Keys to maximizing reproductive efficiency in a beef herd

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Key Points:

- Timed AI and ET are effective methods to establish pregnancy in beef cows
- In order to increase the chance of pregnancy establishment and maintenance, cows must effectively respond to stimulus to synchronize a follicular wave; we must be able to control luteal lifespan, induce ovulation and deposit semen in the appropriate location.
- Decreasing progesterone prior ovulation followed by increasing post-ovulation increase pregnancy rates.
- Increased proestrus length prior to FTAI increased pregnancy rates.
- Increased estradiol prior ovulation, increased pregnancy rates.
- Estrus behavior, between CIDR removal and AI, increase pregnancy rates and decrease embryo mortality.

Introduction

The synchronization of estrus/ovulation and artificial insemination (AI) remain the most influential technologies available to cattle producers for genetic improvement and reproductive management [1]. Fixed-time AI (FTAI) or TAI protocols eliminate the need for estrus detection and permit insemination of heifers and cows at a predetermined time. In addition, timed embryo transfer, or TET, is another method, which allows for direct transfer of embryos at a predetermined time without the need for estrus detection. Resulting pregnancy rates from FTAI are similar to insemination following detection of estrus (see Figure 1). Furthermore, FTAI and estrous synchronization protocols increase the proportion of heifers and cows that conceive early in the breeding season which has important benefits for reproductive management and beef production [3].

An efficient synchronization protocol requires the following physiological processes: 1.) Synchronization of a follicular wave following an ovulatory stimulus (e.g. gonadotropin releasing hormone [GnRH] injection) or induction of dominant follicle turnover (e.g. administration of estradiol or progesterone) culminating in development of a physiologically mature dominant follicle at insemination, 2.) Control of luteal lifespan via prostaglandin F2α (PGF)-induced luteolysis, 3.) GnRH-induced ovulation of a physiologically mature dominant follicle, and 4) deposition of semen at the appropriate time relative to induction of ovulation. The hormone injection sequence is based on the premise that the initial GnRH will induce the ovulation of dominant follicle resulting in synchrony of follicular wave followed by an injection of PGF 7 days later to induce the luteolysis. A second injection of GnRH is administered 48 hours after the PGF to induce ovulation of a new dominant follicle and insemination occurs 24 hours later to the second GnRH injection [4]. Almost all FTAI protocols in the USA are a variation of GnRH-PGF-GnRH injection sequence with some differences in timing of insemination [3]

Alternatively, a treatment with estradiol and progesterone-releasing devices result in synchronous emergence of new follicular wave by induction of dominant follicle turnover. On the first day of protocol, cows receive a progesterone-releasing device, such as a CIDR, and an estradiol injection. Seven to nine days later the device is removed and an injection of PGF is given. In addition, a second injection of estradiol is administered at a similar time to induce ovulation of a physiologically mature dominant follicle.

Approximately 30-56 hours following the second estradiol injection, the insemination is performed [5]. The estradiol and progesterone approach and its variations are the most frequently used synchronization protocols in Brazil and other countries that permit the use of estradiol.

In the last 20 years, many researchers have conducted studies to improve efficiency in both protocols[6-11]. Their research contributed to a better understanding of reproductive physiology in cows and led to the current FTAI and TET protocols. This review will outline the physiology behind synchronization protocols and how they have manipulated it to improve pregnancy rates.

Follicle Size

Current fixed-timed artificial insemination (FTAI) protocols are 80-90% successful in synchronizing ovulation[12]. However, ovulatory follicle size at GnRH-induced or spontaneous ovulation is variable in cattle (Table 1). In postpartum beef cows, ovulatory follicle size was 15.0 ± 0.3 mm (mean ± std) with a range of ≤ 12mm to ≥ 18mm [13]. In the preceding study, there was a significant decrease in pregnancy rate following GnRH-induced ovulation of follicles ≤ 12.0 mm regardless of treatment. In dairy cows, GnRH-induced ovulation of dominant follicles resulted in a quadratic relationship between follicle size and pregnancy establishment in which pregnancy rate increased with dominant follicle size to a certain follicle size and then decreased [14]. Other investigators have also reported that induced ovulation of small physiological immature follicles reduced pregnancy rates in both beef and dairy cattle [7, 8, 15-19].

The ovulation of a physiological immature dominant follicle has been associated with reduced concentration of estradiol at the insemination and lower rate of luteal progesterone secretion after insemination [3]. Perry et al., [16] reported a decrease in pregnancy rate and increased late embryonic mortality following GnRH-induced ovulation of small ovulatory size follicles (≤11 mm diameter; Figure 2). The author attributes the decrease in fertility with lower circulating concentrations of estradiol on the day of insemination, a slower increase in progesterone after insemination, and a decreased circulating concentration of progesterone. However, the follicle size had no apparent effect on fertility when ovulated spontaneously (Figure 2).

It is important to note that it is not the size of the dominant follicle that affects the pregnancy rate but the physiological maturity of the dominant follicle in postpartum beef cows following fixed-time artificial insemination. A dominant follicle that is physiologically mature may be defined as follows: 1) contains a competent oocyte, 2) secretes adequate amounts of estradiol during the preovulatory period, and 3) has the ability to form a corpus luteum capable of secreting adequate amounts of progesterone for establishment and maintenance of pregnancy.

There is evidence that follicle age can affect pregnancy rates in cattle. Cerri et al. [20] reported greater embryo quality at d 6 in dairy cows that were induced to ovulate follicles approximately 1.5 days earlier than peak follicular dominance. Additionally, it has been demonstrated that lactating dairy cows with 3 follicular waves spontaneously ovulate younger follicles and have a greater conception rate than cows experiencing 2 follicular waves during the estrous cycle [21]. In beef cattle, the age of the ovulatory follicle was investigated indirectly by Bridges et al. [6]. In that study, induced ovulation of follicles that were estimated to be approximately 1.5 d younger in cows resulted in greater pregnancy rates when cows with younger follicles were afforded an extended proestrus. However, Abreu et al. [15] reported no differences in pregnancy rates, follicle size at AI and progesterone after AI in cows induced ovulation of a follicle approximately 3 days younger. Thus, it remains unclear as to what the exact effect age of the follicle has on pregnancy success. Recent evidence from Dias et al., [22], suggest a potential effect of progesterone on LH receptors within the follicle that may play a role in some of these results.

Progesterone

The estrous cycle in cows can be divided in two phases, follicular and luteal. The follicular phase begins with pro-oestrus, followed by oestrus and ends at ovulation. The luteal phase covers the metoestrus and diestrus and ends with luteolysis. During the luteal phase, progesterone is the predominant hormone. The concentration of progesterone during the estrus cycle regulates the secretion of GnRH from hypothalamus, which regulates the secretion of gonadotropins (FSH and LH) from the anterior pituitary. FSH is responsible for the follicle recruitment and LH leads the final stages of follicular growth, oocyte maturation and promotes the production and secretion of estradiol from the dominant follicle [23-26]. Progesterone concentrations prior to ovulation have been found to suppress LH pulse frequency, which can affect oocyte maturation, follicular growth and estradiol production [27]. Furthermore, the probability of conception is positively associated with serum concentration of progesterone 7 days after FTAI [28]. Martins et al.,

[28] also reported higher serum concentrations of progesterone 7 days after AI in heifers treated with low doses of progesterone during the estrus synchronization protocol (Figure 3). The authors also reported greater follicle diameter at FTAI, better ovulation rates and higher estrus detection rates, between CIDR removal (d9) and FTAI, in heifers treated with low progesterone when compared to those treated with high progesterone during the estrus protocol. Moreover, cows that were induced ovulated a larger follicle and have greater serum concentration of progesterone after AI [16] (Figure 4).

High progesterone concentration during the hormonal protocols has been shown to have a negative effect in follicle growth, decreasing preovulatory estradiol and post ovulatory progesterone specifically in Nelore heifers. In addition, as mentioned above, high progesterone concentrations prior to ovulation have been found to suppress LH pulse frequency, which can affect oocyte maturation, follicular growth and estradiol production [27]. Recent evidence suggest that LH can impact the expression of LH receptor in the granulosa cells thus directly effecting fertility [29]. Preliminary data from Dias et al., [22] indeed demonstrates that high progesterone concentrations during the synchronized period does decrease LH receptor expression in the granulosa cells of the dominant follicle in Nelore heifers. Future research is needed to confirm these observations but it seems that P4 leading to changes in pulsatility of LH could be a major driver of fertility in these FTAI protocols.

Estradiol

Initiation of estrus occurs following a rise in serum concentrations of estradiol [30]. There have been multiple studies in different breeds of cattle and environments that have demonstrated an increase in estradiol has a direct correlation to an increase in fertility, fertilization rates, and pregnancy rates. Preovulatory estradiol coordinates several physiological processes that contribute to the establishment and maintenance of pregnancy. Increased circulating estradiol levels have shown a positive correlation with follicle size [31], which has resulted in great fertility to FTAI. Cows exhibiting estrus within 24 hrs of TAI have also been reported to have increases in fertility [32, 33]. Estradiol's role during this period is multifaceted but the direct effect on follicular cells within the maturing follicle is critical. It has been reported that increased levels of estradiol have a positive effect on preparation of follicular cells ability to luteinize and secretion of progesterone (Progesterone's role reviewed above).

Increasing circulating concentrations of estradiol may also facilitate fertilization through more efficient transport of the ova and sperm [34, 35]. A potential explanation of how estradiol affects sperm transport is by altering uterine pH around the time of estrus. Recent work by Perry and Perry [36, 37] have focused on how exogenous estradiol administration and standing estrus changes uterine pH. Cows in estrus or supplemented with estradiol had increased concentrations of estradiol and decreased uterine pH compared to cows not displaying estrus (pH 6.7 vs. 7.0 respectively). In addition, work from Roper et al. [38] has provided preliminary data that uterine and vaginal pH at the time of AI or ET has a positive correlation with pregnancy success.

Serum concentrations of estradiol prior to the LH surge and ovulation appear to regulate changes in the uterine environment in cattle. Circulating concentrations of estradiol have been shown to peak around 36 h before ovulation [39] and these increases in preovulatory estradiol have been reported by multiple groups to increase pregnancy success. Jinks et al., [1] reported donor cows with greater circulating concentrations of estradiol were more likely to yield an embryo than an unfertilized oocyte. In the same study recipient cows with greater estradiol at GnRH2 also had an increased pregnancy establishment [1]. In addition when estradiol concentrations at GnRH2 were <8.4 pg/ml a decrease in pregnancy was also observed.

It is clear that estradiol plays a critical role in the establishment and maintenance of pregnancy, however, that exact role remains unclear. Data reported above seems to primarily point to estradiol's ability to improve the maternal environment in the reproductive system but correlations with follicle size, embryo quality, etc cannot be discounted. Overall, increasing preovulatory estradiol or increasing the proportion of females exhibiting estrus leads to increased pregnancy success. Thus increasing the preovulatory estradiol and estrus expression in a breeding program could help improve fertility rates in a cattle herd and reduce reproductive wastage.

Proestrus and Estrus

Proestrus is generally defined as the period from initiation of luteolysis to the onset of estrus during which a dominant follicle and oocyte continue the maturation process. There is accumulating evidence that the length of proestrus can affect the establishment of pregnancy in cattle. Regardless of follicular diameter, luteal function and embryo development were reduced when bovine follicles ovulated following a short verses a long proestrus period [6, 40-42]. Reducing the length of proestrus resulted in inadequate luteal function following ovulation independent of follicle diameter [42]. In the same study, pregnancy rates following embryo transfer were lower in cows with a shorter proestrus compared to cows with a longer proestrus [42]. The preceding data provides further support that it is the physiological maturity of the follicle and not simply size that contributes to establishment and maintenance of pregnancy. In the CO-Synch + CIDR protocol removing a CIDR after 5 days instead of 7 days will increase the length of proestrus and increased pregnancy rates in beef cows [6]. Meneghetti et al., [7], reported an increase on pregnancy rates when length of proestrus is 2 days longer. In this experiment, the researchers increased the proestrous administering prostaglandin two days before the withdrawal of CIDR. In addition, recent evidence from Dias et al., 2015 [22] has demonstrated that increasing proestrus period in Nelore heifers mitigates the negative effects of high vs low progesterone by evaluating the LH receptor in the dominant follicle.

Estrus behavior has been positively correlated with pregnancy success for decades. Although as the industry has moved to more FTAI and TET the need for estrus detection and record keeping has decreased. Based on research data collected, it is clear that FTAI and TET work with a high degree of success, however, in all cases cows exhibiting estrus prior to FTAI or TET tend to perform a few percentage points better or decrease pregnancy loss. Abreu et al., [15] reported greater ovulatory follicle diameter at AI and higher pregnancy rates in cows that exhibit estrus behavior. Pohler et al., [43, 44] reported an increase in pregnancy rates and bPAG concentration on d 28 after FTAI in cows that expressed estrus prior to FTAI (Figure 5). In the same study, the pregnancy rates and bPAG concentration increase as the intensity of estrus increased in Nelore beef cows. Similar data has also been observed in dairy cows undergoing TET. Pereira et al., [45] reported that dairy cows receiving an embryo that had exhibited estrus prior to TET had decreased pregnancy loss compared to those that did not. Therefore, even in timed AI or ET protocols the added benefit of animals exhibiting estrus cannot be discounted.

Summary

In cattle, fertilization generally occurs following > 90% of the time following insemination, but pregnancy rate at the earliest possible detection (day 27) is generally <70%. In this paper, we described some strategies to increase the pregnancy rates during synchronization protocol. The data provided in this paper demonstrates the numerous variables that contribute to successful establishment of pregnancy in beef cattle. It is also evident based on the data that the current FTAI protocols in beef cows are effective at generating pregnancies, but increasing success to a single ovulation is still an area that needs to be investigated.

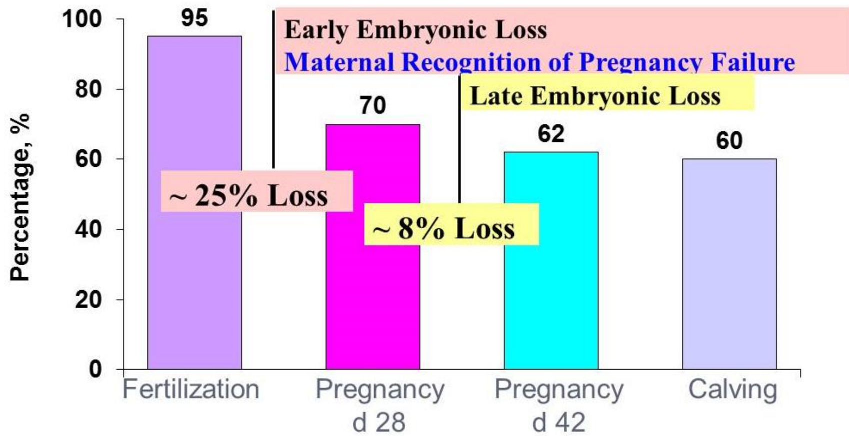

Figure 1 – Pregnancy rates during different times of gestation in beef cows (*Bos Taurus* and *Bos indicus* cattle may differ slightly following fertilization but overall trend is similar.)

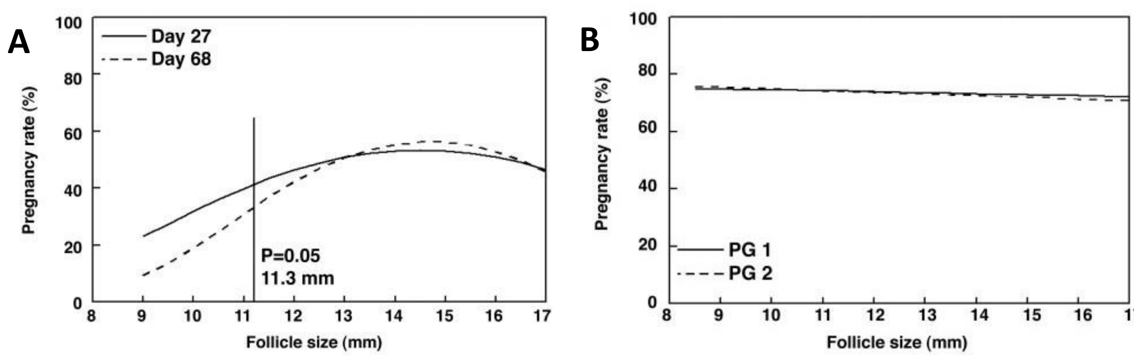

Figure 2 - Regression analysis of the effect of ovulatory follicle size on pregnancy rates. Follicle sizes at which pregnancy rates were decreased (P < 0.05) below the maximal pregnancy rates are indicated with vertical lines (Panel A). The size of follicles that ovulated spontaneously had no effect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

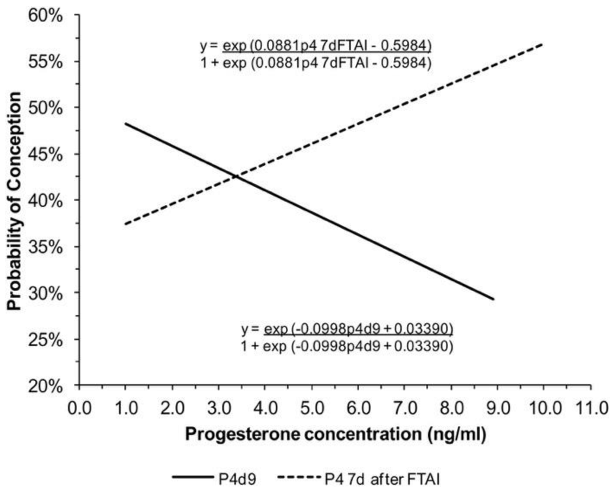

Figure 3 - Relationship between serum P4 concentrations (ng/mL) at time of controlled internal drug release device (CIDR) removal (Day 9; solid line; P ¼ 0.08; n ¼ 449) or 7 days after fixed timed artificial insemination (FTAI; hatched line; P ¼ 0.01; n ¼ 926) and probability of conception in postpubertal Bos indicus (Nellore) heifers submitted to a FTAI protocol [28].

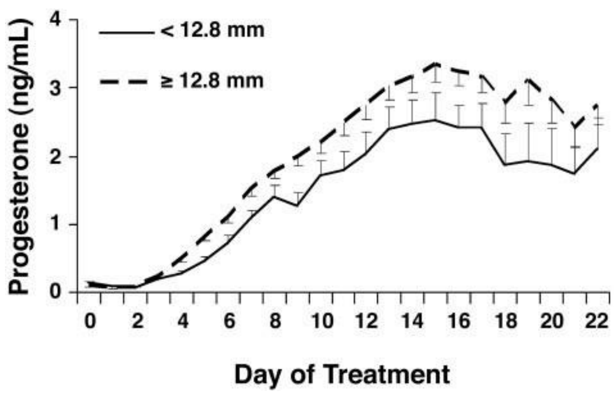

Figure 4 - Effect of ovulatory follicle size on serum concentrations of progesterone from day 0 (insemination) through 22 and the effect of ovulatory follicle size on rate of progesterone rise from day 0 through 16 [32].

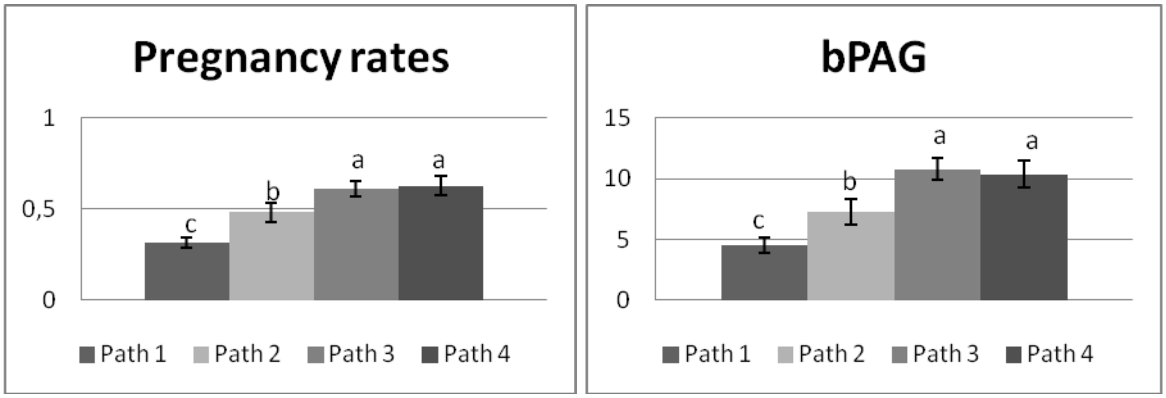

Figure 5 – Pregnancy rates and serum concentrations of pregnancy associated glycoproteins of cows that exhibited different estrus intensities. Cows that exhibited stronger estrus by means of Estrotect heat detectors patch score had higher bPAG concentration on day 28 [44].

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

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

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

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