ABSTRACT

MUTH, ASHLEIGH MARIE. Comparison of Pregnancy Rates in Beef Cattle Following a 7-day Co-Synch Treatment with Once- or Twice-used CIDR Devices. (Under the direction of Dr. C. S. Whisnant).

The use of fixed-time artificial insemination (FTAI) provides producers with numerous benefits including the use of superior genetics, shorter breeding and calving seasons and a more uniform calf crop however the cost of implementing estrus synchronization and FTAI protocols is hindering its use in the beef industry. The objective of these experiments was to determine whether or not a twice-used controlled internal drug releasing (CIDR) device could be implemented in a 7-day Co-Synch + CIDR protocol without adversely affecting pregnancy rates. In experiment 1, nulliparous beef heifers at the Tidewater Research Station were randomly assigned to the control group (*n = 12*) receiving a once-used CIDR device or the treatment group (*n = 12*) receiving a twice-used CIDR device. The CIDR devices were removed and the injection of PGF was administered on d 7 and the heifers were artificially inseminated by one trained technician in the following 60-66 h. The heifers were diagnosed as pregnant 30-40 days after FTAI via transrectal ultrasonography by trained veterinarians. Pregnancy rates were 91.7 ± 9.9 % and 83.3 ± 9.9 % (*P = 0.56*) in control and treatment animals, respectively. In experiment 2, purebred Angus nulliparous females (*n = 99*) between 13 and 27 months of age and multiparous cows (*n = 43*) between 48 and 74 months of age at the Upper Piedmont (Reidsville) Research Station were synchronized for estrus using the same protocol as experiment 1. Females at Reidsville Research Station were also randomly assigned to have their injection of PGF given SubQ or

IM. CIDR devices were removed and the injection of PGF was administered on d 7 and all animals were artificially inseminated in the following 60-66 h by one of two trained AI technicians and were diagnosed as pregnant or open 30-40 d after FTAI via transrectal ultrasonography performed by veterinarians. There was no significant effect of parity *(P = 0.82)*, AI technician *(P = 0.60)*, route of administration of PGF *(P = 0.83)* or treatment *(P = 0.67)* on pregnancy rates to AI in control and treatment animals which were 75.4 ± 6.0% and 71.7 ± 6.4% respectively. Whole blood was also taken in a random subset of nulliparous females at the Reidsville Research Station *(n = 52)* via coccygeal venipuncture just prior to device removal and the plasma was collected and assayed for progesterone concentration via radioimmunoassay. Plasma progesterone concentrations were higher (*P = 0.01*) in the control animals that received that once-used devices than the treatment animals that were administered a twice-used CIDR device (3.4 ± 0.5 ng/mL and 1.4 ± 0.5 ng/mL respectively). This study provides evidence that although plasma progesterone concentrations are lower in animals treated with a twice-used CIDR device, there is still a sufficient concentration of progesterone present in twice-used devices to effectively synchronize estrus without adversely affecting the fertility of a herd.

Comparison of Pregnancy Rates in Beef Cattle Following a 7-day Co-Synch Treatment with Once- or Twice-used CIDR Devices

by
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DEDICATION

This thesis is dedicated to my parents Todd and Tracy Muth who made countless, selfless sacrifices to ensure that my younger sister and I had every opportunity to reach our potential, and by example, instilled in me the values of honesty, hard-work and perseverance; and to my fiancé Seth for not only helping me forward but for graciously taking the back seat while I completed this program. It is because of your enduring support and encouragement that I was able to continue my education and obtain this degree – and I will never be able to express how thankful I am for that and for all of you.

BIOGRAPHY

Ashleigh Marie Muth was born January 26, 1990 to Todd and Tracy Muth in Fayetteville, North Carolina. After the honorable discharge of her father, the family moved to Westerville, Ohio in order to be closer to family. Various school assignments from elementary through high school provide ample evidence of Ashleigh's love of animals and desire to become a veterinarian.

Following graduation from Westerville South High School she attended THE Ohio State University's College of Food, Agricultural and Environmental Sciences for animal science. During her undergraduate career at OSU she was active in the Animal Science Club and held an executive position within the Pre-Veterinary Medical Association. After taking several classes she became increasingly interested in food animal production and set her sights on becoming a food animal veterinarian. It wasn't until spring quarter of her sophomore year after taking an introduction to reproductive physiology course that she decided to switch her focus to research within the field of reproductive physiology. Upon graduation from Ohio State in 2011 Ashleigh immediately applied to North Carolina State University's animal science graduate program to study reproductive physiology under the direction of Dr. Scott Whisnant focusing on reproductive endocrinology and estrus synchronization protocols.

Ashleigh will receive a Master's of Science in Animal Science in the summer of 2013 and will continue her education in the field of reproduction as a candidate for a Doctorate of Philosophy from Mississippi State University under the guidance of Dr. Jamie Larson.

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REVIEW OF LITERATURE

Estrus synchronization and fixed-time artificial insemination (FTAI) protocols are assisted reproductive technologies that provide producers effective means of rapidly increasing the genetic progress of a herd. These technologies were easily integrated into the intensive management practices of the dairy industry, however, according to the National Animal Health Monitoring Systems (NAHMS) only 3.3% of beef heifers in the United States were being synchronized (NAHMS, 1994). The benefits of using estrus synchronization and FTAI include the use of superior genetics, shortened breeding and calving seasons and more uniform calf crops, however the benefits of these technologies are often outweighed by the misperception of these newer technologies in the eyes of the producer. A survey of Illinois producers revealed that the seemingly difficult programs, the questionable profitability, increased time and labor requirements, the high cost of the programs and/or little to no success with the older estrus synchronization programs were the most prominent reasons for not adopting the newer estrus synchronization programs (Kesler, 2003).

The implementation of a controlled internal drug releasing (CIDR) device, although effective in synchronizing estrus, is expensive, costing approximately $10.00 per animal. Originally, CIDR devices had been used from 14 to 21 days in length and were effective at suppressing estrus. Therefore it is presumed that a single CIDR could be used for at least two 7-day synchronization treatments. The objective of these experiments was to determine the effectiveness of used CIDR devices in synchronizing estrus without reducing fertility in beef cattle. In order to understand the significance of progesterone on the female reproductive system and in estrus synchronization protocols, this review of the literature was conducted.

The Estrous Cycle

The estrous cycle is a series of reproductive events that begin at estrus, also referred to as heat, and end at the onset of the subsequent estrus. Cows as well as rodents and sows are females with estrous cycles that occur regularly throughout the entire year and are classified as polyestrous animals. Cows have an estrous cycle length that can range between 17 and 24 days, but are most commonly 21 days in length (Wishart, 1972) with an estrus of approximately 12-18 hours (Nalbandov and Casida, 1942).

The estrous cycle is divided into two phases, the follicular and luteal phases, so named for the dominant structures present on the ovaries during those times. The follicular phase is the shortest phase of the estrus cycle and consists of the period from regression of the corpus luteum (CL) to ovulation which typically occurs 18-30 hours after the onset of estrus (Brewster and Cole, 1941). The luteal phase encompasses approximately 80% of the estrous cycle and consists of the period from ovulation to regression of the CL (Kojima, 2003).

The estrous cycle can be further subdivided into four stages, two within each phase. Within the follicular phase is proestrus, characterized by the formation of ovulatory follicles and estradiol secretion, and the estrus stage when the ovulatory follicle is secreting a maximal concentration of estradiol (E2) resulting in sexual receptivity. Ovulation occurs during metestrus in cattle followed by the formation of the CL and secretion of progesterone (P4). Diestrus is characterized by a mature CL and the sustained P4 secretion by the CL (Senger, 2005).

Endocrine Regulation of the Estrous Cycle

Gametogenesis, folliculogenesis, ovulation, development and function of the corpus luteum and steroidogenesis are all dependent upon the complex and sequential order of secretion of reproductive hormones, and all of these processes can be disrupted from an imbalance of these hormonal interactions.

Follicular emergence occurs in 2 or 3 waves during the luteal phase of the estrous cycle in cattle (Matton et al., 1981) and is prompted by the release of follicle stimulating hormone (FSH) from the anterior pituitary (Adams et al., 1992a; Ginther et al., 1996; Souza et al., 1998). The rise in FSH that stimulates the selection of small antral follicles begins to decline once a follicle has reached approximately 4-5 mm (Ginther, 2000). This follicle will attain dominance and the granulosa cells within this follicle will develop LH receptors (Beg et al., 2001), no longer dependent upon FSH for further development. The dominant follicle also secretes estrogen and inhibin which are potent inhibitors of FSH production (Kaneko et al., 1997), causing subordinate follicles to undergo atresia (Adams et al., 1992a; Ginther et al., 2001). Sustained production of progesterone by the CL prevents stimulation of a GnRH and LH surge sufficient enough to induce ovulation and therefore the dominant follicle in the first wave (in a two wave cycle), or the first and second waves (in a three wave cycle) undergo atresia. The last pool of follicles is selected by a subsequent surge of FSH and the dominant follicle develops around day 18 of the estrous cycle (Ireland et al., 1979).

At this time progesterone concentrations are low due to the absence of a functioning corpus luteum and the preovulatory follicle on the ovary is secreting large quantities of estradiol, the hormone responsible for estrus behavior. The increased concentration of

estradiol elicits a cascade of events in the hypothalamus and pituitary that increases the number of gonadotropin releasing hormone (GnRH) receptors and directly stimulates GnRH secretion (Karsch, 1987). The hypothalamic surge of GnRH prompts the secretion of luteinizing hormone (LH) from the anterior pituitary (Schally et al., 1971) stimulating estradiol production by the ovulatory follicle. LH and estradiol create a positive feedback loop, increasing the amplitude of the LH pulses (Stumpf et al., 1989) until LH concentration is sufficient to cause ovulation of the preovulatory follicle (Abdel-Sater, 2011; Stumpf et al., 1991).

Upon ovulation the theca and granulosa cells of the post-ovulated Graafian follicle undergo biochemical and morphological changes to become the steroidogenic small and large luteal cells of the corpus luteum (Farin et al., 1986; Fitz et al., 1982; Niswender et al., 1986) responsible for the secretion of progesterone and subsequently, the maintenance of pregnancy in all mammals (Allen and Meyer, 1935; Peters et al., 1994). Small luteal cells secrete low basal levels of progesterone, which increase when stimulated by LH, however large luteal cells are responsible for most of the progesterone production by the CL and do not increase progesterone production in response to LH (Farin et al., 1986; Fields and Fields, 1996). During the early post-ovulatory period pulses of LH occur frequently but with low amplitude, due to the presence of estradiol and low levels of progesterone from the newly developed CL (Rahe et al., 1980). However, during the late luteal phase, as the small and large luteal cells of the CL continue to grow and develop, progesterone production increases dramatically causing LH pulses to occur less frequently, but with greater amplitude than during the early luteal phase (Echternkamp and Hansel, 1973; Walters et al., 1984). Should

that oocyte be fertilized the CL will remain and continue to produce progesterone throughout pregnancy, however, if the oocyte is not fertilized, the process of luteolysis will ensue (Kojima, 2003).

Luteolysis is the regression of the CL and is mediated by prostaglandin ($PGF_{2\alpha}$, PGF). Toward the end of the luteal phase the corpus luteum is producing lower concentrations of progesterone allowing the dominant follicle to synthesize estrogen. Estrogen prompts the synthesis of oxytocin (OT) by the hypothalamus as well as the formation of endometrial oxytocin receptors. Oxytocin is released from the posterior pituitary, binds to its endometrial receptors and stimulates uterine secretion of PGF (McCracken et al., 1999). Prostaglandin acts locally, diffusing from the uterine vein to the closely associated ovarian artery and stimulates luteal secretion of OT. A positive feedback between OT and PGF creates irreversible structural damage to the CL (Baird et al., 1976; McCracken et al., 1984; McCracken et al., 1999; Silvia et al., 1991), causing a rapid decline in progesterone concentration.

Estrus synchronization

Synchronization of estrus is the phrase used in which the estrous cycle of an animal is manipulated in order to induce estrus in females at a desirable time. Ideally, estrus synchronization will elicit a highly fertile estrus in the majority of females in the herd within a matter of days, so as to breed them within the same time-frame (Odde, 1990). Estrus synchronization is an effective technology and potential benefits include shortened breeding and calving seasons and also allows for a more uniform calf crop to be produced. This can be

done by one of three methods: using exogenous progestogens such as the orally active compound melengestrol acetate (MGA) or in the form of a controlled internal drug releasing (CIDR) device continually for a minimum of 14 days, regression of the CL in cycling animals using an injection of PGF or using a CIDR short-term 5-7 days followed by an injection of PGF.

Progesterone

Progesterone (P4) is an unsaturated diketone with an empirical formula $C_{21}H_{30}O_2$ and, concomitantly with the other steroid hormones is derived from cholesterol (Ruzicka, 1936). Progesterone was so named by Willard M Allen for being a **proge**stational **ster**oidal ket**one** and was isolated from corpus luteum extracts in 1934 simultaneously by four independent laboratories (Allen, 1970). Progesterone is the predominant hormone within its class of hormones referred to as progestogens and is essential in the regulation of the reproductive functions in all mammalian females.

Effects of Progesterone on the Female Reproductive System

Gustav Jacob Born believed the corpus luteum to be the endocrine gland responsible for uterine proliferation, placentation and the maintenance of pregnancy in mammals (Frobenius, 1998). In 1903 his student, Ludwig Fraenkel, tested the hypothesis by excising the CL from the ovaries of newly pregnant rabbits and observed the termination of pregnancy. In 1928, Allen and Corner mated rabbits and removed the CL 18 hours after mating and found that the embryos would develop for only the four days spent in the

oviducts but would cease growth by day 5. It was assumed that these embryos became atretic because the uterus had not been primed for implantation. Allen and Corner continued their studies by formulating corpus luteum extract and injecting the fluid into castrated adult female rabbits and observed uterine proliferation. Furthermore, if the females were mated 18 hours prior to castration, the pregnancies were carried to term with daily injections (Allen, 1935).

Research on the corpus luteum and progesterone progressed and scientists reported many different physiological changes of the female reproductive organs under the influence of progesterone. Long term daily injections of corpus luteum extracts or progesterone were reported to cause a prolonged interestrus period in mice (Patel, 1930; Phillips, 1937; Selye et al., 1936), rabbits (Makepeace et al., 1936), sheep (Dutt and Casida, 1948), dairy cattle (Christian and Casida, 1948; Willett, 1950) and pigs (Ulberg et al., 1951). Interestrous intervals were also reported as being shortened by approximately 4 days in ewes and cows when progesterone was administered daily for 6 days (sheep) or 10 days (cows) on the afternoon of the first day of observed estrus (Woody et al., 1967). Interestrous intervals have also similarly been decreased by approximately 4 days in cattle when daily injections of progesterone are administered for 3 or 4 days immediately after the observation of estrus up to 36 h after estrus (Battista et al., 1984; Garrett et al., 1988a; Ginther, 1970; Harms and Malven, 1969).

Loutradis et al. (1991) examined the role of progesterone on ovulation by administering the progesterone antagonist mifepristone (RU486) at various times during a gonadotropin-ovulatory treatment (PMSG-hCG). Inhibition of ovulation was observed when

RU486 was given simultaneously with the injection of hCG providing strong evidence that progesterone is necessary in order for ovulation to occur. Ovulation was also inhibited in rhesus monkeys given trilostane (TRL) a progesterone synthesis inhibitor at midcycle providing more evidence in favor of an ovulatory action of progesterone (Hibbert et al., 1996). Progesterone may play a role in regulating the secretion of plasminogen activator, collagenase, and other proteolytic enzymes, responsible for the breakdown of the collagenous follicular wall, necessary for ovulation to occur (Graham and Clarke, 1997; Iwamasa et al., 1992).

Progesterone receptor knock-out (PR KO) mice developed by Lydon et al. (1995) allowed for the definitive characterization of physiological responses specifically caused by progesterone. Although the adult female PR mutant mice did not appear morphologically different from their heterozygous littermates or wild-type controls, these PR KO mice were unable to ovulate mature preovulatory follicles and the granulosa cells did not show any signs of luteinization even after treatment with gonadotropins (Lydon et al., 1995). The uteri of the homozygous mice were hyperplastic with a highly disorganized system of luminal and glandular epithelial cells after treatment with estrogen and progesterone and a marked increase in the inflammatory response in the uterus of the PR KO mouse supporting claims that estrogen is responsible for uterine proliferation and progesterone exhibits anti-inflammatory anti-proliferative effects in uterine tissue. Mammary gland development of the PR mutant mice was compared to ovariectomized wild-type mice, and upon estrogen-progesterone treatment the wild-type females developed normal glandular tissue in the mammary fat pad whereas a more basic ductal branching with a complete absence of lobular

alveoli necessary for lactation was observed in the homozygous females. The PR mutant females also lacked a lordosis response when introduced to a sexually experienced wild-type male even after the females were primed with estrogen and progesterone (Conneely et al., 2001; Conneely et al., 2003; Lydon et al., 1995; Lydon et al., 1996).

Role of Progesterone on Fertility

Progesterone is most commonly known for its role in pregnancy but progesterone also plays a large role in determining the fertility of females through several actions both pre- and post-insemination.

Progesterone concentration has been shown to be positively correlated to the number of antral follicles (antral follicle count, AFC) present during follicular waves, and the animals with greater AFC and therefore, a high concentration of progesterone, had higher pregnancy rates than those animals with a low AFC (Cushman et al., 2009; Mossa et al., 2010). Furthermore, low-AFC cows, although the CL were of similar size, exhibited decreased expression of LH receptors and StAR activity in the luteal cells compared to high-AFC cows (Jimenez-Krassel et al., 2009). During the follicular waves the negative feedback of progesterone on LH prevents the dominant follicle from ovulating and therefore it undergoes atresia (Savio et al., 1993a). High plasma progesterone during this time results in a dominant follicle that is smaller in size and shorter-lived, allowing for an earlier surge in FSH, and an earlier emergence of the next follicular wave (Adams et al., 1992b). However, low plasma progesterone does not suppress LH to the same extent, allowing an increased frequency in LH pulses, extending the lifespan of the dominant follicle beyond normal limits (Savio et al.,

1993b; Sirois and Fortune, 1990) thereby forming a persistent follicle. This persistent follicle continues to produce estradiol (Fortune and Rivera, 1999), delaying the FSH surge for the next follicular wave, which delays the next emergence of follicles.

The ovulation of a persistent follicle is also a cause for decreased fertility. The prolonged exposure of the uterus to estradiol produced by the follicle may alter the uterine environment making it unsuitable for the embryo (Butcher and Pope, 1979), the increased frequency of LH pulses may cause the premature resumption of meiosis (Mattheij et al., 1994) and/or yield an oocyte at a more mature stage of development at ovulation (Diskin et al., 2006; Mihm et al., 1999).

The concentration of plasma progesterone is important, as is the timing of the post ovulatory rise in progesterone. The concentration of progesterone should begin to rise by day 4 following ovulation and reach a maximum concentration around days 8-10 in cattle and sheep (Niswender et al., 2000). A delay in the post ovulatory rise, even as little as a day, results in impaired embryo development, due in part to the asynchrony of the needs of the developing conceptus with the various secretions of the endometrium (Mann et al., 1998a; Mann and Lamming, 1999), decreased embryo survival (Larson et al., 1997) and therefore result in a reduction in pregnancy rate in mated animals (Lamming and Darwash, 1995). Mann et al. (1998a) reported poorly developed embryos producing little to no interferon tau (IFNT), the hormone required for maternal recognition of pregnancy (Roberts, 1996), in cows that displayed a late post ovulatory rise in progesterone, but well elongated embryos producing large quantities of IFNT in cows that exhibited an early rise in progesterone on day 16 following insemination. An increase in progesterone from days 2-5 was shown to

cause significantly advanced embryo development (10 fold increase in conceptus length) on day 14 (Garrett et al., 1988b), whereas Kerbler et al. (1997) induced accessory corpora lutea around day 8 in cows and only observed a slight increase in IFNT production by day-18 embryos, suggesting that the onset of the post ovulatory rise in progesterone and not the final concentration of progesterone attained is most important in controlling embryo development in the cow. IFNT production by day-16 embryos was significantly increased in cows supplemented with progesterone from days 5-9, but not in cows supplemented from days 12-16 (Mann et al., 1998b). Progesterone is initially responsible for down regulating the synthesis of oxytocin receptors in the uterine luminal epithelium which halts uterine production of $PGF_{2\alpha}$ until IFNT, produced by the developing embryo, is able to take over (Lamming and Mann, 1995; Silvia et al., 1991; Wathes et al., 1996). An initial rise in oxytocin receptors can be observed around days 15-17 immediately preceding luteolysis however, the developing conceptus begins to secrete IFNT at the morula and blastocyst stages (Kubisch et al., 1998) and reaches peak production around day 14 just prior to elongation (Robinson et al., 2006) preventing the synthesis of oxytocin receptors (Robinson et al., 1999). IFNT also induces a prostaglandin synthesis inhibitor (Thatcher et al., 1995) and has also been shown to stimulate synthesis of Prostaglandin E_2 (PGE2) a recognized luteotrophic agent (Arosh et al., 2004). Several studies have shown that a delay in the luteal phase rise in progesterone induces a premature up regulation of estrogen receptor-α and an earlier, stronger release of $PGF_{2\alpha}$ that the developing embryo cannot inhibit, causing early embryonic death (Cerri et al., 2011; Mann and Lamming 1995; Mann and Lamming, 1999).

Exogenous Progesterone Sources

Progesterone is the key hormone in the regulation of the estrous cycle and is equally important for follicular growth as well as development and survival of the embryo, making it the key hormone of interest to manipulate for estrus synchronization protocols. Scientists have developed several exogenous sources of progestins with varying modes of dispersal, some of which have been discontinued and others that are still used today.

6-chloro-6-dehydro-17-acetoxy progesterone (CAP), 6-methyl-17 acetoxy-progesterone (MAP), dihydroxyprogesteroneacetophenide (DHPA) and 6-methyl-17-alpha-acetoxy-16 methylene-pregn-4,6-diene-3,20-dione (MGA, melengestrol acetate) are all orally active progestational steroids that are fed to animals at various doses for 14-19 days to produce a tightly synchronized ovulation and estrus. These progestogens are affordable and easily administered however the fertility of treated animals was significantly reduced at first estrus compared to the untreated control animals (Hansel et al., 1966; Odde, 1990; Wiltbank et al., 1967) and are therefore no longer widely used with the exception of MGA which is now mainly used in feedlots to suppress estrus and improve feed efficiency and rate of gain in heifers (Patterson et al., 1989).

Synchro-Mate B was a progesterone-estrogen synchronization treatment in which an implant containing 6 mg of norgestomet was inserted for 9 days followed by an injection of 5 mg of estradiol valerate and 3 mg of norgestomet after implant removal. Brown et al. (1988) conducted a study comparing two estrus synchronization protocols; MGA fed for 14-16 days followed by an injection of PGF 17 days following the last feeding, or Synchro-mate B. The percentage of heifers in estrus within 120 hours after treatment was similar between the

MGA treated animals and the implanted animals as was their degree of synchrony. Synchronized conception rates and synchronized pregnancy rates however were significantly greater in animals treated with MGA than in animals treated with Synchro-Mate B (Brown et al., 1988). The low conception and pregnancy rates as well as the increased labor necessary for use of the Synchro-mate B treatment were disadvantageous for producers and the product was discontinued in 2000.

Controlled Internal Drug Release (CIDR) Device

Controlled Internal Drug Release (CIDR) devices were developed in New Zealand in 1987 and were approved for the use of estrus synchronization of cattle in the United States by the Food and Drug Administration (FDA) in 2002. The CIDR-B is a vaginal insert for cattle (bovine) and consists of a T-shaped nylon spine that is coated with a silicon elastomer impregnated with 1.38g of progesterone (1.9g of progesterone in other countries) with a thin, exteriorized nylon tail for easy removal (Mapletoft et al., 2003). Plasma progesterone concentrations in ovariectomized cows reached near luteal levels (5 to 7 ng/mL) within 24 hours after insertion of CIDR-B and decreased to 2 to 3 ng/mL by day 3 or 4, remaining at this concentration until device removal on day 7, and reaching a baseline concentration within 12 hours after device removal (Martinez, 2002). This rapid rise and fall in serum progesterone concentrations may more effectively regulate LH secretion and follicle development and prevent the ovulation of aged follicles seen in cows treated with MGA (Kinder et al., 1996).

In 1984, Smith et al. conducted two experiments to compare PGF-estrus intervals and pregnancy rates among various progesterone-prostaglandin treatments. In experiment one, 242 Holstein heifers were randomly assigned to receive one of three treatments; 1) control (no treatment), 2) progesterone-releasing intravaginal device inserted for 6 days followed by an injection of 25 mg of PGF at device removal (PRID-6 + PGF-6) or 3) a PRID inserted for 7 days followed by an injection of 25 mg of PGF 24 hours prior to device removal (PRID-7 + PGF-6). Both of the PRID + PGF treatments effectively synchronized estrus as 99% of the treated animals were detected in estrus within 168 hours after the injection of PGF. A tighter synchrony of the animals in the PRID-7 + PGF-6 treatment group was observed although the interval from PGF to estrus was shorter in the PRID-6 + PGF-6 treated animals. Pregnancy rates were not different between PRID-6, PRID-7 or control groups. In experiment 2, 274 Holstein heifers were randomly assigned to one of three treatment groups; 1) control (no treatment), 2) two injections of 25 mg of PGF IM at 11-d interval (2 x PGF) or 3) PRID-7 + PGF-6. All of the animals were inseminated at a pre-determined time (2 x PGF at 80-h and PRID-7 at 84-h after the last injection of PGF). The interval to estrus was longer among the PRID-7 treated animals but more of the PRID-7 treated animals were observed in estrus within 120 hours after PGF injection. The pregnancy rates of the PRID-7 heifers did not differ from the untreated controls but was higher than the pregnancy rates of the 2 x PGF treated heifers (Smith et al., 1984) suggesting that intravaginal progesterone releasing devices can synchronize estrus effectively enough to obtain similar pregnancy rates as non-treated animals when inseminated at a pre-determined time. In a series of experiments conducted by Lucy et al. (2001), cows of varying breed, parity, and body condition score

were bled on days -7 and 0 and the serum was analyzed for progesterone content. If both of the blood samples had a plasma progesterone concentration below 1 ng/mL the animals were considered anestrous (cows) or prepubertal (heifers). The animals were randomly assigned to one of three treatments; 1) control (untreated), 2) a single injection of 25 mg of PGF or 3) a CIDR device inserted for 7 days and an injection of 25 mg of PGF on day 6 and all animals were inseminated approximately 12 hours after the first observation of estrus. In experiment 1, a higher percentage of beef cows in the CIDR + PGF treatment group were observed in estrus within the first three days after PGF injection compared to animals in the PGF and control groups. After treatment with PGF or a CIDR + PGF, cows that were previously diagnosed as anestrous were observed in estrus within 3 days after PGF injection. Although the days until first estrus was observed was longer in anestrous cows than cyclic cows, treatment with PGF or treatment with a CIDR + PGF significantly reduced the days until first observed estrus compared to the control animals. In experiment 2, a higher percentage of beef heifer in the CIDR + PGF treatment group were observed in estrus within 3 days of the last PGF injection compared to the control and PGF treated animals. CIDR + PGF treated heifers exhibited estrus several days prior to control heifers. Heifers treated with a CIDR + PGF also had higher 31-day pregnancy rates compared to PGF treated and control heifers. In experiment 3, dairy heifers were exposed to either the PGF treatment or the CIDR + PGF treatment. The percentage of heifers in estrus within 3 days of the PGF injection was great in the CIDR + PGF treated animals compared to the PGF treated animals. No significant differences among treatments in the dairy experiment might have been caused by a low number of dairy heifers in the experiment, a difference in management or breed differences

between beef and dairy cattle or the majority of treated heifers were already cycling and the addition of a CIDR was therefore not as beneficial. This study provides evidence that synchronizing estrus in beef cows using a CIDR + PGF or PGF alone may be beneficial to producers. Treatment with PGF or a CIDR + PGF reduced the days until first estrus in anestrous cows. A greater percentage of cyclic cows treated with PGF or CIDR + PGF were observed in estrus compared to control cows. The heifers in these experiments benefitted more from synchronization with a CIDR + PGF than PGF alone. A higher proportion of beef and dairy heifers treated with a CIDR + PGF in experiments 2 and 3 were observed in estrus within 3 days compared to the PGF and control animals and the beef heifers in experiment 2 had higher 31-d pregnancy rates compared to the PGF treated and control animals (Lucy et al., 2001).

CIDR devices are also effective in producing a tightly synchronized estrus in a large number of treated animals when used for longer periods (>14 days) without the use of other hormones. MacMillan and Peterson (1993) conducted an experiment using 748 heifers to compare three synchrony treatments; 1) a CIDR device inserted for 7 days followed by an injection of PGF at device removal (7 day CIDR + PGF), 2) CIDR device insertion for 14 days with no luteolytic treatment at device removal (14 day CIDR) and 3) CIDR device insertion for 21 days with no luteolytic treatment at device removal (21 day CIDR). The 21 day CIDR treatment produced the most precise synchrony compared to the 14 day CIDR and 7 day CIDR+PGF treatments within 48 hours after device removal. Conversely, calving rates to first insemination were highest among animals in the 7 day CIDR + PGF treatment group which had less synchrony compared to the longer 14 day CIDR and 21 day CIDR treatments

with more precise synchrony (MacMillan and Peterson, 1993). A decrease in fertility at synchronized estrus during longer treatments with progestogens has been well documented (De Bois and Bierschwal, 1970; Henricks et al., 1973; Hill et al., 1971; Odde, 1990; Roche, 1978; Zimbleman and Smith, 1966) and is believed to be caused by the increased incidence of persistent follicles (Kojima et al., 1995).

Although a poorly synchronized herd or a herd with low fertility as a result of poor management are downfalls to utilizing CIDR devices without an injection of prostaglandin or any other luteolytic factor, it is beneficial for resynchronizing estrus in a herd with cows of unknown pregnancy status because there is no risk of terminating any established pregnancies through the induction of luteolysis. In 1999 a series of experiments were conducted by El-Zarkouny and Stevenson in order to determine the effects of CIDR insertion on resynchronized returns to estrus, conception rate of the first synchronized estrus and fertility of cows impregnated before treatment. Whether or not the addition of estradiol to CIDR after timed-AI (TAI) would cause a more precise synchrony of estrus than the CIDR and what effect it would have on established pregnancies and fertility of non-pregnant cows at the subsequent synchronized estrus was also studied. In experiment 1, ovulation was previously synchronized using either the 1) Ovsynch or 2) Ovsynch + CIDR protocols and cows were inseminated 16 to 20 hours after the second injection of GnRH. Half of the cows from each pre-breeding treatment were then assigned to one of the two post-breeding treatments; 1) insertion of a once-used CIDR 13 days after TAI for 7 days or 2) no further treatment. The addition of CIDR devices increased the synchrony of estrus in animals after device removal however the addition of the CIDR device did not increase the overall rate of

return to estrus in non-pregnant cows between 20 and 26 days after TAI. Synchronized conception rates did not differ between the control animals or the CIDR treated animals and did not compromise established pregnancies. Animals pregnant following the first synchronization protocol that were treated with a CIDR had a higher rate of embryo survival than their control counterparts. During experiment 2, cows were synchronized using the Ovsynch + CIDR protocol and inseminated 16 to 20 hours after the second injection of GnRH. After TAI the CIDR was reinserted on day 13 and removed on day 20. The cows were randomly assigned to one of four treatment groups; 1) CIDR control (no further treatment), 2) 1 mg of estradiol benzoate (EB), 3) 0.5 mg of estradiol cypionate (ECP) or 4) 1 mg of ECP on day 13 after TAI (day of CIDR reinsertion) and a second injection of estrogen at the same dose in each cow 24 hours after CIDR removal. Regardless of estrogen treatment, rates of return to estrus did not differ from control animals nor did synchronized conception rates. The combination of progesterone-estrogen treatment was not detrimental to fertility of subsequent estrus or established pregnancy (El-Zarkouny and Stevenson, 2004). Estrus synchronization protocols can be used to increase the use of artificial insemination in animals, such as beef herds and dairy heifers, that are less intensively managed (Mauleon, 1974) and a major downfall to synchronization protocols using a CIDR plus a luteolytic agent, as opposed to natural service mating, is the need to observe the animals for signs of estrus. More recent approaches to estrus synchronization protocols focus on control of the luteal phase as well as synchronization of follicular emergence and ovulation of the dominant follicle that allow animals to be bred by fixed-time artificial insemination (FTAI) (Larson et al., 2006; Mapletoft et al., 2003; Martinez et al., 2000; Martinez et al., 2012).The two most

common FTAI estrus synchronization protocols are the Ovsynch and Co-synch programs (Bridges et al., 2008a).

The Ovsynch program is a 10-day protocol that begins with an injection of gonadotropin releasing hormone (GnRH) on day 0, an injection of PGF on day 7, a second injection of GnRH on day 9 followed by insemination 8-16 hours after the second GnRH injection. There are three Co-synch protocols; 5-day Co-Synch + CIDR, 7-day Co-Synch and 7-day Co-Synch + CIDR, which are 8, 10 and 10 days in duration respectively. An initial injection of GnRH on day -7 or -5, is followed by an injection of PGF on day 0 (a second injection of PGF 12 hours after CIDR removal in the 5-day protocol) and a second injection of GnRH given concurrently with insemination 60-66 hours after the last PGF treatment in the 7-day programs or at 72 hours after the last injection of PGF in the 5-day program. The major downfall to the 7-day Co-Synch treatment is its inability to initiate cyclicity in peripubertal heifers and anestrous cows, however, the addition of a CIDR device, from the first injection of GnRH to the first injection of PGF, prevents spontaneous ovulation and can progress the onset of estrous in non-cycling animals (Bridges et al., 2008a).

Martinez et al. (2012) conducted an experiment comparing estrus and ovulation synchronization of the Ovsynch protocol or the Ovsynch + CIDR protocol in beef heifers. All of the heifers were treated following the Ovsynch protocol. Half of the animals were assigned to the control group and received no further treatment and half of the heifers were assigned to the Ovsynch + CIDR group and received a CIDR device from day 0 to day 7. There was no difference among treatment with or without a CIDR device in the diameter of the dominant follicle at the time of the first injection of GnRH, CIDR removal or second

GnRH injection. There was also no difference in the area of the CL at the time of first GnRH injection or CIDR removal. The diameter of the dominant follicle was greater prior to ovulation in progesterone treated heifers compared to control animals. The Ovsynch + CIDR treated heifers also showed a reduced variability in the interval from PGF to ovulation in treated animals compared to control heifers (Martinez et al., 2012).

The most commonly used FTAI estrus synchronization protocol in beef cattle is the Co-synch program with or without a progestin which requires the animals to be handled only three times (Geary and Whittier, 1998). A collection of experiments were conducted by Bridges et al. (2008b) comparing the pregnancy rates achieved by FTAI using the Co-synch protocols. In experiment 2, postpartum beef cows were assigned to receive one of two treatments; 1) 7-day Co-Synch + CIDR (7CO-60) or 2) 5-day Co-Synch + CIDR (5CO-60). Timed-AI was performed in all animals 60 hours after the last injection of PGF. Pregnancy rates from TAI and breeding season pregnancy rate of cyclic cows did not differ between the 7-day or 5-day Co-synch however the breeding season pregnancy rate in previously anestrous cows was higher in animals treated with the 7-day Co-synch protocol compared to the 5-day Co-Synch protocol. A third and fourth experiment was conducted in order to compare the 7-day Co-synch TAI 60 hour protocol to the 5-day Co-synch TAI 72 hour protocol. Postpartum beef cows were assigned to one of two treatments; 1) the 7-day Co-Synch + CIDR protocol with TAI performed at 60 hours after PGF (7CO-60) or 2) the 5-day Co-Synch + CIDR protocol with TAI performed at 72 hours after PGF (5CO-72). The pregnancy rates to TAI were higher in the 5CO-72 protocol compared to the 7CO-60 protocol and no difference was detected between the breeding season pregnancy rate, which provides strong evidence that

the modified 5-day Co-Synch + CIDR (5CO-72) protocol most effectively synchronizes estrus and ovulation in both cyclic and acyclic females in order to be bred by FTAI, compared to the 7CO-60 or 5CO-60 protocols but each of the protocols are effective in inducing cyclicity during the breeding season (Bridges et al., 2008b).

Although beef producers concede that assisted reproductive strategies are effective methods to rapidly advance the genetic progress of their herd, only 7.1% of all beef females are artificially inseminated and an ever lesser percentage of animals undergo estrus synchronization (Lamb, 2001).

Cost of Estrus Synchronization Protocols and Artificial Insemination

There are numerous benefits to utilizing estrus synchronization and fixed-time artificial insemination protocols, in livestock production systems. The use of FTAI allows producers with the means of disseminating superior genetics throughout their herd. Producers can purchase semen from bulls with a variety of proven traits to cater to their operation. Low birth weights, high weaning and yearling weights, along with muscle and marbling are desirable characteristics in terminally bred animals. Increased milk production, temperament, mothering and longevity are among some of the desirable traits in replacement heifers. Synchronizing estrus in the herd allows for shorter breeding and calving seasons that begin earlier creating a more uniform calf crop (Johnson et al., 2003; Rogers et al., 2012).

The benefits of estrus synchronization and FTAI are often overlooked by the cost of implementing the programs, along with the time and labor requirements, complexity of the

programs and questionable profitability, among others, according to Illinois and North Carolina producers (Joseph, 2013; Kesler, 2003).

The costs of natural service are often overlooked but are a necessary tool for comparison when determining the potential benefit of adopting estrus synchronization protocols and FTAI. Purchase prices of seedstock bulls are highly variable and averages have been reported between $2,200.00 and $3,000.00 (Johnson et al., 2003; Miller, 2011; Parish and Riley, 2011). Assuming an average purchase price of $2,600.00, a salvage value of $1100.00 and 4 years of service, the bull costs $1,500.00 or $375.00 per year. Additional costs from bull ownership include feed, necessary facilities, and veterinary costs which add an additional $400.00 per year for a total of $775.00 per year (Miller, 2011). A mature bull can breed between 25 and 35 females in a pasture-based management system (Gadbury and Powell, 2009). Assuming an average of 30 females and a 91% breeding season pregnancy rate (Johnson et al., 2003; Miller, 2011; Parish and Riley, 2011), the cost averages out to $28.39 per pregnancy from natural mating.

As previously mentioned, the most commonly used estrus synchronization-FTAI in beef cattle is the 7-day Co-Synch + CIDR program. This program requires two injections of GnRH, a CIDR device and an injection of PGF. A 30 mL vial of GnRH (Cystorelin) costs $41.10 and contains 15 doses, for a total of $2.74 per dose or $5.48 per cow synchronized. A package of ten CIDR devices costs $121.00 or $12.10 per cow. Prostaglandin $F_{2\alpha}$ (Lutalyse) costs $59.10 for a 100 mL vial containing 25 doses for a total of $2.36 per dose. The cost of synchronization per cow totals $19.94 (Agtechinc.com). Assuming $15.00 per straw of frozen semen and $5.00 per animal AI technician fee, and a 65% conception rate, it costs

$61.45 per pregnancy when animals are synchronized with the 7-day Co-Synch + CIDR program and bred by timed artificial insemination. Calving rates, percentages of cows calving in the first 30 days of the season, percentage of calf crop weaned, weaning age and weaning weight were reported higher in synchronized-FTAI animals than animals from natural matings (Anderson and Deaton, 2003; Rodgers et al., 2012). The average weaning weights of calves from synchronized-FTAI (ES/FTAI) females and calves from natural matings were reported to be 577 pounds and 505 respectively, a 72 pound difference (Anderson and Deaton, 2003). According to the United States Department of Agriculture National Agricultural Statistics Service (NASS) weaned calves were worth an average of $1.42 per pound in early 2012 (NASS, 2012). A $101.24 increase in sale price per calf from ES/FTAI females was obtained by investing an additional $33.06 in estrus synchronization and FTAI for a total return of investment of $68.18 per weaned calf from an ES/FTAI cow excluding the savings expected from reduced bull costs. The profitability of estrus synchronization and FTAI programs can be increased by another $6.05 when the CIDR device is used a second time and by $8.07 when the CIDR device is used for a third time, provided multiple uses do not negatively affect pregnancy rates of the herd.

The Reuse of Controlled Internal Drug Release Devices

Macmillan and Peterson (1993) reported that 96.0% of all females (237/247) treated with a CIDR device for 21 days were observed in estrus within 48 hours of device removal resulting in the most precise synchrony of all other treatments performed. Although the fertility of these animals were reduced and long-term treatment with progestogens is not

recommended, this experiment provides evidence that CIDR devices containing 1.9g of progesterone release sufficient progesterone for 21 days to prevent estrus and may therefore be reused at least once more to effectively synchronize estrus. Getting two or three uses of the CIDR would have a significant effect since the CIDR is the most costly item (approximately $10.00 per device) in the synchronization procedure. Studies have found that used CIDR devices do release progesterone in similar concentrations to new devices (Cerri et al., 2009; Colazo et al., 2004; Long et al., 2009; Zuluaga and Williams, 2008) and have produced similar results for synchronization.

Cerri et al. (2009) reported higher average mean plasma progesterone concentrations in cycling dairy cows treated with a new CIDR device compared to animals treated with a previously used-autoclaved CIDR, but both below 1 ng/mL which was attributed to the use of lactating dairy cows that metabolize progesterone at a much higher rate due to increased blood flow. Cows bearing a corpus luteum on one of the ovaries were presynchronized and 48 hours after first injection of PGF the animals were randomly assigned to receive a new or used CIDR device. There was no difference in the percentage of control and treated cows that displayed estrus after insert removal. In a second experiment, it was found that an increased number of anestrous dairy cows resumed estrus after treatment with a new or previously used CIDR than in the control animals but no difference was observed between CIDR treated groups. The incidence of a shorter than normal estrous cycle was also lesser for cows receiving a new or used CIDR compared to control animals. These results suggest that an effective synchronization of a herd can be obtained with once-used CIDR devices in cyclic and acyclic females. In beef cattle, mean plasma progesterone concentrations during a 7-day

treatment period with a new, 7-day previously used autoclaved or a 7-day previously used disinfected CIDR devices were compared and found to be higher in the new and used-autoclaved devices than for the used-disinfected devices but were always at concentrations above 1 ng/mL (Zuluaga and Williams, 2008) which was further supported by the findings of Cerri et al. (2009) indicating a concentration of progesterone sufficient enough to suppress and synchronize estrus and ovulation two times per CIDR. Long et al. (2009) performed an experiment comparing the plasma progesterone concentration of ovariectomized beef cows after the use of a new, once- or twice-used CIDR device. The final plasma progesterone concentration (PPC) of twice-used CIDR was below 1 ng/mL, indicating that a CIDR used a total of three times may reduce pregnancy rates to TAI.

In order to determine whether or not twice-used CIDR devices were effective in synchronizing estrus for FTAI a collection of experiments were performed by Colazo et al. (2004). In experiment 1, heifers were examined for the presence of luteal tissue by ultrasonography to determine the number of prepubertal and pubertal animals. Heifers were randomly assigned to receive a new or once-used CIDR and 1 mg of estradiol cypionate (ECP) alone, or in addition to 100 mg of progesterone. Pregnancy rate did not differ between prepubertal and pubertal animals. Pregnancy rates did not differ between new CIDR with or without 100 mg of progesterone and once-used CIDR with or without 100 mg of progesterone. Experiment 2 was designed to compare pregnancy rates obtained from animals treated with a once- or twice-used CIDR. On day 0 females were randomly assigned to receive either a once-used or a twice-used CIDR and an injection of 1 mg estradiol benzoate (EB) alone, or in addition to 100 mg progesterone. Heifers had a higher pregnancy rate than

cows and females treated with a once-used CIDR had a higher pregnancy rate than the animals treated with a twice-used CIDR (Colazo et al., 2004) suggesting that a CIDR could be used for 2 7-day synchronization protocols (14 days total) but the use of a CIDR for 3 7-day protocols (21 days total) decreases the herds pregnancy rate.

The reviewed literature cites numerous benefits when a CIDR device is added to an estrus synchronization protocol, especially in herds that may have peripubertal heifers and/or anestrous cows. Several studies using plasma progesterone concentration or pregnancy rates to compare used CIDR devices to new devices have been conducted, concluding that once-used devices release similar progesterone concentrations to new CIDR devices. There have been very few studies conducted comparing progesterone release or pregnancy rates obtained when new, once-used or twice-used devices were administered. However, of these studies, the animals were not synchronized using newer programs such as 7-day Co-Synch + CIDR protocol which utilize multiple hormones to not only control regression of the CL but follicular emergence and turnover as well. In order to compare the efficacy of a twice-used CIDR device to a once-used device in synchronizing estrus and maintaining the fertility of nulliparous and multiparous beef animals, the following experiments were conducted.

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INTRODUCTION

Artificial insemination has been commercially available for over 75 years and is the most popular assisted reproductive technology because of its ease of application, its relative affordability and most importantly its effectiveness at rapidly increasing the genetic progress within herds (Vishwanath, 2003). However, the use of artificial insemination was limited until the development of estrus synchronization protocols which allowed producers to manipulate the estrous cycle of their females and induce ovulation at a time of their choosing. Current estrus synchronization protocols employ hormones such as prostaglandin to regulate luteolysis and gonadotropin-releasing hormone to control the emergence of follicular waves and ovulation of a viable dominant follicle. The addition of exogenous progesterone in the form of CIDR devices to these synchronization protocols further enhances fixed-time artificial insemination (FTAI) in beef cattle (Busch et al., 2007). The benefits of employing estrus synchronization are numerous and include comparable pregnancy rates to AI following estrus detection, shortened breeding and calving seasons and a higher degree of calf uniformity. It may also decrease labor costs as well because there is no need for daily estrus detection and breeding. Beef producers have been reluctant to adopt these technologies for several reasons including the complexity of synchronization protocols, the increased time and labor requirements, cost of implementing the programs and questionable profitability (Joseph et al., 2013; Kesler, 2003).

The 7-day Co-Synch + CIDR treatment is one of the more popular synchronization protocols used by beef producers and has been reviewed by numerous scientists that have reported pregnancy rates of 43-66%, 45-68% and 51% when animals underwent FTAI 48,

54-66 and 72 hours after the injection of prostaglandin respectively (Bridges et al., 2008; Busch et al., 2007; Dobbins et al., 2006; Kasimanickam et al., 2006; Kasimanickam et al., 2008; Lamb et al., 2001; Larson et al., 2006; Martinez et al., 2002; Stevenson et al., 2003). The program begins on day 0 with an injection of GnRH concurrently with the insertion of a CIDR device. On day 7 an injection of PGF is administered and the CIDR device is removed. A second injection of GnRH and Fixed-Time Artificial Insemination (FTAI) is performed in 60-66 h after the injection of PGF. The CIDR device is the most expensive supply needed for this protocol, costing approximately $10.00 per animal for a 7 day use. CIDR devices have been implemented in protocols lasting 21 days (Macmillan and Peterson, 1993) and although fertility was reduced the device was still effective at suppressing estrus and creating a tightly synchronized group of females, indicating that a used CIDR device could still be effective in several shorter estrus synchronization protocols. Studies have, in fact, demonstrated that once-used devices release progesterone at concentrations similar to that of new devices (Cerri et al. 2009; Colazo et al., 2004; Zuluaga and Williams, 2008;) and result in similar pregnancy rates, but plasma progesterone concentrations in animals treated with a twice-used insert were lower than animals treated with a new or once-used device (Colazo et al., 2004; Long et al., 2009) which resulted in lower pregnancy rates. According to Roeber et al. (2001), among the top five quality challenges in both beef and dairy market animals, reported by the National Market Cow and Bull Beef Quality Audit – 1999, is the incidence of injection-site lesions in the muscle. The development of lesions from injections of reproductive hormones has not been investigated however, the option of administering PGF subcutaneously as a preventative measure has been studied (Chebel et al., 2007; Colazo et al., 2002a; Colazo et

al., 2002b) and concluded that the CL regresses after a subcutaneous injection of the recommended dose of PGF. These are the first experiments to the author's knowledge that have been conducted to compare the efficacy of a twice-used CIDR vs. a once-used device, implemented in a 7-day Co-Synch + CIDR protocol, as well as a SubQ versus IM injection of PGF, in synchronizing estrus without adversely affecting pregnancy rates to FTAI in nulliparous and multiparous beef animals.

MATERIALS AND METHODS

The North Carolina State University Institutional Animal Care and Use Committee approved all of the procedures described in the following experiments.

Experiment 1

Animals

The animals used in this study were purebred Angus and Angus cross heifers (n = 24) between 13 and 15 months of age housed at the Tidewater Research Station located in North Carolina maintained in pastures separate from the cows. The heifers were fed coastal Bermuda grass hay and pasture and had ad libitum access to water.

Intravaginal Insert Preparation

The CIDR devices used in this study had been used previously either once or twice before in animals at the Tidewater Research Station. The once-used CIDR devices were previously used in animals synchronized in a 7-day Co-Synch + CIDR protocol and then washed, air dried and stored at room temperature. The twice-used CIDR devices were previously used for two 7-day Co-Synch + CIDR protocols, for a total of 14 days. The CIDR devices were washed after the first treatment and again after the second treatment, air dried and stored at room temperature. The CIDRs were washed by first being rinsed with water and washed in diluted Novalsan® solution (chlorohexidine diacetate, Fort Dodge Laboratories, Fort Dodge, IA) and rinsed again with water.

Treatment Groups

The beef heifers were randomly assigned to the control or treatment groups; control animals were administered a once-used CIDR device and treatment animals received a twice-used CIDR. All of the animals were synchronized using the 7-day Co-Synch + CIDR protocol (Figure 1). Briefly, an injection of 100 μg GnRH (Cystorelin®, gonadorelin diacetate tetrahydrate, Merial, Duluth, GA) was given on d 0 concurrently with the insertion of a once-used or twice-used Eazi-Breed™ CIDR®(progesterone, Zoetis, Madison, NJ) and an injection of 25 mg PGF (Lutalyse®, dinoprost tromethamine, Zoetis, Madison, NJ) and device removal 7 d later. A second injection of GnRH and FTAI was performed in all animals 60-66 h after PGF by one trained AI technician.

Pregnancy Diagnosis

Pregnancy was diagnosed in all animals via transrectal ultrasonography 30-40 days after fixed-time artificial insemination by veterinarians from North Carolina State University College of Veterinary Medicine.

Statistical Analyses

Statistical Analyses were performed using the mixed procedure in SAS version 9.3 (PROC MIXED, Cary, NC) and examined the effect of treatment (once- vs. twice-used CIDR device) on pregnancy rates to AI (Table 1). Pregnancy rates obtained using the 7-day Co-Synch + new CIDR protocol from 2010-2012 in nulliparous animals at the Tidewater Research Station were compared to the pregnancy rates obtained from the current experiment

(2013) (Table 2) were analyzed using the chi-square test in SAS version 9.3 (CHISQ, Cary, NC).

Experiment 2

Animals

The animals used in this study were purebred Angus yearling heifers and 2-year old heifers, held from their first breeding season, (n= 99) between 13 and 27 months of age and cows (n = 43) between 48 and 74 months of age housed at the Upper Piedmont (Reidsville) Research Station located in North Carolina. The nulliparous and multiparous animals were maintained on pasture separate from each other. The animals were fed coastal Bermuda grass hay and pasture and had ad libitum access to water.

Intravaginal Insert Preparation

The CIDR devices used in this study had been used previously either once or twice before in animals at the Reidsville Research Station. The once-used CIDR devices were previously used in animals synchronized in a 7-day Co-Synch + CIDR protocol and then washed, air dried and stored at room temperature. The twice-used CIDR devices were previously used for two 7-day Co-Synch + CIDR protocols, for a total of 14 days. The CIDR devices were washed after the first treatment and again after the second treatment, air dried and stored at room temperature. The CIDRs were washed by first being rinsed with water and washed in diluted Novalsan® solution (chlorohexidine diacetate, Fort Dodge Laboratories, Fort Dodge, IA) and rinsed again with water.

Treatment Groups

The animals were randomly assigned to the control or treatment groups; control animals were given a once-used CIDR device and treatment animals received a twice-used CIDR. All of the animals were synchronized using the 7-day Co-Synch + CIDR protocol (Figure 2). Briefly, an injection of 100 μg GnRH (Cystorelin®, gonadorelin diacetate tetrahydrate, Merial, Duluth, GA) was given on d 0 concurrently with the insertion of a once-used or twice-used Eazi-Breed™ CIDR®(progesterone, Zoetis, Madison, NJ) and an injection of 25 mg PGF given either intramuscularly (IM) or subcutaneously (SubQ) (Lutalyse®, dinoprost tromethamine, Zoetis, Madison, NJ) and device removal 7 d later. A second injection of GnRH and FTAI was performed in all animals 60-66 h after PGF by one of two trained AI technicians.

Pregnancy Diagnosis

Pregnancy was diagnosed in all animals via transrectal ultrasonography 30-40 days after fixed-time artificial insemination by veterinarians from North Carolina State University College of Veterinary Medicine.

Sample Collection and Analysis

Whole blood samples were taken in a random subset of heifers at the Reidsville Research Station by coccygeal venipuncture and collected into Vacutainer® (Becton, Dickson and Company, Franklin Lakes, NJ) tubes just prior to device removal and stored at 4 °C. The

blood was centrifuged for 15 min at 2500 rpm and serum was collected and stored frozen at -20 °C until assayed for progesterone concentration.

Progesterone Analysis

A Coat-A-Count® radioimmunoassay kit (Siemens Medical, Los Angeles, CA) previously validated in our laboratory for use with bovine serum (Whisnant and Burns, 2002) was used to determine the concentration of plasma progesterone in the blood samples collected from the Reidsville heifers according to manufacturer's instructions. The kit works through competitive binding; a high concentration of progesterone in the serum will quickly bind to the antibodies, preventing the radio-labeled progesterone from binding, producing a low count. Conversely, low serum progesterone will allow the radio-labeled progesterone to bind to all of the unbound antibodies, producing a high count. The intra-assay coefficient of variation was 1.35%.

Statistical Analyses

Statistical Analyses were performed using the mixed procedure in SAS version 9.3 (PROC MIXED, Cary, NC). The mixed procedure was used to examine the relationship between treatment and plasma progesterone concentration (Figure 3). The mixed procedure also examined the effects of all measured variables - treatment, AI technician, route of administration and parity – as well as all 2-, 3- and 4-way interactions for the combined dataset (Table 3). Pregnancy rates obtained using the 7-day Co-Synch + new CIDR protocol from 2010-2012 at the Reidsville Research Station were compared to the pregnancy rates

obtained from the current experiment (2013) (Table 4) were analyzed using the chi-square test in SAS version 9.3 (CHISQ, Cary, NC).

RESULTS

Experiment 1

Pregnancy rates to FTAI were 91.7 ± 9.9% and 83.3 ± 9.9% in control and treatment animals respectively (Figure 4) which were not significantly different *(P = 0.56)*. Females at the Tidewater Research Station are regularly synchronized using the 7-day Co-Synch protocol + new CIDR. Pregnancy rates obtained by the same technician in the nulliparous animals at Tidewater did not differ between years (Table 2).

Experiment 2

A significant difference *(P = 0.01)* in plasma progesterone concentration was observed in heifers assigned to the control group receiving the once-used CIDR device and heifers assigned to the treatment group receiving the twice-used CIDR devices (3.4 ± 0.5 ng/mL vs. 1.4 ± 0.5 ng/mL) (Figure 3) however pregnancy rates to FTAI in control and treatment animals (Figure 5A) were 75.4% and 71.7% *(P = 0.67)*. There was no main effect (*P = 0.60)* of AI technicians (Figure 5B) on pregnancy rates (Technician 1, 75.9% vs. Technician 2, 71.3%). Animals receiving PGF injection intramuscularly had pregnancy rates of 72.6% and animals receiving PGF subcutaneously had pregnancy rates of 74.5% *(P = 0.83)* (Figure 5C). There was also no main effect *(P = 0.82)* of parity (Figure 5D) on pregnancy rates to FTAI (nulliparous 72.6% vs. multiparous 74.6%). The 2- and 3-way interactions were also examined and no significant difference was observed, however there was a significant difference *(P = 0.04)* observed with the 4-way interaction (Table 3).

Pregnancy rates obtained by the same two AI technicians in the nulliparous and multiparous animals at Reidsville were not different between years although there was a 16% increase in pregnancy rate from 2012 to 2013 (58.0% vs. 74.6% respectively) which could be attributed to cows greater than 7 years of age being culled from the herd (Table 4). A possible location effect was examined by comparing the pregnancy rates of the nulliparous animals at each location from 2010-2013 and were not different.

DISCUSSION

Artificial insemination and estrus synchronization have been adopted by the majority of dairy operations and have been an effective means to improve the genetic progress within a herd. Beef producers have been reluctant to incorporate this technology due in part to the short-term cost of the implementation of these reproductive protocols and the questionable profitability from their use (Joseph, 2013; Kesler, 2003). Numerous experiments ranged from using unaltered or ovariectomized females receiving used CIDR devices to analyze plasma progesterone concentration without breeding and pregnancy detection (Cerri et al., 2009; Long et al., 2009; Zuluaga and Williams, 2008) and breeding and diagnosing pregnancy in animals without the collection of blood for analysis of progesterone concentration (Colazo et al., 2004).

Bull ownership costs approximately $775.00 per year when its sale price was $2,600.00 (Miller, 2011). In a pasture-based management system a bull can breed 30 females a season and with a 91% breeding season pregnancy rate (Johnson et al., 2003; Miller, 2011; Parish and Riley, 2011), the cost averages out to $28.39 per pregnancy from natural mating. The cost of synchronization per cow totals $19.94 when the 7-day Co-Synch + CIDR program is used. Assuming $15.00 per straw of frozen semen and $5.00 per animal AI technician fee, and a 65% conception rate, it costs $61.45 per pregnancy when animals are synchronized and bred by timed artificial insemination. The average weaning weights of calves from synchronized-FTAI (ES/FTAI) females were reported to be 72 pounds heavier than calves from natural matings (Anderson and Deaton, 2003). An average of $1.42 per pound in early 2012 (NASS, 2012) yields a $101.24 increase in sale price per calf from

ES/FTAI females by investing an additional $33.06 in estrus synchronization and FTAI for a total return of investment of $68.18 per weaned calf, excluding the savings expected from reduced bull costs. Reducing the short-term cost associated with these technologies may provide beef producers with an incentive to begin adopting these practices into their herds. One method of reducing the cost of estrus synchronization is the reuse of CIDR devices which would decrease the cost of CIDR to $6.05 per cow when the CIDR device is used a second time and to $4.03 per cow when the CIDR device is used for a third time.

Several studies have concluded that once-used CIDR devices release progesterone at concentrations similar to new devices and do not decrease pregnancy rates. Plasma progesterone profiles revealed that once-used CIDR release progesterone at or above 1.0 ng/mL during its second 7-day use but twice-used devices only released a subluteal concentration of 0.9 ng/mL on the last day of its third use. The plasma progesterone profiles suggest that twice-used CIDR devices may not be effective at suppressing estrus (Long et al., 2009) which supported the findings of Colazo et al. (2004). Pregnancy rates in animals administered a twice-used CIDR device were lower than the pregnancy rates in animals receiving a new or once-used device. These findings contradict the results obtained in the current experiments. A progesterone concentration of at least 1 ng/mL is indicative of a basal luteal phase progesterone concentration, necessary to suppress estrus, LH secretion and ovulation. The plasma progesterone concentration prior to device removal from the 26 nulliparous animals receiving the twice-used device averaged 1.4 ng/mL, suggesting a concentration sufficient enough to suppress ovulation until the CIDR device was removed

and PGF was administered to lyse the CL, which was confirmed by similar pregnancy rates among animals in the two treatment groups.

The discrepancy in plasma progesterone concentration and pregnancy rates may have arisen from the differing methods of sanitation. Both Long et al. (2009) and Colazo et al. (2004) autoclaved their CIDR devices after each use. Zuluaga and Williams (2008) reported an enhanced rise in plasma progesterone, greater than that observed in animals receiving new CIDR devices, in animals receiving a once-used autoclaved CIDR, during the first two days of insertion. Plasma progesterone then decreased, below that of animals receiving the used-disinfected devices, by day 4. This rapid increase and decrease in plasma progesterone could be caused by the formation of crystalline progesterone on the surface of the device, which was observed in progesterone-releasing intravaginal devices (PRID) after being autoclaved (Mcphee et al., 1983). High-pressure steam sterilization could alter the structural integrity of the progesterone-impregnated silicone moulding, and in doing so, decrease the effectiveness of a CIDR device after each sterilization.

It can be assumed that the scientists autoclaved the used CIDR devices to prevent the possibility of transmitting diseases to other unaffected animals within the herd. However, producers don't often have autoclaves on-site. Disinfecting devices is an important objective when multiple uses are being recommended and further research into feasible disinfecting methods should be investigated.

One of the top five beef quality assurance challenges is injection-site lesions in the muscle (Roeber et al., 2001). Vaccinations and antibiotics are often given intramuscularly in the neck, avoiding the hindquarters, and the possibility of creating lesions in higher priced

retail cuts. Reproductive hormones, however, are often given in the hindquarters. Research into injection-site lesions has not focused on the possibility of IM hormone injection causing muscle tissue damage. Animals in experiment 2 were randomly assigned to receive their injection of PGF in the muscle or under the skin, to determine the efficacy of a subcutaneous injection of PGF in regression of the CL. Previous experiments have investigated the possibility of SubQ injections of PGF (Chebel et al., 2007; Colazo et al., 2002a, Colazo et al., 2002b) and have reported similar rates of luteolysis between IM and SubQ injections which is also consistent with these findings.

The experiments conducted by our laboratory, namely experiment 2, were conducted in a way that closely resembled what can be found on a typical farm; animals of varying parity, at various days post-partum, being bred by multiple technicians, and devices disinfected with substances such as an iodine or chlorohexidine solution instead of high-steam pressure sterilization (autoclave), and provides strong evidence that in a practical farm setting CIDR devices can be used a total of three times in 7-day estrus synchronization protocols without adversely affecting the pregnancy rate to AI, making these protocols more economical for the producers that utilize them.

DIRECTION FOR FUTURE RESEARCH

Further research into the effect of treatment on parity may indicate that the reuse of once- or twice-used CIDR devices may be most effective/least detrimental in heifers, or cows that are past a certain day postpartum. Blood collection at various times through CIDR insertion would provide an accurate profile of plasma progesterone and blood collected from animals of all parities should be collected and analyzed to discern a possible parity effect. Ultrasonography of ovaries may also be beneficial in order to detect the presence of a corpus luteum which may be the cause for high plasma progesterone concentration observed in some of the animals. Ovarian mapping via ultrasonography would also be beneficial in order to determine whether treatment has an effect on the incidence of polycystic ovaries and to examine follicular turnover. Research could also be conducted using shorter estrus synchronization protocols such as the 5-day Co-Synch + CIDR to determine if fertility rates are higher and if a CIDR could be used four times without adversely affecting fertility.

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Tidewater Estrus Synchronization Protocol

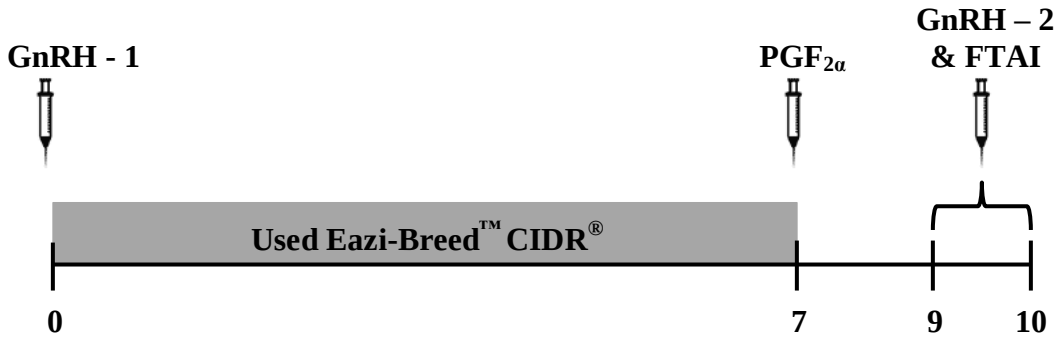

Figure 1: 7-day Co-Synch + Progestin estrus synchronization protocol. The used CIDR device was inserted vaginally concurrently with an injection of GnRH on day 0. The CIDR was removed on day 7 followed by an injection of $PGF_{2\alpha}$. A second injection of GnRH was given with Fixed-time Artificial Insemination (FTAI) 60-66 hours after the injection of $PGF_{2\alpha}$.

Experiment 1 Final Model Used for Analysis of Treatment of Nulliparous Tidewater Animals

Table 1: Statistical model used for analysis of treatment effect on pregnancy rates of the nulliparous animals at the Tidewater Research Station.

	DF	F VALUE	P VALUE
TOTAL (N - 1)	**23**		
MODEL	**1**		
EFFECT			
Treatment (TRT)	1	0.3	0.56
ERROR	**22**		

Four Year Pregnancy Rates of Nulliparous Animals at Tidewater Research Station

Table 2: Pregnancy rates to AI of the nulliparous animals at Tidewater from 2010 – 2013. All animals were synchronized following the 7-day Co-synch + CIDR protocol. Animals from 2010 – 2012 received new CIDR devices and animals from 2013 were involved in the current experiment and received either a once- or twice-used device. Pregnancy rates were not different between years (*P = 0.41*).

Tidewater		
	Nulliparous	
2010	19/27	70.0%
2011	15/22	68.0%
2012	17/23	74.0%
2013	21/24	87.5%

Reidsville Estrus Synchronization Protocol

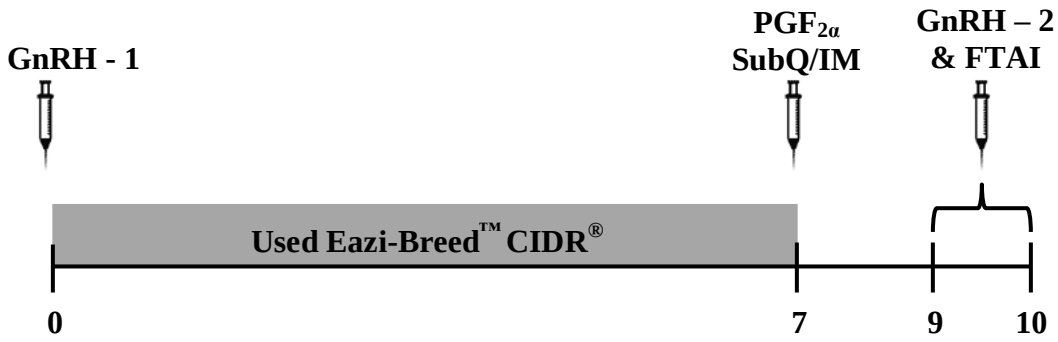

Figure 2: 7-day Co-Synch + Progestin estrus synchronization protocol. The used CIDR device was inserted vaginally concurrently with an injection of GnRH on day 0. The CIDR was removed on day 7 followed by an injection of $PGF_{2\alpha}$ given either subcutaneously (SubQ) or intramuscularly (IM). A second injection of GnRH was given with Fixed-time Artificial Insemination (FTAI) 60-66 hours after the injection of $PGF_{2\alpha}$.

Progesterone Concentration (ng/mL) by Treatment

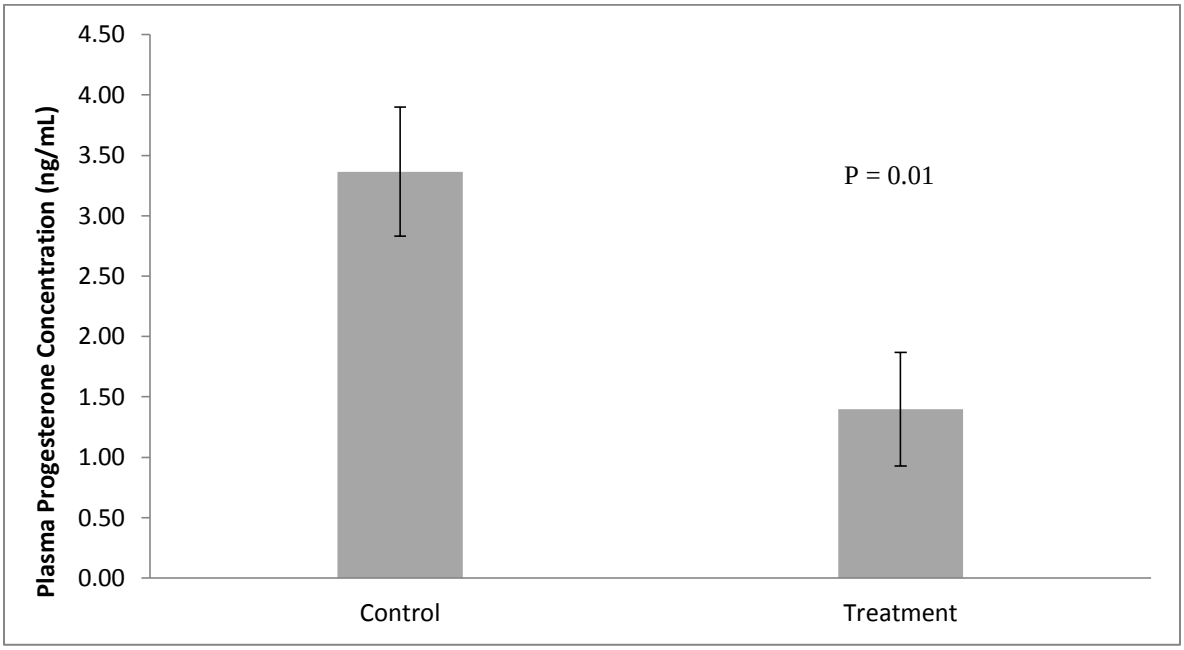

Figure 3: Progesterone concentration by treatment. Control animals *(n = 26)* receiving the once-used CIDR devices had plasma progesterone concentrations (PPC) significantly higher *(P = 0.01)* than treatment animals *(n = 26)* receiving the twice-used CIDR devices (3.4 ± 0.5 ng/mL vs. 1.4 ± 0.5 ng/mL).

Experiment 2 Final Model Used for Statistical Analysis of Treatment, AI Technician, Route of Administration and Parity of Reidsville Animals

Table 3: Statistical model used for analysis of the main effects of treatment, AI technician, route of administration and parity and all 2-, 3- and 4-way interactions on pregnancy rates of the nulliparous and multiparous animals at the Reidsville Research Station

	DF	F VALUE	P VALUE
TOTAL (N - 1)	**141**		
MODEL	**15**		
EFFECT			
Treatment (TRT)	1	0.2	0.67
Technician (AI)	1	0.3	0.60
Route of Administration (INJ)	1	0.0	0.83
Parity (P)	1	0.0	0.82
TRT*AI	1	0.0	0.85
TRT*INJ	1	1.3	0.25
TRT*P	1	0.1	0.74
AI*INJ	1	0.1	0.80
AI*P	1	2.1	0.14
INJ*P	1	0.0	0.53
TRT*AI*INJ	1	0.4	0.52
TRT*AI*P	1	0.1	0.78
TRT*INJ*P	1	0.0	0.91
AI*INJ*P	1	0.4	0.55
TRT*AI*INJ*P	1	4.5	0.04
ERROR	**126**		

Four Year Pregnancy Rates of Nulliparous and Multiparous Animals at Reidsville Research Station

Table 4: Pregnancy rates to AI of the nulliparous and multiparous animals at Reidsville from 2010 – 2013. All animals were synchronized following the 7-day Co-synch + CIDR protocol. Animals from 2010 – 2012 received new CIDR devices and animals from 2013 were involved in the current experiment and received either a once- or twice-used device. Pregnancy rates of the nulliparous (*P = 0.74*) and multiparous (*P = 0.13*) did not differ between years.

Reidsville				
	Nulliparous		Multiparous	
2010	40/58	69.0%	77/132	58.0%
2011	43/61	70.5%	69/128	54.0%
2012	36/56	64.0%	76/131	58.0%
2013	72/99	72.6%	32/43	74.6%

Effect of Treatment on Pregnancy Rate to AI in Tidewater Heifers

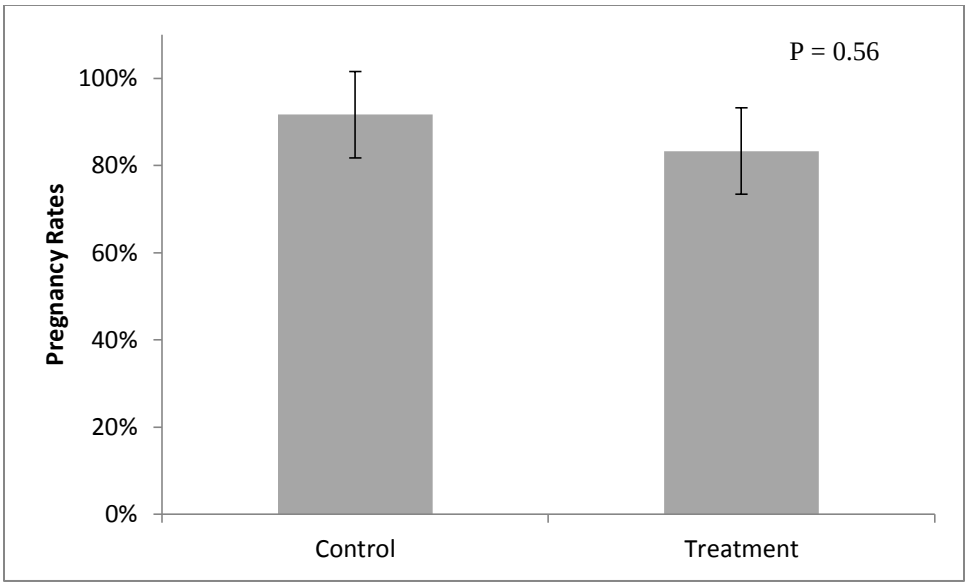

Figure 4: The effect of treatment (once- vs. twice-used CIDR device) on pregnancy rate to FTAI in beef heifers *(n = 24)* at Tidewater Research Station.

Main Effects on Pregnancy Rates to FTAI in Reidsville Animals

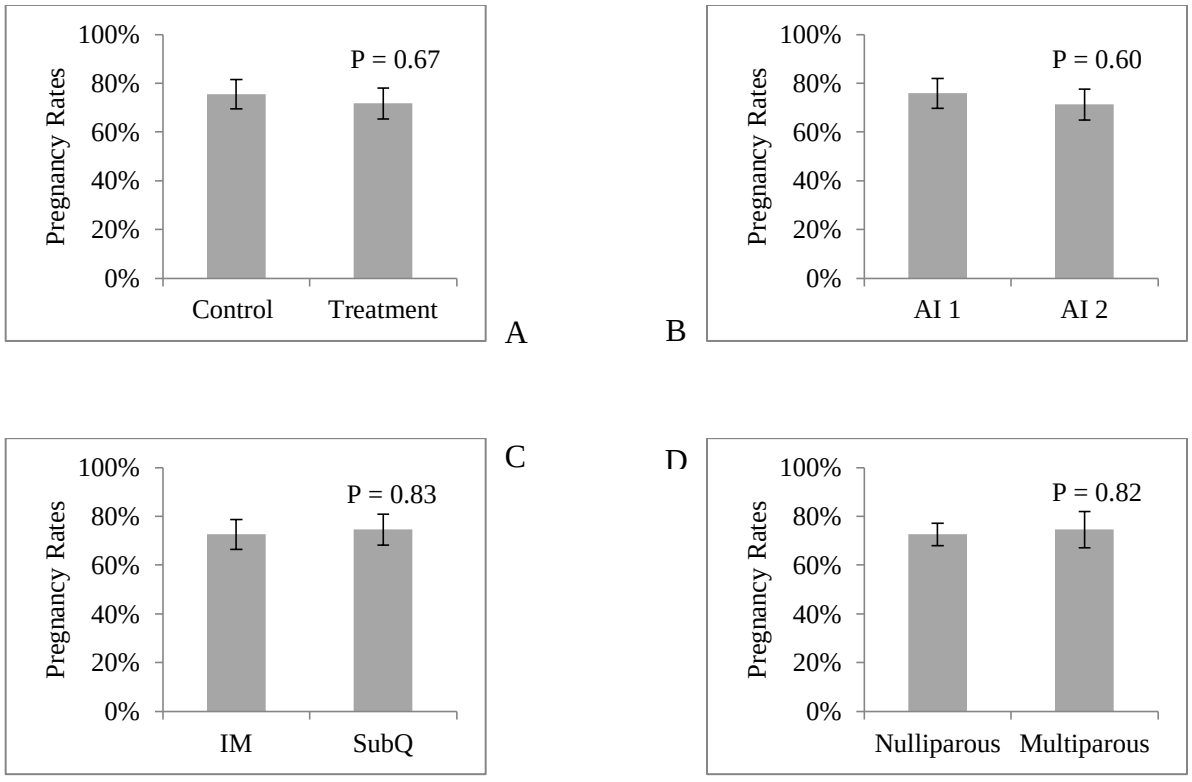

Figure 5: The main effects of treatment (A), AI technician (B), route of PGF administration (C) and parity (D) on pregnancy rates of the Reidsville animals *(n = 99)*.