et al. (2008) suggested that normal cows should have a sterile uterus two to four weeks after parturition. Obtaining valid information on this subject becomes critical when evaluating potential diagnostic tools and the inflammation process that characterizes uterine diseases.

4. Definition of postpartum uterine diseases

Most cows experience uterine bacterial infection during, or in the days following, parturition (Gier and Marion, 1968; Griffin et al., 1974; Williams et al., 2005). Thus, why do not all cows develop uterine diseases? This raises the question as to how uterine diseases should be defined. When reviewing the literature, it becomes obvious that there is a lot of variation in the definitions of diseases and terms used in various studies. This variation is a major weakness for comparing these studies (Gilbert, 1992). Metritis and endometritis are commonly used terms, but often their definition is missing in study reports, or they sometimes are taken as equivalent or interchangeable. Histological and clinical diagnoses are also difficult to compare because they use the same terminology, but their definitions may be different (Bondurant, 1999). In order to improve this situation, Sheldon et al. (2006) proposed standardized definitions of metritis, clinical endometritis, and subclinical endometritis. Although it could be argued that these definitions might not be totally accurate, they will be used in this thesis because they represent the current state of the art. Uterine diseases are difficult to define, since such conditions are only of interest if detrimental to the health of the cow or future reproductive performance.

Metritis is defined as an acute systemic illness due to infection of the uterus with bacteria, usually within 20 days after parturition. It is characterized by a fetid brown-red watery uterine discharge, and usually pyrexia with reduced milk yield, inappetence, dullness, and possible dehydration (Drillich et al., 2001; Sheldon et al., 2006). It is known that some cases of severe bacterial infection may cause systemic signs without pyrexia, which is independent of the species of bacteria found in the uterus (Sheldon et al., 2004b). For this reason, as well as the high prevalence of fever of unknown origin in fresh cows, pyrexia monitoring as the only tool for the detection of metritis is not accurate (Sheldon et al., 2004b; Benzaquen et al., 2007). Based on this disease definition, diagnosis of metritis on farms can be somewhat subjective due to the lack of formal objective criteria.

Endometritis is defined as inflammation of the uterus without systemic illness (LeBlanc, 2008). Clinical endometritis or purulent vaginal discharge is diagnosed by physical examination, and is defined as the presence of purulent or mucopurulent vaginal discharge, 21 days or more after parturition, without any systemic signs of illness (Sheldon et al., 2006). This definition was primarily based on a large scale study investigating clinical criteria having a negative impact on reproductive performance (LeBlanc et al., 2002a). In this study, cows between 20 and 33 days after parturition having purulent vaginal discharge or cervical diameter >7.5 cm, or cows >26 days after parturition with mucopurulent or purulent vaginal discharge, had reduced reproductive performance. It was suggested that an abnormally enlarged cervix could reflect delayed involution and potential infection (LeBlanc et al., 2002a). Since this study was published, other investigations found similar results, which validate this definition (McDougall et al., 2007; Runciman et al., 2008b; Gautam et al., 2009). The source of purulent material found in the cranial vagina in cows positive for clinical endometritis is unclear. It has been assumed to be from the endometrium, and would drain through the cervix (Kasimanickam et al., 2005b). However, this theory has not been validated yet. Other conditions, such as cervicitis and vaginitis, could be involved (LeBlanc et al., 2011; Deguillaume et al., 2012). Further research is needed to address this issue.
Subclinical endometritis is based on the concept that cows can have uterine disease without any visually observable signs at clinical examination. It is defined as the presence of uterine inflammation, having a negative impact on reproductive performance, in cows unaffected by clinical endometritis (Kasimanickam et al., 2004). It is detected by endometrial cytology sampling using a brushing device, a low-volume saline flush, or a biopsy (Bonnett et al., 1991; Gilbert et al., 1998; Kasimanickam et al., 2004). The cut-off for the level of inflammation having a negative impact on reproduction varies between studies, based on the cow population and the time in postpartum period at sampling. A study found that cows between 20 and 33, and 34 and 47 days after parturition had decreased pregnancy rate with the proportion of polymorphonuclear (PMN) cells >18 % and >10 %, respectively (Kasimanickam et al., 2004). Other studies found lower thresholds (Gilbert et al., 1998; Gilbert et al., 2005; Barlund et al., 2008), but used a different cow population, including all cows without systematically evaluating their clinical endometritis status. It should be considered that these studies were evaluating endometritis diagnosed cytologically and not subclinical endometritis, as in the former study (Kasimanickam et al., 2004). Overall, classification of endometritis, as clinical or subclinical, might not be the most relevant way to present it. It may be more precise to focus on the method of diagnosis (clinical and cytological). Comparison of clinical and cytological endometritis diagnostic results, performed simultaneously, showed a poor correlation and additional impact on reproduction (Dubuc et al., 2010a,b). These results suggest that clinical and cytological endometritis may be two different conditions having additive detrimental impact on subsequent reproduction (Dubuc et al., 2010a, 2011).

5. Metritis

5.1 Epidemiology and risk factors

Since essentially all cows experience uterine infection in the postpartum period, but only some of them develop uterine diseases, such as metritis and endometritis, there must be risk factors explaining why some cows cannot avoid the pitfall. Overall, these risk factors can be arbitrarily divided into two categories: factors increasing the contamination of the uterus and factors decreasing the innate immune response of the cow.

Metritis is often associated with retained placenta (Bartlett et al., 1986; Gröhn et al., 1990; Correa et al., 1993). Retained placenta (RP) is defined as retention of fetal membranes 24 hours after parturition, and is diagnosed by visual or vaginal inspection (Kelton et al., 1998). If retained, the membranes will usually remain for an average of seven days (Eiler, 1997). The incidence of RP can vary between herds, but the median reported is 8.6 % (Kelton et al., 1998). The proposed contribution of retained placenta in the pathogenesis of metritis is in providing an ideal environment for bacterial growth, because of the large amount of necrotic tissue present, delay of lochia expulsion, and potential lesions to the uterus from manual removal (Kaneene et al., 1986; Bolinder et al., 1988; Sheldon, 2004). The consistent association between RP and metritis could also be partially explained by the impaired immune response occurring in both circumstances, which may presumably be linked (Gunnink, 1984; Gilbert et al., 1993; Hammon et al., 2006).

The pathogenicity of E. coli and the systemic effects of LPS during metritis were investigated recently (Williams et al., 2008; Silva et al., 2009). It was suggested that some strains of E. coli may be associated with the incidence of metritis (Williams et al., 2008), but there are conflicting data on this question (Sheldon et al., 2009a; Silva et al., 2009). Bovine herpes virus 4 has been suggested to be linked with uterine diseases, but further research is necessary to support this theory (Sheldon et al., 2009a).

Other reported risk factors for metritis include twins, dystocia, stillbirth, abortion, milk fever, negative energy balance, and deficiency in hygiene (Bartlett et al., 1986; Gröhn et al., 1990; Correa et al., 1993). Unfortunately, most of the epidemiological studies investigating risk factors used non-standardized disease definitions for metritis, which makes their findings difficult to interpret and compare. This could also explain the large variation of the reported incidence of the disease between studies.
Dry matter intake was shown to be decreased, as early as two weeks prior to parturition, in cows that developed puerperal metritis (Urton et al., 2005; Huzzey et al., 2007). It is not clear if this reduction of intake is a direct cause of metritis, but it might more likely be linked with changes in energy and lipid metabolism and insufficient intake of antioxidants (Hammon et al., 2006; Bourne et al., 2007). A reduction in dry matter intake was also noted as early as 48 hours prior to parturition in cows that experienced dystocia, which has been identified as a risk factor for metritis (Proudfoot et al., 2009).

5.2 Diagnosis

A case of metritis is defined as a systemically ill cow, presenting fetid brown-red watery uterine discharge with pyrexia (>39.5 °C), during the first 20 days after parturition. This cow presents signs of systemic disease, such as decreased appetite and milk production (Sheldon et al., 2006). This definition is somewhat subjective for on-farm application because the only objective criterion used is pyrexia. Seeking an objective method for early detection of metritis, studies have found that rectal temperature monitoring alone during the postpartum period is inaccurate (Sheldon et al., 2004b; Benzaquen et al., 2007). Rectal palpation of the cervix and uterus for estimating the size, symmetry, and consistency can be used, but is also a subjective and inaccurate method (Miller et al., 1980). Vaginal examination with a hand, a speculum, or the Metrichek device are helpful to visualize vaginal discharge (Miller et al., 1980; Dohmen et al., 1995; Pleticha et al., 2009), and are more sensitive to detect vaginal discharge than transrectal palpation of the uterus (LeBlanc et al., 2002a). Bacterial culture can be performed. However, since most cows are contaminated during this period with a broad range of non-specific bacteria, this procedure does not seem promising (Gier and Marion, 1968; Griffin et al., 1974; Williams et al., 2007). There is a lack of data regarding the use of endometrial cytological examination in early postpartum period. Perhaps the cows that develop metritis have an insufficient innate immune response, which could lead to their identification early the postpartum period. Future research is needed in this area. Ultrasound is a good standardized and objective method to evaluate the uterine involution (Kamimura et al., 1993; Sheldon et al., 2003) and might be used to diagnose metritis, but there is currently a lack of information on this subject.

5.3 Impact

Although metritis can be a severe illness for the cow, its longer term impact on reproduction, production, and culling parameters have not been well studied. Studies have investigated some effects, but not all together in a well designed large scale study. Again, a problem when comparing studies is the lack of standardized definition. A frequently cited study reported an overall economic negative impact of $106 for each case of metritis (Bartlett et al., 1986). Unfortunately, the disease definition of puerperal metritis in this case was based on a diagnosis by rectal palpation and included cows up to 30 days after parturition, which limits its validity. A recent study used a more standardized definition and found the cost of a case of puerperal metritis to between $329 and $386 (Overton and Fetrow, 2008). Although the cost was calculated from one herd in California, which may not be representative of the Canadian situation, the major interest in this study was its evaluation of the overall impact of metritis. It evaluated milk production, reproductive performance, culling risk, antibiotic use, and disposal of waste milk.

Metritis was shown to have a detrimental impact on subsequent reproductive performance (Fourichon et al., 2000; Overton and Fetrow, 2008). Unfortunately, studies investigating the impact of metritis on reproduction usually do not consider if the cows are affected by endometritis. It is known that endometritis is detrimental to reproductive performance and that cows with metritis are at higher risk of developing endometritis (LeBlanc et al., 2002a; Kasimanickam et al., 2004; Gilbert et al., 2005). It is also known that uterine infection can influence the return of normal ovarian function, and that anovulation is detrimental to subsequent reproductive performance (Walsh et al., 2007; Williams et al., 2007). Overall, it is not clear if metritis causes a reduction of reproductive performance by itself, or by increasing the incidence of conditions associated with poor reproductive performance.
Metritis is detrimental to milk production. Studies found a decrease in milk production varying in duration, in magnitude, and by parity (Fourichon et al., 1999; Overton and Fetrow, 2008; Wittrock et al., 2009).

It is unclear if metritis increases culling risk and if it does, whether it is caused by the disease itself, or by other related factors, such as endometritis and subsequent poorer reproductive performance. A study conducted on 7523 cows in 7 herds found no effect of the disease (Gröhn et al., 1998), whereas another investigation conducted in 1 herd found an increased risk (Overton and Fetrow, 2008). Further research is needed to better define this relationship.

5.4 Treatment and prevention

Two major approaches are considered for the treatment of metritis: local and systemic therapy. Local treatment of a uterine disease generally implies infusing a substance in the uterus or draining it (Roberts, 1956). Various drugs and disinfectants have been used such as oxytetracycline and iodine. Little information from well designed studies is available regarding their efficacy and safety. Some studies have found them to be harmful, or detrimental to future reproduction (Nakao et al., 1988; Stevens et al., 1995).

Studies of systemic treatments for metritis have mostly focussed on antibiotic administration, but other drugs have been tested. For an efficacy study to be valid, some critical points are the definition of disease and cure, and the presence of a negative control group. Many studies have failed to meet these criteria (Masera et al., 1980; Gustafsson, 1984; Smith et al., 1998; Drillich et al., 2001; Zhou et al., 2001; Chenault et al., 2004). Interestingly, few studies have focussed on the pharmacokinetic, bacterial susceptibility, and residue aspects of these antibiotics (Bretzlaff et al., 1983a,b; Gilbert and Schwark, 1992; Dinsmore et al., 1996; Okker et al., 2002; Sheldon et al., 2004a; Drillich et al., 2006a).

Hormones have also been tested as therapy for metritis cases. The effect of PGF treatments given twice, eight hours apart, on day eight after parturition, in cows having RP or metritis, and treated with ceftiofur for five days, was investigated (Melendez et al., 2004). In this case, PGF treatment had a positive effect on uterine involution and conception risk at first service. Interestingly, although the disease was not always well defined, PGF was found to have very little success as a therapy for RP and for the prevention of metritis (Archbald et al., 1990; Risco et al., 1994; Stevens and Dinsmore, 1997). Other drugs, such as estradiol cypionate, cloxacillin, ampicillin, and gonadotropin releasing hormone, were found to have poor success for reducing the incidence of metritis or for improving subsequent reproductive performance (Overton et al., 2003; Risco and Hernandez, 2003; Drillich et al., 2007).

Ceftiofur has been studied several times for the treatment of metritis and is labelled in Canada for metritis at a dosage of 2.2 mg/kg, IM or SC, q24, for three to five days. These studies were conducted in two situations: treatment or prevention of the disease. Unfortunately, the validity of these studies can be questioned since their definition of cure was variable, and many did not have a negative control group (Smith et al., 1998; Drillich et al., 2001; Schmitt et al., 2001; Zhou et al., 2001; Drillich et al., 2003; Risco and Hernandez, 2003; Chenault et al., 2004; Drillich et al., 2006b; Drillich et al., 2006c; Drillich et al., 2007). Ceftiofur was found efficacious for the treatment of cows with RP for reduction of the incidence of metritis (Risco and Hernandez, 2003), with or without monitoring of rectal temperature during the postpartum period (Drillich et al., 2003; Drillich et al., 2006b; Drillich et al., 2006c). Although the minimum inhibitory concentration (MIC) values for relevant uterine pathogens are available (Sheldon et al., 2004a), little information is available regarding the diffusion of ceftiofur in uterine tissue, and most studies have based their classification of success of treatment on clinical examination. Only two studies (one in healthy cows and one in cows with RP) have investigated the diffusion of ceftiofur in uterine tissue at a dosage of 1 mg/kg, with partial success in maintaining MIC90 levels against E. coli (Okker et al., 2002; Drillich et al., 2006a). Future research investigating the efficacy of ceftiofur should focus on obtaining better pharmacokinetic and uterine distribution data, as well as using standardized definitions of disease and cure (Sheldon et al., 2006).
Future research should also investigate the effects of ceftiofur on subsequent reproductive performance and milk production.

6. Endometritis

6.1 Epidemiology and risk factors

Endometritis refers to inflammation of the uterus without systemic illness 21 days or more after parturition (Sheldon et al., 2006; LeBlanc, 2008). Its definition is linked to its detrimental impact on subsequent reproduction (LeBlanc et al., 2002a; Gilbert et al., 2005; Sheldon et al., 2006). Retained placenta is a major risk factor for endometritis, but it is unclear if this link is direct or through a higher risk of developing metritis (Correa et al., 1993; Runciman et al., 2008b; Gautam et al., 2009). Animals affected by RP suffer from immunosuppression and have increased bacterial contamination of the uterus (Sheldon et al., 2009b). This impaired ability of the immune system could presumably reduce the rate of clearance of bacteria in the uterus and lead to uterine inflammation later in lactation (Kim et al., 2005). Calving problems including dystocia, stillbirth, and twinning are associated with increased risk of endometritis (Markusfeld, 1987; Correa et al., 1993; Kaneene and Miller, 1995). Energy balance indicators such as increased NEFA and decreased dry matter intake before parturition, and increased BHBA early postpartum have been shown to increase the risk of endometritis (Hammon et al., 2006). Metabolic disorders, such as abomasal displacement, hypocalcemia, and ketosis also increase the risk (Markusfeld, 1987; Correa et al., 1993; Whiteford and Sheldon, 2005). Interestingly, there is little evidence of the potential link between metritis and endometritis in the literature, since most studies have considered them separately. This situation should be addressed in future research to better understand the link between retained placenta, metritis, and endometritis.

6.2 Diagnosis

Reports of the incidence of endometritis from valid large-scale multi-herd studies are not abundant in the literature. Some studies have performed such an investigation, but it may be difficult to compare them because the diagnostic techniques used were different (LeBlanc et al., 2002a; Kasimanickam et al., 2004; Gilbert et al., 2005; McDougall et al., 2007; Barlund et al., 2008). Diagnostic techniques for endometritis can be divided into two main approaches: techniques suitable to be part of a routine clinical examination, or techniques that may be impractical for routine use in clinical practice.

6.2.1 Routine clinical techniques

Clinical techniques refer to procedures that can be performed as part of a routine clinical examination of cows in veterinary practice. LeBlanc et al. (2002) were the first to define clinical endometritis using an objective outcome, the decreased risk of pregnancy, and not a subjective outcome like delayed uterine involution, as done previously (Tennant and Peddicord, 1968; Griffin et al., 1974). Clinical endometritis was then defined as a cervix diameter ≥ 7.5 cm by transrectal palpation (≥ 20 days after parturition), or as the presence of purulent, or mucopurulent or purulent vaginal discharge detected by vaginoscopy after 20 and 26 days after parturition, respectively (LeBlanc et al., 2002a). Cows with clinical endometritis had a hazard ratio of 0.73 for pregnancy compared with unaffected cows after accounting for parity, herd, and ovarian status. Since then, other studies found similar disease definitions (McDougall et al., 2007; Gautam et al., 2009; Runciman et al., 2009).

Evaluations of vaginal discharge by vaginoscopy or with the Metricheck device technique provide similar results (McDougall et al., 2007; Pleticha et al., 2009; Runciman et al., 2009). Manual examination of vaginal discharge is comparable with vaginoscopy (Pleticha et al., 2009). Rectal palpation of the cervix and uterus, and visual
inspection of the perineal region for detection of discharge, have a lower sensitivity compared with vaginoscopy (LeBlanc et al., 2002a).

The reported prevalence of clinical endometritis, approximately 30 days after parturition, is typically between 17 % and 23 % (LeBlanc et al., 2002a; Kasimanickam et al., 2006; McDougall et al., 2007; Runciman et al., 2008b; Gautam et al., 2009). Spontaneous cure of clinical endometritis occurring between two examinations was reported in 75 % of cows first examined between 15 to 60 days after parturition, and again between 61 and 150 days after parturition (Gautam et al., 2010). This finding is similar to 77 % from another study investigating the spontaneous cure between 20 and 33, and 34 and 47 days after parturition (LeBlanc et al., 2002b).

6.2.2 Non-routine clinical techniques

Several non-routine clinical techniques (i.e. methods that are impractical to use as part of a routine clinical examination procedure in veterinary practice) can be used to diagnose endometritis. Endometrial biopsy is a reliable method to evaluate inflammation in endometrium, and its result has been linked to subsequent reproductive performance (Studer and Morrow, 1978; Bonnett et al., 1991; Bonnett et al., 1993).

Although it is not easy to pass guarded swabs trans-cervically, uterine bacteriology sampling was investigated as a predictor of reproductive performance (Dohmen et al., 2000; Földi et al., 2006; Azawi et al., 2007). The main problem with bacteriology in postpartum period is the lack of definition of a normal cow, as far as the optimal rate of elimination of uterine bacteria. In this context, a diagnosis of contaminated uterus in a cow 30 days after parturition is likely undesirable, but significance of the pathogens found may be unclear based on current literature.

Endometrial cytology is used in horses to diagnose endometritis (Couto and Hughes, 1984). Similar methods in cattle have been proposed to be good indicators of endometritis (Gilbert et al., 1998; Kasimanickam et al., 2004). Low-volume uterine lavage and the cytobrush technique have been compared and were found equivalent (Kasimanickam et al., 2005a; Barlund et al., 2008). Endometrium cytological sampling is most common, but some data suggest that cervical sampling may be useful (Ahmadi et al., 2004; Ahmadi et al., 2006; Yavari et al., 2009). Diagnosis of endometritis based on endometrial cytology is currently considered as the gold standard technique (Kasimanickam et al., 2004; Gilbert et al., 2005; Barlund et al., 2008). The proportion of PMN, among epithelial cells and other leukocytes, is used as an indicator of inflammation. Endometritis is defined as an increased presence of inflammatory cells, associated with a detrimental effect on future reproductive performance (Gilbert et al., 1998; Kasimanickam et al., 2004; Barlund et al., 2008). The threshold of PMN at which the animal is declared diseased is determined using reproductive performance as an outcome (Gilbert et al., 1998; Kasimanickam et al., 2004; Barlund et al., 2008). The reported thresholds varied between studies, mostly because of the time of examination after parturition (Gilbert et al., 1998; Kasimanickam et al., 2004; Barlund et al., 2008).

From 20 to 33 days after parturition, the reported threshold was >18 % PMN in a population of cows presenting no clinical signs of endometritis (Kasimanickam et al., 2004). If the population studied included all cows, without regard to their clinical signs, the thresholds were >6 to 8 % PMN, and >4 to 5 % PMN, in different studies performed 28 to 41 days, and 40 to 60 days after parturition, respectively (Gilbert et al., 2005; Barlund et al., 2008; Galvão et al., 2009a). In beef cows, a similar study found the threshold to be >6 % PMN when examined 26 days before the start of breeding (Santos et al., 2009). Taken together, these studies show that there is a decrease in PMN counts over time after parturition, and that cows affected with cytological endometritis seem to have a greater proportion of PMN than normal cows, which highlights the fact that normal cows (defined as having acceptable reproductive performance) do not need to have zero PMN (Kasimanickam et al., 2004; Gilbert et al., 2005; Barlund et al., 2008). Unfortunately, the bacterial aspect of endometritis was not taken into account in such studies, but would provide useful information regarding the relationship between bacterial pathogens and uterine inflammation over time. It is unclear how long uterine inflammation remains after elimination of bacterial pathogens, and this question deserves some attention in the future.
Ultrasonography can be used for diagnosis of endometritis by looking for the presence of fluid in the uterus ≥20 days after parturition, and offers the advantage of an immediate result (Dhaliwal et al., 2001; Mateus et al., 2002b; Kasimanickam et al., 2004). It is a specific method (92 %), but it has a poor sensitivity (31 %) compared to cytology (Barlund et al., 2008). Cows diagnosed with uterine fluid have an inconsistent impact on reproductive performance compared to cows without visible fluid (Kasimanickam et al., 2006; Barlund et al., 2008). Little information is available regarding the relationship between cows found with uterine fluid and cows found with positive cytology (increased proportion of PMN). Information were retrieved from personal communication with the first author of Kasimanickam et al. (2004) to obtain data that were not presented in the article. Among cows found with uterine fluid at ultrasonography 20 days after parturition, 55 % had a positive cytology, whereas of the cows with positive cytology, 46 % had uterine fluid. These findings are somewhat surprising if the presence of uterine fluid were a reflection of inflammation. Perhaps, these two conditions reflect partially different uterine conditions, which could explain why the impact on reproduction is inconsistent (Kasimanickam et al., 2006; Barlund et al., 2008).

The reported prevalence of cytological endometritis, at approximately 30 to 60 days after parturition, was highly variable between studies, with a study average between 11 and 53 % of cows (Gilbert et al., 2005; Kasimanickam et al., 2006; Barlund et al., 2008; Galvão et al., 2009a). In a population of cows without clinical signs of endometritis, the prevalence of cytological endometritis was 34 % (Kasimanickam et al., 2004). This variation of prevalence is mainly influenced by the time of examination after parturition, and whether cows with clinical endometritis were excluded.

6.3 Impact

Because endometritis is a localized uterine disease, it causes no direct loss of milk production or mortality (Fourichon et al., 1999). However, no standardized definition of uterine diseases was used in the latter review paper, which makes its validity questionable. Presumably, endometritis would not affect milk production.

It is clear that endometritis has a negative impact on subsequent reproductive performance (LeBlanc et al., 2002a; Gilbert et al., 2005; Barlund et al., 2008). The magnitude of this impact varies between studies. For clinical endometritis, this impact was observed as a reduction of pregnancy risk at first service ranging from 21 to 62 %, and by an increase of 32 to 205 days in median time to pregnancy (LeBlanc et al., 2002a; Gautam et al., 2009). For clinical endometritis in seasonal calving systems, there was an increase of 19 to 49 days in the median interval from start of the breeding season to pregnancy (McDougall et al., 2007; Runciman et al., 2008b). For cytological endometritis, there was a relative decrease of 32 to 69 % in pregnancy risk at first service, and an increase of 24 to 88 days in median time to pregnancy (Kasimanickam et al., 2004; Gilbert et al., 2005; Barlund et al., 2008). Endometritis is presumably detrimental to reproduction because of its negative effect on return to ovarian activity (Herath et al., 2006; McDougall et al., 2007; Senosy et al., 2009), on embryo survival, and by causing endometrial damage (Moraitis et al., 2004; Hill and Gilbert, 2008). Further research is required to confirm these hypotheses.

6.4 Treatment and prevention

Many therapies have been investigated for the treatment of endometritis but several of these clinical trials have suffered from major flaws, such as a lack of standardized definition of the disease, a lack of objective and appropriate measurement of success, a lack of negative control groups, a lack of randomization of treatment, and a lack of study power with only a small number of animals tested. Intra-uterine antibiotic infusion treatments for endometritis are used to attempt to obtain a high concentration of drug at the site of infection (Gustafsson, 1984; Gilbert and Schwark, 1992). Common substances used for intrauterine infusion includes oxytetracycline (Thurmond et al., 1993), penicillin (Thurmond et al., 1993), chloramphenicol (Steffan et al., 1984), iodine (Callahan and Horstman, 1987; Nakao et al., 1988), cephalirin (McDougall, 2001; LeBlanc et al., 2002b; McDougall, 2003), and ceftiofur (Galvão et al., 2009b). Overall, there is little evidence that intrauterine infusion treatments are
beneficial, except for cephapirin which had a consistent positive impact for mitigating the effect of endometritis on subsequent reproductive performance (McDougall, 2001; LeBlanc et al., 2002b; McDougall, 2003; Kasimanickam et al., 2005b; Runciman et al., 2008a). A major concern with intrauterine infusion is the establishment of a withdrawal period, since most of these treatments are not approved for such use (Kaneene et al., 1986; Dinsmore et al., 1996). However, cephapirin is labelled in Canada for use as intra-uterine infusions with a withdrawal of zero hours for milk, and 48 hours for meat.

6.4.1 Prostaglandin F2α

The efficacy of PGF has been evaluated in numerous trials for the treatment of endometritis with inconsistent results (Etherington et al., 1984; Young et al., 1984; Young and Anderson, 1986; Etherington et al., 1988; Armstrong et al., 1989; Mcclary et al., 1989; White and Dobson, 1990; Morton et al., 1992; Etherington et al., 1994; Gay and Upham, 1994). Poor measures of reproductive performance or inadequate statistical analyses, such as linear regression for number of service per conception, have been used in these studies, which decreased their validity. Major study flaws as reported in the previous section are also commonly found in these studies.

Dinoprost and cloprostenol are the two major PGF reported in the literature. Although the purpose of this review is not to compare their efficacy to induce luteolysis, little evidence suggests that they would have different impacts on reproductive performance (Donaldson, 1984; Etherington et al., 1994).

It is known that PGF causes luteolysis in cows having a responsive corpus luteum (CL), which leads to a decrease in progesterone concentration, and subsequent estrus with uterine contraction (Larson and Ball, 1992). It has been shown that under some circumstances PGF has a positive effect on uterine contraction, but it is unknown if this finding is clinically relevant in cows <60 days after parturition (Rodríguez-Martínez et al., 1987; Hirsbrunner et al., 1998; Hirsbrunner et al., 2010). Presumably, estrus would lead to physical expulsion of bacteria and fluid, and to a period when uterine immunity may be enhanced because of the low level of progesterone (Kasimanickam et al., 2005b). This hypothesis, frequently mentioned in the discussion section of papers, has surprisingly never been proven in cattle (Lewis, 1997).

Although many papers have investigated the effect of PGF in the postpartum period, most of them did not consider uterine disease. A trend can be seen regarding the positive impacts of PGF treatment ≥15 days after parturition for reducing the interval between calving and observation of first estrus, and is of benefit for herds having a low pregnancy rate (Etherington et al., 1984; Young, 1989; Etherington et al., 1994). This finding makes biological sense considering the luteolytic effect of PGF (Larson and Ball, 1992). Unfortunately, this estrus synchronization effect of PGF has not always been considered in studies using first service pregnancy risk as an outcome.

Some studies have included uterine disease as an outcome in their statistical analyses. For both clinical and cytological endometritis, PGF treatment ≥20 days after parturition had a negligible effect on the subsequent prevalence of the disease (LeBlanc et al., 2002b; Hendricks et al., 2006; Galvão et al., 2009a). In most of those studies, PGF treatment had a positive impact on reproductive performance, such as median days to pregnancy and pregnancy risk at first service (LeBlanc et al., 2002b; Hendricks et al., 2006; Galvão et al., 2009a). Among cows having cytological endometritis, cows with low body condition score (≤2.5) had increased reproductive performance with PGF treatment (Galvão et al., 2009a). Among cows with clinical endometritis, PGF treatment had no effect on reproduction in cows having a CL, and had a detrimental effect in cows without a CL (LeBlanc et al., 2002b).

Overall, even if several studies have been conducted and given inconsistent results, the impact of PGF for treating endometritis, as defined and understood currently, appears negligible. On the other hand, PGF treatment appears to be beneficial for improving reproductive performance, and is likely linked to an estrus synchronization effect. Excellent recent comprehensive studies should be used as examples for future study design (LeBlanc et al., 2002b; Kasimanickam et al., 2005b; Hendricks et al., 2006; Galvão et al., 2009a). A better understanding of the
relationship between cytological and clinical endometritis might help to better target the subpopulations of cows, if any, that can benefit from PGF treatment in postpartum period.

7. Conclusion

Postpartum uterine diseases are detrimental to dairy cows and costly to dairy producers. Dairy practitioners can help to reduce the impact of uterine diseases with appropriate cow-level diagnosis, treatment, and prevention strategies. Herd-level surveillance of uterine diseases is also useful and may serve at evaluating their prevalence and establish mitigation strategies.

8. References


Dr. Michael Day:
Dr. Michael Day earned his BS in Animal Husbandry from the University of Missouri, Columbia, in 1980. He subsequently earned his MS and Ph.D. degrees in reproductive endocrinology in the Department of Animal Sciences at the University of Nebraska- Lincoln, and joined the faculty at The Ohio State University in the Department of Animal Sciences in 1985. He is currently a Professor and Graduate Studies Chair in the Department. In 1996-97, Dr. Day took a sabbatical to work in collaboration with Dr. Jock MacMillan at the Dairying Research Corporation in Hamilton, New Zealand.

The focus of Dr. Day’s research is the endocrine regulation of pubertal, follicular and uterine processes in cattle. Research targeting development and improvement of estrous cycle control in beef cattle has been a constant component of his program. He currently has ongoing and active research collaborations in Brazil in addition to his program in Ohio. Dr. Day has authored/coauthored over 75 journal articles, 125 abstracts and 30 papers in proceedings. His research has been primarily funded by USDA-NIFA and the AI industry. He contributes to many extension programs on a State, national, and international level.

Dr. Jocelyn Dubuc:
Dr. Dubuc completed a veterinary degree in 2005 at the Université de Montréal. He then completed a master degree at the Université de Montréal in 2007 and a doctoral degree at the University of Guelph in 2010. His doctoral degree focused mainly on postpartum uterine diseases. During his graduate studies, he worked as a dairy practitioner in 2 practices. He currently works as a faculty for the ruminant field service clinic of the Université de Montréal. He focuses on dairy herd management and preventive medicine. His main interests in research and practice are postpartum uterine diseases, transition cow diseases, and milk fat depression.

Dr. Randall Hinshaw:
Randall Hinshaw received his DVM degree from the University of Georgia in 1982 and moved to Harrisonburg, VA to begin his veterinary career. The practice currently is bovine exclusive and employs seven veterinarians. Since 2000 his practice has been limited to bovine embryo transfer and ultrasonography. He has given numerous presentations and training seminars across the U.S. and served as the president of the American Embryo Transfer Association. In 2006 he was given the bovine practitioner of the year award from the American Association of Bovine Practitioners. He and his wife, Linda, have three adult daughters and three grandchildren.

Dr. Robert McCorkell:
Dr. McCorkell received his DVM from the Western College of Veterinary Medicine in 1983. He then spent many years as a mixed animal practitioner, much of it in rural central Alberta, before returning to graduate school to study the reproductive physiology of some of Canada’s native ungulates (elk, and white-tailed deer). After receiving his PhD from the University of Saskatchewan in 2006 he was a lead researcher in a project investigating the reproductive physiology of Wood Bison. He is a member of the Wood Bison Reproductive Research Group working to adapt assisted reproductive technologies to bison in order to provide a possible solution to the diseased wood bison in Wood Buffalo National Park. In August of 2008 he joined the new Faculty of Veterinary Medicine at the University of Calgary as a Senior Instructor.
Dr. Garrett R. Oetzel:

Dr. Garrett R. (Gary) Oetzel, DVM, MS, is an associate professor in the Food Animal Production Section, Department of Medical Sciences, School of Veterinary Medicine at the University of Wisconsin-Madison. Dr. Oetzel grew up on a beef cow-calf farm in southwestern Ohio. He completed his undergraduate and veterinary medical training at The Ohio State University, receiving his BS in 1978 and DVM in 1981. From Ohio he went to the University of Illinois where he completed an internship, residency, and graduate program. He received his MS degree in Veterinary Clinical Medicine in 1985. Dr. Oetzel then spent three years as an assistant professor at Colorado State University where he refined his interests in dairy nutrition and dairy production medicine. After one year in private dairy practice in Reedsville, WI, Dr. Oetzel joined the Food Animal Production Medicine Section at the University of Wisconsin-Madison School of Veterinary Medicine in September, 1989. Dr. Oetzel's research interests are in applied dairy nutrition and metabolic diseases such as milk fever, ruminal acidosis, ketosis, and displaced abomasum. He teaches veterinary clinical nutrition to the first- and third-year veterinary medical students and applied dairy nutrition to the fourth-year veterinary medical students. His clinical interests include troubleshooting techniques and the application of herd-based tests for metabolic disease problems in dairy herds.

Dr. Robert Tremblay:

Rob Tremblay is a Bovine and Equine Specialist with Boehringer Ingelheim (Canada) Ltd. He graduated from the Ontario Veterinary College in 1982 and worked in large animal practice in New Brunswick. After receiving a DVSc degree in 1988, he remained at OVC as a faculty member in the large animal clinic until 1992. He spends much of his time working on the control of infectious diseases of cattle.

Brian Van Doormaal:

Brian Van Doormaal has dedicated his professional career of 26 years involved in the genetic improvement of dairy cattle in Canada. Most significantly, he has been the General Manager of Canadian Dairy Network (CDN) for the past 17 years, a position he has held since the company was first established in 1995. For a period of two years starting in June 2010, he was also the Chief Executive Officer of Holstein Canada and managed both companies during a time when the industry was embracing genotyping and genomics for dairy cattle improvement. Over the course of his career at CDN he has been directly involved in the implementation of several genetic evaluation advancements including national genomic evaluations for Canada in 2009. At Holstein Canada, focus was on the establishment of a world leading breed association offering a full portfolio of genotyping services for all dairy breeds in Canada and abroad. Internationally, Brian Van Doormaal has been the Canadian representative on the Steering Committee of Interbull for over 16 years. In addition, he is well-known for his numerous extension articles and public speaking engagements in both official languages aimed at educating dairy producers and industry personnel on a wide variety of topics associated with dairy cattle improvement and genetic selection.

Dr. Richard Whitaker:

Richard O. Whitaker, DVM, MBA. Whit did his undergraduate studies at Washington and Lee University and The University of Virginia; graduated from the University of Georgia - College of Veterinary Medicine in 1979, and earned an MBA from the University of Southern Maine in 2004. Dr. Whitaker has been a Bovine Embryo Transfer practitioner for over 30 years. Whit formed New England Genetics in 1987 and became Certified by the American Embryo Transfer Association in 1988. He presently serves AETA’s Board of Directors, and chairs the AETA Government Liaison Committee. Dr. Whitaker also owns and operates a small animal practice, Central Maine Veterinary Hospital in Turner, Maine.