COMPARISON OF SHORT-TERM VS. LONG-TERM ESTROUS SYNCHRONIZATION PROTOCOLS USING CIDR DEVICES IN SHEEP AND GOATS DURING AND OUTSIDE THE NATURAL BREEDING SEASON

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Abstract

Controlling reproductive cycles during active cyclicity and seasonal anestrous in small ruminants is critical for profitability. The objective of this study was to evaluate the effect on estrous response and interval to estrus of two CIDR protocols in sheep and goats during breeding and non-breeding seasons. In experiment 1, 133 ewes were randomly assigned to 1 of 3 treatments during the breeding season. In the CIDR-7 group, ewes received a CIDR insert for 7 d. In the CIDR-7 + PGF treatment, ewes received a CIDR insert for 7 d and 20 mg of prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) upon CIDR removal. Ewes in the CIDR-14 treatment received a CIDR insert for 14 d. Following CIDR removal all ewes were exposed to a ram every 12 h until breeding. There was a shorter interval from CIDR removal to estrus in the CIDR-14 treatment compared to the CIDR-7 and CIDR-7 + PGF treatments ($P<0.05$). There was no difference in number of ewes per treatment displaying estrus. In experiment 2, 54 ewes were randomly assigned to one of two treatment groups during the anestrous season. Ewes in CIDR-7 and CIDR-14 treatments received a CIDR insert for 7 d and 14 d, respectively. Upon CIDR removal ewes were exposed to a ram every 12 h until breeding. There was a significantly shorter interval from CIDR removal to estrus in CIDR-14 ewes when compared with CIDR-7 ewes ($P<0.05$). For experiment 3, 37 Boer does were randomly assigned to one of two treatments. In the CIDR-10 treatment, does received a CIDR insert for 10 d and 20 mg of $PGF_{2\alpha}$ at time of CIDR removal. In the CIDR-19 treatment, does received a CIDR insert for 19 d. Upon CIDR removal, does were exposed to a buck fitted with a marking harness and chalk marks were recorded every 12 h. The number of does displaying estrus was not different (CIDR-7, 85%; CIDR-14, 95%). There was no difference in interval from CIDR removal to estrus between treatments. Results from experiments 1 and 2 supported the hypothesis that long-term protocols yield a shorter interval to estrus when compared with short-term protocols.

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Dedication

I dedicate this work to my lord and savior Jesus Christ, for whom I strive to do all my work. I would also like to dedicate my thesis to my parents, John and Suzanne Harl, and my brother Dylan. Thank you all for the blessings and support that made this possible.

Chapter 1 - General Review of Literature

Introduction

Efficient reproductive management of livestock is critical for producers attempting to meet market demands (Jackson et al., 2014). In small ruminants, reproductive management is difficult due to seasonality of the estrous cycle, and is problematic for producers trying to meet the demands of a year-round market (Legan & Karsch, 1980; Haibel, 1990; Lehman et al., 1997; Amoah et al., 1996). Sheep and goats are short-day seasonal breeders, naturally cycling in the fall, with anestrous occurring in the spring and summer (Haibel, 1990). Control of the cycle during the breeding season, as well as induction of cyclicity out of season, is important for producers to maximize operation profits (Malpaux et al., 1996).

Estrous Cycle in Small Ruminants

The estrous cycle is a series of hormonal cascades that change the morphology of the female reproductive system to prepare for pregnancy (Fatet, et al., 2011). Sheep and goats experience a seasonally polyestrous reproductive cycle. This seasonal period of reproductive cyclicity ensures offspring are born at an optimal time for survival (Abecia et al., 2011). The breeding season of small ruminants begins in late summer after the summer solstice and ends in late winter after the winter solstice (Evans et al., 2000). Sheep display a 17 d estrous cycle, with estrus lasting between 18-48 h, and ovulation occurring an average of 24 h after the onset of estrus (Abecia et al., 2011). Goats exhibit a 21 d estrous cycle with an estrus duration of 24-48 h, and ovulation occurring, on average, 24 h after the onset of estrus (Rahman et al., 2008). Transitional periods from anestrous to active cyclicity generally occur during the mid-summer months as days begin to shorten (Abecia et al., 2011).
The estrous cycle is controlled by a series of hormones that target specific tissues. Near the end of the estrous cycle, GnRH is released in increasing frequency as progesterone concentrations decrease (Lehman et al., 1997). Increased pulsatile secretion of GnRH causes the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary (Turzillo et al., 1998). Basal levels of FSH and LH cause the development of antral follicles on the ovary (Fatet et al., 2011). Antral follicles undergo a series of changes which include recruitment, selection, dominance, and atresia (Fatet et al., 2011). Recruited follicles begin to grow and secrete minimal levels of estradiol, and follicles that do not undergo atresia (cell death) are selected (Amoah et al., 1996). One to two follicles

are further selected for dominance (Fatet et al., 2011). Follicles that do not become dominant also undergo atresia (Evans et al., 1999). Follicles that grow to dominance secrete an increasing amount of estrogen which acts on the surge center of the hypothalamus, and inhibin, which inhibits FSH release from the anterior pituitary (Evans et al., 1999). When the feedback of estrogen reaches threshold levels and there are low levels of circulating progesterone, the surge center of the hypothalamus releases a burst of GnRH (Rubianes & Menchaca, 2003). This induces a surge of LH from the anterior pituitary, which causes ovulation of the dominant follicle(s) (Fatet et al., 2011).

Recruitment and development of follicles occurs in a series of waves, with ovulation of the dominant follicle(s) occurring during periods of high estrogen and low progesterone (Abecia, 2011). Sheep and goats have an average of 3 to 4 follicular waves per cycle, with the final wave producing the ovulatory follicle (Fatet et al., 2011). The ovulated follicle becomes a corpus hemorrhagicum and undergoes a process known as luteinization (Amoah et al., 1996).

Luteinization is the transformation of follicular cells into luteal cells which form the corpus luteum (CL). The CL produces concentrations of progesterone necessary to sustain pregnancy (Arashiro et al., 2010). Specifically, thecal cells from outer lining of the follicle are mixed with granulosa cells from the interior lining of the follicle immediately after ovulation. Both these cell types become luteal cells of the CL. (CL; Fatet et al., 2011). The corpus luteum begins to secrete progesterone at an increasing level, until peak secretion is reached, approximately 5 d after ovulation (Fatet et al., 2011).

The CL functions (secretes progesterone) for an average of 13 d in sheep and 16 d in goats, during which follicular waves continue to recruit and develop follicles. Ovulation is prevented during peak CL function due to the negative feedback of progesterone on the secretion of GnRH from the hypothalamus (Fatet et al., 2011). Approximately 13 d in sheep and 16 d in goats after estrus, $PGF_{2\alpha}$ is secreted from the non-pregnant uterus and lyses the CL (Fatet et al., 2011). The uterus begins to secrete pulses of $PGF_{2\alpha}$ near the end of the luteal phase. The mechanism triggering the increased secretion of $PGF_{2\alpha}$ is not well understood, but it is thought that prolonged exposure to increased levels of progesterone plays a major role (Fierro et al., 2011). As progesterone decreases, its inhibition on GnRH and subsequently, gonadotropin release is removed and a new cycle begins (Fierro et al., 2011).

Seasonality

Small ruminants experience seasonal periods of active cyclicity. The seasonality of estrus prevents offspring from being born during periods of the year that survival chances are low (Lehman et al., 1997). The natural breeding season of small ruminants is controlled by photoperiod and changes in hormone receptors (Malpaux et al., 1996; Reiter et al., 2009). Changes in day length alter hormonal triggers that induce or inhibit active cyclicity (Goodman et al., 1981; Reiter et al., 2009). In order for active cyclicity to occur, the hypothalamus must be stimulated to secrete sufficient amounts of GnRH stimulate the release of LH in the anterior pituitary (Caraty & Skinner, 1999). A decrease in light is perceived in the retinal ganglion cells of the eye, which sends signals to the superchiasmatic nuclei (SCN) located in the hypothalamus (Reiter et al., 2009). Signals from the SCN are transmitted to the superior cervical ganglion where norepinephrine stimulates the pineal gland to release melatonin, (Malpaux et al., 1996). During periods of high melatonin release, there is a decrease in the neurotransmitter RFRP-3 from RFRP neurons (Senger, 2012). In short-day breeders, like sheep and goats, a decrease in RFRP-3 stimulates the secretion of kisspeptin-10 which acts on GnRH neurons to inhibit secretion of follicle-stimulating hormone and luteinizing hormone from the anterior pituitary (Senger, 2012). When small ruminants are cycling, follicular growth and ovulation takes place as well as sexual receptivity (Lehman et al., 1997; Reiter et al., 2009). During seasonal anestrous, sheep and goats experience follicular development, but because GnRH pulses do not occur, sexual behavior and ovulation does not occur (Malpaux et al., 1996). Surges of GnRH dramatically decrease during seasonal anestrous due to the changes in the ability of GnRH neurons to respond to positive feedback from estradiol (Lehman et al., 1997). GnRH neurons do not have estrogen receptors, and require mediators to convey signals from estradiol (Lehman et al., 1997). With the decrease in GnRH pulses, the rise of estradiol during the follicular phase of the estrous cycle cannot occur (Lehman et al., 1997). The exact role melatonin plays in changing the feedback ability of estrogen on these mediators is not known, and is still the subject of research (Reiter et al., 2009).

Estrous Synchronization in Small Ruminants

Synchronization of the estrous cycle is important for predicting lambing and kidding seasons, to ensure a steady supply of product (Carlson et al., 1989). Initial research in estrous synchronization utilized progesterone to lengthen the luteal phase and inhibit estrus. The ability to manipulate the estrous cycle in the sheep was first discovered in 1948 and required 14 daily subcutaneous (SQ) injections of progesterone in (Dutt et al., 1948). The treatment narrowed the range of estrous display

to 8 d after the end of treatment, but due to the persisting effects of progesterone on the female reproductive tract after treatment, low fertility in ewes was a serious concern (Abecia et al., 2011). Researchers determined that a treatment method which would completely eliminate the effect of exogenous progesterone upon removal was needed in small ruminants (Abecia, et al., 2011).

Vaginal inserts impregnated with progesterone became the subject of research beginning in 1965 with progesterone sponges becoming a common method of progesterone delivery in sheep (Robinson, 1965). However, vaginal irritation and sponge adhesion to the vaginal wall made sponges difficult to use (Rahman et al., 2008). In 2009 the FDA announced the approval of controlled internal drug release (CIDR) devices for sheep in the U.S. (FDA, 2009). The CIDR device is made from silicone elastomer impregnated with progesterone (Zoetis). Due to the ease of use and ease of availability, the CIDR has become the gold standard for progesterone delivery in sheep and goats (Jackson et al., 2014). Estrus in animals treated with CIDRs typically occurs within 48 h after withdrawal of the device (Jackson et al., 2014).

Follicular development during the estrous cycle can be controlled with hormone manipulation. Equine chorionic gonadotropin (eCG), formerly known as pregnant mare serum gonadotropin (PMSG), was found to induce follicular development in ewes and increased the number of dominant follicles (Licht et al., 1979; Wildeus, 2000). Administration of eCG can induce ovulation in ewes and does during anestrous (Wildeus, 2000; Rahman et al., 2008; Olivera-Muzante, 2011). A major difficulty associated with the administration of eCG is prolonged biological life (Wildeus, 2000). Prolonged biological life of eCG allows for continual recruitment of antral follicles, resulting in an increased number of unovulated follicles (Armstrong, 1983). The administration of eCG in small ruminants is used for increasing ovulation rate in attempts to increase fecundity of females (Espinosa-Márquez, et al., 2004).

Prostaglandin-$F_{2\alpha}$ and its analogues have also been used to synchronize estrus by controlling luteal function (Abecia et al., 2011) since $PGF_{2\alpha}$ was discovered to have a luteolytic effect in sheep (McCracken et al., 1972). During the estrous cycle, $PGF_{2\alpha}$ is secreted by the non-pregnant uterus 13 d (sheep) to 16 d (goats) after estrus. Administration of $PGF_{2\alpha}$ after removal of a CIDR mimics the secretion of $PGF_{2\alpha}$ by the uterus, causing lysis of the CL and the onset of a new follicular phase (Fatet et al., 2011). Administration of $PGF_{2\alpha}$ is effective from approximately d 3 to d 14 of the estrous cycle in sheep (Abecia et al., 2011). Analogues of prostaglandins can also induce luteolysis and are often more cost-effective (Light et al., 1994). The effectiveness of $PGF_{2\alpha}$ is limited to the active period of

cyclicity in small ruminants. The lack of ovulation of the follicle during seasonal anestrous causes a lack of luteal development (McCracken et al., 1972).

Synchrony of ovulation can also be an important aspect of synchronization protocols, particularly if animals are being synchronized in preparation for embryo transfer (ET) or artificial insemination (AI) purposes (Menchaca et al., 2010). Gonadotropins released from the pituitary are responsible for the control of ovulation. Luteinizing hormone (LH) stimulates ovulation, and is important for maintenance of the corpus luteum (CL; Rahman et al., 2008). LH is released from the pituitary in a surge, and acts on the dominant follicle(s) to prepare them for ovulation approximately 24 h prior to actual ovulation (Rahman et al., 2008). Levels of circulating LH in the bloodstream rise and fall rapidly, causing the surge of hormone (Lehman et al., 1997). The LH surge is controlled by GnRH released by the hypothalamus (Lehman et al., 1997). GnRH administration induces the release of LH from the anterior pituitary (Turzillo et al., 1996) which acts on the follicle to induce ovulation. Using GnRH to induce ovulation can lead to an increase in conception for animals bred via AI, and increased embryo yield in animals being used in ET programs (Cameron et al., 1988).

Estrous Detection

Sexual receptivity in the female is expressed in a short period of time known as estrus. Estrus is the period during which the female will stand to be bred by the male (Fatet et al., 2011). Females undergo behavioral changes during the estrous period that indicate sexual excitement and receptivity to the male (). Sheep exhibit an estrous period lasting an average of 30 h, ranging from 18 to 48 h (Abecia et al., 2011). Goats experience an estrous period lasting an average of 36 h with a range from 19 to 48 h (Abecia et al., 2011).

Females in all species exhibit an increase in physical activity as the estrous period approaches, which is generally observed as increased locomotion (Senger, 2012). In addition to increased movement, females will also display increased vocalization, urination, tail flagging (goats) and aggressive behavior towards other females (Abecia et al., 2011). During estrus, mounting of other females can be observed, especially in goats (Fatet et al., 2011). The use of males with marking harnesses equipped with marking chalk can be used to determine when females stand to be bred by the male (Fierro et al., 2011).

Protocols for Estrous Synchronization Utilizing CIDR Inserts

Research protocols for CIDR inserts have been focused on short-term (5-7 d) and long-term (12-19 d) length in small ruminants (Carlson et al., 1989; Abecia et al., 2011; Vilariño et al., 2011; Jackson et al., 2014). One of the benefits to short-term progesterone protocols is the ability to synchronize females in a short period of time. This can be beneficial to producers in planning timed AI or ET programs. Short-term protocols typically combine the use of progesterone with multiple follicle-controlling hormones such as follicle-stimulating/ovulation inducing PG 600® (Intervet INC. Milsboro, DE), a combination of equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG), and $PGF_{2\alpha}$. Using multiple hormonal controls in short-term synchronization protocols gives an increased ability to control luteal and follicular dynamics (Vilariño et al., 2011). Previous research indicates that serum progesterone concentrations are maintained at an increased level when compared with a long-term progesterone insert protocol (Vilariño et al., 2011). The label-recommended protocol for CIDR insert in ewes is 5 d, and has been proven to induce ewes and does to exhibit estrus during active cyclicity and during anestrous (Jackson et al., 2014). Concerns with short-term CIDR protocols include inconsistency in estrus response and increased interval to estrus (Abecia et al., 2008). Estrus cannot be precisely predicted, and the interval from CIDR removal to estrus can range from 60-108 h (Carlson et al., 1989; Jackson et al., 2014). Long-term synchronization protocols in sheep and goats have proven to result in shorter intervals from CIDR removal to estrus when compared with short-term protocols (Ungerfeld & Rubianes, 2002; Hashemi et al., 2006).

Conception rate in synchronization

Conception in small ruminants subjected to estrous synchronization protocols is a major concern in production. If animals are successfully synchronized but fail to conceive after breeding, there is no benefit to subjecting females to synchronization. Results reported by Ungerfeld et al. (2002) indicated that ewes treated with short-term CIDR inserts only (6 d) in seasonal anestrous experienced a 60% conception rate, which did not differ when compared with FGA implanted ewes. Additionally, Jackson et al. (2014) reported that ewes during anestrous treated with a CIDR for 5 d experienced a 55% conception rate, which did not differ when compared with ewes that received a 5d CIDR plus $PGF_{2\alpha}$ upon CIDR removal, or control (untreated) treatments. A study by Vilariño et al. (2011) reported that does synchronized with CIDRs for 5 d during the non-breeding season did not experience decreased fertility. Does demonstrated a 75% conception rate, which did not differ when compared with does that received both CIDRs and additional exogenous hormones such as $PGF_{2\alpha}$ or eCG

(Vilariño et al., 2011). The use of short-term CIDR protocols does not appear to have a negative effect on fertility during the natural breeding period. A study by Menchaca et al. (2007) reported that does treated with CIDRs alone for 6 d did not experience adverse fertility when compared with does that were treated with a 5 d CIDR, then $PGF_{2\alpha}$ or eCG upon CIDR removal.

Long-term CIDR protocols do not appear to experience adverse fertility effects as a result of the protocols. Results in a study by Fukui et al. (1999) reported that ewes synchronized using a 12 d CIDR only protocol during seasonal anestrous experienced a 56% conception rate. Similar results were reported by Ozyurtlu et al. (2010). In anestrous ewes treated with CIDRs for 12 d, then administered eCG upon CIDR removal, conception rate achieved 70% and was higher than ewes that were not treated %?) (Ozyurtlu et al., 2010). In ewes treated with CIDRs for 12 d, 92% conceived and lambed (Carlson et al., 1989). Goats treated with long-term CIDR protocols did not appear to experience adverse effects on fertility. In a study by Romano (2003), does synchronized with CIDRs for 12d, then artificially inseminated experienced a 63% conception rate, and did not differ from does synchronized with subcutaneous progesterone devices implanted in the base of the ear, or progesterone feed additives such as MGA. Similar results were reported in does synchronized during the breeding season with CIDRs for 14 d (Cetin et al., 2009). Cetin et al. (2009) reported that does exhibited a 91% conception rate which did not differ from animals synchronized with subcutaneous progesterone implants or untreated does.

The use of double $PGF_{2\alpha}$ injections has been shown to bring cyclic females into estrus approximately 48 h after second injection, however, predictability of estrus onset is varied, and fertility after double $PGF_{2\alpha}$ injections is approximately 60% during the breeding season when compared with 80 to 90 % in progesterone- synchronized animals during the breeding season (Carlson et al., 1989; Abecia et al., 2011; Fierro et al., 2012). Combinations of CIDR + $PGF_{2\alpha}$ to synchronize females can bring females into estrus during the natural breeding season and during anestrous, without sacrificing fertility (Vilariño et al., 2011; Jackson et al., 2014).

Pregnancy Diagnosis

Accurate diagnosis of pregnancy at an early stage is an important aspect of production. Incorrect diagnosis of pregnancy during the natural breeding season can lead to missed opportunity for re-breeding, and loss of an entire breeding season for the ewe (Ganaie et al., 2009). Multiple techniques can be implemented in the early diagnosis of pregnancy, and fetal counts in small ruminants (Karen et al., 2001; Ganaie et al., 2009).

Ultrasonography can be used to detect pregnancy in a quick and accurate manner (Ishwar, 1995). Pregnancy diagnosis can be accomplished with ultrasound in either a trans- abdominal or trans-rectal techniques (Ganaie et al., 2009; Grizelj et al., 2013). Trans-abdominal ultrasound in small ruminants are categorized as A-mode, B-Mode, and Doppler (Ganaie at al., 2009). A-mode ultrasonography measures amplitude of either echo or depth and compares it with time (Ishwar, 1995). Ultrasonic waves are emitted from a hand-held transducer placed in direct contact with the skin (Ishwar, 1995). Waves are reflected off multiple tissue types at various depths and converted to either an audible or visual signal (Ishwar, 1995). Signal is displayed on an oscilloscope when a fluid-filled structure is detected (Ishwar, 1995; Ganaie et al., 2009). False positive diagnoses of pregnancy can occur when the urinary bladder, or if hydrometra (fluid or pus accumulation in the non-pregnant uterus) is occurring in the female (Ishwar, 1995). A-mode ultrasonography is reliable in ewes and does that are at least 50 d post-mating (Ganaie et al., 2009). False negatives can occur in early gestation females due to a decrease in uterine fluid to fetal tissue ratio (Ishwar, 1995). Accuracy of pregnancy diagnosis in ewes 60 to 151 d gestation has been reported to be 83% (Lindahl, 1969). The largest benefit of A-mode ultrasonography is its ability to be used when transport of large amounts of equipment or use of electricity is not possible (Ishewar, 1995).

Real-time, B-mode ultrasonography was developed in Australia to diagnose pregnancy and determine fetal numbers (Ishwar, 1995). B-mode ultrasound uses waves to produce a moving 2-D image of fluids, tissues, and bone (Ganaie at al., 2009). Pregnancy diagnosis is confirmed by the presence of placentomes, placental fluid, or fetal image and is accurate from 30 d after mating (Haibel, 1988). Techniques for pregnancy diagnosis can be quickly learned, and experienced technicians can exhibit accuracy in diagnosis from 90 to 100 % (Haibel, 1990). False positives in B-mode ultrasound are rare, but can be a result of early embryonic death, unobserved abortion, or mistaking the urinary bladder for uterine fluid (Ishwar, 1995). While B-mode scans are typically trans-abdominal, they can also be accurately used trans-rectally to detect early pregnancy (<25 d; Haibel, 1988). An advantage of B-mode ultrasonography is the ability to detect fetal numbers and fetal sex (Ishwar, 1995). Optimal time to determine fetal numbers is between 45 to 90 d post-mating (Haibel, 1990). Fetal age can also be determined from 40 to 100 d post-mating in small ruminants with B-mode ultrasound (Ishwar, 1995). False negatives are typically associated with operator error (Ishwar, 1995). In addition to pregnancy diagnosis, B-mode ultrasound can assist in the diagnoses of disease in the female reproductive tract (Ishwar, 1995).

Doppler ultrasonography focuses on the detection of movement such as fetal heartbeat, fetal circulation, and fetal movement as a positive indicator for pregnancy (Ishwar, 1995). First used in 1964 for pregnancy diagnosis in humans, it was applied to sheep in 1967 (Callagan et al., 1964; Fraser and Robertson, 1967). Fetal heartbeat, or pulse faster than maternal heartbeat and pulse, or fetal movement is considered a positive indicator of pregnancy. Doppler ultrasonography has an accuracy of 100%, but is not effective in detecting pregnancy in animals less than 50 d post-mating (Ishwar, 1995).

Hormone assays can also be used in pregnancy diagnosis for small ruminants. Measurement of steroid hormones such as progesterone provide an accurate method of pregnancy diagnosis in sheep and goats (Ishwar, 1995). Radioimmunoassay (RIA) allows for tests to detect hormone levels in blood, milk, and urine (Ishwar, 1995). Measurement of progesterone in blood and milk can be done as early as 18 d post-mating (Ishwar, 1995). Accuracy of diagnosis in females has been found to be approximately 84 %, with later gestation resulting in increased accuracy (Tsang, 1978). A plasma progesterone level of ≥ 1.75 ng/mL in ewes is considered indicative of pregnancy (Ganaie at al., 2009

Published research has reported methods of estrous synchronization that are effective in both sheep and goats. As the use of assisted reproductive techniques (artificial insemination, embryo transfer) increases in small ruminants, predictability in estrous behavior and fertility are expected by producers implementing synchronization protocols. While the use of CIDRs in sheep has been approved for use, research on various lengths of protocols in sheep and goats have not been directly compared for efficiency in estrous response or subsequent fertility.

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Chapter 2 - Effect of Short-term vs. Long-term CIDR Protocols on Synchronization of Estrous in Ewes during the Breeding Season

Introduction

Inefficient reproductive performance in sheep is one of the biggest detriments to profitability in the industry (Jackson et al., 2014). The ability to control reproduction in ewes and meet the demands of a year-round market is critical for maximizing the profitability of a sheep operation (Jackson et al., 2014). Sheep are seasonal short-day breeders, cycling in the fall while becoming anestrus in the spring, which does not allow for the year-round production of lamb (Abecia et al., 2011). One possible approach to overcome this limitation is the use of exogenous hormones to induce and synchronize estrus outside the natural breeding season in sheep. (Lehman et al., 1997).

Controlled internal drug release devices (CIDRs) contain progesterone and were approved for estrous synchronization in sheep in the U.S. in 2009 (FDA, 2009). The use of progesterone as an inhibitor of estrus to synchronize breeding females is well-established in farm animal species. The ability to successfully synchronize estrus with CIDRs has been well documented for in-season and out-of-season breeding programs in sheep, but the protocols are varied and there have been few direct comparisons of treatments in controlled studies (Carlson et al., 1989; Knights et al., 2001; Abecia et al., 2011; Jackson et al., 2014). Label-use for synchronization of estrus calls for CIDRs to be inserted for 5 consecutive days in anestrous ewes, but there is no in-season label protocol for synchronizing ewes (Zoetis, 2014).

The objective of this study was to compare three CIDR protocols on their effectiveness of inducing estrus and subsequent fertility within the same flock.

Materials and Methods

Animals

Two experiments were conducted using Rambouillet, Southdown, Hampshire X Suffolk, Columbia, Romanov X Katahdin, Romanov X White Dorper, and Romanov X Katahdin X White Dorper ewes. Experiment 1 utilized wool and hair ewes averaging 57.8 kg and compared three progesterone-based estrous synchronization protocols. Experiment 2 utilized hair ewes averaging 51.8 kg and compared two progesterone-based estrous synchronization protocols. All ewes in both experiments were fed a whole corn/ protein pellet diet at a rate of 0.91 kg·hd·d. In addition to grain, all

ewes were fed free choice brome hay and had access to automatic water tanks. All ewes were maintained in 6.1 X 18.29 X 0.54 m dry lot pens and grouped according to day of treatment. Bunk space measured 0.46 X 7.62 m. Trials were conducted at the Kansas State University Sheep and Meat Goat Center. Animals were cared for in accordance with Institutional Animal Care and Use Committee protocol.

Experiment 1: In-Season Estrous Synchronization

Experiment 1 was conducted from October 2012 to November 2012. One hundred and thirty-three ewes of various breeds were used that included Southdown, Rambouillet, Columbia, Suffolk X Hampshire, and Easy Care (Romanov X White Dorper X Katahdin). Ewes were blocked by breed, and weight, and then randomly assigned to 1 of 3 treatment groups.

Treatment groups were divided into 5 subgroups across 5 d. Animals were assigned so all treatments were evenly represented on each day.

Ewes in the CIDR-7 treatment (n=44) received a controlled internal drug release insert (CIDR®, EAZI-BREED™, Zoetis, Florham Park, NJ) for 7 d and received no other exogenous hormones (Fig 1). Ewes in the CIDR-7 + PGF treatment (n=44) (Fig 1) received a CIDR insert for 7 d and 10 mg of prostaglandin-$PGF_{2\alpha}$ (Lutalyse®, Zoetis, Florham Park, NJ; i.m.) at the time of CIDR removal. Animals in the CIDR-14 (n=44) treatment received CIDR inserts for 14 d and no other exogenous hormones were administered (Fig 1).

Ewes were exposed to an intact ram beginning 24 h after the withdrawal of CIDR inserts and every 12 h thereafter until mating was observed. Ewes displaying estrus continued to be exposed to a ram every 12 h until mated a second time, or until the ewe would no longer stand for the ram. Ram exposure occurred at 0630 and 1830 daily.

Experiment 2: Out-of-Season Estrous Synchronization

Experiment 2 was conducted from March 3rd through March 24th, 2014. Fifty-four ewes of three different breed compositions were used consisting of Romanov X Katahdin, Romanov X White Dorper, and Romanov X Katahdin X White Dorper. Animals were blocked by breed, age, and weight, and then randomly assigned to 1 of 2 treatment groups.

Treatment groups were divided into 5 subgroups across a 5 d period. Animals were assigned so both treatments were evenly distributed over all 5 d. Treatments were spaced over 5 d to extend the lambing season. Ewes in the CIDR-7 (n= 27) received CIDR inserts for 7 d and no other exogenous

hormones were administered (Fig 4). Ewes in the CIDR-14 treatment (n= 27) received CIDR inserts for 14 d with no other exogenous hormones administered (Fig 4).

Beginning 24 h after CIDR withdrawal all ewes were exposed to a ram at 0700 and 1900 daily. Ram exposure continued every 12 h until ewes were mated twice, or would no longer stand for the ram.

Pregnancy Diagnosis

Pregnancy diagnosis was made at 30 d post-breeding via abdominal ultrasonography (ALOKA SSD-500V). Open ewes were separated from pregnant ewes and re-exposed to an intact male.

Statistical Analysis

Continuous data (hours until estrus) were analyzed using the PROC MIXED model in SAS data analysis software (SAS Inst. Inc., Cary, NC). Fixed effects in the model included treatment, breed, weight, and age. The random effect in the model was day of assigned treatment. Categorical data collected (estrous display, pregnancy diagnosis) were analyzed using PROC LOGISTIC, a regression model designed for binary categorical data.

Results and Discussion

Experiment 1

There was no difference (P= 0.5838) in the estrous response rate among the treatments. Thirty-nine of 44 ewes (89%) in the CIDR-7 treatment exhibited estrus compared to 42 of 45 ewes (93%) in the CIDR-7 + PGF treatment and 41 of 44 ewes (93%) in the CIDR-14 treatment. When compared with CIDR-7 (59.67 ± 6.27 h) and CIDR-7 + PGF (64.38 ± 9 h) treatments, ewes in the CIDR-14 treatment exhibited a shorter (P<0.01) interval between CIDR removal and onset of estrus (25.0857 ± 1.53 h; Table 1). There was no difference in interval to estrus in CIDR-7 ewes when compared with CIDR-7 + PGF does (P=0.5963). Pregnancy rate was 93%, 95% and 95% for CIDR-7, CIDR-7+PGF and CIDR-14, respectively, and was not different (P=0.7993).

Ewes in our CIDR-7 + PGF treatment had a shorter interval to estrus when compared to those reported in previous studies of ewes treated with a 5 d CIDR + $PGF_{2\alpha}$ protocol (141.6 h ± 25.2) as reported by Jackson and coworkers (2014). Those investigators suggested that CIDRs inserted for 5 d have a longer interval to estrus compared to ewes treated with ewes treated with eCG, GnRH, or $PGF_{2\alpha}$

in addition to a 5 d CIDR. Ewes in the current study did exhibit estrus, but the interval from CIDR removal to estrus was more variable, in ewes treated with CIDRs for 7 d (59.7h) compared with ewes treated with CIDRs for 14 d (25.1h).

Variation in synchrony of estrus for the CIDR-7 + PGF treatment may be due to stage of the estrous cycle in each ewe at time of CIDR insertion. Exogenous progesterone from a CIDR can prevent ovulation and subsequent estrus through negative feedback on the hypothalamus, limiting GnRH secretion. The effectiveness of a short-term CIDR protocol is dependent on the day of the cycle the ewe receives a CIDR. The insertion of a CIDR in females that were between d1 and 10 of their cycle would not be affected as the CL would have time to grow and regress normally. When a CIDR is removed from a female after the CL has regressed, estrous behavior should follow within 24-48 h. However, interval to estrus may be longer if a CL is still present. Prostaglandin-2α is only effective if a functional CL (d 13-17 of the cycle) is present at the time of injection. Therefore an injection of $PGF_{2\alpha}$ has no effect during anestrous or during proestrus, estrus and metestrus of the active cycle.

Similar to our results, ewes treated with a CIDR for 12 d exhibited a shorter interval to estrus (30.1 h ± 7.6; Hashemi et al., 2006) than ewes treated with a 5 d CIDR protocol, (39.9 h ± 2.1; Ungerfeld et al., 2002) or with ewes left untreated (156 h ± 25.2; Jackson et al., 2014). The shorter interval to estrus in our study could be explained by the 2 d of increased duration of CIDR treatment compared to Carlson and coworkers (Carlson et al., 1989). Increased duration of CIDR insertion increases the probability that a ewe's current CL will regress during treatment. Results of our CIDR-7 treatment showed a shorter interval to estrus compared to previously reported data in ewes treated with a CIDR device for 5 d. Jackson et al (2014) reported that ewes treated with CIDRs for 5 d and receiving $PGF_{2\alpha}$ upon CIDR removal experienced an extended interval to estrus (156 h ± 25.2) Ewes displayed estrus, but interval to estrus was significantly different when compared to untreated ewes (228 h ± 26.4). The decreased interval to estrus in our study could be attributed to season of breeding. Jackson and co-workers conducted their study in August, during the transition period from anestrous to active cyclicity, while our study was conducted in October, during the middle of the natural breeding season.

Experiment 2

Twenty-three of 27 ewes (85%) in the CIDR-7 treatment exhibited estrus compared with 25 of 27 ewes (92.59%) in the CIDR-14 treatment (P=0.3950). When compared with CIDR-7 ewes (63.2 h ± 8.7), there was a significantly shorter interval to estrus in CIDR-14 ewes (34.2 h ± 7.9; P<0.01).

Conception did not differ between treatments (P=0.9538). Twenty-two of 27 ewes (84.62%) in the CIDR-7 treatment conceived after exposure to the ram while 25 of 27 ewes (92.59%) in the CIDR-14 treatment conceived.

Results from this study agree with results reported by Knights et al. (2001) that out-of-season ewes treated with a 5 d CIDR protocol exhibited a 48% estrus response within 48 h after CIDR removal. The difference in estrus in the current study (85 vs 77 % overall estrous response) may be a result of increased length of CIDR insert protocol (7 vs 5 d). The increased protocol length may increase the length of priming in the hypothalamus by elevated progesterone levels from the CIDR which may elicit a stronger estrous response. Exposure to elevated levels of progesterone during seasonal anestrous "primes" the brain to enable maximum response to estradiol and display estrous behavior (Turzillo et al., 1998). Increased length of progesterone exposure may increase the probability of behavioral estrus. Estrous response in our CIDR-14 treatment was similar to responses found in a study by Hashemi et al. (2006) who reported that ewes subjected to a CIDR insert for 12 d exhibited 93.3 % estrus response within 48 h after CIDR removal. These results indicate that a 12 d protocol is just as effective as a 14 d CIDR protocol in anestrous ewes.

Implications

The use of CIDR inserts for estrous synchronization can improve the efficiency of reproductive management in ewes. The current results demonstrated that ewes receiving a 14 day CIDR treatment experienced a shorter interval to estrus when compared to ewes receiving a 7 day CIDR treatment. This shortened and predictable interval can better assist producers in planning the breeding and lambing season.

Chapter 3 - Effect of Short-term vs. Long-term CIDR Protocols for Synchronization of Estrus in Boer Does During the Non-Breeding Season

Introduction

Estrous synchronization is the most widely used method to manage reproduction in livestock (Chao et al., 2008). Current methods of estrous synchronization in goats are inconsistent, and currently no hormones are approved in the U.S. for goats (Fajt, 2011). Off-label use of exogenous hormones requires the approval of a veterinarian to synchronize estrus in goats is often permitted (Fajt, 2011).

Exogenous progesterone has been used to synchronize estrus in goats via vaginal sponges (Abecia et al., 2011).. Although sponges are effective in synchronizing goats, they are not preferred due to the difficulty in placement and the frequency of vaginal irritation and adhesion to the vaginal wall (Rahman et al., 2008). A way to reduce these problems with sponges is the use of controlled internal drug release (CIDR) devices for the delivery of progesterone in estrous synchronization programs. The use of CIDR devices are currently the preferred method of progesterone administration in goats.

Ideally, synchronization protocols need to be effective during the natural breeding season of the doe as well as during seasonal anestrous (Wildeus, 2000). Goats are seasonal breeders and naturally begin to cycle in the fall, with periods of anestrous occurring in the spring and summer (Lehman et al., 1997). The objective of this study was to evaluate short and long estrous synchronization protocols on the effectiveness of estrous synchrony in out-of-season does.

Materials and Methods

Trials were conducted at the Kansas State University Sheep and Meat Goat Center. All does were maintained in 6.10 X 18.29 X 0.54 m dry lot pens. Bunk space measured 0.46 X 7.62 m. Animals were allowed free access to pasture during the day, and were confined to pens at night. Animals were cared for in accordance with Institutional Animal Care and Use Committee protocol.

Out-of-season Breeding in Boer Does

The trial was conducted June-July 2014 using 40 does of the Boer breed. Does were randomly assigned to one of two treatments. Does in the CIDR-10 treatment received CIDR inserts for 10

consecutive days (n=19; Fig. 5) and at CIDR removal received 10mg of $PGF_{2\alpha}$, i.m. Does in the CIDR-19 treatment received CIDR inserts for 19 d (n=19; Fig. 5) and received no additional exogenous hormones. Does were exposed to a vasectomized buck, at 0700 and 1900 daily, beginning 24 h after CIDR removal. Once an animal exhibited estrus, it was moved into a pen with constant exposure to an intact male. The buck in the constant exposure pen was fitted with a marking chalk harness, and all does were observed for breeding marks at 0900 and 2100 daily.

Two does were removed from the study. One doe was eliminated from the CIDR-19 group because a CIDR insert could not be safely inserted into the vagina. A second doe was eliminated from the CIDR-19 group when she was determined to be already pregnant from a previous breeding.

Blood Sample Collection

Two blood samples were taken from the jugular vein and assayed for serum progesterone concentration. Concentrations of serum progesterone were classified as high (≥1 ng/mL) and low (<1 ng/mL) (Saharrea et al., 1998). Blood samples were taken on d -29 (19 d CIDR) or d -20 (10 d CIDR) prior to CIDR insertion, and d -19 or d -10 immediately prior to CIDR insertion. Animals with low progesterone in both samples were considered to be in anestrous and not actively cycling.

Pregnancy Diagnosis

Pregnancy diagnoses were made 30 d post-breeding via abdominal ultrasound (ALOKA SSD-500V). Open does were separated from pregnant does and re-exposed to an intact male.

Statistical Analysis

Continuous data collected during this study (serum progesterone concentration, estrous interval) were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment was the fixed effect in the MIXED model, while day of treatment was a random effect. Categorical data collected (estrous display, conception) were analyzed using the LOGISTIC procedure to analyze the binary data.

Results and Discussion

In the CIDR-10 treatment, (55.89% of the does were considered to be anestrous as defined by having blood progesterone <1 ng/mL for both blood samples (Table 5). Sixty-eight percent displayed estrus after CIDR removal, with the average interval from CIDR removal to estrus of 40.62 h ±1.59h. Two out of twenty does were diagnosed as pregnant at 30 d post-mating. Seventy-two percent of does

in the CIDR-19 treatment were anestrous. Seventy-two percent of does in the CIDR-19 treatment displayed estrus after CIDR removal. The average interval from CIDR removal to estrus was 28.0 h ± 2.54.

Only 26% of does were diagnosed as pregnant 30 d post-mating. The single buck used for all matings passed a breeding exam at the beginning of the trial but was infertile upon a recheck after the conclusion of the trial. Because of this setback, pregnancy response data were not analyzed.

There was not a significant difference in cyclicity between treatments (P=0.6783). There was no difference in interval from CIDR removal to standing heat in the CIDR-10 treatment when compared to the CIDR-19 treatment (P=0.9077). Two does were removed from the study during data collection. The first doe was removed due to inability to insert the CIDR device into the vagina. The second doe was removed from the study after she was determined to be bred after the trial start date.

Estrous response during the non-breeding season is controlled by multiple factors, and response to synchronization protocols is directly affected. Influences can include progesterone priming, body condition, temperature, and nutrition. Short-term estrous synchronization with CIDRs increased estrus display 68% in goats synchronized with CIDRs for 5 d and receiving $PGF_{2\alpha}$ upon CIDR removal (Vilariño et al., 2011) when compared with the current study. Potential causes for the decreased estrous response in the current study could be body condition and nutrition prior to the start of the trial. Does in the current study were delivered to the trial location approximately 5 d prior to the start date of the trial. Although plain of nutrition was increased upon arrival, does may not have been provided with adequate nutrition prior to arrival that would allow for maximum reproductive function. Similar to our results, Cetin et al. (2009) reported that does synchronized with CIDRs for 14 d exhibited a 75% estrus response.

Implications

Induction of estrus in anestrous does is possible using CIDR devices. Results of this study did not demonstrate a difference in short-term or long-term protocols on interval from CIDR removal to standing heat.

Figures and Tables

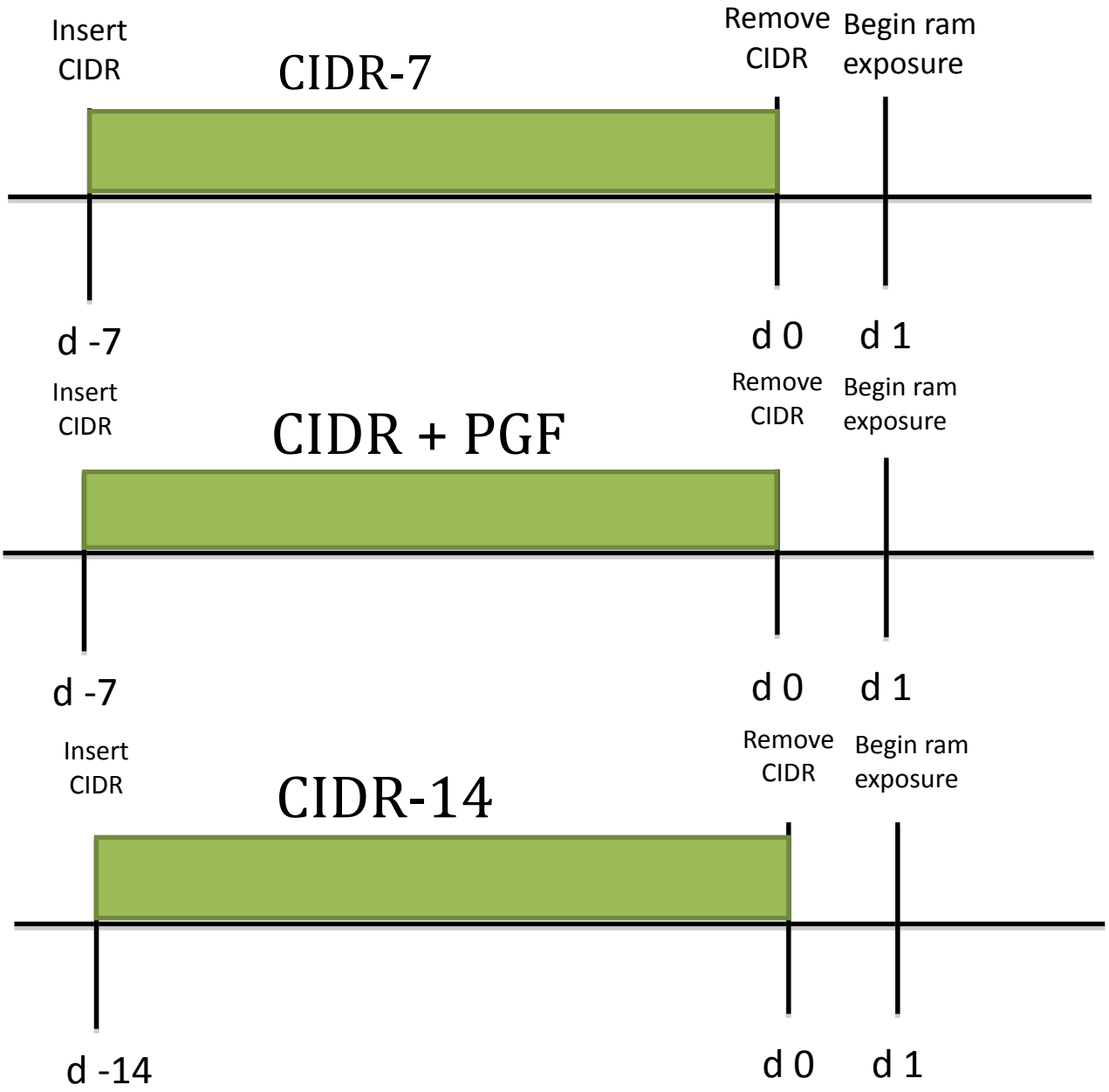

Figure 1. Experimental Design for all Treatment Groups in Experiment 1

Treatment	Hours to Estrus[a]	Estrus Display[b]	Overall Pregnancy[c]	Fecundity[d]	Number of Animals
CIDR-7	59.67 ± 6.27	42/49 (85.71 %)	43/45 (95.56)	1.7	45
CIDR-7+PGF	64.38 ± 9.60	39/49 (79.59%)	41/44 (93.18)	2	44
CIDR-14	25.09 ± 1.53*	41/49 (83.67%)	43/44 (97.73)	1.5	44
P-Value	*0.0028	0.3154	0.7993	0.3721	

Table 1. Hours to Estrus, Estrus Display, Fecundity, and Pregnancy Rate by Treatment

a. Interval from CIDR removal to standing heat
b. Number of ewes displaying estrus/number of ewes per treatment
c. Number of ewes bred/number of ewes per treatment
d. Average number of lambs/ewe/treatment

*Indicates significance at $P<0.05$

Day[a]	Average Hours to Estrus[b]	Estrus Display[c]	Pregnancy Rate[d]	Fecundity[e]	Number of Animals
1	25.28 ± 5.80	26/28 (92.86%)	25/28 (89.29%)	*1.6	28
2	40.53 ± 8.84	28/28 (100%)	27/28 (96.64%)	*1.6	28
3	25.59 ± 5.58	23/26 (88.46%)	24/26 (92.29%)	*1.7	26
4	34.79 ± 8.70	26/28 (92.86%)	28/28 (100%)	1.8	28
5	33.62 ± 8.99	20/23 (86.96%)	23/23 (100%)	2	23
P-Value	0.3356	0.7107	0.6756	0.0240	_

Table 2. Hours to Estrus and Pregnancy Rate by Day

a. Represents each treatment in 5 consecutive days
b. Number of ewes in standing heat/number of ewes per day of treatment
c. Number of ewes bred/number of ewes per day of treatment
d. Interval from CIDR removal to standing heat
e. Number of lambs/ewe/day of treatment
*Indicates significance at $P<0.05$

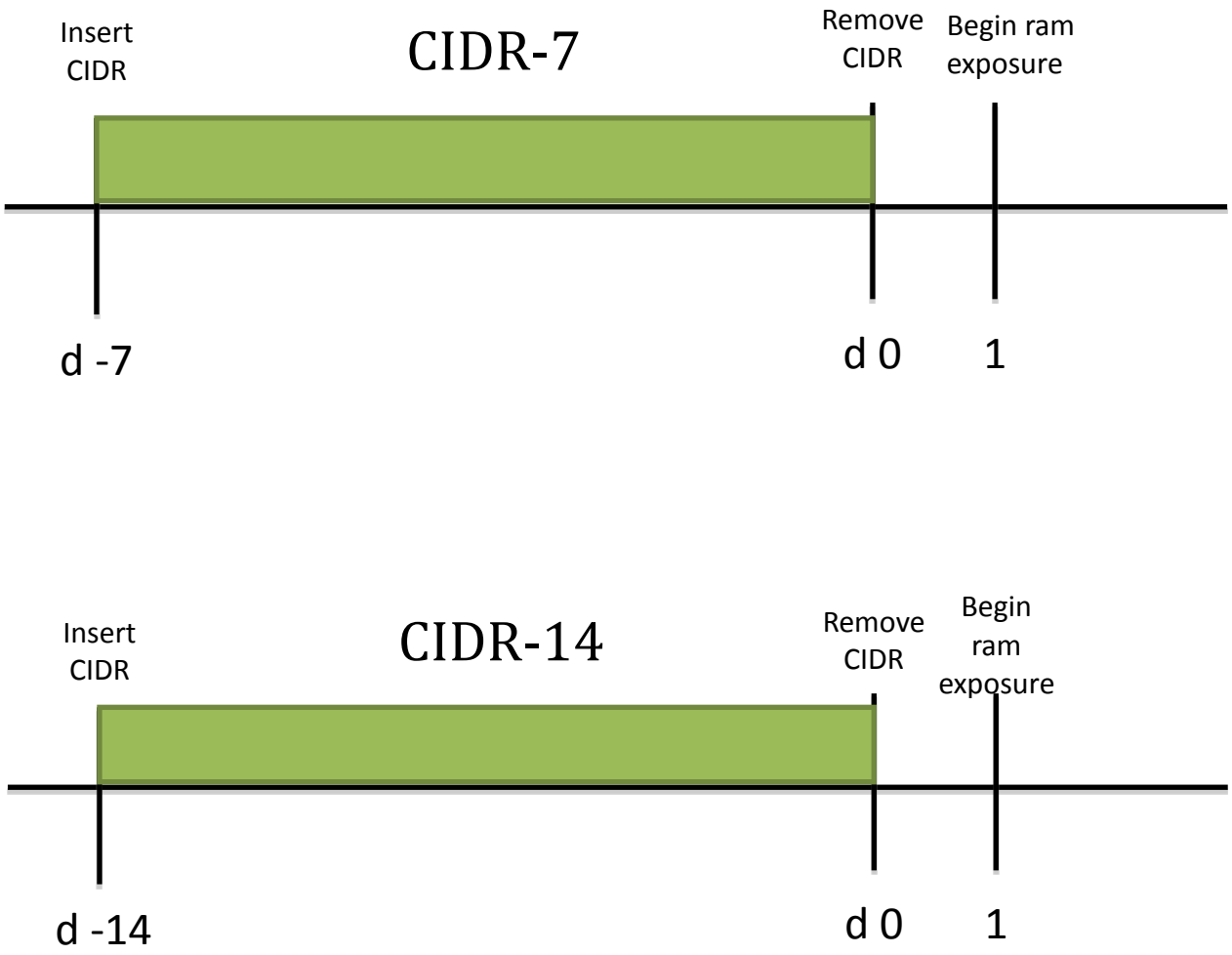

Figure 2. Experimental Design for all Treatment Groups in Experiment 2

Treatment	Average Hours to Estrus [a]	Estrus Display [b]	Pregnancy Rate [c]	Number of Animals
CIDR-7	63.2264 ± 8.7452	23/27 (85.19%)	22/27 (81.48%)	27
CIDR- 14	34.2264 ± 7.9617	25/26 (96.15%)	23/26 (88.46%)	26
P-Value	0.0213	0.3950	0.9538	_

Table 3. Hours to Estrus, Estrus Display, Fecundity, and Pregnancy Rate by Treatment

a. Interval from CIDR removal to standing heat
b. Number of ewes in standing heat/number of does per treatment
c. Number of ewes pregnant/number of ewes per treatment

Day[a]	Hours to Estrus[b]	Estrus Display[c]	Pregnancy Rate[d]	Number of Animals
1	69.8 ± 7.1672	11/12 (91.67%)	10/12 (83.33%)	12
2	54.6 ± 16.4625	11/12 (91.67%)	9/12 (75.0%)	12
3	54.6 ± 19.6222	8/9 (88.89%)	9/9 (100%)	9
4	49.5 ± 14.9248	9/10 (90.0%)	8/10 (80%)	10
5	32.4 ± 9.7690	9/10 (90.0%)	9/10 (90%)	10

Table 4. Hours to Estrus, Estrus Display, Fecundity, and Pregnancy Rate by Day

a. Represents each treatment over 5 consecutive days
b. Interval from CIDR removal to standing heat
c. Number of ewes in standing heat/number of ewes per day of treatment
d. Number of lambs/ewe/day of treatment
e. Number of ewes pregnant/number of ewes per treatment

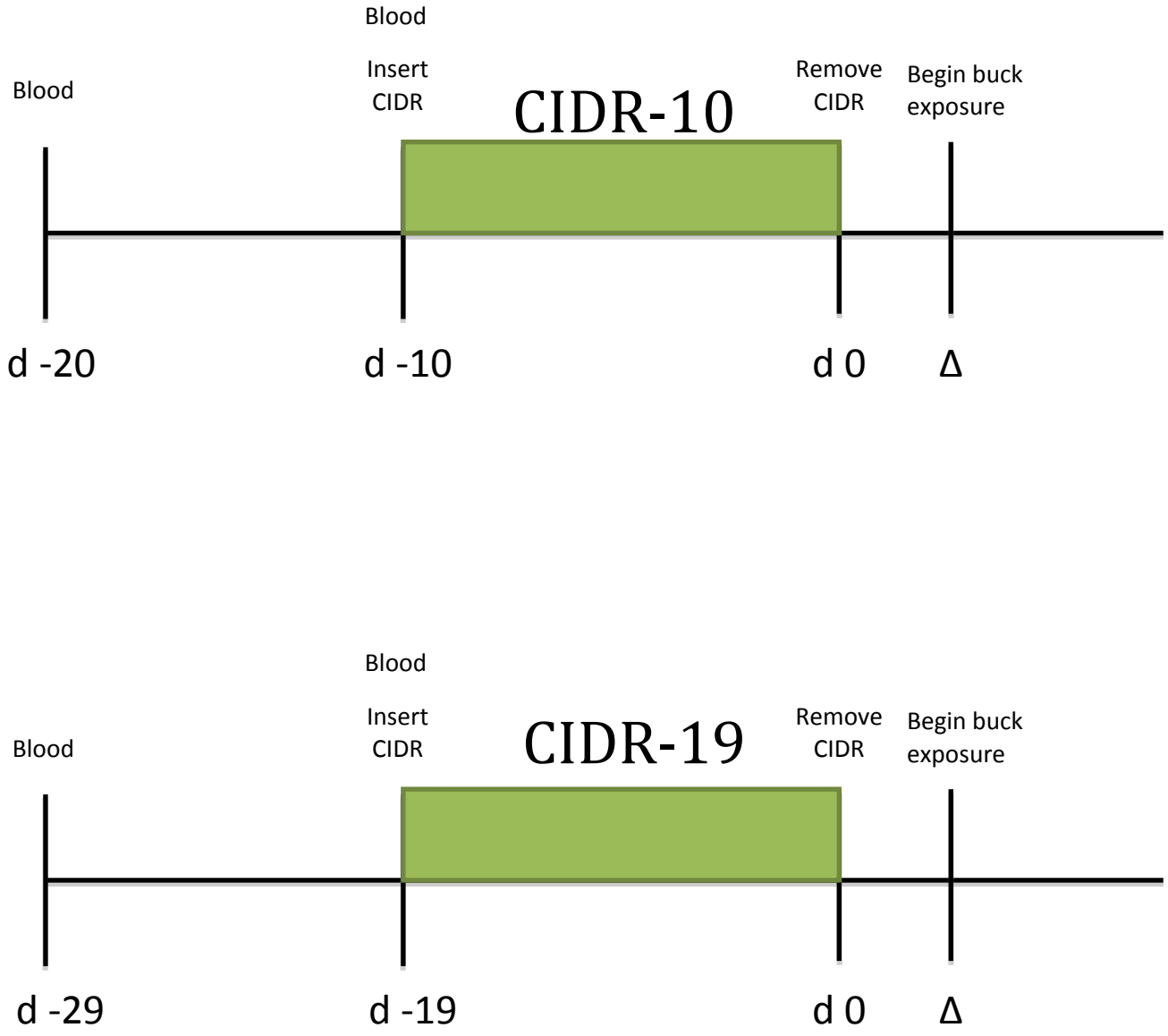

Figure 3. Experimental Design for all Treatment Groups in Experiment 3

Treatment	Cyclicity [a]	Average Hours to Estrus [b]	Estrus Display [c]	Pregnancy Rate [d]	Number of Animals
CIDR-10	8/19 (44.11%)	40.62 ± 1.58	13/19 (68.42%)	2/19 (10.52%)	19
CIDR-19	5/18 (27.78%)	28.0 ± 2.54	13/18 (72.22%)	5/18 (27.78)	18
P-value	0.6783	0.8557			

Table 5. Cyclicity, Hours to Estrus, Estrus Display, and Pregnancy Rate by Treatment

a. Does with blood progesterone > 1 ng/mL for one or both blood samples
b. Interval from CIDR removal to standing heat
c. Number of does exhibiting estrus/number of does per treatment
d. Number of does bred/number of does per treatment

Day [a]	Hours to Estrus [b]	Cyclicity [c]	Estrus Display [d]	Pregnancy Rate [e]	Number of Animals
1	48	25 (2/8)	62.50 5/8	2/8 (25%)	8
2	38 ± 1.8516	28. 57 (2/7)	85.71 6/7	2/7 (28.57%)	7
3	42 ± 12.7481	0 (0/7)	85.71 6/7	2/7 (28.57%)	7
4	30 ± 3	25 (2/8)	25.0 2/8	0/7	8
5	28 ± 2.3422	71.43 (5/7)	85.71 6/7	1/7 (14.29%)	7
P-value	0.4535	0.6896			

Table 6. Cyclicity, Hours to Estrus, Estrus Display, and Pregnancy Rate by Day

a. Treatments broken evenly into 5 consecutive days
b. Interval from CIDR removal to standing heat
c. Blood progesterone ≥ 1 ng/mL for at least one blood sample = active cyclicity
d. Number of animals in standing heat/number of animals per treatment
e. Number of animals bred/number of animals per treatment

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