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COMPARISON OF CONCEPTION RATES IN BEEF CATTLE INSEMINATED WITH EITHER SEXEDULTRA™ SEX-SORTED SEMEN OR CONVENTIONAL SEMEN IN FIXED-TIME ARTIFICIAL INSEMINATION (FTAI) PROTOCOLS

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THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food and Environment at the University of Kentucky

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2017

ABSTRACT OF THESIS

COMPARISON OF CONCEPTION RATES IN BEEF CATTLE INSEMINATED WITH EITHER SEXEDULTRA™ SEX-SORTED SEMEN OR CONVENTIONAL SEMEN IN FIXED-TIME ARTIFICIAL INSEMINATION (FTAI) PROTOCOLS

Estrous synchronization and artificial insemination (**ESAI**) are reproductive technologies that cattlemen can use to improve the reproductive performance of their herds. Controlling the gender ratio of the calf-crop can also improve the opportunity for increased revenue and profit. Producers are able to shift and/or control the gender ratio of their calf crop by incorporating sex-sorted semen into their AI programs. However, decreased conception rates to AI have been previously observed when sex-sorted semen was used in comparison to conventional semen of the same sires. The objective of the first study was to determine if conception rates will differ in females inseminated with conventional semen or SexedULTRA™ sex-sorted semen when estrus is synchronized using an industry-standard, 7-d CO-Synch + Controlled Internal Drug Release (**CIDR**) protocol for fixed-time artificial insemination (**FTAI**). The objective of the second study was to determine if conception rate to FTAI differs between SexedULTRA™ sex-sorted and conventional semen when yearling beef heifers are synchronized using a 14-d controlled internal drug release (**CIDR**) - PGF2α (**PGF**) protocol modified to optimize the control of ovulation and timing of insemination.

KEYWORDS: estrous synchronization, fixed-timed artificial insemination, sex-sorted semen

Benjamin R. Crites

November 21, 2017

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Chapter 1

Literature Review

Reproductive Anatomy

The Uterus

The bovine uterus is composed of a uterine body and two uterine horns. The body of the bovine uterus is quite small (4 to 5 cm) while the uterine horns may be 15 to 25 cm in length (Erickson et al., 1985). Generally, the size of the entire reproductive tract for a non-pregnant female is 30.5 to 45.7 cm in length (Beverly and Sprott, 1995). However, in heifers the tract may only be 20 cm long, while mature cows may have tracts that are closer to 61 cm in length (Beverly and Sprott, 1995). A study conducted in 1976, indicated that the uterus of non-pregnant females weighed around 250 g. In the same study, uterine weight increased greatly during gestation. Around mid-gestation, at 134 days they indicated a weight of almost 2 kg. Whereas at 264 days the weight increased to 7 kg (Ferrell et al., 1976).

The uterus is composed of three layers of tissue and is supported by the mesometrium, a portion of the broad ligament. The endometrium is the most inner layer and responds to cyclic hormonal changes, receives the fertilized embryo, helps form the placenta, and is essential for reproductive functions. The middle layer, myometrium, is composed of smooth muscle cells and makes up most of the uterine volume. The perimetrium is a thin layer of tissue that is the outer layer of the uterus. Proper uterine function is required to establish and maintain pregnancy (Bridges et al., 2014). The uterus is also involved with sperm viability and transport, expelling the fetus, and hormone synthesis, such as prostaglandin (Cooke 2009).

The Cervix

The uterus is connected to the vagina by the cervix. The cervix is composed of connective tissue and is used as a landmark during rectal palpation and artificial insemination (Beverly and Sprott, 1995). Typically, the cervix contains three rings, or folds, which aid in the main function of the cervix, which is to isolate the uterus from the external environment (Cooke 2009). During pregnancy, the cervix fills with a thick mucus known as the cervical plug and protects the uterus from infections entering from the vagina (Whittier 1993). However, during estrus, the cervical mucus is often seen flowing out of the vulva (Beverly and Sprott, 1995) and lubricates the vagina for copulation (Cooke 2009). Cervical mucous filters out sperm with poor morphology and motility and only a minority of sperm enter the cervix (Suarez and Pacey, 2006). Along with serving as a sperm filter, the cervix also acts as a reservoir for healthy sperm that has been deposited (Morton and Glover, 1973).

Differences between cervix size and shape have been reported to exist between Bos Indicus/Bos Indicus-cross cattle and Bos Taurus cattle (Varner et al., 1985). Cervix diameter was larger ($P<0.05$) for Angus cattle (3.1 cm) compared to Santa Gertrudis (Bos Indicus-cross; 5.7 cm). A larger ($P<0.01$) percentage of Bos Taurus cattle (84%) had a cylindrical cervical shape, compared to only 29% of Santa Gertrudis cattle (Varner et al., 1985). Regardless of breed influence, cattle with a conical shaped cervix exhibited lower ($P<0.01$) pregnancy rates (Varner et al., 1985).

The Oviducts and Ovary

The oviducts, also known as fallopian tubes, extend from the uterine horns to the ovaries and are joined at the utero-tubal junction. Typically, the length of the oviducts are around 19.7 cm (Crisman et al., 1980) and 1.6 mm to 3.2 mm in diameter (Beverly and Sprott, 1995). Similar to the uterus, oviducts are composed of several layers, which include the mucosa, muscular, and serosa (Aviles 2015). The oviducts can be divided into four segments; the isthmus, ampulla, infundibulum, and fimbria. The infundibulum is a funnel-shaped that surrounds the ovary and capture the ovum that is released during ovulation (Whittier 1993). The ampulla serves as the site of fertilization (Aviles 2015) while the isthmus serves as a reservoir for healthy sperm (Coy et al., 2012).

The ovaries in a beef female are bean-shaped, generally 2.5 to 3.8 cm long, and are located within the abdominal cavity (Whittier 1993). The predominant functions of the ovaries are to produce the female gamete and two primary reproductive hormones, estradiol and progesterone (Whittier 1993). Two main structures reside on the ovaries, corpora lutea and follicles, and are responsible for the production of progesterone and estradiol, respectively (Whittier 1993). The function of both structures are discussed in greater detail in the description of the estrous cycle.

Estrous Cycle

Overview

Estrus has been defined as the period of time when the female is sexually receptive to the male (Rae et al., 1998 and Perry 2004). The length of estrus can range from 6 to 24 h (Perry 2004). However, the typical length of sexual receptivity is around 15 to 18 h (Whittier 1993, Rae et al., 1998, and Perry 2004). A definitive sign of estrus is when a female will stand to be mounted. Additional signs of estrual activity include mounting other animals, clear mucous discharge from the vagina, and a swollen vulva (Perry 2004). The estrous cycle is the period of time between when estrus is expressed. In cattle, the length of the estrous cycle is typically around 21 days, but can range between 18-24 d (Whittier 1993, Rae et al., 1998, Perry 2004, Parish et al., 2010). Typically, estrus is referred to as day 0 of the estrous cycle (Whittier 1993). The average length of gestation in beef cattle is around 283 days. However, Bos Indicus cattle tend to have longer gestation periods compared to Bos Taurus cattle (Knapp et al., 1940). Additionally, increased cow's age, male fetuses, and growing fetus weight all prolonged gestation in Holstein-Friesian cows (Nogalski and Piwczynski, 2012).

Estradiol released from a preovulatory follicle induces the period of sexual receptivity (Parish et al., 2010). The high levels of estradiol stimulate a surge release of gonadotropin releasing hormone (**GnRH**) from the hypothalamus. This release of GnRH induces a surge release of luteinizing hormone (**LH**) and follicle stimulating hormone (**FSH**) from the anterior pituitary. The surge release of LH induces the ovulatory follicle to rupture and subsequently release the ovum (Acosta 2007). This process is known as ovulation and occurs around 30 h after the onset of

estrus (White et al., 2002), but can range from 24 to 32 h after estrus is initiated (Perry 2004). The mature follicle will rupture once it reaches its maximum size of greater than 10 mm and the follicle is producing sufficient levels (>5.0 pg/ml) of estradiol to induce the preovulatory surge of gonadotropins (Noseir 2003).

It appears that both progesterone and prostaglandins are essential mediators of ovulation (Fortune et al., 2009). A 2001 study indicated increases in progesterone concentrations in the follicular fluid of ovulatory follicles by about 4.5-fold around 1.5 h after the peak in the LH surge (Fortune et al., 2001). In addition, near the time of ovulation, levels of PGE_2 and $PGF_{2\alpha}$ increase dramatically in follicular fluid (Fortune et al., 2009). The secretion of prostaglandins by granulosa cells is increased by both LH and FSH (Bridges et al., 2006). The theca and granulosa cells of a perivovulatory follicle are targets for both PGE_2 and $PGF_{2\alpha}$ that are produced by the granulosa cells (Fortune et al., 2009).

After ovulation, a corpus luteum (**CL**), forms from the granulosa and theca interna cells of the ruptured follicle (O'Shea, 1987). These follicular cells transform into luteal cells through the process of luteinization. The granulosa cells transform into large luteal cells, while the theca cells are transformed into small luteal cells (O'Shea 1987). The small luteal cells respond to LH with increased secretion of progesterone via activation of the protein kinase A second messenger pathway, whereas the large luteal cells contain receptors for PGF2α and mediate the luteolytic actions (Niswender et al., 2000). Progesterone is synthesized from cholesterol and is transported into the mitochondria using steroidogenic acute regulatory protein (**StAR**).

Cholesterol is converted to pregnenolone by cytochrome P-450 side-chain cleavage enzyme (**P-450$_{scc}$**), transported out of the mitochondria, and 3β-hyrdroxysteroid dehydrogenase (**3β-HSD**) converts it into progesterone (Niswender et al., 2000).

The pulsatile release of LH appears to be necessary for luteal development in cows and is required to maintain normal expression of mRNA and presumably proteins, encoding StAR, P-450$_{scc}$, and 3β-HSD (Niswender et al., 2000). The process of luteinization is induced by the preovulatory surge of LH (Murphy 1999 and Schams and Berisha, 2004. The granulosa and theca cells undergo vascularization and hypertrophy to form the CL. Luteinization involves the transformation of a preovulatory follicle into a highly vascularized CL that is capable of producing large amounts of progesterone (Smith et al., 1994).

It has been suggested that LH increases progesterone production by facilitating transport of cholesterol through the cell (Niswender et al., 2000). Growth hormone (**GH**) also directly effects luteal function by binding to its receptor and activating the membrane-associated tyrosine kinase JAK2 (Niswender et al., 2000). Additionally, GH influences luteal function indirectly by increasing expression of IGF-I which may stimulate secretion of progesterone (Niswender et al., 2000). The binding of PGE_2 to its receptor in large luteal cells has also shown to increase progesterone production (Niswender et al., 2000). The CL is responsible for the production of progesterone (**P4**) and is required for the establishment and maintenance of pregnancy (Schams and Berisha, 2004). Progesterone also downregulates receptors for estradiol, acts as a differentiation factor on the endometrium, induces quiescence of the myometrium, prevents

uterine contractions, and in-part governs the length of reproductive cycles (Niswender et al., 2000).

Between day 5 and 8 there is a dramatic increase in CL weight, while concentrations of P4 increased from day 5 to 8 and also from day 8 to 16 (Mann 2009). The CL reaches full maturity around day 16 of the estrous cycle (Mann 2009) and on day 15 of the cycle a CL normally produces nearly 300 μg of P4 (Staples and Hansel, 1961 and Staples et al., 1961). In a non-pregnant female, the CL will undergo luteolysis at this point (Perry 2004) and prostaglandins (PGF2α; **PGF**) are released from the uterus in a pulsatile manner (Kindahl et al., 1981). It appears that tumor necrosis factor-α (**TNF-α**) and oxytocin may play a role in inducing the release of PGF from the uterus (Okuda et al., 2002). As luteolysis continues and the CL regresses, P4 concentrations decline, and the female is able to return to estrus (Spencer 2013). An early CL (< d 5) may not be responsive to PGF perhaps due to incomplete vascularization, incomplete differentiation in luteal cells (Wiltbank et al., 1995), and changes in gene expression, especially PGHS-2 (Tsai and Wiltbank, 1998). Regression of the CL is essential for normal cyclicity as it allows for the final development of a preovulatory follicle, while prevention of luteolysis is essential to establish pregnancy (Okuda et al., 2002).

In a pregnant female, the conceptus signals its presence and prolongs the lifespan of the CL in a process known as maternal recognition of pregnancy (**MRP**; Bazer et al., 1991). In bovine females, MRP begins on day 16 of the estrous cycle (Hansel and Blair, 1996 and Spencer 2013). Elongation of the conceptus is associated with increased production of interferon tau (**IFNT**).

Adequate IFNT secretion from the conceptus is required for MRP. The oxytocin receptors are blocked by IFNT and as a result the uterus does not produce the pulsatile release of PGF (Spencer 2013) initiating regression of the CL. Additionally, IFNT may inhibit the TNF-α and oxytocin action and therefore stop the release of PGF (Okuda et al., 2002).

Follicular Waves

Ovarian follicles are composed of granulosa and thecal cells (Young and McNeilly, 2010). During the estrous cycle, follicles grow and develop in a series of waves (Sirois and Fortune, 1988 and Noseir 2003). In cattle, the growth of follicles occurs in a pattern of two to three waves (Sirois and Fortune, 1988, Ginther et al., 1989, Noseir 2003, Acosta 2007, and Lucy 2007). A wave of follicular growth involves the synchronous recruitment of a group, or cohort, of follicles (Ginther et al., 1989 and Lucy 2007) and a peak in FSH precedes each wave (Lucy 2007). The release of FSH from the anterior pituitary is controlled by the release of GnRH from the hypothalamus (Taya et al., 1996).

Follicular growth begins with a large pool (>100,000) of primordial follicles (Lucy 2007). The store of primordial follicles is not renewable and serves the entire reproductive lifespan of the female (Picton 2000). Primordial follicles are composed of an oocyte that is surrounded by a mixture of flattened and cuboidal granulosa cells and are ≤45µm (Picton 2000). Primordial follicles develop into primary follicles which are characterized by one complete layer of cuboidal granulosa cells (Picton 2000). The growth of preantral follicles is dependent on growth factors;

such as insulin growth factor (**IGF**) and epidermal growth factor (**EGF**) via endocrine mechanisms (Webb et al., 2004). Preantral follicular growth is slow and may take >30 d in cattle for follicles to develop into the preantral stage (Picton 2000).

During each follicular wave, a group of three to six follicles are recruited and simultaneously begin to grow (Quirk et al., 2004). Increases in FSH concentrations drives the current FSH dependent follicles to grow and promotes estradiol synthesis by the follicles (Lucy 2007). This group of small follicles, 4-5 mm, will grow similarly for three days and when the largest follicle reaches 8.5 mm, the other growing follicles regress (Acosta 2007) and the majority of growing follicles will undergo atresia (Quirk et al., 2004). Follicle selection occurs at the end of the common growing phase and the dominant follicle grows at a continuing rate (Ginther et al., 2001). It has been suggested that a decrease in FSH secretion may be key in follicle selection (Webb et al., 2004). It appears that estradiol-17β (Acosta 2007) and LH receptors are involved with the separation of follicular growth (Ginther et al., 1996 and Acosta 2007).

The largest, dominant follicle is identified when it reaches a size of 10 mm (Acosta 2007) and acquires LH receptors in the granulosa and theca cells (Acosta 2007 and Lucy 2007). The dominant follicle becomes dependent on LH and it appears that the shift from FSH to LH dependence is pivotal in the development of the dominant follicle (Lucy 2007). The granulosa cells of follicles produce inhibin and estradiol (Taya et al., 1996). Inhibin that is released by the follicles, blocks the release of FSH from the pituitary (Taya et al., 1996). In the presence of a CL, a dominant follicle will regress as the LH surge cannot occur (Quirk et al., 2004) because high

levels of progesterone restrict LH pulse frequency and concentration (Niswender et al., 2000). Progesterone inhibits the LH pulse frequency and there is insufficient LH for the follicle to live. As the dominant follicles regresses, inhibin levels decrease, and thus results in another peak of FSH initiating a new follicular wave.

The underlying factors that influence the proportion of two- or three-wave cattle is unknown (Ginther et al., 1989). However, the incidence of the number of follicular waves may depend on follicular size and estradiol concentration (Noseir 2003). In two-wave cattle, follicular waves occur on days 0 and 10 (Ginther et al., 1989 and Noseir 2003). While in three-wave cattle, waves occur on days 0, 9, and 16 (Ginther et al., 1989 and Noseir 2003). Cattle that display two follicular waves tend to have shorter estrous cycle lengths (20 d versus 22 d) compared to cattle with three waves of follicular growth (Ginther et al., 1989). Additionally, for three-wave cattle the ovulatory follicle emerges later (d 16 versus d 10), thus a shorter interval to ovulation (7 d versus 11 d), and a possess a smaller preovulatory diameter (14 mm versus 16 mm) (Ginther et al., 1989). The dominant follicle in two-wave cattle appears 10 d before ovulation, while the dominant follicle in three-wave cattle appears 6 d before (Sirois and Fortune, 1988).

Dominant follicles that develop during luteal regression can fully develop and ovulate (Quirk et al., 2004). Declining levels of progesterone allows for an increase in LH pulse frequency (Fortune et al., 2009). This promotes the development of the dominant follicle into a preovulatory follicle that is capable of making enough estradiol (Fortune et al., 2009). The ovulatory follicle produces enough estradiol that results in a surge release of LH (Noseir 2003). Ovulation can occur when

the follicle is >10 mm in diameter and when there is sufficient (>5 pg/mL) estradiol concentrations to induce the preovulatory surge of gonadotropins (Noseir 2003). Ovulation of the Graafian follicle and subsequent release of the oocyte occurs from the LH surge (Acosta 2007).

Fertilization

In cattle, the bull deposits several billion spermatozoa into the anterior vagina of the female (Lopez-Gatius, 1999). The cervix is a major obstacle, with usually around 1% of the deposited sperm reaching the uterine body (Lopez-Gatius, 1999). Along with serving as a sperm filter, the cervix plays an important role as a reservoir for healthy sperm (Morton and Glover, 1973). Sperm may leave the cervix by their own motility or be transported by cervical and uterine contractions (Morton and Glover, 1973). Sperm is transported through the uterus to the oviduct via muscular contractions (Suarez and Pacey, 2006). Transport of sperm through the cervix and uterus to the oviducts occurs rapidly and a small population of sperm are present in the oviducts within minutes of insemination (Rodriguez-Martinez 2007).

The isthmus region of the oviduct serves a sperm reservoir (Coy et al., 2012 and Aviles 2015). Sperm are held on the surface of mucosal folds in the isthmus and remain there until the time of ovulation nears (Ikawa et al., 2010). The process of capacitation appears to be a prerequisite before sperm can fertilize an oocyte (Rodriguez-Martinez 2007 and Ikawa et al., 2010). During capacitation, cholesterols and other sterols are removed from the sperm surface (Ikawa et al.,

2010). The oviductal fluid contains glycosaminoglycans, either sulfated or non-sulfated, and could trigger the process of capacitation (Rodriguez-Martinez 2007).

The site of fertilization occurs in the ampulla portion of the oviduct (Betteridge and Flechon, 1988 and Aviles 2015). Sperm cells are hyperactivated and begin to swim straight and vigorously and this hyperactivity is essential for fertilization to occur (Coy et al., 2012). When ovulation occurs, the oocyte is released from the follicle, surrounded by a large number of cumulus cells, and is captured by the infundibulum (Aviles 2015). The oocyte travels to the ampulla by coordinated contractions of the smooth muscle cells and the ciliary beat of the epithelial cells (Coy et al., 2012 and Aviles 2015). The sperm locate the cumulus-cell oocyte complex (**COC**) as it arrives in the ampulla and sperm penetrate the cumulus-cell matrix soon after interacting with COCs (Ikawa et al., 2010).

The capacitated sperm are able to penetrate the cumulus layers and bind to the zona pellucida (Rodriguez-Martinez 2007). The major components of the zona pellucida are three glycosylated proteins, Zp1, Zp2, and Zp3 (Ikawa et al., 2010). The penetration of the cumulus layer and binding to the zona pellucida stimulates the occurrence of the acrosome reaction (Rodriguez-Martinez 2007). The acrosome reaction, or acrosomal exocytosis, is a prerequisite for sperm to fuse with an egg and only occurs in capacitated sperm and is induced by a calcium influx (Ikawa et al., 2010). It appears that Zp3 is the primary sperm receptor and can induce the acrosome reaction (Ikawa et al., 2010). The acrosomal enzyme, Acr, is responsible for proteolytic cleavage

of the ZP and allows for sperm penetration (Ikawa et al., 2010). Immediately after penetrating the ZP, sperm meet and fuse with the plasma membrane of the egg (Ikawa et al., 2010).

Fertilization rates in cattle are quite high and approach 90% (Bridges et al., 2014). The now fertilized embryo will stay in the oviduct 4 to 5 days before traveling to the uterus (Aviles 2015). As previously discussed, the female recognizes that a pregnancy is present (MRP), which occurs beginning around day 16 of the estrous cycle (Hansel and Blair, 1996 and Spencer 2013). Although fertilization is near 90%, there is about a 35% incidence of embryonic mortality (Diskin et al., 2006). In addition, about 70 to 80% of total embryonic loss occurs during the first few weeks after insemination, particularly between days 7 and 16 (Spencer 2013). Progesterone maintains the endometrium functions of the uterus that are necessary for conceptus growth, implantation, placentation, and development to term (Dorniak et al., 2013). In the uterus, progesterone acts as a differentiation factor in the endometrium and induces quiescence of the myometrium (Niswender et al., 2000).

Postpartum Anestrus

Factors that Impact the Anestrus Period

Reproductive efficiency has a major impact on production in beef cow-calf operations (Rhinehart et al., 2016). The postpartum anestrus period is a major limiting factor on the reproduction efficiency in a herd (Montiel and Ahuja, 2005) and is a source of economic loss to producers (Yavas and Walton, 2000). Regulating the anestrus period is essential to maintain beef cattle productivity and profitability (Anderson 2006). Postpartum anestrus, or the

postpartum interval, is the period of time from calving to first estrus (Short et al., 1990). In order for beef cows to calve in a 365-day interval, a cow must rebreed within 80-85 days after calving (Yavas and Walton, 2000). Therefore, the anestrus period becomes a critical management period in order to maintain the 365-day calving interval. Several factors influence the length of the postpartum anestrus, some of which include: suckling of a calf, nutritional levels, parity, and incidence of dystocia (Short et al., 1990, Montiel and Ahuja, 2005, Yavas and Walton, 2000, Anderson 2006, and Rhinehart et al., 2016).

Postpartum anestrous is initiated by the presence of a calf (Rhinehart et al., 2016). The average length of the postpartum anestrus period is 50 to 100 days (Williams 1990). However, cows in poor body condition may have anestrus periods that exist for over 100 days (Williams 1990). Two of the major regulators of the postpartum anestrus period are the presence of a suckling calf and the nutritional levels of the cow (Short et al., 1990, Montiel and Ahuja, 2005, Stevenson et al., 2003, Martins et al., 2012). The frequency and duration of the suckling periods appear to effect the anestrus period length (Williams 1990). However, when calves were weaned at birth, females returned to estrus around 14 days after parturition (Williams 1990).

Ovarian function begins rapidly after calving (Rhinehart et al., 2016). Before the first ovulation, there are periods of growth and regression of follicles that fail to ovulate, that occur in wave-like patterns (Murphy et al., 1990). These wave-like patterns consist of one, two, or three periods of follicular growth and the size of the dominant follicle increases for each period prior to the first ovulation (Murphy et al., 1990). The first ovulation postpartum can occur if the dominant follicle can produce enough estradiol to induce the preovulatory gonadotropin surge (Anderson 2006

and Rhinehart et al., 2016). However, the majority of the first dominate follicles fail to ovulate likely due to the deficiency of LH (Crowe et al., 1993).

After the first ovulation, cows exhibit a short luteal phase (Perry et al., 1991). The research from Perry et al., (1991) observed a duration of 8.5 days between first and second ovulations in postpartum beef cows and Murphy et al., (1990) reported an average length of 9.7 days after the first ovulation. These findings agree with Odde et al. (1980) who found that most short cycles lasted for 7-10 days between the first and second ovulations.

During the first 10 to 20 days after calving, the level of available LH in the pituitary is lower and less LH is released in response to GnRH (Short et al., 1990). The frequency of LH pulses is lower (<1 pulse/4 h) during postpartum anestrus, however the frequency increases (1 pulse/1-2 h) prior to estrus (Short et al., 1990). In addition, suckling appears to interfere with the release of GnRH and/or the pituitary gland is unable to respond to GnRH (Williams 1990) and reduces the frequency of pulsatile LH release (Murphy et al., 1990). The first postpartum estrus can be delayed by only two to three suckles per day (Williams 1990). The maternal bond appears to be essential for prolonging the postpartum anovulation period that is induced by a suckling calf (Montiel and Ahuja, 2005).

The nutritional status of an animal is evaluated through body condition scores, on a 1-9 scale (Montiel and Ahuja, 2005). Both pre- and postpartum energy balance affect the postpartum anestrus interval in beef cows (Hess et al., 2004). However, differences in body condition scores (BCS) that exist prior to calving may be more important than those that exist after calving (Short

et al., 1990, Montiel and Ahuja, 2005). Research suggests that there is a significant ($P<0.001$) relationship between BCS and the percentage of cows that are cycling (Stevenson et al., 2003). In their study they reported that for every one unit change in BCS, the percentage of cows cycling increased by 18 ± 2% (Stevenson et al., 2003). The effect of BCS on the postpartum interval appear to be greatest with scores less than 4, while there appears to be little effect when scores are above 7 (Short et al., 1990). Thin cows with a BCS <4 had lower ($P<0.001$) pregnancy rates (59%) than cows with a BCS score greater than 5 (90%) (Rae et al., 1993). Cows that calved with a BCS <5 (93.1 d) had longer ($P<0.01$) postpartum intervals than cows that calved with a BCS >5 (63.5 d) (Lents et al., 2008). A recommended body condition score at calving is between 5 and 7 (Short et al., 1990).

Cow age also appears to influence the length of the anestrus period. In a study of 3,269 beef heifers and cows, the percentage of primiparous cows (55%) that were cycling at the beginning of the breeding season were lower ($P<0.001$) than the percentage of multiparous cows (64%) (Stevenson et al., 2003). Younger cows, 2- and 3-year olds, have longer postpartum intervals (Short et al., 1990).

The incidence of dystocia is also more prevalent in 2-year olds than in mature cows (Laster et al., 1973, Bellows and Short, 1978). Females that experienced dystocia exhibited 15.9% lower overall conception rates (Laster et al., 1973). Cows that experienced dystocia calved an average of 5.8 days later the following year than those females that did not experience calving difficulties (Laster et al., 1973). Other studies have reported an increase of 28 days between calving intervals in females that experienced dystocia compared to those that did not (Gaafar et

al., 2011). Intervening early when calving assistance is needed greatly reduces the postpartum interval (Geary 2003).

Strategies to Reduce the Anestrus Period

Several protocols have been developed that can successfully initiate a fertile estrus in anestrous females (Rhinehart et al., 2016) and will be discussed in further detail. Reducing the length of the breeding season is something that can be done by producers to improve postpartum fertility (Short et al., 1990). Reducing the length of the breeding season results in a more uniform calf crop and allows producers to sell calves in larger lot sizes. Selling a group of five calves resulted in an increase of $11/cwt over selling an individual animal (Halich and Burdine, 2014). Breeding seasons of 45 days increases the pregnancy potential in all cows as they are past the effects of uterine involution, short estrous cycles, and the main effects of anestrus (Short et al., 1990).

Maintaining a BCS of at least a 5 at calving has been recommended (Short et al., 1990). Cows that were fed to calve with a BCS >5 compared to a BCS <5 and those that were fed larger amounts of a 42% protein supplement (2.5 kg/d vs. 1.2 kg/d) had shorter postpartum intervals (63.5 vs. 93.1 d and 77.4 d vs. 83.5 d, respectively) and also larger dominant follicles (15.6 vs. 13.4 mm and 15.1 vs. 13.6 mm, respectively) (Lents et al., 2008). This data is similar to Ciccioli et al (2003), who reported shorter ($P<0.01$) postpartum intervals and larger ($P<0.01$) dominant follicles when cows were fed to gain more (0.90 kg/day vs. 0.45 kg/d) (Ciccioli et al., 2003).

Some research suggests that exposing cows to a sterile bull prior to the breeding season can improve postpartum fertility (Short et al., 1990). A study conducted in 2010 consisted of 39

primiparous beef females and four epididymectomized bulls to determine the effect on the duration of daily bull exposure starting around 50 d after calving (Tauck et al., 2010). In this study, groups of cows were randomly assigned to treatment; bull exposure for 6 h (**BE6**), bull exposure for 12 h (**BE12**, or no exposure to the bulls (**NE**). This study reported that the interval from calving to ovulatory activity was shorter ($P<0.05$) for the BE12 and BE6 cows (87.7 and 90.0 d, respectively) compared to cows in the NE group (101.2 d) (Tauck et al., 2010). The percentage of cows that resumed ovulatory activity was also greater ($P<0.05$) for the BE12 and BE6 group (60.0% and 64.3%, respectively) compared to the females in the NE group (Tauck et al., 2010). Miller and Ungerfeld (2007) examined the impact that rotating bulls during the breeding season had on overall pregnancy rates and the percentage of cows cycling. Ninety-one multiparous cows were randomly assigned to treatment; continuous exposure (**C**) to two bulls or rotating two bulls every week (**R**) for a seven week breeding season. Cows in the R treatment had an increase percentage of cows cycling at four weeks ($P=0.024$) as well as between five and 7 weeks ($P<0.001$) (Miller and Ungerfeld, 2007). The pregnancy rates were also greater ($P=0.045$) for the R treatment (56.2%) than the C treatment (35.6%). However, these pregnancy rates are much lower than what would be expected after a 50-day breeding season. Even with a conception rate of 70%, within two cycles (42 days) there would be 91% of the animals bred.

The negative effect from a suckling calf can be reduced by using alternative weaning methods; such as complete weaning, limiting nursing to once or twice daily, or weaning for 48 h with an estrous synchronization program (Short et al., 1990). However, these weaning options need to occur before the start of the breeding season (Short et al., 1990). Cows that have their calves removed at birth have shown to resume estrus activity as early as 14 days after (Williams, 1990).

However, weaning a calf at birth is not feasible for commercial cattlemen, therefore alternative options are needed. Martins et al. (2012) assessed the impact of early weaning and 48-hour calf removal on reproductive performance in 112 primiparous cows. Treatments consisted of early weaning (EW approximately 95 d after birth), interval weaning (IW – 48 h removal, five times, 20 d apart), and control (CON, single 48 h calf removal). Females in the EW (137 d) treatment tended (P=0.15 and 0.14, respectively) to have shorter postpartum intervals than the IW (144 d) and CON (159 d) cows (Martins et al., 2012). Additionally, overall pregnancy rates were higher (P=0.05) for the EW treatment (92.0%) compared to the IW and CON treatments (88.6 and 70.4%, respectively) (Martins et al., 2012).

Follicular growth appears to be well established before the first postpartum ovulation (Day 2004). In a 1993 study, the first dominate follicle formed 10-11 days after calving (Crowe et al., 1993). Of the proportion of females in the growing phase of the dominant follicle, 100% ovulated after an injection of GnRH (Crowe et al., 1993). Anovulation in the early postpartum period is due to inadequate LH secretion (Roche et al., 1992) and not the available supply of LH (Crowe et al., 1993). Treatments that increase the level of progesterone prior to ovulation enhanced the pulsatile release of LH and are key components to initiate fertile estrous cycles in anestrual females (Day 2004). A study by Fike et al. (1997) included 362 anestrus cows that were 25 to 50 d postpartum and evaluated the impact of progesterone using an Eazi-Breed™ Controlled Internal Drug Release (**CIDR**) device for 7 days. A greater (P<0.001) percentage of cows formed a CL with a typical lifespan that received only a CIDR (55%) compared to the non-treated group (16%) (Fike et al., 1997). Additionally, a larger (P<0.05) percentage of cows that received only a CIDR device showed signs of standing estrus (45%) whereas only 24% of the non-

treated group displayed signs of estrual activity (Fike et al., 1997). Furthermore, the cows that exhibited estrual behavior all did so between two and four days after removal of the CIDR device (Fike et al., 1997). In another study, the percentage of estrus activity in the first 3 d of the breeding season was higher ($P<0.001$) in mature cows that received a CIDR device for 7 d and an injection of PGF2α on d 6 (59%) was higher than in cows that received a single injection of PGF2α (33%) and in the control females (15%) (Lucy et al., 2001). Although the anestrus period can be problematic for producers at times, several options are available to help overcome the negative effects and increase the proportion of cycling cows at the beginning of the breeding season.

Controlling the Estrous Cycle

At any given time, females in a herd can be at very different stages in the estrous cycle. Also, cows may either be cyclic or anestrous. Controlling the estrous cycle can be advantageous for producers. Controlling the estrous cycle, called estrous synchronization, can be utilized in both natural service settings and in combination with AI. Incorporation of AI into beef cattle operations is low in the United States, with less than 8% of all operations utilizing AI (USDA 2009). Both time and labor were found to be the most common reasons that AI was not used (USDA 2009). Therefore, estrous synchronization protocols need to minimize the number of animal handlings while also considering the times at which they need to occur.

Products Commonly Used to Control Estrus

Currently in the U.S., there are three main types of products that are used in the control and synchronization of estrus in cattle. These products include progestins, prostaglandins, and GnRH and are commercially available in several forms.

Prostaglandins

A common method to synchronize estrus is using a single injection of prostaglandin $F_{2\alpha}$ (**PGF**). However, a single injection of PGF has its limitations. Administration of PGF has limited efficacy when administered to females in the early stages of the estrous cycle and females that are anestrous. The efficacy is limited in anestrous females as they are not cycling and do not have a corpus luteum to be responsive to PGF. Additionally, early CL (<5 d) produce the highest levels of PGI_2, a luteotropic prostaglandin (Wiltbank and Ottobre, 2003). Only a small portion of females will respond to a single injection of PGF until she is around day 5 to 7 of the estrous cycle. A previous study demonstrated that PGF was less effective at regressing a CL between days 4 and 9 of the estrous cycle (King et al., 1982). Using the single injection method, around 70% of females should exhibit estrus within 5 days following the administration of PG (Odde 1990). This method relies heavily on estrous detection and insemination occurs accordingly. Much of the success or failure of conception rates relies on the accuracy of the individual that is detecting estrus. This method is not suitable for individuals where observation of the cattle is not possible for multiple time periods during the day.

Two injections of PGF can also be used to synchronize estrus in cattle. Using this method, injections are usually separated by 10 or 11 days. With this protocol, approximately 70% of

females will respond to the initial injection as previously discussed. These females observed in estrus can be inseminated or can also receive the second injection with the remaining females that did not express estrus. Females that expressed estrus after the first shot will be on day 6 to 9 of their cycle. Those that did not express estrus after the first shot should be on day 11 to 18 of their cycle. After the second injection on day 10 or 11, a majority of the females should exhibit estrus within 2 to 5 days of PGF administration. Research has demonstrated that the interval from the second PGF injection to estrus is shorter ($P<0.01$) for heifers (54 h) than for cows (62 hours) (King et al., 1982). With a two-shot PGF method, a large amount of heat detection is required, however, time breeding may also occur at 80h after the second injection. Pregnancy rates after 5 days were similar between cattle inseminated after the presence of estrus or time bred at 80 h (Odde 1990).

Progestogens

The use of PGF alone will not synchronize estrus in females that are currently not cycling and also in females that are at an early stage of their cycle (before day 5 to 7). To maximize the estrous synchronization response, it becomes necessary to find a method that is capable of synchronizing cycling and anestrous females. Including a progestin into an estrous synchronization protocol can induce estrus in anestrous females and can be used in combination with PG (Lucy et al., 2001 and Stevenson et al., 2003b). A progestogen mimics the biological action of a CL and inhibits ovulation from occurring. Two forms of progestogens are commercially available today and come in the form of a vaginal insert or feed additive. A controlled internal drug release (**CIDR**) device is a vaginal insert that contains 1.38 g of the natural hormone, progesterone. Melengestrol acetate (**MGA**) is a progestin, a synthetic version

of progesterone, and is available as a feed additive. Typical recommendations for MGA are to feed it at a level of 0.5mg/hd/day for a period of 7 to 14 days depending on the protocol. It has been found that MGA has a higher binding affinity than progesterone for the progesterone receptors and therefore lower circulating concentrations are needed (Perry et al., 2004).

Administering progestogen for 7 days before PG ensures that a CL is allowed to develop for at least 7 days and will respond to the PG and regress (Lucy et al., 2001). Addition of a progestin may be essential to maximize pregnancy rates in young, thin, and late calving cows (Stevenson et al., 2003b). Combining a CIDR device and PGF led to a greater percentage of females in estrus during the first 3 days of a breeding season compared to a single injection of PGF (59% and 33%, respectively; $P<0.001$) and also tended to increase pregnancy rates after a 30-day breeding season (Lucy et al., 2001).

Females that were fed MGA for a period of 14 days and received PGF 17 later were found to have higher estrous response and pregnancy rates compared to a single injection of PGF (Patterson et al., 2003). The addition of a CIDR tended to increase ($P=0.11$) pregnancy rates compared to the addition of MGA in the CO-Sync protocol (100 µg GnRH, followed by 25 mg PG 7 d after, and a second injection of GnRH 48 h later) (51.3% and 39.1%, respectively (Stevenson et al., 2003b). In anestrous females, the addition of a CIDR to the CO-Sync increased ($P<0.05$) pregnancy rates from 39% to 59% (Lamb et al., 2001). Since MGA is a feed additive, it is hard to control a consistent intake amongst females. This may have an impact on the effectiveness of MGA compared to a CIDR device.

Gonadotropin Releasing Hormone

Gonadotropin releasing hormone (**GnRH**) is commonly used in conjunction with PG in synchronization protocols. In synchronization protocols, GnRH is used to initiate a new follicular wave or cause ovulation of a dominant follicle around the time of AI. A well-known synchronization protocol known as Ov-Sync, developed by Pursley et al. 1995, uses both GnRH and PGF to synchronize estrus and ovulation. In the Ov-Sync protocol, GnRH is administered on day -7 with the purpose of ovulating a follicle and starting a new follicular wave. Seven days later PGF is administered to cause luteolysis of a CL. Forty-eight hours after PGF, females receive a second administration of GnRH to induce ovulation. Ovulation should occur 24-32 hours after the administration of GnRH. Timed-AI then occurs 24 hours after the second injection of GnRH. The timing of injections involved with the Ov-Sync protocol are easy for a producer to schedule.

Additionally, when GnRH is administered to females in random stages of the estrous cycle, not all follicle sizes are responsive to GnRH. It appears that a follicle must be >10 mm in order to ovulate (Noseir 2003). Perry et al. 2003, observed significant ($P<0.04$) differences in pregnancy rates at day 68 when females were induced to ovulate after receiving GnRH. Females that had GnRH-induced ovulation of follicles <11 mm had lower pregnancy rates compared to females that ovulated follicles that were 14.5 to 15 mm (18% and 62% respectively; $P<0.04$) (Perry et al., 2003). In that same study, all embryonic mortality occurred in females that ovulated a follicle <11 mm. Improper uterine environment and follicular and oocyte development could potentially attribute to the lack of pregnancy maintenance of the females that ovulated smaller follicles.

Based upon the discussion above, it appears that the use of GnRH, progesterone, and PGF can be used effectively to synchronize and control estrus in both cows and heifers.

Gender Sorted Semen

Progressive cattlemen work towards improving their herd's productivity level each and every year. For those operations that implement artificial insemination already, a potential step to improve their production efficiency would be to control the gender ratio of their calf crops. Controlling the gender ratio of a calf crop can be accomplished by incorporating sex-sorted semen into their breeding programs. Several scenarios and breeding programs have been outlined below to further describe situations where controlling the gender of a calf-crop is desirable.

Potential Reasons to Utilize Female-Sorted Semen

Producers that are looking to expand the size of their operations rapidly could do so by increasing the number of female calves born. This eliminates the need to purchase any replacement females and reduces the risk of bringing in any outside diseases (Seidel 2011). Both seedstock and commercial cattleman could have a potential market for selling yearling heifers and bred females to other producers. Research has demonstrated that heifer calves usually come smaller at birth (Laster et al., 1973). It has also been found that by breeding a heifer to have a smaller calf reduces the incidence of dystocia (Morris et al., 1986 and Dematawewa and Berger, 1997)

It may be the case that certain sires produce females that make extremely good cows (i.e. phenotypically, high fertility, performance) that work in a particular environment. Additionally, it might also be that a heifer calf is more desirable from a certain cow family. Perhaps these females are good structured, sound on their feet and legs, display high udder quality, wean heavier calves, exhibit increased fertility, better fit the environment, and ultimately make superior dams as a mature cow. Using female-sorted semen in these instances allows producers to decide the matings that they would like to generate daughters from and can apply to both the beef and dairy industries. Also, in the dairy industry, increasing the number of females born is more desirable, as males generally have lower economic value (Karakaya et al., 2014).

The use of female-sorted semen could be used in a beef production system that does not involve mature cows. Most of the nutrients used in beef production systems are for cow herds that produce calves (Ferrell and Jenkins, 1985). Seidel and Whittier (2015) discussed the possibility of beef production without mature cows. In this scenario, female-selected sex-sorted semen would be used so that a heifer replaces themselves with a heifer calf (Seidel and Whittier, 2015). In this system, the feed and pasture requirements per unit of beef decreases, two-year old females no longer have to be rebred, and the genetic interval is greatly reduced (Seidel and Whittier, 2015). However, a higher incidence of dystocia may exist, but hopefully reduced as female-selected semen will be used. Additionally, there is a possibility for carcasses to be discounted due to ossification that may occur (Seidel and Whittier, 2015).

Potential Reasons to Utilize Male-Sorted Semen

A seedstock producer's main goal is to generate and sell bulls for commercial cow-calf operations and to other seedstock breeders as well. Selecting and using sons from proven, high-

accuracy sires can make rapid improvements in the genetic progress of herds. Increasing accuracy can enhance the rate of genetic change (Betz 2007). Additionally, by using male-selected semen, bulls can be produced from the best dams for different marketing scenarios and in some large cases, it is easy to market several full brothers (Seidel 2011).

Not only is a feeder steer worth more money per pound (Barham and Troxel, 2007), but male calves also gain more from calving to weaning then female calves (Marlowe and Gaines, 1958). Bull calves were found to gain 5% faster than steers and steers were found to gain 8% faster than heifers prior to weaning (Marlowe and Gaines, 1958).

In the dairy industry, conventional herds generate a surplus of male dairy calves (Murphy et al., 2016). It is estimated that 0.1% of all male dairy born in the United States are selected to become dairy sires (De Vries et al., 2008). A beef and dairy crossbred calf is usually more valuable for meat production than a purebred dairy calf (Hohenboken 1999), a proposed method is to breed a proportion of dairy females using semen from beef sires. By utilizing male sorted beef semen, dairy producers could increase the value of non-replacement calves by capturing the added value of beef sires for meat production, plus the growth and feed efficiency advantages that a male calf has over female calves (Hohenboken 1999).

Incorporating Both Genders

A proposed method to utilize sex sorted semen of both genders in a crossbreeding system known as Two-Breed Rotational and Terminal-Sire, that was previously described by Gregory and Cundiff (1980). This breeding system involves breeding all replacement females and 25% of

the mature cows to female-selected semen of maternal sires. All of the remaining cows in the herd would then be inseminated with male-selected semen using a terminal sire. This breeding scenario would capture the maximum advantage of breed differences for maternal and terminal roles and also maximum advantage of individual and maternal heterosis (Hohenboken 1999).

Summary

The aforementioned scenarios describe several potential avenues for incorporating sex-sorted semen into breeding programs for both commercial and purebred cattlemen. Previous economic analyses have estimated that the more valuable sex be worth $200.00 more to make economic sense (Seidel 2003). However, each farm and ranch operation experiences different input costs and marketing opportunities. While sex-sorted semen may not be appropriate for every operation, it has the potential to increase the production efficiency in the cattle industry.

Semen sorting technology

Sorting semen into X- and Y- bearing sperm is due to the size differences that exist between chromosomes. Studies indicate that the X-chromosome is 3.7-4.22% larger than the Y-chromosome (Garner and Seidel, 2008 and Sharpe and Evans, 2009). Currently, no other sorting technique is as effective as flow cytometry (Sharpe and Evans, 2009 and Vishwanath 2016). The process of flow cytometry has previously been described by Sharpe and Evans, (2009). Flow cytometry involves 21 steps prior to cryopreservation, compared to 3 or 4 for conventional semen (Vishwanath 2014). During the sorting process, the physiology of sperm could be altered which could contribute to reduced fertility of sex-sorted sperm (Seidel 2003 and Garner and

Seidel, 2008). This altered physiology to the sperm may limit longevity in the female reproductive tract (Thomas et al., 2014).

Significant advancements have been made in sperm sorting technology, allowing sex-sorted semen to become commercially available. In the early 1990's, sorting speeds were 200-400 cells/second, sorting accuracy of 83%, and 70% fertility of conventional semen (Vishwanath 2016). However, in the last few years, sorting speeds are now 7,000 – 10,000 cells/second with greater than 90% sorting accuracy, and conception rates are 92-98% of conventional semen (Vishwanath 2016). Typical concentrations of sex-sorted semen have been 2.0×10^6 (Thomas et al., 2017), however SexingTechnologies (Navasota, TX) is currently marketing sex-sorted semen at a concentration of 4.0×10^6, under the tradename SexedUltra 4M. Schenk et al. (2009) reported no differences ($P>0.1$) between concentrations of sex-sorted sperm at 10×10^6 and 2×10^6 (Schenk et al., 2009).

Previous Research

Several studies have indicated reduced conception rates using sex-sorted semen compared to conventional semen of the same sires (Dejarnette et al., 2011, Filho et al., 2012, Thomas et al., 2014, Sales et al., 2011, Dejarnette et al., 2009, Funston and Meyer, 2012, Hall et al., 2017). However, some research has reported increased conception rates with sex-sorted semen when females expressed estrus prior to insemination (Filho et al., 2012, Funston and Meyer, 2012, Hall et al., 2017). Additionally, increased conception rates with sex-sorted semen were observed when insemination occurred 0-12 h before ovulation (Sales et al., 2011). A method to increase conception rates using sex-sorted semen has been to delay insemination in the non-estrus

females. In this process known as split-time AI (**STAI**), females that exhibit estrus are inseminated as schedule and the non-estrus females receive GnRH and breeding is delayed 20 h. Several studies conducted in 2014 observed increased conception rates in non-estrus females when using sex-sorted semen (Thomas et al., 2014 and Thomas et al., 2014b). When STAI was used in heifers, the conception rates for non-estrus heifers increased ($P=0.02$) from 34% to 49%. However, when the method of STAI was applied to cows a greater increase was observed. Cows that failed to express estrus and were inseminated 20 h after an administration of GnRH had increased ($P<0.0001$) conception rates compared to non-estrus cows inseminated at the normal time (36% vs. 3%, respectively) (Thomas et al., 2014). A concern with STAI is that it requires an additional handling and a second insemination time. This may not be conducive to operations that hire breeding technicians as they would have to schedule two consecutive breeding dates. In a previous study of United States beef producers, labor and time were the two most common reasons that AI was not utilized (USDA 2009). Therefore, a protocol that increases both time and labor for producers and professional AI technicians, may limit the adoption of the STAI protocol in commercial beef operations.

Statement of the Problem

Overcoming Production Inefficiencies

There are several instances in both the beef and dairy industries where a producer would be interested in controlling the gender ratio of the calves born in their herds. When using conventional semen, a producer has a 50/50 chance of the calf being the desired sex. However, sex-sorted semen could overcome the possibility of having the less desired sex born and therefore improve the efficiency of the operation. Depending on the individual goals of the operation, there are numerous advantages for incorporating gender-sorted semen and potential scenarios are outlined below.

Potential Reasons to Utilize Female-Sorted Semen

Producers that are looking to expand the size of their operations rapidly could do so by increasing the number of female calves born. This eliminates the need to purchase any replacement females and reduces the risk of bringing in any outside diseases (Seidel 2011). Both seedstock and commercial cattleman could have a potential market for selling yearling heifers and bred females to other producers. Research has demonstrated that heifer calves usually weigh less at birth compared to bull calves (Laster et al., 1973). It has also been found that by breeding a heifer to have a smaller calf reduces the incidence of dystocia (Morris et al., 1986 and Dematawewa and Berger, 1997)

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females are good structured, sound on their feet and legs, display high udder quality, wean heavier calves, exhibit increased fertility, better fit the environment, and ultimately make superior dams as a mature cow. Using female-sorted semen in these instances allows producers to decide the matings that they would like to generate daughters from and can apply to both the beef and dairy industries. Also, in the dairy industry, increasing the number of females born is more desirable, as males generally have lower economic value (Karakaya et al., 2014).

The use of female-sorted semen could be used in a beef production system that does not involve mature cows. Most of the nutrients used in beef production systems are for cow herds that produce calves (Ferrell and Jenkins, 1985). Seidel and Whittier (2015) discussed the possibility of beef production without mature cows. In this scenario, female-selected sex-sorted semen would be used so that a heifer replaces themselves with a heifer calf (Seidel and Whittier, 2015). In this system, the feed and pasture requirements per unit of beef decreases, two-year old females no longer have to be rebred, and the genetic interval is greatly reduced (Seidel and Whittier, 2015). However, a higher incidence of dystocia may exist, but hopefully reduced as female-selected semen will be used. Additionally, there is a possibility for carcasses to be discounted due to ossification that may occur (Seidel and Whittier, 2015).

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Incorporating Both Genders

A proposed method to utilize sex sorted semen of both genders in a crossbreeding system known as Two-Breed Rotational and Terminal-Sire, that was previously described by Gregory and Cundiff (1980). This breeding system involves breeding all replacement females and 25% of the mature cows to female-selected semen of maternal sires. All of the remaining cows in the herd would then be inseminated with male-selected semen using a terminal sire. This breeding

scenario would capture the maximum advantage of breed differences for maternal and terminal roles and also maximum advantage of individual and maternal heterosis (Hohenboken 1999).

Summary

Sex-sorted semen may be used to overcome production inefficiencies in both the beef and dairy industries. Previously, limited research has been conducted with sex-sorted semen using industry-standard fixed-time AI (**FTAI**) protocols. Fixed-time AI protocols allows for mass breeding of females and can eliminate the need for estrus detection. For FTAI protocols to be effective, the number and frequency of handlings needs to be reduced and the need for estrus detection be minimized or eliminated (Busch et al., 2008). Synchronization protocols should not only be effective, but also practical and economical (Wilson et al., 2010). Currently there are not any single, FTAI synchronization protocols for use specifically with sex-sorted semen. Therefore, the objective of our first experiment, further explained in Chapter 2, was to determine if conception rates differ between conventional and sex-sorted semen when estrus is synchronized using the industry-standard 7-d CO-Sync + CIDR protocol in both cows and heifers.

As previously mentioned, increased conception rates with sex-sorted semen were observed when insemination occurred closer to ovulation (Sales et al., 2011). Perhaps later insemination with sex-sorted semen relative to observed estrus more closely synchronized sperm function and ovulation (Schenk et al., 2009). Therefore, it may be possible to increase conception rates with sex-sorted semen if FTAI occurs in relation to a synchronized ovulation. Ov-Sync, a well-known synchronization protocol in the dairy industry, utilizes both GnRH and PGF2α, and has been shown to synchronize ovulation within an 8 h window (Pursley et al., 1995). Our second

experiment, described in Chapter 3, analyzed the conception rates of sex-sorted semen in heifers enrolled in a synchronized ovulation FTAI protocol.

Chapter 2.

Comparison of Conception Rates in Beef Cattle Inseminated with either SexedUltra™ sex-sorted semen or Conventional Semen After Estrus Is Synchronized for Fixed Time Artificial Insemination Using 7-Day Co-Synch.

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Abstract

The objective of this study was to compare conception rates of female beef cattle inseminated at a fixed-time with either conventional (**CON)** or SexedUltra™ sex-sorted (**SU)** semen. Treatments included CON or SU with two sires represented within each treatment. Cows (n=316) and heifers (n=78) from six locations were randomly assigned to treatment. Estrus was synchronized in all females using the industry-standard 7-d CO-Synch + controlled internal drug release (CIDR) protocol (100 μg GnRH + CIDR [1.38 g progesterone] on d 0, 25 mg PGF2α at CIDR removal on d 7, and 100 μg GnRH on d 10, 54 h (heifers) or 66 h (cows) after CIDR removal). Estrotect™ estrus detection aids were applied at CIDR removal and estrus expression was recorded at insemination. Animals were assumed estrual if greater than 50% of the patch coating was removed. Conception rates to fixed-time artificial insemination (**FTAI**) were analyzed using PROC GLIMMIX in SAS 9.4 and significance was determined at $p<0.05$. The results from this study indicated no main effects of treatment (*P= 0.34*), location (*P=0.79*), sire (*P= 0.65*), or age (*P=0.8*) on AI conception rates. However, a significant sire by treatment interaction was observed (*p=0.05*). Conception rates were similar (*p=0.43)* between CON (56.4%) and SU (54.8%) for Sire A, while conception rates with SU (44.9%) were lower (*p<0.05)* compared to CON (59.6%) for Sire B. Conception rates did not differ (*p=0.25)* between sires for CON (56.4% vs 59.6%), however, rates tended to differ (*p=0.09*) for SU between Sire A (54.8%) and Sire B (44.9%). Conception rate for estrual females (62.8%) was higher (*p<0.0001*) than non-estrual females (38.7%) at FTAI regardless of treatment. The interaction between estrus expression and treatment tended (*P=0.11*) to impact conception rates. In the SU treatment, conception rates differed (*P<0.0001*) between estrual (63.8%) and non-estrual females (27.3%). In contrast, no differences (*P=0.8*) were observed between CON (61.9%) and SU (63.8%) in estrual females.

Larger studies are warranted to determine appropriate timing of insemination with sex-sorted semen in FTAI protocols.

Key words: sex-sorted semen, fixed-time AI, beef cattle

Introduction

Estrous synchronization and artificial insemination (**ESAI**) are reproductive technologies that beef cattle producers are progressively using to improve the reproductive performance of their herds. Benefits of ESAI include a more condensed calving season, increased pregnancy rates, increased calf uniformity, and increased weaning weights (Lucy et al., 2001, Busch et al., 2008, and Rodgers et al., 2012). Fixed-time artificial insemination (**FTAI**) allows all females to be inseminated at the same time and reduces labor requirements. Protocols for FTAI are designed to maximize the number of females that are in estrus prior to the time of breeding and eliminate the need for estrus detection. Condensing the calving season increases calf uniformity and allows producers to sell calves in larger lot sizes. Selling a group of five calves resulted in an increase of $11/cwt over selling an individual animal (Halich and Burdine, 2014). Cows enrolled in a FTAI program observed increased weaning percentages, weaning weight per cow exposed, and a net advantage of $49.14 compared to natural service without synchronization (Rodgers et al., 2012).

Controlling the gender of the calf can also improve the opportunity for increased revenue and profit. In the dairy industry, increasing the number of females born is desirable, as males generally have lower economic value (Karakaya et al., 2014). Commercial dairies have targeted use of sex-sorted semen in heifers rather than cows, as they have greater opportunity for conception (Dejarnette et al., 2011). In commercial beef production, steer calves are more valuable than their heifer counterparts (Barham and Troxel, 2007). Producers are able to shift and/or control the gender ratio of their calf crop by incorporating sex-sorted semen into their AI

programs. However, decreased AI conception rates have been observed when sex-sorted semen was used in comparison to conventional semen of the same sires (Sales et al., 2011, Sá Filho et al., 2012, and Thomas et al., 2014). Recent research suggests that SexedUltra™ sex sorted semen can be effectively used with good conception rates in a split-timed AI program in beef heifers (Thomas et al., 2014 and Thomas et al., 2017). In a split-timed AI program, FTAI occurs around 66 h for females that have expressed estrus and insemination is delayed by 20 h after GnRH is administered for females that fail to express estrus. Delayed insemination for non-estrus females requires an additional handling of those females and therefore increases labor requirements. Currently, research is insufficient comparing conception rates between conventional and sex-sorted semen using industry-standard protocols for FTAI. The objective of this study is to determine if conception rates will differ in females inseminated with conventional semen or sex-sorted semen when estrus is synchronized using the 7-d CO-Synch + Controlled Internal Drug Release (**CIDR**) protocol for FTAI.

Materials and Methods

The experimental procedures in this project were approved by the Institutional Animal Care and Use Committee at the University of Kentucky, protocol number 2016-2546.

Animals

Crossbred lactating beef cows and yearling beef heifers (n=394) across six locations in Kentucky were subjected to estrus synchronization using the 7-d CO-Synch + CIDR protocol (Figure 2.1).

Animals were administered GnRH (100 μg, i.m.; Factrel®, Zoetis, Parsippany, NJ) and an Eazi-Breed™ CIDR insert (1.38 g progesterone, Zoetis, Parsippany, NJ) on day 0. The CIDR device was removed, animals were injected with dinoprost tromethamine (25 mg, Lutalyse®, Zoetis, Parsippany, NJ) and estrus detection aids were applied on day 7 (Estrotect™, Rockway Inc., Spring Valley, WI). The condition of the patches was observed at insemination and they were recorded as: estrual (patch > 50% activated; orange), proestrual (patch < 50% activated; orange), non-estrual (not activated; gray), or unknown (missing). Fixed-time AI occurred at 54 h for yearling heifers (n=78) and 60 h for mature cows (n=316) following CIDR removal. All females were administered GnRH (100 μg, im) at FTAI. Within age group (yearling heifer, two-year-old, mature cow) animals were randomly assigned to treatment. Treatments consisted of conventional semen (**CON**) or SexedUltra™ 4M sex-sorted semen (**SU**). Two sires (A and B) were used in each treatment.

Semen treatments

Semen from two bulls from contemporaneous ejaculates were processed as either sex-sorted semen using the SexedULTRA™ procedure or as conventional semen. The conventional semen was packaged at 25-40 x 10^6 live cells per 0.5 ml straw. SexedULTRA™ 4M sex-sorted semen (Sexing Technologies, Navasota, TX) was processed using the technology described by Gonzalez-Marin et al., 2017 and using the flow cytometry process previously described by Sharpe and Evans, 2009 and Gonzalez-Marin et al., 2017. Sex-sorted semen was packaged at 4 x 10^6 cells per 0.25 ml straw and male-sorted semen was used with a sorting accuracy of 95%. All inseminations were performed by the same technician across all locations.

Pregnancy Diagnosis

Conception rate to AI was determined via transrectal ultrasonography (Ibex Pro equipped with an 8-5MHz, E.I. Medical Imaging, Loveland, CO) at 47 to 58 days post FTAI.

Statistical Analysis

All statistical analyses were conducted using the SAS (version 9.4; SAS Inst. Inc.) statistical program. The experiment was designed as a randomized complete block. Animals were blocked by age within location and randomly assigned to treatment. Conception rates to AI were analyzed using the GLIMMIX procedure. The model consisted of location, age within location, sire, treatment, and the sire by treatment interaction. Estrus expression was included in the model as a covariate. All variables were analyzed as fixed factors except for age. An additional model for pregnancy status was created using the GLIMMIX procedure and included the variables of sire, treatment, estrus expression, and all higher order interactions. In order to analyze the significance of age, an additional model was designed using the GLIMMIX procedure as a split plot design. The fixed factors in this model included age, sire, treatment, and their interactions with random factors being location and age within location. Differences in means were assessed using pairwise comparisons. For all statistical analyses, differences were considered significant at $P<0.05$.

Results

The number of females represented in each treatment within location are shown in Table 2.1. Sizes of these operations ranged from 11 to 184 head; with an average herd size of 66 head. No main effects of treatment (*P= 0.34*), location (*P=0.79*), sire (*P= 0.65*) or age (*P=0.8*) on AI conception rates were observed.

A significant sire by treatment interaction was observed (*P=0.05*). Within Sire A, conception rates were similar (*P=0.43*) between CON (56.4%) and SU (54.8%), however conception rates with SU (44.9%) were lower (*P<0.05)* compared to CON (59.6%) for Sire B (Table 2.2). Conception rates in the CON treatment did not differ (*P=0.25*) between Sire A (56.4%) and Sire B (59.6%), while SU conception rates tended to differ (*P=0.09*) between Sire A (54.8%) and Sire B (44.9%).

The Estrotect™ patch status appeared to influence AI conception rates (*P=0.0001*). The four Estrotect™ patch categories were activated (assumed estrual; **EST**), partial (assumed proestrual; **PRO**), gray (assumed non-estrual; **ANE**), and unknown (missing). Cows (n=13) and heifers (n=16) with missing patches were removed from data analyses that included estrus expression in the model. The conception rate for EST females (62.8%) was higher (*P<0.0001*) than ANE females (38.7%) at FTAI regardless of treatment or sire (Table 2.3). Conception rates for PRO females (45.2%) were not significantly different (*P=0.25 and 0.29, respectively*) from EST and ANE females.

Interestingly, the interaction between estrus expression and treatment tended (*P=0.11*) to impact conception rates (Table 2.3). Conception rates tended to differ (*P=0.12*) between ANE (50%) and EST females (61.9%) in the CON group. In the SU treatment, conception rates differed (*P<0.0001*) between EST (63.8%) and ANE females (27.3%). Within the ANE females, conception rates differed (*P=0.02*) between the CON (50%) and SU (27.3%) treatments. Conception rates also tended to differ (*P=0.14*) in the SU treatment between ANE (27.3%) and PRO (40.9%) females. In the EST females, conception rates did not differ (*P=0.8*) between SU (63.8%) and CON (61.9%).

Conception rates to FTAI did not vary among female age (*P=0.8*). Conception rates averaged 51.3%, 55%, and 53.3% for heifers, two-year olds, and mature cows respectively (Table 2.4).

Discussion

Incorporating FTAI into a breeding program can provide many benefits to a producer, including improving genetics rapidly, shortening the calving season length, increasing pregnancy rates, and increasing calf age and weight at weaning (Lucy et al., 2001, Busch et al., 2008, and Rodgers et al., 2012). Although the technology to AI cattle has been commercially available in the United States since the mid-1930's, producers have been slow to adopt this practice. According to a 2007-2008 survey conducted by the USDA National Animal Health Monitoring Services, only 7.8% of all operations in the U.S. incorporate AI (USDA, 2009). Labor and time were the most

common reasons that AI was not utilized (USDA, 2009). Fixed-time AI protocols allows for mass breeding of females and can eliminate the need for estrus detection. For FTAI protocols to be effective, the number and frequency of handlings needs to be reduced and the need for estrus detection be minimized or eliminated (Busch et al., 2008). Synchronization protocols should not only be effective, but also practical and economical (Wilson et al., 2010).

The use of sex-sorted semen in AI programs enables producers to shift the gender ratio of their calf crops. Operations that are looking to expand their herd size would benefit from having more heifer calves, whereas a producer selling calves for beef, would desire more male calves. Not only do steers weigh more at weaning than their heifer counterparts, but they were also found to be worth almost $12/45.5 kg more (Barham and Troxel, 2007).

Semen is sorted into X- and Y- bearing sperm using flow cytometry based on the size difference between chromosomes. Currently, no other sorting technique is as effective as flow cytometry (Sharpe and Evans, 2009 and Vishwanath, 2015). Studies indicate that the X-chromosome is 3.7-4.22% larger than the Y-chromosome (Garner and Seidel, 2008 and Sharpe and Evans, 2009). Significant advancements have been made in sperm sorting technology, allowing sex-sorted semen to become commercially available. In the early 1990's, sorting speeds were 200-400 cells/second, sorting accuracy of 83%, and 70% fertility of conventional semen (Vishwanath, 2015). However, in the last few years, sorting speeds are now 7,000 – 10,000 cells/second with greater than 90% sorting accuracy, and conception rates are 92-98% of conventional semen (Vishwanath, 2015).

In this study, we compared conception rates using conventional and sex-sorted semen in cows and heifers when estrus was synchronized using an industry standard 7-d CO-Synch + CIDR protocol. This protocol was selected because it was identified as the most widely used FTAI protocol based upon conversations with several AI industry professionals. The results from these data indicated no main effects of treatment, location, sire, or age on AI conception rates. The similar conception rates between CON and SU semen in this study differs from previous research that reported decreased conception rates with sex-sorted semen compared to conventional semen (Dejarnette et al., 2009, Dejarnette et al., 2011, Sales et a., 2011, Funston and Meyer, 2012, Sá Filho et al., 2012, Thomas et al., 2014, Thomas et al., 2017, and Hall et al., 2017). Perhaps the number of females included in this study allowed for differences in treatments to remain undetected. Similar conception rates between treatments has the potential to dramatically increase the use of sex-sorted semen in the beef and dairy industries.

Economically, there are several considerations to take into account when using sex-sorted semen. Reduced fertility of sex-sorted semen has considerable costs and the additional costs are also very sensitive to the fertility level of the herd (Seidel, 2003). It has been estimated that the desired sex be worth $200 more in order for sex-sorted semen to make sense economically (Seidel, 2003). Additionally, in a three-year study using sex-sorted semen, on average 22.5% fewer cows ($P<0.05$) calved in the first 21 d period (Hall et al., 2017). Heifers that calved during the first 21 d period of the calving season had increased longevity and weaning weight through their first six calves compared to heifers that calved later in the season (Cushman et al., 2013). A

shift in calving distribution can impact seedstock and commercial cattlemen. More cows calving later in the calving season would lead to younger bulls to sell and heifers that are younger at the start of the breeding season (Hall et al., 2017).

During the sorting process, the physiology of sperm could be altered which could contribute to reduced fertility of sex-sorted sperm (Garner and Seidel, 2008 and Seidel, 2003). This altered physiology to the sperm may limit longevity in the female reproductive tract (Thomas et al., 2014). It has been observed that changing the timing of insemination did improve fertility of sex sorted semen in FTAI programs (Thomas et al., 2017). Similar to other reports (Dejarnette et al., 2009, Healy et al., 2013, and Hall et al., 2017) we observed a treatment by sire interaction. A recent study indicated significant variability between 19 sires represented, with conception rates ranging from 19.3% to 55.6% with sex-sorted semen (Hall et al., 2017). Perhaps sires respond to the sorting process in different, currently undetected ways. Responding differently to the sorting process could potentially explain the differences that were observed in the sire by treatment interaction in this study. Studies to further investigate any fertility differences from the sorting process would require a large number of bulls.

Currently no research has been reported comparing SU and CON in industry standard protocols for FTAI. The females in this study were synchronized using the 7-d Co-Synch protocol with insemination occurring at 54 h for heifers and 60 h for cows. Perhaps the conception rates observed were lower due to the timing of insemination. This protocol was designed for use with conventional semen with normal FTAI for heifers occurring at 54 h and cows at 60-66 h after

CIDR removal (BRTF, 2017). Busch et al. (2008) reported increased pregnancy rates when breeding occurred at 66 h rather than 54 h in mature cows synchronized with this protocol (Busch et al., 2008). However, the optimal time of insemination with sex-sorted semen may be different than with conventional semen (Thomas et al., 2017). Several studies have suggested delaying insemination when using sex-sorted semen. Schenk et al. (2009) observed increased AI conception rates with sex-sorted semen when breeding was delayed 18-24 hours after the onset of estrus in beef heifers (Schenk et al., 2009). This is similar to Sales et al. (2011) and Thomas et al. (2017) who observed higher conception rates with sex-sorted semen when AI was performed closer to ovulation (Sales et al., 2011 and Thomas et al., 2017). Perhaps later insemination with sex-sorted semen relative to observed estrus more closely synchronized sperm function and ovulation (Schenk et al., 2009). Inseminating at the normal time for conventional semen could potentially account for the reduced AI conception rates with the sex-sorted semen from Sire B in the current study.

In the current study, a tendency was observed for an interaction between estrus expression and treatment. Conception rates were similar between CON (61.9%) and SU (63.8%) for EST females. These results differ from Thomas et al. (2014) who observed lower conception rates with SU (42 and 51%) than CON (77%) in EST females (Thomas et al., 2014). Conception rates were significantly lower for SU (27.3%) than CON (50%) for females that were non-estrual (no signs of estrus activity). These data support the observation of Thomas et al., 2014, who saw significantly greater conception rates using conventional semen (37%) rather than sex-sorted semen (3%) in cows that failed to express estrus (Thomas et al., 2014). Additionally, in the SU

treatment, conception rates were higher for EST females (63.8%) compared to those who were ANE (27.3%). These data are similar to multiple studies that have also reported increased AI conception rates with sex-sorted semen if estrus was expressed prior to breeding (Funston and Meyer, 2012, Thomas et al., 2014, and Hall et al., 2017). Hill et al. (2016), noticed that cows showing estrus by 75 h had greater pregnancy rate to AI with conventional semen (61.3% vs. 37.9%) than cows not showing estrus (Hill et al., 2016).

One method to increase conception rates to FTAI using sex-sorted semen has been to delay insemination in heifers that fail to exhibit estrus. Thomas et al., 2014 observed increased conception rates when GnRH was administered to non-estrus females and breeding was delayed 20 hours compared to breeding with sex-sorted semen at the normal time (Thomas et al., 2014). A concern with the split-time breeding is the increased number of animal handlings. With the proposed method, females that have not expressed estrus would have to be sorted off an additional time for the delayed breeding. The extra handling and second time of breeding would not be suitable for single appointment breeding. As suggested by others, a proposed method is to inseminate all estrual females with sex-sorted semen and use conventional semen for the remaining females (Dejarnette et al., 2009 and Thomas et al., 2014).

No differences in conception rates existed between heifers, two-year olds, and mature cows in the current study. With relatively small number of animals in each age group, these results should be interpreted cautiously. However, previous studies have reported no differences in conception rates between cows and heifers inseminated with sex-sorted semen (Hall and Glaze, 2014).

Conclusions

These results indicate that conception rates of females synchronized using the industry standard 7-d CO-Synch + CIDR protocol did not differ between conventional and SexedUltra™ sex-sorted semen. However, a significant interaction existed between treatment and sire. Although the conception rates between the two sires represented did not differ with conventional semen, differences were observed using the sex-sorted semen for one of the sires. Perhaps this interaction between semen type and sire could be attributed to how sires respond to the sorting process or to the timing of insemination. In addition, estrual females had significantly greater AI conception rates than those that were non-estrual. These results support previous research that demonstrated the importance of estrus expression prior to breeding with sex-sorted semen. Currently, insemination times in FTAI protocols are based upon the time of estrus expression. Perhaps timing of insemination should occur in relation to the time of ovulation rather than estrus expression when sex-sorted semen is used. Further studies are needed to explore alternative FTAI synchronization protocols with respect to ovulation for use with sex-sorted semen as well as to examine any differences in sire fertility that may be present after the sorting procedure.

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Derek Wise, and Lauren Wood, and a special thank you to Mr. Tommy Yankey from the Anderson County Cooperative Extension office for his coordination between the producers. The information reported in this article (publication no. XXXX) is part of a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

Table 2.1.
Number of females based on location, treatment [a], and sire

Location	Semen Type	Sire A	Sire B
1	Conventional	3	3
	SexedULTRA	3	3
2	Conventional	3	3
	SexedULTRA	3	2
3	Conventional	49	45
	SexedULTRA	45	45
4	Conventional	8	8
	SexedULTRA	9	8
5	Conventional	5	5
	SexedULTRA	5	3
6	Conventional	33	35
	SexedULTRA	34	33
Overall	Conventional	102	99
	SexedULTRA	99	94

[a] Females in the conventional treatment received conventional, non-sex-sorted semen and females in the SexedULTRA treatment received SexedULTRA 4M sex-sorted semen.

Table 2.2.

Conception rates [a] to fixed-time artificial insemination (FTAI)[b] based on treatment [c] and sire

	Treatment [c]			
	Conventional		SexedULTRA	
	Proportion	Percentage	Proportion	Percentage
Sire A	53/94	56.4%	51/93	54.8%[x]
Sire B	53/89	59.6%[a]	40/89	44.9%[b,y]
Overall	106/183	57.9%	91/182	50.0%

[ab] Conception rates with different superscripts within rows are different, *P<0.05*

[xy] Conception rates with different superscripts within column are different, *P=0.09*

[a] Conception rates to AI determined by transrectal ultrasonography 47 – 58 d after FTAI.

[b] Refer Fig. 2.1. for synchronization protocol.

[c] Females in the conventional treatment received conventional, non-sex-sorted semen and females in the SexedULTRA treatment received SexedULTRA 4M sex-sorted semen.

Table 2.3.
Conception rates [a] to FTAI by treatment and estrus expression [b]

	Estrotect Patch Color at FTAI [c]					
	Non-estrual		Proestrual		Estrual	
Treatment	Proportion	Percentage	Proportion	Percentage	Proportion	Percentage
Conventional	28/56	50.0%[a,y]	5/9	55.6%	73/118	61.9%[x]
SexedULTRA	15/55	27.3%[b,d]	9/22	40.9%[c]	67/105	63.8%[a]
Overall	43/111	38.7%[b]	14/31	45.2%	140/223	62.8%[a]

[ab] Conception rates with different superscripts within rows and columns are different, *P<0.05*
[xy] Conception rates with different superscripts within rows are different, *P=0.12*
[cd] Conception rates with different superscripts within rows are different, *P=0.14*
[a] Conception rates to AI determined by transrectal ultrasonography 47 – 58 d after FTAI.
[b] Refer Fig. 2.1. for synchronization protocol. Females in the conventional treatment received conventional, non-sex-sorted semen and females in the SexedULTRA treatment received SexedULTRA 4M sex-sorted semen.
[c] Expression of estrus was determined by the status of the Estrotect estrus detection aids at FTAI.

Table 2.4.
Conception rates [a] to FTAI [b] based on female age

	Age [c]					
	Yearling Heifers		Two-Year Olds		Mature Cows	
	Proportion	Percentage	Proportion	Percentage	Proportion	Percentage
Overall	40/78	51.3%	22/40	55%	147/276	53.3%

[a] Conception rates to AI determined by transrectal ultrasonography 47 – 58 d after FTAI.
[b] Refer Fig. 2.1. for synchronization protocol.
[c] Females were classified as yearling heifers, two-year olds, or mature cows.

Figure 2.1. 7-d CO-Sync + CIDR protocol

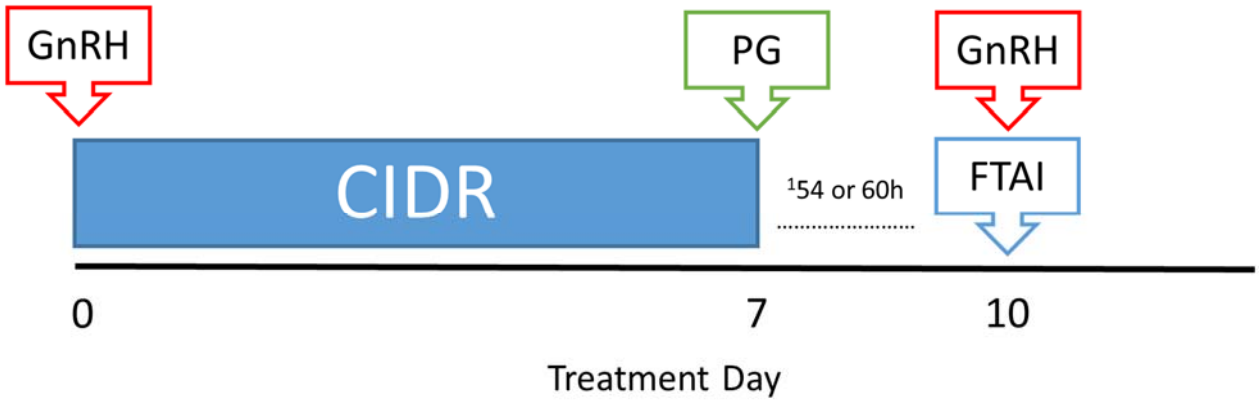

Fig. 2.1. Treatment schedule for the 7-d CO-Sync + CIDR protocol. Animals were administered GnRH (100 µg, i.m.; Factrel®, Zoetis, Parsippany, NJ) and an Eazi-Breed™ CIDR insert (1.38 g progesterone, Zoetis, Parsippany, NJ) on day 0. The CIDR device was removed, animals were injected with dinoprost tromethamine (25 mg, Lutalyse®, Zoetis, Parsippany, NJ) and estrus detection aids were applied on day 7 (Estrotect™, Rockway Inc., Spring Valley, WI). [1]Fixed-time AI occurred at 54 h for yearling heifers and 60 h for mature cows following CIDR removal. All females were administered GnRH (100 µg, im) at FTAI.

Chapter 3.

Conception Rates in Yearling Beef Heifers Inseminated with either SexedULTRA™ or Conventional Semen After Ovulation Is Synchronized for Fixed Time Artificial Insemination Using a Modified 14-Day CIDR-PG Protocol.

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Abstract

The objective of this study was to determine if conception rates to fixed-time artificial insemination (**FTAI**) differs between SexedULTRA™ sex-sorted (**SU**) and conventional (**CON**) semen when ovulation is synchronized using a 14-d controlled internal drug release (**CIDR**) - PGF2α (**PG**) protocol modified to optimize the timing of FTAI with respect to ovulation. Treatments included CON or SU with three sires represented within each treatment. Heifers (n=218) from one location were randomly assigned to treatment. Ovulation was synchronized in all females using a modified 14-d CIDR-PG protocol. Heifers received a CIDR insert from d 0 to 14, followed by 25 mg PG 16 d later (d 30). All females were administered GnRH 54 h after PG and FTAI occurred 18 h after GnRH (72 h after PG administration). Estrotect™ estrous detection aids were applied at PG administration and estrous expression was recorded at insemination. Animals were assumed estrual if greater than 50% of the patch coating was removed. Heifers were exposed to natural service sires 7-10 days post AI for the remainder of the breeding season. Conception rates to FTAI were determined on day 49 via transrectal ultrasonography and analyzed using PROC GLIMMIX in SAS 9.4 and significance was determined at $p<0.05$. Conception rates were similar (*P=0.47*) between heifers inseminated with CON semen (53/103 = 51.5%) and SU semen (53/115 = 46.1%). Regardless of treatment, the expression of estrous did not affect conception rates (*P=0.25*); estrual females had similar conception rates (50/91 = 54.9%) to non-estrual females (21/42 = 50%). Additionally, no effect of technician (*P=0.78*), sire (*P=0.43*), sire by treatment interaction (*P=0.88*), or a treatment by estrous expression interaction (*P=0.98*) was observed. In conclusion, the 14-d CIDR-PG protocol modified to control ovulation is an effective method for FTAI with sex-sorted semen. More research is warranted to

determine the long-term economic impact of controlling the calf crop gender ratio in beef cow-calf operations.

Key words: sex-sorted semen, fixed-time artificial insemination, synchronized ovulation

Introduction

Estrus synchronization and artificial insemination (**ESAI**) are reproductive technologies that cattlemen can use to improve the reproductive performance of their herds. Benefits of ESAI include a more condensed calving season, increased pregnancy rates, increased calf uniformity, and increased weaning weights (Lucy et al., 2001, Rodgers et al., 2012, Busch et al., 2008). Fixed-time artificial insemination (**FTAI**) allows all females to be inseminated at the same time and reduces labor requirements. Protocols for FTAI are designed to maximize the number of females that are in estrus prior to the time of breeding and eliminate the need for estrus detection. Condensing the calving season increases calf uniformity and allows producers to sell calves in larger lot sizes. Cows enrolled in a FTAI program observed increased weaning percentages, weaning weight per cow exposed, and a net advantage of $49.14 compared to natural service without synchronization (Rodgers et al., 2012).

Controlling the gender of the calf can also improve the opportunity for increased revenue and profit. In the dairy industry, increasing the number of females born is more desirable, as males generally have lower economic value (Karakaya et al., 2014). Commercial dairies have targeted use of sex-sorted semen in heifers rather than cows, as they have greater opportunity for conception (Dejarnette, 2011). In commercial beef production, steer calves are more valuable than their heifer counterparts (Barham and Troxel, 2007, Halich and Burdine, 2014). Producers have the opportunity to control the gender ratio of their calf crop by incorporating sex-sorted semen into their AI programs. However, decreased AI conception rates have been observed when sex-sorted semen was used in comparison to conventional semen of the same sires (Filho

et al., 2012, Thomas et al., 2014, Sales et al., 2011). Recent research suggests that SexedULTRA™ sex-sorted semen can be effectively used with good conception rates in a split-timed AI program in beef heifers (Thomas et al.,2014 and Thomas et al., 2017). In a split-timed AI program, FTAI occurs around 66 h for females that have expressed estrus and insemination is delayed by 20 h after GnRH is administered for females that fail to express estrus. Delayed insemination for non-estrus females requires an additional handling of those females and therefore increases labor requirements. Currently, insufficient research has been performed comparing conception rates between conventional and sex-sorted semen using synchronization protocols for single appointment breeding. Previous studies with sex-sorted semen have utilized protocols designed to synchronize estrus with the timing of insemination to occur in relation to estrous expression. The objective of this study was to determine if conception rate to FTAI differs between SexedULTRA™ sex-sorted and conventional semen when yearling beef heifers are synchronized using a 14-d controlled internal drug release (**CIDR**) - PGF2α (**PG**) protocol modified to optimize the control of ovulation and timing of insemination. The 14-d CIDR-PG protocol does not rely on GnRH to control follicular dynamics as compared to the 7-d CO-Sync + CIDR protocol (Busch et al., 2007 and Kasimanickam et al., 2015). The long term progestin treatment in the 14-d CIDR-PG protocol facilitates a tight synchrony of estrous expression after PGF administration (Leitman et al., 2009a,b).

Materials and Methods

The experimental procedures in this project were approved by the Institutional Animal Care and Use Committee at the University of Kentucky, protocol number 2016-2546.

Animals

Crossbred yearling beef heifers (n=218) at one location in Kentucky were subjected to a FTAI synchronization protocol using a modified 14-d CIDR-PG protocol (Figure 3.1). Animals received an Eazi-Breed™ CIDR insert (1.38 g progesterone, Zoetis, Parsippany, NJ) from d 0 to 14, followed by an injection of dinoprost tromethamine (25 mg, i.m.; Lutalyse®, (**PG**) Zoetis, Parsippany, NJ) 16 d later (d 30). Estrous detection aids (Estrotect™, Rockway Inc., Spring Valley, WI) were applied at PG administration. All females were administered GnRH (100 µg, i.m.; Factrel®, Zoetis, Parsippany, NJ) 54 h after PG. The condition of the patches was observed at insemination and were recorded as: estrual (patch > 50% activated; orange), proestrual (patch < 50% activated; orange), non-estrual (not activated; gray), or unknown (missing). Fixed-time AI occurred at 18 h after GnRH administration (72 h post PG). Inseminations were performed by two technicians. Treatments consisted of conventional semen (**CON**) or SexedULTRA 4M™ sex-sorted semen (**SU**) and three sires were used in each treatment. Heifers were exposed to natural service sires 7-10 days post FTAI for the remainder of the breeding season.

Semen treatments

Semen from three bulls from contemporaneous ejaculates were processed as either sex-sorted semen using the SexedULTRA™ procedure or as conventional semen (Sexing Technologies, Navasota, TX). The conventional semen was packaged at 25-40 x 10^6 live cells per 0.5 ml straw. SexedULTRA™ sex-sorted semen (Sexing Technologies, Navasota, TX) was processed using the

technology described by Gonzalez-Marin et al., 2017 and using the flow cytometry process previously described by Sharpe and Evans, 2009. Sex-sorted semen was packaged at 4 x 10^6 cells per 0.25 ml straw and male sorted semen was used at a sorting accuracy of 95%.

Pregnancy Diagnosis

Conception rate to AI was determined via transrectal ultrasonography (Ibex Pro equipped with an 8-5MHz, E.I. Medical Imaging, Loveland, CO) at 49 days post-FTAI. Final pregnancy rate was determined via transrectal ultrasonography at 91 days after FTAI occurred.

Statistical Analysis

All statistical analyses were conducted using the SAS (version 9.4; SAS Inst. Inc.) statistical program. The experiment was designed as a completely randomized design and animals were randomly assigned to treatment. The effects of technician and final pregnancy rates by treatment were analyzed using the ANOVA procedure. Conception rates to AI were analyzed using the GLIMMIX procedure using the binomial distribution, link logit function. The model consisted of sire, treatment, and the sire by treatment interaction. An additional model was designed to analyze the impact of estrous expression on conception rates using the GLIMMIX procedure. This model consisted of sire, treatment, sire by treatment, estrous expression, and the treatment by estrous expression interaction. Those with missing data for estrous expression were removed from this analysis. All variables were analyzed as fixed factors. For all statistical analyses, differences were considered significant at $P<0.05$.

Results

Conception rates for the treatments were similar (*P=0.47*) between heifers inseminated with sex-sorted semen (46.1%; 53/115) or conventional semen (51.5%; 53/103). No differences in conception rates were observed (*P=0.42*) between the three sires represented in this study. Additionally, the interaction between sire and treatment was insignificant (*P=0.89*) (Table 3.1). Conception rates to FTAI were also similar (*P=0.78*) between the two technicians.

The four Estrotect™ patch categories were activated (assumed estrual; **EST**), partial (assumed proestrual; **PRO**), gray (assumed non-estrual; **ANE**), and unknown (missing). Heifers (n=4) with missing patches were removed from data analyses that included estrous expression in the model. The Estrotect™ patch status and the interaction between treatment and estrous expression did not affect conception rates (*P=0.25 and 0.98, respectively*) (Table 3.2).

The final breeding season pregnancy rates were similar (*P=0.14*) for heifers inseminated with SU (76.5%; 88/115) and CON (85.5%; 87/103) semen. At the end of the breeding season 80.3% (175/218) of the heifers were pregnant with 19.7% (43/218) females not conceiving (Table 3.3).

Discussion

Incorporating FTAI into a breeding program can provide many benefits to a producer, including improving genetics rapidly, shortening the calving season length, increasing pregnancy rates, and increasing calf age and weight at weaning (Lucy et al., 2001, Busch et al., 2008, Rodgers et

al., 2012). Although the technology to AI cattle has been commercially available in the United States since the mid-1930's, producers have been slow to adopt this practice. According to a 2007-2008 survey conducted by the USDA National Animal Health Monitoring Services, only 7.8% of all operations in the U.S. incorporate AI (USDA 2009). Labor and time were the most common reasons that AI was not utilized (USDA 2009). Fixed-time AI protocols allows for mass breeding of females and can eliminate the need for estrus detection. For FTAI protocols to be effective, the number and frequency of handlings needs to be reduced and the need for estrus detection to be minimized or eliminated (Busch et al., 2008).

Semen is sorted into X- and Y- bearing sperm using flow cytometry due to the size difference that exists between the chromosomes. Studies indicate that the X-chromosome is 3.7-4.22% larger than the Y-chromosome (Sharpe and Evans, 2009, Garner and Seidel, 2008). Currently, no other sorting technique is as effective as flow cytometry (Sharpe and Evans, 2009, Vishwanath 2015). In the early 1990's, sorting speeds were 200-400 cells/second, sorting accuracy of 83%, and 70% fertility of conventional semen (Vishwanath 2015). However, significant advancements have been made in sperm sorting technology, allowing sex-sorted semen to become commercially available.

The use of sex-sorted semen in AI programs enables producers to shift the gender ratio of their calf crops. Operations that are looking to expand the size of their herd would benefit from having more heifer calves, whereas a producer selling calves for beef, would desire more male calves. Not only do steers weigh more at weaning than their heifer counterparts, but they were

also found to be worth more at market (Barham and Troxel, 2007 and Halich and Burdine, 2014).

A proposed method for incorporating sex-sorted semen is to develop a strategic breeding plan consisting of both maternal- and terminally-oriented sires. A crossbreeding system known as, Two-Breed Rotational and Terminal-Sire, previously described by Gregory and Cundiff (1980), combines individual, maternal, and terminal heterosis along with breed complementarity. In this scenario, a small population of mature cows (approximately 25%) and all yearling heifers would be mated to maternal oriented sires with female-sorted semen to generate replacement females. The remaining portion of the cowherd would be mated to terminal sires using male-sorted semen. Not only are first-parity females more likely to experience calving difficulty, but a smaller portion of female calves born require assistance at parturition (Dematawewa and Berger, 1997). Breeding yearling heifers to female-sorted semen should help reduce the incidence of dystocia in the younger females. The calves produced from the terminal sires will represent all three breeds utilized in the crossbreeding system. Calves generated from a two-breed rotational and terminal-sire crossbreeding system are estimated to have a 20.8% increase in weight marketed per cow exposed (Gregory and Cundiff, 1980). By using this breeding strategy in conjunction with sex-sorted semen, producers are able to select the population of females they wish to generate replacements from, while maximizing the profit potential for those remaining in the herd.

In this study, we compared conception rates using conventional and sex-sorted semen in yearling beef heifers when ovulation was synchronized using a modified 14-d protocol CIDR-PG protocol. The results from these data indicated no main effects of treatment (*P=0.47)*, sire (*P= 0.42*), or estrous expression (*P= 0.25*) on FTAI conception rates. These data are similar to Crites et al. (2017) who observed similar conception rates between CON and SU semen when estrus was synchronized using the industry-standard 7d CO-Synch + CIDR protocol (Crites et al., 2017). However, similar conception rates between CON and SU semen in these studies differs from previous research that reported decreased conception rates with sex-sorted semen compared to conventional semen (Dejarnette et al., 2009, Dejarnette et al., 2011, Sales et al., 2011, Filho et al., 2012, Funston and Meyer, 2012, Thomas et al., 2014, Hall et al., 2017). Similar conception rates between treatments has the potential to dramatically increase the use of sex-sorted semen in the beef and dairy industries.

Economically, there are several considerations to take into account when using sex-sorted semen. The cost of sex-sorted semen can range from $10-50 more per dose than conventional semen (Seidel 2003). Reduced fertility of sex-sorted semen has considerable costs and the additional costs are also very sensitive to the fertility level of the herd (Seidel 2003). Previous economic analyses have estimated that the more valuable sex be worth $200.00 more in order for sex-sorted semen to make economic sense (Seidel 2003). Additionally, in a three-year study using sex-sorted semen, on average 22.5% fewer cows (*P<0.05)* calved in the first 21 d period (Hall et al., 2017). Heifers that calved during the first 21 d period of the calving season had increased longevity and weaning weight through their first six calves compared to heifers that

calved later in the season (Cushman et al., 2013). A shift in calving distribution can impact the productivity of seedstock and commercial cattlemen. More cows calving later in the calving season would lead to younger bulls to sell and heifers that are younger at the start of the breeding season (Hall et al., 2017).

During the sorting process, the physiology of sperm could be altered which could contribute to the reduced fertility of sex-sorted sperm (Seidel 2003, Garner and Seidel 2008). This altered physiology to the sperm may limit longevity in the female reproductive tract (Thomas et al., 2014) and it has been noticed that changing the timing of insemination did improve fertility of sex-sorted semen in FTAI programs (Thomas et al., 2017). Contrary to other reports (Dejarnette et al., 2009, Healy et al., 2013, Hall et al., 2017, Crites et al., 2017) we did not observe a treatment by sire interaction. However, a recent study indicated significant variability between 19 sires represented; with conception rates ranging from 19.3% to 55.6% (Hall et al., 2017).

Recent research with sex-sorted semen has focused primarily on synchronizing estrus and breeding based upon observed estrous response (Thomas et al., 2014 and 2017, Hall et al., 2017). In this study, we synchronized ovulation using a modified 14-d protocol CIDR-PG protocol. In a retrospective analysis, higher conception rates were observed with sex-sorted semen when AI was performed closer to ovulation (Sales et al., 2011). The synchronization protocol used in the current study combines the 14-d CIDR-PG protocol previously described by Kasimanickam et al., 2015 and also a modified version of Ovsynch, previously described by Pursely et al., 1995. The 14-d CIDR-PG protocol does not rely on GnRH to control follicular

dynamics as compared to the 7-d CO-Sync + CIDR protocol (Busch et al., 2007 and Kasimanickam et al., 2015). Research has demonstrated that delaying PGF administration until d 10-15 of the estrus cycle improved conception rates compared to heifers that are early (d 5-9) in their cycle (King et al., 1982). Additionally, heifers administered PGF on d 10-15 of their cycles exhibited estrus around 60 h compared to 48 h for early cycle heifers (King et al., 1982) which is conducive for FTAI to occur at 72 h post PGF administration.

In the Ovsynch protocol, females received GnRH on d 0, PGF2α 7 d later, and a second dose of GnRH administered 48 h after PGF2α with timed insemination occurring 24 h after the final GnRH (Pursley et al., 1995). It was reported that ovulation was synchronized in an 8 h window with cows and heifers ovulating between 24 and 32 h after the second injection of GnRH (Pursley et al., 1995). Greater conception rates were observed when AI occurred 16 h after the second GnRH injection (Pursley et al., 1998).

In the current study, heifers received GnRH 54 h after an injection of PG with FTAI occurring 18 h after GnRH administration. The purpose of the GnRH administration after the injection of PG was to induce ovulation of a preovulatory follicle. When GnRH was administered 48 h after an injection of PG in dairy heifers, 75% ovulated between 26 and 30 h (Pursley et al., 1995). The GnRH administration was delayed 54 h after PG to induce ovulation in a larger percentage of heifers. Increased conception rates were observed when insemination occurred between 0 and 12 h before ovulation compared to 12 to 24 h and >24 h before ovulation (Sales et al., 2011). With FTAI occurring 18 h after the injection of GnRH, ovulation should occur 6 to 14 h after

insemination based upon the previous research of Pursley et al., 1995 which also corresponds to the window of time that Sales et al., 2011 reported to have the highest AI conception rates. Conception rates to AI are improved as the timing of insemination and ovulation are optimized (Saacke 2008). The respected durations of time for administration of PG and GnRH along with the timing of FTAI are easily implemented in the industry setting. The synchronization protocol used in this study allows for convenient scheduling of a single appointment breeding with a technician.

In the present study, the effect of estrous expression and the treatment by estrous expression was not significant. Conception rates were similar between CON (56.9%) and SU (52.5%) for EST females. Similar conception rates between CON and SU for EST females support previous findings from our lab. Our previous research indicated similar conception rates between CON (61.9%) and SU (63.8%) if estrus was expressed prior to insemination (Crites et al., 2017). However, these results differ from Thomas et al., 2014 who observed lower conception rates with SU (42 and 51%) than CON (77%) in EST females (Thomas et al., 2014). Conception rates were also similar for SU (50%) and CON (50%) for females that were non-estrual (no signs of estrus activity). These data are different from the observation of Thomas et al., 2014, who saw significantly greater conception rates using conventional semen (37%) rather than sex-sorted semen (3%) in cows that failed to express estrus (Thomas et a., 2014). Additionally, in the SU treatment, conception rates were similar for EST females (52.5%) compared to those who were ANE (50%). These data differ from multiple studies that reported increased AI conception rates with sex-sorted semen if estrus was expressed prior to breeding (Funston and Meyer, 2012,

Thomas et al., 2014, Hall et al., 2017, Crites et al., 2017). The results from these data suggest that estrous expression at FTAI does not impact conception rates when insemination occurs in relation to a synchronized ovulation.

One method to increase conception rates to FTAI using sex-sorted semen has been to delay insemination in heifers that fail to exhibit estrus. Thomas et al., 2014 observed increased conception rates when GnRH was administered to non-estrus females and breeding was delayed 20 hours compared to breeding with sex-sorted semen at the normal time. A concern with the split-time breeding is the increased number of animal handlings. With the proposed method, females that have not expressed estrus would have to be sorted off an additional time for the delayed breeding. The extra handling and second time of breeding would not be suitable for single appointment breeding. It has also been suggested by others (Thomas et al., 2014, Dejarnette et al., 2009) to inseminate all estrual females with sex-sorted semen and use conventional semen for the remaining females.

Conclusion

In conclusion, these results indicate that conception rates of females inseminated with conventional and SexedUltra™ sex-sorted semen did not differ when ovulation was synchronized using a modified 14-d CIDR-PG protocol. These results also demonstrated that conception rates were similar for both CON and SU semen regardless of estrous expression. The modified 14-d CIDR-PG protocol for yearling beef heifers was an effective synchronization

protocol with sex-sorted semen. The similar conception rates between semen types in a single appointment breeding could lead to a dramatic increase in the use of sex-sorted semen in both the beef and dairy industries. Having the capability to control the gender ratio without sacrificing conception rate has the potential to increase producer revenue and improve production efficiency. Further studies are needed to determine the long-term economic impact of whole herd insemination with sex-sorted semen and to determine the appropriate timing of insemination with respect to ovulation.

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Table 3.1.
Conception rates [a] to fixed-time artificial insemination (FTAI)[b] based on treatment [c] and sire

	Treatment [c]			
	Conventional		SexedULTRA	
	Proportion	Percentage	Proportion	Percentage
Sire A	20/37	54.1%	20/39	51.3%
Sire B	13/30	43.3%	16/39	41.0%
Sire C	20/36	55.6%	17/37	45.9%
Overall	53/103	51.5%	53/115	46.1%

[a] Conception rates to AI determined by transrectal ultrasonography 49 d after FTAI.
[b] Refer Fig. 3.1. for synchronization protocol.
[c] Females in the conventional treatment received conventional, non-sex-sorted semen and females in the SexedULTRA treatment received SexedULTRA 4M sex-sorted semen.

Table 3.2.
Conception rates [a] to FTAI by treatment and estrus expression[b]

	Estrotect Patch Color at FTAI [c]					
	Non-estrual		Proestrual		Estrual	
Treatment	Proportion	Percentage	Proportion	Percentage	Proportion	Percentage
Conventional	6/12	50.0%	16/38	42.1%	29/51	56.9%
SexedULTRA	15/30	50.0%	17/43	39.5%	21/40	52.5%
Overall	21/42	50.0%	33/81	40.7%	50/91	54.9%

[a] Conception rates to AI determined by transrectal ultrasonography 49 d after FTAI.
[b] Refer Fig. 3.1. for synchronization protocol. Females in the conventional treatment received conventional, non-sex-sorted semen and females in the SexedULTRA treatment received SexedULTRA 4M sex-sorted semen.
[c] Expression of estrus was determined by the status of the Estrotect estrus detection aids at FTAI.

Table 3.3.
Final breeding season pregnancy rates [a] by treatment [b]

	Pregnant		Open	
Treatment	Proportion	Percentage	Proportion	Percentage
Conventional	87/103	85.5%	16/103	15.5%
SexedULTRA	88/115	76.5%	27/115	23.5%
Overall	175/218	80.3%	43/218	19.7%

[a] Breeding season pregnancy rates determined by transrectal ultrasonography 91 d after FTAI.
[b] Refer Fig. 3.1. for synchronization protocol. Females in the conventional treatment received conventional, non-sex-sorted semen and females in the SexedULTRA treatment received SexedULTRA 4M sex-sorted semen.

Figure 3.1. Modified 14-d CIDR-PG protocol

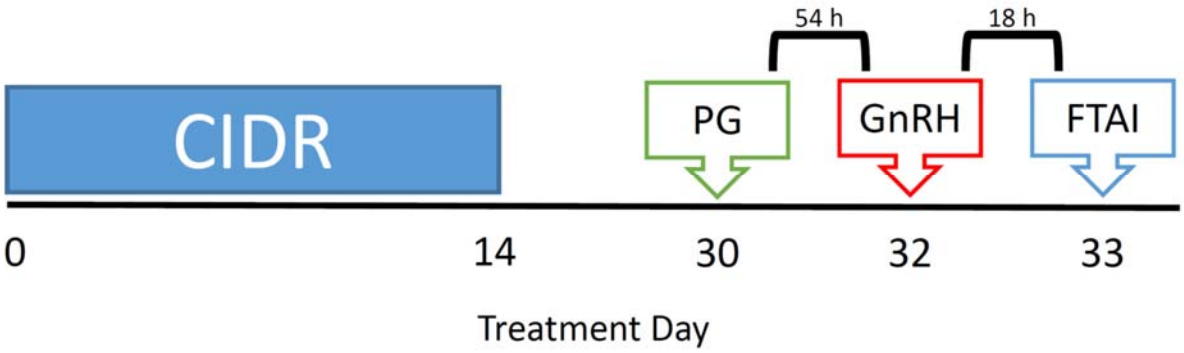

Fig. 3.1. Treatment schedule for the modified 14-d CIDR-PG protocol. Animals received an Eazi-Breed™ CIDR insert (1.38 g progesterone, Zoetis, Parsippany, NJ) from d 0 to 14. On d 30 animals were injected with dinoprost tromethamine (25 mg, i.m.; (**PG**) Lutalyse®, Zoetis, Parsippany, NJ) and estrus detection aids were applied. Heifers received an injection of GnRH (100 µg, i.m.; Factrel®, Zoetis, Parsippany, NJ) 54 h after PG administration. Fixed-time AI occurred at 18 h after GnRH administration (72 h after receiving PG).

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