Physiological factors affecting success to reproductive technologies

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Introduction

At the time of publication of this proceedings, prices are at a relatively low point in the long-term cattle cycle. During such times, beef cattle producers are often tempted to lower input costs by strategically eliminating certain management practices. However, a more prudent approach to decreasing input costs per unit of production is by improving the other side of the equation. In other words, for commercial and purebred cow/calf producers, increasing the pounds of calf weaned per cow exposed. Reproductive efficiency has the most dramatic impact on production per cow.

Applied reproductive technologies consistently improve production per cow through access to better genetics from both the sire and dam. But, an often overlooked and arguably more important effect of these technologies, is their associated improvements in percentage calf crop and forward shift in calving distribution during the calving season. To take advantage of both of these positive attributes, care should be taken to select and manage cows and heifers to optimize the number of calves resulting from these technologies. The following list of physical and chemical processes that affect the success of applied reproductive technologies will be discussed in light of both their impact on improved genetics and calving distribution.

Factors

Pre-pubertal and postpartum anestrous

Anestrus has long been recognized as the primary factor reducing reproductive efficiency in beef cow-calf operations (Short, 1990). Unfortunately, anestrus occurs annually in productive females; heifers are anestrus prior to puberty and cows undergo a period of anestrus after each calving. The length of the anestrous period is governed by many factors including presence of a calf, nutritional status, cow age, and degree of calving difficulty. Regulation of anestrus is essential to maintaining productivity and profitability in beef cattle production.

Early conception is limited by the proportion of suckled cows not exhibiting regular estrous cycles (anestrus) at the beginning of the breeding season (Short et al., 1990). The incidence of anestrus at the initiation of the breeding season is significant in beef cow-calf operations. In an experiment that included 851 postpartum cows from six states (Lucy et al., 2001), 53% of cows were anestrous 7 days before the beginning of the breeding season (range 17-67%). In other experiments the incidence of anestrus ranged from 44% (Gasser et al., 2003) to 46% (Stevenson et al., 2003). Reproductive rate in beef cattle can only be maximized when methods are used that successfully synchronize a fertile estrus in anestrous beef cows.

The factors regulating anestrus have been reviewed extensively (Short et al., 1990; Stevenson et al., 1997; Yavas and Walton, 2000; Rhodes et al., 2003). Anestrus is initiated by the presence of a suckling calf; weaning at birth results in the resumption of estrous cycles in about 14 days (Williams, 1990). Nutritional status greatly influences the length of the anestrous period. Reduced body energy reserves or inadequate energy or protein intake at calving can delay the return to estrus. In a recent summary, Stevenson and coworkers (2003) identified three key issues (body condition, parity, and days postpartum) that were associated with the incidence of anestrous and pregnancy failure.

The endocrine changes that precede first estrus must be imitated to successfully induce estrus in anestrous cows. Ovarian function begins rapidly after calving. A wave-like pattern of follicle growth begins approximately two weeks postpartum and multiple waves of growth occur prior to the first ovulation (Murphy et al., 1990). Diameter of the dominant follicle increases with each successive follicular wave up to either ovulation (Murphy et al., 1990) or the fourth or fifth wave in cows with longer postpartum intervals (Stagg et al., 1995). The first ovulation postpartum is preceded by an increase in the pulsatile secretion of LH (Walters et al., 1982).

The initial postpartum ovulation occurs if the dominant follicle can produce sufficient estradiol to induce the preovulatory gonadotropin surge. The initial postpartum ovulatory event results in the formation of a corpus luteum (CL) that is short-lived (7-10 days). The short cycle is a normal occurrence at the first spontaneous postpartum ovulation in beef females (Day et al., 1990). The first CL postpartum is short lived because of the premature release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus (Cooper et al., 1991) due to the low concentrations of progesterone (Zollers et al., 1993) and estradiol (Mann and Lamming, 2000) before ovulation. Low concentrations of progesterone and estradiol before ovulation alter endometrial progesterone and oxytocin receptor concentrations leading to the premature release of $PGF_{2\alpha}$ and the early regression of the CL. Protocols that increase progesterone before ovulation and enhance pulsatile LH secretion and follicle growth are important components of any program to successfully induce fertile estrous cycles in anestrous cows.

Administration of a progestin to anestrous cows for a short time period (5-9 days) can successfully induce estrus in many anestrous cows (Day, 2004) and is the core treatment used to induce resumption of estrous cycles in most protocols to synchronize estrus. Treatment of postpartum anestrous cows with a progestin not only induces estrus (Fike et al., 1997) but also the duration of the subsequent estrous cycle is typically of normal length (Ramirez-Godinez et al., 1981; Hu et al., 1990). Progestin treatment likely induces estrus by increasing LH secretion in postpartum cows (Garcia-Winder et al., 1986), seasonal dairy cows (Rhodes et al., 2002) and prepubertal heifers (Anderson et al., 1996; Hall et al., 1997; Imwalle et al., 1998). The secretion of LH increased both during progestin exposure and after removal of the progestin.

Progestin treatment has direct ovarian effects in postpartum anestrous cows. Both systemic and intrafollicular estradiol concentrations were increased by progestin exposure (Garcia-Winder et al., 1986; Inskeep et al., 1988). Progestin treatment appears to enhance follicle growth in postpartum anestrous cows (Rhodes et al., 2003).

Administration of gonadotropin releasing hormone (GnRH) 7 days before treatment with $PGF_{2\alpha}$ has been shown to increase estrus and ovulation rate in anestrous cows (Stevenson et al., 2003). Induction rate appears to be highest when cows receive a combination of progestin and GnRH (Stevenson et al., 2003). More anestrous cows ovulated after GnRH treatment if they were exposed to a progestin either before or at the same time as the GnRH injection (Stevenson et al., 2003). The induction of ovulation was increased linearly with BCS in multiparous cows and primiparous cows with a BCS greater than 5. The ability to induce ovulation in anestrous primiparous cows was limited (< 20%) when BCS was less than 5.

To calve at 24 months of age, heifers must reach puberty and conceive by approximately 15 months of age. Several factors influence the age at puberty including breed composition, nutrition, body weight, bull exposure and the environment (Patterson et al., 1992b). Lifetime productivity of a female is affected by age at puberty. Heifers that conceive early in the first breeding season are more likely to conceive early in subsequent seasons and thus become more productive cows (Lesmeister et al., 1973).

Similar to postpartum anestrous in cows, the proportion of heifers that are pubertal at the beginning of the breeding season influences reproductive rate. The incidence of pubertal anestrous at the onset of the breeding season has been estimated. In earlier work, Patterson and coworkers (1992b) indicated that approximately 35% of heifers were prepubertal at the onset of the breeding season. In more recent studies, 88% (1,245 heifers total; Larson et al., 2004b), 83% (203 heifers total; Lamb et al., 2004), and 57% (724 heifers total; Lucy et al., 2001) were prepubertal on the first day of the breeding season.

Anestrus, whether prepubertal or postpartum, greatly influences successful reproduction in beef cow-calf operations. Protocols have been developed that effectively induce a fertile estrus in anestrous females. Incorporation of these protocols will enable beef cow-calf operations to maximize reproductive potential.

Estrus and ovulation

The chronological order in which ovulation and insemination take place is important for the success of artificial insemination (AI). Throughout the early development and adoption of AI, estrous detection was used to mark the impending ovulation. Research to find the most appropriate time to inseminate relative to estrus yielded the AM/PM rule that was an industry standard for many years. More recently, improvements in the synchronization of follicular growth and, thereby, a more synchronous occurrence of ovulation, has enabled more widespread adoption of timed AI (aka, appointment breeding). This, in turn, led to simplified implementation of AI without significant sacrifice in pregnancy rate.

Pregnancy rate is calculated as the number of females pregnant relative to the number inseminated (either by displayed estrus or timed AI). So, if 95% of a herd is inseminated by estrous detection and 70% of them become pregnant to that insemination, the pregnancy rate is 67%. Alternatively, if an entire herd is inseminated based on time (regardless of displayed estrus) and 70% of them become pregnant to that insemination, the conception rate and pregnancy rate are 70%.

Taking advantage of the improved ability to synchronize follicular wave dynamics and ovulation has led to the ability to improve the number of calves resulting from AI because cows and heifers are bred to timed insemination that might not be inseminated based on display of behavioral estrus. While the conception rate in those females is often lower than breeding on estrus, the resulting increase in the total number of pregnancies often offsets the increased cost of additional semen. That is especially true considering that the pharmaceuticals used for the synchronization protocol have already been applied to every female.

Adhearing to the timing for application of pharmaceuticals and insemination is critical to obtain optimum results from synchronization and AI. The protocols published by the Beef reproduction Task Force, and printed in the major genetics companies' sire directories, are rigorously tested with at least hundreds (and usually thousands) of heifers and/or cows to arrive at the published protocol. They are vetted by scientists throughout the U.S. and across the country for effectiveness and repeatability. So, deviating from that timing most often does more harm than good.

Embryonic loss

Reports of fertilization rates have varied but, on average, were similar for lactating and non-lactating dairy cows (76.2% and 78.1% respectively; reviewed by Santos et al., 2004). However, the wide range of successful fertilization in that review (55.3 to 98.0%) indicates that environmental impacts and management strategies likely affect the ability of the male and female gametes to establish a two-cell embryo. Fertilization rates tend to be less variable and more successful for beef cows. Sreenan and Diskin (1980) reported a 90% fertilization rate for beef heifers following a single AI while Maurer and Chenault (1983) reported 100% fertilization rate for parous beef cows pen-mated at the onset of estrus. In each of the reports cited above, fertilization rate was considerably greater than calving rate. Regardless of relative fertilization rate and breed type, the disparity between fertilization rate and number of calves born to a single insemination indicates an extremely high occurrence of pregnancy wastage from the time a zygote is created to parturition.

Pregnancy loss can be defined broadly as death of the conceptus at any point from syngamy to parturition. However, as investigation of this phenomenon increased, terminology has been applied that delineates developmental periods during pregnancy. According to the Committee on Bovine Reproductive Nomenclature (1972), embryonic mortality is considered to be "the death or loss of conceptus during the embryonic period". This same committee recommended that the definition for the embryonic period set forth by Dennis (1969) be accepted: "the period from conception to the end of the stage of differentiation".

The embryonic period has been divided further into more concise periods of development. However, unlike much of the nomenclature used to describe periods of gestation, these terms have not yet been recognized formally but have advanced into common use in the literature. Early embryonic development refers to the period from fertilization to approximately day 20 of pregnancy. Late embryonic development indicates the period from days 20 to 45 post-fertilization. Fetal development is specified as the period of development from day 45 to term. More specifically, early fetal development refers to the period of gestation from completion of differentiation to day 60 post-fertilization (Committee on Bovine Reproductive Nomenclature, 1972).

As reviewed by Aylon (1978), much of the early data collected for investigation of embryonic loss relied on determination of whether the post-insemination estrous cycle was longer than the previous or longer than what would be considered average (17 – 25 days; (Erb and Holtz, 1958). More precise determination of actual embryonic loss, independent of fertilization failure, came about with the implementation of timed slaughter of inseminated cows. Tanabe and Casida (1949) slaughtered repeat-breeding (anatomically normal with a minimum of four infertile services) cows at 3 and 34 days post-AI and found that 66.1% presented a fertilized ovum at day 3 while only 23.1% had a normal embryo at day 34. Normal cows exhibited less fertilization failure and less embryonic loss at day 35 (16 and 14.5%, respectively; Ayalon, 1978).

Inskeep and Dailey (2005) reviewed the distribution of pregnancy failure from fertilization through transition to the fetal period. These authors concluded that the majority (57%) of failed pregnancies occur during early embryonic development, within one estrous cycle after insemination. In a review of the literature compiled upon request by the Commission of the European Communities, Sreenan and Diskin (1986) suggested that, for normal cows, "while embryo losses occur gradually from fertilization onwards, the greatest increment of losses would seem to occur between about days 15 and 18". They reached this conclusion by summarizing published fertilization rates for cows and heifers as "the number of normal cleaved ova or embryos as a proportion of all ova (fertilized or unfertilized) recovered" up to and including day 8 post-insemination. The fertilization rates for heifers and cows to natural mating or AI were 88% and 90%, respectively (Sreenan and Diskin, 1986). They then compiled data from several other authors who employed oocyte and embryo recovery at various times after insemination. When considered together, these reports supported the authors' conclusion that most of the embryonic loss occurred between days 15 and 18 (Sreenan and Diskin, 1986).

The period of development between approximately days 25 and 60 post-insemination is often referred to as late embryonic/early fetal development (Inskeep, 2004). Pregnancy loss during this developmental period constitutes a lower percentage of the total than earlier loss. However, the economic impact is significant because it results in an extended inter-estrous interval and more days in milk before a successful pregnancy is established (reviewed under heading "Economic Impact of Pregnancy Loss"). The frequency of late embryonic / early fetal pregnancy failure is easier to establish than early embryonic loss because the pregnancy can be observed by transrectal ultrasonography on gestation day 25 or detected by manual palpation on day 35.

During the late embryonic/early fetal period of development, one of the most critical processes is placentation. Formation of an efficient system of nutrient and waste transfer between dam and fetus is required to take over support of fetal growth as the demand for nutrition becomes more than can be supplied by histotrophic maintenance from the uterus (Roberts and Bazer, 1988). Failure to form a proper and efficient placental transfer has been suggested as the factor leading to a majority of late embryonic loss that occurs between days 30 and 45 of gestation (Dailey et al., 2002). This concept was supported by Starbuck and coworkers (2004) who reported that most pregnancy loss after day 30 in dairy cows and heifers had occurred before day 42 post mating. To understand pregnancy wastage during placentation, placental development should be considered.

Several reviews have compiled data from field trials that describe the rate of pregnancy failure from pregnancy diagnosis (gestation days 25 or 30) until the early fetal period (gestation days 45, 60, 80 and/or 100). Smith and Stevenson (1995) reported an average "late embryonal loss" of 12.4% for cows and heifers bred after several different estrous synchronization protocols. Pregnancy survival was not altered by method of synchronization but tended to be higher in heifers than in multiparous cows (Smith and Stevenson, 1995).

Milk progesterone has been used to identify luteal phases presumed to have been extended (> 24 days) by early pregnancy. Using this method, several authors have reported the frequency of pregnancy loss up to day 100 post-insemination (Bulman and Lamming, 1979; Grimard et al., 2006; Kummerfeld et al., 1978). These estimates range from 7% to 25.2% and agree with the ranges reported in the reviews by Inskeep (2004) and Dailey et al. (2002). Humbolt (2001) reported that late embryonic mortality ranged between 8 and 17%, with a mean of 14%. More recently, Grimard et al. (2006) reported that season and body condition score (BCS) affected pregnancy loss between pregnancy days 21 and 100 despite not having affecting conception rates.

While the timing of embryonic and fetal death has been well characterized and the frequency of pregnancy failure in cattle has been associated with several hormones, changes in the molecular biology of conceptuses that lead to late embryonic or early fetal death have not been determined. Other situations that lead to poor reproductive efficiency might prove to be useful tools for developing hypotheses to be tested. One such situation is *in vitro* production of bovine embryos.

Calving rates from transfer of *in vitro*-produced bovine embryos to apparently normal recipient cows or heifers are poor. The degree to which reproductive success is altered depends on the method of fertilization and type of culture system used. Even though the amount of loss is substantially more, the timing of pregnancy failure for these situations often resembles the timing of both early embryonic and late embryonic / early fetal losses for AI-derived pregnancies. Therefore, studying pregnancy failure data from embryos produced by *in vitro* fertilization (IVF) might provide new insight into pregnancy loss for AI pregnancies.

Management that influences physiological factors

Calving season

Of all the management practices that are discussed to create the ideal physiological environment for successful applied reproductive strategies, having a defined calving season is the most important. In fact, it is better to think of it as a gateway tool that should be implemented to allow other management practices to be used. The term "defined" or "controlled" calving season simply means managing the cow herd to calve within a relatively short period of time (usually 45, 60 or 90 days). Most of the other practices cannot be easily done if calves are not similar in age and cows are not at the same place in their production cycle.

One example of a management practice that is difficult to apply to year-round calving herds is a proper vaccination protocol. Imagine having 7-month-old calves in the same pasture with 3-month-old claves and newborn calves. These calves are not all ready to be given the same vaccinations at any one point in time. Gathering just a few calves at a time when they reach the appropriate age for a given vaccination is inefficient and is rarely maintained diligently. So, health of the cows and calves suffer in year-round calving herds.

Similarly, consider nutritional management. Providing the right nutrition (not too much and not too little) to a dry cow that is in the same pasture with a cow nursing a 2-month-old calf is practically impossible. Either the dry cow is getting more nutrition than it needs - stocking rate could be increased or it could be on lower quality hay/pasture – or the cow in peak lactation is getting less nutrition than she needs and will lose body condition. To say it another way, supplementing lactating cows in the same pasture with dry cows (that do not need extra nutrition) wastes feed/money.

With that evidence, it seems logical that most cattle producers would have a defined calving season. But, the National Animal Health Monitoring System (NAHMS; a USDA source for cattle production statistics) reports that less than half of the small herds across the country had a defined calving season in 2008. The most likely reason is that small herds are not often the primary source of income for a producer – resulting in less incentive to increase revenue. The two most common arguments against having a controlled breeding and calving season are: "I do not have anywhere to put the bull when it is not with the cows" and "I like having a calf ready to sell whenever I need it throughout the year."

It is true that the bull needs to be out of the pasture for a period of time. But, it does not have to be the entire balance of the time outside of the breeding season. The bull should be separate from the cows when they are calving and until the breeding season starts again. But, the bull can remain in the pasture after you plan to end your calving season. For example, if it remains in the pasture longer than your planned 90-day breeding season, ask the veterinarian to tell you which pregnant cows will not calve in your calving season and market them as bred replacements that might fit into someone else's calving season. This flexibility could make it easier to make use of bull leasing programs or buying bulls together with another producer that uses a different breeding season.

To the point about a continuous stream of revenue for year-round calving by having staggered availability of weaning aged calves; recall the discussion about limited management options for health and nutrition. Having a calf available to sell at any given time is less important than increasing the overall profitability and ease of management. Also consider the opportunity to concentrate the time spent on calving management. If all the cows are calving within a defined period of time, it is easier to watch them diligently, assist when needed and reduce death loss (both calves and cows/heifers). In other words, labor can be scheduled for the calving season whereas year-round calving leads to missing more calving difficulties. That can result in thousands of dollars lost if a cow and calf die calving while the producer is out of town or not expecting calves to arrive at random times.

Finally, consider the example of soybean production. When planting row crops, a producer does not plant one pass with the planter one week, another pass a week later and so on until the field is completely planted. The entire field is planted at the same time and managed as a single unit throughout the growing season; fertilizing, applying insecticide and herbicide and harvesting the entire field at the same time. Calving year-round is comparable to the idea of staggering the planting of a field over the entire growing season. If cows are at different places in their production cycle, and calves are at variable ages from newborn to weaning age, many of the management practices cannot be done to all the cattle at the same time.

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