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Effect of Time of PGF2alpha Application on Reproductive Outcome in Ewes

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ABSTRACT

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Traditionally, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been included in short-term progesterone-based estrous synchronization (STPBES) protocols but its inclusion has been associated with a reduction in fertility at the synchronized estrus. Also, previous studies have shown that there are differences in the endocrine status and follicular dynamics of ewes at different stages of the estrous cycle, although effects have not been sufficiently evaluated regarding the reproductive outcomes when luteolysis is induced on different days of the cycle. To evaluate the effect of time of application of $PGF_{2\alpha}$ relative to the progesterone treatment on fertility, ewes (*N*=442) from 4 farms located in WV and PA were randomly assigned to receive controlled internal drug-releasing devices (CIDR-g; 0.3 g progesterone) for 5 days alone (*n*=123; treatment 1), in combination with 25 mg $PGF_{2\alpha}$ (5 mL Lutalyse; Dinoprost Tromethamine; Zoetis) at CIDR insertion (*n*=103; treatment 2) or removal (*n*=100; treatment 3), or 25 mg $PGF_{2\alpha}$ alone (*n*=116; treatment 4) prior to being joined with sexually mature rams. To compare reproductive performance in ewes treated with $PGF_{2\alpha}$ at different stages of the estrous cycle, ewes (*N*=148) from 1 farm located in southwestern PA were pre-synchronized using treatment with a CIDR device for 7 days. Ewes were randomly assigned to receive a 25 mg intramuscular injection of $PGF_{2\alpha}$ 7 (*n*=48), 10 (*n*=50), or 13 (*n*=50) days following CIDR removal which was projected to be equivalent to day 5, 8, and 11 of the estrous cycle. Ewes were then joined with sexually mature rams on the day of $PGF_{2\alpha}$ injection. Data were analyzed using analysis of variance with the model consisting of the main effects of treatments, farms and their interactions and additionally, least square means for treatment effects were determined. Estrous response did not differ among ewes receiving progesterone-based treatments but was significantly lower in ewes receiving $PGF_{2\alpha}$ only. Pregnancy rate to first service was highest in ewes receiving CIDR only. The percent of ewes lambing to first service was greater in ewes treated with CIDR only than in ewes treated

with $PGF_{2\alpha}$ ($p=0.05$). In experiment 2, the mean estrous response was 85.1% and did not differ with treatment. Conception rate was higher in ewes treated with $PGF_{2\alpha}$ at predicted day 5 than those injected at day 11 ($p<0.05$) and tended to be higher than ewes injected on day 8 ($p<0.1$). Pregnancy rate to first service tended to be higher in day 5 than day 11 ewes ($p=0.07$), and more ewes lambed to first service when treated at day 5 than at either day 8 or 11 ($p=0.05$). First service prolificacy was also greater in ewes treated at predicted day 5 than those injected on day 11 ($p=0.05$) and tended to be higher than that of ewes injected with $PGF_{2\alpha}$ at predicted day 8 ($p=0.14$). In conclusion, application of $PGF_{2\alpha}$ at the beginning or end of progesterone pretreatment did not enhance synchrony of estrus or other reproductive outcomes and synchronization of estrus with a 5 day treatment with progesterone was sufficient to synchronize estrus with higher fertility. Additionally, application of $PGF_{2\alpha}$ earlier in the estrous cycle resulted in improved reproductive performance.

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REVIEW OF LITERATURE

I. Estrous Synchronization

Estrous synchronization is an important component for optimization of livestock reproductive management that has been used commonly since the 1940's (Abecia et al., 2011). The operative goal of an efficient estrous synchronization program is to have a simple, cost-effective method to produce offspring at a desired timepoint, either through natural service or artificial insemination, in order to maximize farm profitability. Synchronization or induction of estrus is utilized to time as well as to synchronize mating and parturition in the ewe as well as the time of weaning of lambs. Synchronization of estrus can also be used to decrease unproductive intervals and provide a more continuous supply of lamb (Carlson et al., 1989; Contreras-Solis et al., 2009). The outcomes from estrous synchronization include better assignment of labor, reduction in neonatal mortality, and improved strategic marketing of lambs leading to increased production and profitability. Weaning of offspring that are born at a similar timepoint also leads to more uniform lamb crop for market and allows scheduling of lambing at times that are advantageous in terms of supplies, labor, facilities, and market price trends (Carlson et al., 1989). Synchronization of estrus also allows for optimization of efficiency of producers' time and facilities by shortening the span of lambing ewes, whereby reduction of lamb mortality can also be achieved with greater observation of lambs during their first three days of life when mortality is highest (Knights et al., 2003).

With the continuous decline in the sheep industry over the past several years, an increase in reproductive efficiency could reduce the cost of lamb and help revive the state

of the industry (Knights et al., 2003). Use of reproductive technologies to synchronize estrus in ewes can also allow the producer to select a time at which to breed a higher number of ewes so the time of lambing can be pre-determined. Ewes whose estrus periods are synchronized lamb at a time that is more convenient to the producer, and additionally those lambs can be sold at pre-defined marketing periods. Application of these techniques also serves to shorten the duration of lambing, which saves the producer both time and money with greater efficiency of their lambing system. Additionally, because sheep are seasonal breeders, there is a seasonal pattern of production for meat and milk, so ewes that are bred to have their lambs weaned at a time when the seasonal supply is low have the potential to garner higher market prices for meat (Abecia et al., 2011).

A number of beneficial assisted reproductive technologies rely on the utilization of estrous synchronization methods to synchronize components of the estrous cycle of female animals. Artificial insemination (AI) is one such application that is made more feasible through estrous synchronization (Fierro et al., 2011). Traditionally, it had been impractical to accomplish in sheep due to difficulty of detecting estrus, but with more efficient methods of synchronizing estrus, AI has become a more successful and commonplace practice in sheep (Abecia et al., 2011). Artificial insemination is an important tool to allow for genetic improvement and diversity in a flock (Amiridis et al., 2012). Superovulation and embryo transfer have additionally become more commonplace with the utilization of a pre-synchronized estrus in order to maximize the number of offspring produced by a genetically desirable female or to produce superior males as semen donors for AI (Abecia et al., 2011; Amiridis et al., 2012). Effective

estrous synchronization methods make use of these technologies feasible, and thereby help facilitate genetic improvement and reproductive efficiency in flocks.

II. Ovine Estrous Cycle

The development and use of cost effective estrous synchronization programs are based on a comprehensive knowledge of the ovine estrous cycle. Sheep are seasonally polyestrous and have an estrous cycle that averages 16-18 days in length (Bartlewski et al., 2011). The primary environmental determinant of annual cyclicity is photoperiod but factors such as temperature, nutritional status, social interactions, and lactation period are known to modulate it (Rosa et al., 2003). Ewes typically transition into an estrous state beginning in late summer or early autumn in response to shortening day length, which is perceived by the pineal gland through the retina (Abecia et al., 2011).

The pineal gland is responsible for synthesizing and secreting melatonin nocturnally, so its secretion is inversely related with daylength (Malpaux et al., 1996). Melatonin is involved with regulation of secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus via a multistep neural pathway. An increase in duration of melatonin secretion resulting from a decrease in daylength then leads to an increase in secretion of GnRH from the hypothalamus (Abecia et al., 2011). GnRH induces the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland (Rosa et al., 2003). LH and FSH are also called gonadotropins because they then act on the ovaries, or gonads, of the female to stimulate follicular growth and development (Abecia et al., 2011).

Sheep typically have 2-4 waves of antral follicular emergence per estrous cycle regulated by progesterone and gonadotropins and the final as well as the penultimate

wave yields the ovulatory follicle(s) in a state of weak follicular dominance (Bartlewski et al., 2011; Leyva et al., 1998; Evans et al., 1999). In ewes, ovulable follicles are present throughout the estrous cycle (Houghton et al., 1995). As the follicles grow and mature, they secrete increasing amounts of estrogen (Bartlewski et al., 2011). Estrogen exerts ovarian control on the hypothalamus via both positive and negative feedback mechanisms depending on its circulating concentration (Senger, 2012). During follicular development, it stimulates the hypothalamus to secrete GnRH and subsequently the anterior pituitary gland to secrete LH (Karsch et al., 1993). Once the pulsatile release of LH reaches a threshold amplitude and frequency, ovulation occurs 24-27 hours later (Bartlewski et al., 2011; Inskeep, 2005; Rubianes et al., 2003).

During ovulation, the follicle ruptures and the oocyte is expelled, which leaves a remnant of the follicle on the ovary. LH acts on both the thecal and granulosal cells of the follicular remnant to form the small and large cells of the corpus luteum (CL) respectively through a process called luteinization (Weems et al., 2006). The CL is a transient endocrine gland that synthesizes and secretes increasing amounts of progesterone as it reaches structural and functional maturity (Bartlewski et al., 2011). Progesterone has an inhibitory effect on the hypothalamus and suppresses the release of GnRH, which subsequently leads to a reduction in output of LH and FSH by the anterior pituitary gland (Senger, 2012). In the absence of fertilization, uterine $PGF_{2\alpha}$ is released in increasing amounts 11-12 days following estrus, which induces luteal regression and therefore a decline in progesterone concentration (Baird et al., 1976; Ottobre et al., 1984; Inskeep, 2005). Uterine release of $PGF_{2\alpha}$ is negligible prior to this timepoint, and it has been established that luteolysis in sheep involves a local feedback loop between the

uterus and the corpus luteum (Baird et al., 1976; Scaramuzzi et al., 1977; Wiltbank and Casida, 1956). Also, structural luteolysis is an irreversible process (Baird et al., 1976).

This process has been shown to be regulated by a high concentration of progesterone, which is important for timing the initial peaks of $PGF_{2\alpha}$ (Vincent and Inskeep, 1986). The decreasing concentration of progesterone also modulates the release of $PGF_{2\alpha}$ from the uterus by keeping it at midrange values until luteolysis has begun, where with decreasing concentrations, greater amounts of $PGF_{2\alpha}$ are released from the uterine endometrium to complete the regression of corpora lutea (Vincent and Inskeep, 1986; Griffeth et al., 2002). The inhibitory effect of progesterone is then removed and estrus and ovulation can ensue to initiate a new cycle during which the ewe will have another opportunity to become pregnant.

The estrous cycle is divided roughly into two phases: the follicular and luteal phases. The follicular phase lasts for 2-4 days and is characterized by a predomination of circulating estrogen, which leads to behavioral estrus followed by a surge in luteinizing hormone and subsequently ovulation (Inskeep, 2005). The luteal phase lasts for 12-14 days in the ewe and can be defined as the length of time during which one or more corpora lutea are present on an ovary. This phase is characterized by a high circulating concentration of progesterone, which is released by the corpus luteum that is formed from the post-ovulatory follicular remnant (Knights et al., 2003). As the primary component in defining the length of the estrous cycle, manipulation of the luteal phase length is the most common mode of estrous synchronization. Additionally, a thorough understanding of the mechanics and endocrine variables during these two phases is important for manipulating said variables for estrous synchronization.

III. General Approaches to Synchronization of Estrus

Since the 1940's, a number of different methods for synchronizing the estrous cycle in ewes have been used and can include either natural or artificial/hormonal treatments to impact the cycle (Abecia et al., 2011). Commonly, exogenous hormones such as progesterone and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or their synthetic analogues are used both exclusively as well as in combination to modify the physiologic duration of natural hormonal events to alter the luteal phase of the estrous cycle. Although there are numerous different methods that can be utilized to control the estrous cycle, two main approaches have traditionally been used. One involves utilizing $PGF_{2\alpha}$ or its analogues to eliminate endogenous progesterone and the other uses exogenous progesterone or progestogens.

The main concepts involved in their use are that progestagens exert their effect primarily through mimicking the activity of the corpus luteum in order to extend or enact an artificial luteal phase by suppressing circulating LH and ovulation until the CL is regressed or the exogenous source of progesterone has been removed or the progesterone has been metabolized by the ewe. $PGF_{2\alpha}$ shortens the treated luteal phase by regressing the corpus luteum prematurely so that ewes transition into proestrus and estrus together (Karsch et al, 1977). Progesterone-based approaches are generally preferred by producers over prostaglandin-based protocols, as the fertility obtained from a particular synchronization approach often determines its feasibility (Fierro et al., 2013; Greyling and Brink, 1987). Typical estrus synchronization rates associated with prostaglandin-based (Gonzalez-Bulnes et al., 2002; Godfrey et al., 1997) and progestagen-based protocols (Langford et al., 1983; Simonetti et al., 2000) are approximately 70% and 80%

respectively. During the breeding season, each method can be used either alone or in combination with the other and both hormones modify the natural estrous cycle by altering the length of the luteal phase.

i. Progesterone-Based Approaches

The use of progesterone in control of the ovine estrous cycle was first reported in 1948 to be an effective method to synchronize estrus and ovulation in ewes that were at three different points of cyclicity (Dutt and Casida, 1948). Initially, only long-term progesterone treatments were utilized in sheep, which lasted for 12-14 days or longer (Dutt and Casida; 1948; O'Mary et al., 1950; Robinson, 1964; Gordon, 1975; Allison, 1978). The length of treatment with progesterone was likely maintained for such a duration based on the assumption that progestational treatment must span the length of the natural ovine luteal phase in order to ensure absence of luteal tissue at cessation of treatment (Dutt and Casida; 1948, Woody et al., 1967). Such methods were long-lasting and often resulted in lower fertility.

Several methods of administering progesterone or its synthetic analogues (progestogens) have been developed. Progestogens have been delivered through inclusion in the feed as melengestrol acetate (MGA), implants under the skin (Norgestomet-SynchroMate B), intravaginal sponges containing flurogestone acetate (FGA) or medroxyprogesterone acetate (MAP), and intramuscular injections (Knights et al., 2003; Gordon, 1975; Gordon, 1977). Limiting factors of delivery devices have been their capability to maintain high concentrations of progesterone in the ewe throughout the treatment period, to be retained properly within the animal, and to be delivered in a sanitary manner as hygienically as possible.

Although initially administered via injections, progestational agents have been utilized through intravaginal devices since the '60s through intravaginal sponges (Robinson, 1964; Robinson, 1965) and more currently, controlled internal drug releasing (CIDR) devices (Welch et al., 1984; Wheaton et al., 1993). Intravaginal devices allow for an abrupt cessation of hormonal treatment (Abecia et al., 2011). The CIDR was developed in New Zealand by Dr. Welch and Dr. Miller in 1984 and is made of a progesterone-impregnated silicone elastomer molded over a nylon core (Welch, 1984). CIDR devices provide high circulating levels of natural progesterone with a loaded dosage of 0.30 grams (Abecia et al., 2011; Welch et al., 1984; Carlson et al., 1989), are a more sanitary intravaginal device in comparison with sponges, and neither impede nor absorb vaginal secretions (Welch, 1984; Carlson et al., 1989; Wheaton et al., 1993). It has also been shown to provide mean progesterone concentrations approximately twice that of implants and sponges so it is an effective mode of administering high doses of exogenous progesterone (Hamra et al., 1986).

The mean plasma progesterone content has been observed to rise to peak values of 5.5 ng/ml within two hours of CIDR insertion in ovariectomized ewes, a level of which exceeds the typical mid-luteal plasma concentration of approximately 3 ng/ml, suggesting that it is rapidly released and absorbed effectively through the vaginal wall (Ainsworth and Downey, 1986; Carlson et al., 1989). Additionally, the plasma progesterone concentration returned to basal values within four (Ainsworth and Downey, 1986) to six (Greyling and Brink, 1987) hours of CIDR device removal. Results from prior experiments have shown that progesterone can drift into and out of muscle tissue rapidly, and is maintained at similarly equal concentrations between muscle, fat, and

plasma (Kincl, 1971; Carlson et al., 1989). This is of importance as abrupt cessation of progesterone exposure is an important feature of reproductive management technologies and it illustrates that progesterone originating from CIDR devices is cleared rapidly and residues in meat should not be of concern to consumers of lamb or mutton.

1) Long-Term Progesterone-Based Synchronization Protocols

Traditionally, long-term treatments of progestogens have been utilized in order to synchronize estrus in ewes (Dutt and Casida, 1948, Robinson, 1965). Progestogens have generally produced more favorable estrous response in comparison with prostaglandin-based protocols (Gonzalez-Bulnes et al., 2002; Godfrey et al., 1997; Langford et al., 1983; Simonetti et al., 2000). However, use of progestogens in estrous synchronization can be associated with a degree of sub-fertility compared with the unaltered cycle due to alterations in the pattern of LH release (Scaramuzzi et al., 1988; Haresign, 1985; Letelier et al., 2011) and decreases in sperm transport and survival in the reproductive tract (Hawk and Conley, 1971). One factor that could help elucidate the disparity in fertility often noted between the two approaches could be the increased uterine vascularity observed in progestogen-treated compared with prostaglandin-treated ewes. A study recently conducted by Ruiz-Gonzalez et al. compared the effects on caruncular early angiogenesis between the two approaches and found a higher number of capillary vessels and vascular endothelial growth factor (VEGF) in progestagen-treated ewes and evidence of impaired vascular development and remodeling of caruncular tissue in prostaglandin-treated ewes (Ruiz-Gonzalez et al., 2013).

Whereas some studies have found long-term progesterone treatments to result in fertility comparable with that of spontaneous breeding during the natural breeding season

with an overall lambing percentage of approximately 90-95% (Carlson et al., 1989), other studies have found a reduction in fertility despite a high incidence of ewes showing estrus (Vinoles et al., 2001; Robinson et al., 1970). This decrease in conception rate has been attributed to the modified hormonal milieu leading to asynchrony between estrus and ovulation (Scaramuzzi et al., 1988). Additionally, reduction in fertility at the synchronized estrus following treatment with MAP for 12-14 days might be due to steroid effects on follicular development (Kruip and Brand, 1975). Long-term progestogen treatments could be associated with a lower circulating concentration of progesterone, as the progesterone concentration decreases continually after day 3 in ewes treated with a single CIDR-g (Wheaton et al., 1993). Subluteal progesterone concentrations have been observed to result in persistent follicles in the majority of ewes studied (Johnson et al., 1996), and extension of the first wave dominant follicle's lifespan in turn delays emergence of the following follicular wave (Vinoles et al., 1999).

Decreased conception rates (72 vs. 98%) have also been observed in ewes treated with a subluteal concentration of progesterone in comparison with control ewes (Johnson et al., 1996), whereas ewes treated with supraluteal concentrations have shown a decrease in the growth of the dominant follicle (Rubianes et al., 1997) as well as increased follicular turnover (Noel et al., 1994). Higher pregnancy rates observed with short term versus long-term progesterone treatment have been related to more rapid follicular turnover (Vinoles et al., 2001). It appears then that the degree of follicular senescence and its effects on fertility may shift in relation to dosages of progestogens or duration of treatment. Long-term progestogen treatments have also been implicated in a reduction in the number of total follicles and impaired overall viability of embryos (Gonzalez-Bulnes

et al., 2005) but have produced a high degree of synchrony (Vinoles et al., 2001). Although progestogen-based protocols have generally been preferred by technicians over prostaglandin-based techniques, the poor fertility often associated with long-term treatments as well as the long duration of time required for their use have made long-term treatments' practical use less than ideal.

2) Short-Term Progesterone + Prostaglandin Combination Protocols

More recently, short-term progesterone-based estrous synchronization protocols have been shown to be a successful alternative to long-term treatments (Vinoles et al., 2001; Dixon et al., 2006). Short-term progesterone treatments of approximately 5-7 days block ovulation until the source of progesterone is removed, but may not allow sufficient time for corpora lutea to mature fully (Beck et al., 1993). Therefore, these treatments have been used in combination with $PGF_{2\alpha}$ in order to shorten the treatment period from that of long-term CIDR use as well as to avoid the issue of ewes with corpora lutea that are refractory to $PGF_{2\alpha}$, as may be encountered using single $PGF_{2\alpha}$ injection regimens (Beck et al., 1993). These approaches have been shown to produce comparable and often improved fertility in comparison with longer duration progestagen treatments (Fitzgerald et al., 1985; Ozturkler et al., 2003) as well as prostaglandin-based protocols (Dixon et al., 2006; Beck et al., 1993; Loubser and Van Niekerk, 1981). Combination treatments are effective due to the fact that the progesterone pretreatment prevents formation of corpora lutea for the duration of treatment, and therefore ensures that any existing corpora lutea are a minimum of five days old and therefore sensitive to prostaglandin administration (Acritopoulou et al., 1980; Beck et al., 1993). When prostaglandin is injected at

progestogen removal, all individuals should undergo luteolysis simultaneously (Beck et al., 1993).

The combination protocol reduces the exposure of animals to intravaginal devices and provides a reduction in duration of progestagen pretreatment (Abecia et al., 2011). The length of progestagen treatment has been reduced from 8-9 days in earlier experiments (Greyling et al., 1979; Loubser et al., 1981) to 5-7 days in more recent studies in sheep (Fitzgerald et al., 1985; Beck et al., 1993; Ozturkler et al., 2003; Dixon et al., 2006). Overall, these short-term treatments have produced a high estrus response with 80 (Ozturkler et al., 2003), 82 (Dixon et al., 2006), 89 (Fitzgerald et al., 1985), and 100 percent (Beck et al., 1993) and conception rates of 76-93 percent (Loubser et al., 1981; Ozturkler et al., 2003; Fitzgerald et al., 1985; Dixon et al., 2006). This method has also been demonstrated to be an effective mode of estrous synchronization both in cattle (Beal, 1983; Smith et al., 1984) as well as goats (Corteel et al., 1988; Kusina et al., 2000). However, despite producing a high degree of synchrony, combined treatments of $PGF_{2\alpha}$ and progesterone present an approximately 30% higher cost in comparison with treatment of exogenous progesterone alone.

3) Short-Term Progesterone Treatment without $PGF_{2\alpha}$

Few studies have evaluated the use of short-term progesterone-based protocols without the use of prostaglandin. However, there is some evidence that suggests that inclusion of $PGF_{2\alpha}$ may not be necessary. Higher pregnancy rates have been observed in ewes using 6 day (87%) than 12 day (63%) progestogen treatments, both without the use of prostaglandin (Vinoles et al., 2001). Slower follicular turnover associated with long-term treatments can induce ovulation of persistent dominant follicles, and it was proposed

that the higher pregnancy rate associated with the short-term treatment may be attributed to the ovulation of newly recruited growing follicles (Vinoles et al., 2001). This was supported by the observation that the ovulatory follicle emerged prior to sponge withdrawal in ewes treated with long-term progesterone and around the time of sponge removal in the short-term treatment ewes (Vinoles et al., 2001).

Short term progesterone treatment can provide a higher circulating concentration of progesterone in comparison with long-term treatments, as the highest concentration with CIDR use is reached on day 3 and decreases thereafter (Wheaton et al., 1993). Prior research has illustrated that the dose of exogenous progesterone administered was positively related with the number of ewes exhibiting estrus and lambing (Robinson et al., 1968; Allison and Robinson, 1970). This data is of practical benefit to meeting producers' objectives of utilizing cost-effective and efficient method of reproductive management, as it provides a means of estrous synchronization that does not require a long period of preparation, needs no other exogenous hormones to aid in supporting a fertile outcome, and is 30% cheaper than combination treatments.

ii. Prostaglandin-Based Approaches

Prostaglandin-based estrous synchronization protocols involve the termination of the luteal phase of the ewe by regressing corpora lutea. Prostaglandin $F_{2\alpha}$ is the primary luteolytic hormone in sheep (McCracken et al., 1970; McCracken et al., 1972; Barrett et al., 1971; Light et al., 1994) that is produced by the non-pregnant uterine endometrial glands (Scaramuzzi et al., 1984). $PGF_{2\alpha}$ is metabolized at an extremely rapid rate; for example, 99% of injected $PGF_{2\alpha}$ is metabolized through a single pulmonary passage to 15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (Davis et al., 1980; Piper et al., 1970).

The minimal exogenous dose that has been shown to induce luteolysis in sheep is approximately 2 ug/hour (Chamley et al., 1972). High doses are required in systemic or intramuscular administration in order to be effective, with higher doses of 20-24 mg producing greater estrous response (Scaramuzzi et al., 1984; Fukui and Roberts, 1981; Hackett and Robinson, 1980). It has been well-established that the effectiveness of prostaglandin (measured through the proportion of ewes detected in estrus) is dose-dependent, with ewes having more recently formed corpora lutea requiring a higher luteolytic dose of $PGF_{2\alpha}$ (Fierro et al., 2013; Pope et al., 2004; Loubser et al., 1981; Hackett et al., 1980), but another study found that lower doses to be sufficiently effective using more potent analogues of $PGF_{2\alpha}$ (Contreras-Solis et al., 2009). Additionally, the luteolytic efficacy of prostaglandin is day, frequency of exposure, and route dependent (Pope et al., 2004; Douglas and Ginther, 1973).

The effectiveness of prostaglandin-based approaches is limited to ewes with a mature, active corpus luteum a minimum of 3-5 days old for their efficacy in luteolysis (Hackett et al., 1980; Rubianes et al., 2003; Pope et al., 2004). The refractoriness of newly formed corpora lutea is restricted to the first two days (Rubianes et al., 2003), and has been shown to be associated with strong luteal catabolism of $PGF_{2\alpha}$ by 15-hydroxyprostaglandin dehydrogenase (PGDH); (Silva et al., 2000). Early corpora lutea (day 4) have been found to have increased intraluteal enzymatic activity of PGDH converting $PGF_{2\alpha}$ to 13,14-dihydro-15-keto- $PGF_{2\alpha}$ (PGFM) in comparison with older (day 13) corpora lutea (Silva et al., 2000; Hansen, 1974; Ensor and Tai, 1995). This in turn could prevent uterine $PGF_{2\alpha}$ from gaining access to receptors on large luteal cells and transiently prevent luteolysis (Silva et al., 2000).

When a single injection is applied to a flock of randomly cycling ewes, only 66% of ewes are expected to respond because the corpus luteum of the ewe is only responsive to prostaglandin $F_{2\alpha}$ between days 4-14 of the estrous cycle (Acritopoulou and Haresign, 1980). Ewes that are either in the follicular or early or late luteal phase will be unresponsive to its effect (Pope et al., 2004; Trounson et al., 1976). Whereas most studies have found a general reduction in fertility associated with prostaglandin use, some studies have found no differences when comparing long-term (12 day) progestagen-impregnated sponge use in comparison with an 11 day double injection regimen of $PGF_{2\alpha}$ (Hackett et al., 1981; Allison et al., 1978).

Although treatment with a single injection of $PGF_{2\alpha}$ could produce a high degree of synchrony if all ewes treated are in the luteal phase at the time of injection (Acritopoulou et al., 1977), this is often an unrealistic situation when attempting to synchronize estrus in a flock of randomly cycling ewes. The problem of ewes being refractory to the effect of $PGF_{2\alpha}$ due to treatment at stages of their estrous cycle in which they do not have a CL that is sensitive to exogenous prostaglandin can be avoided by providing two injections of $PGF_{2\alpha}$ 9-12 days apart (Loubser et al., 1981; Acritopoulou et al., 1978; Allison et al., 1978). This ensures that all ewes should be in the mid-luteal phase at the time of the second injection, but fertility will often still be reduced due to poor final maturation of preovulatory follicles from reduced LH (Barrett et al., 2002).

A comparison study of two commonly used prostaglandin-based protocols has produced estrus response and conception rates of 97% and 80% respectively for the 11 day double injection regimen in comparison with a 73% estrus response and 64% conception rate with a single injection utilizing 20 mg of $PGF_{2\alpha}$ (Beck et al., 1987).

However, other experiments have resulted in poor fertility using double injection protocols. One study found that only 45% of ewes administered a prostaglandin analogue (ICI-80,996) had ovulated by 70 hours following treatment in comparison with 90% treated with a progestogen (SC-9880) and of ewes that had ovulated, the progestogen-treated ewes had a significantly higher fertilization rate (69% vs. 7%) (Boland et al., 1978).

A number of factors could help explain the variable fertility often observed with prostaglandin-based synchronization methods. Prostaglandin-based protocols have been shown to alter sperm transport in the reproductive tract (Hawk and Cooper, 1977). Follicles induced to ovulate with prostaglandin $F_{2\alpha}$ have been observed to contain fewer granulosa cells and have resulted in a longer estrous cycle (Wiley et al., 1997). Synchronization of estrus with prostaglandin results in preovulatory follicles with altered steroidogenic capacity which produce subfunctional corpora lutea (White et al., 1987; Wiley et al., 1997; Letelier et al., 2011). Additionally, steroids are essential for preparation of the reproductive tract for fertilization and embryo transport and also serve to induce appropriate myometrial contractions for improvement of fertility (Fierro et al., 2011; Meikle et al., 2001; Sosa et al., 2008). Overall, these factors could help explain the reduction in fertility associated with the use of prostaglandin-based protocols in comparison with progesterone-based treatments.

IV. Effects of Application of Synthetic Progestogens on Components of the Estrous Cycle

i. Effect on Ovulation

Traditional progesterone-based synchronization protocols involved the use of progesterone or, more commonly, its analogues for durations that met or exceeded the length of the natural luteal phase of the ewe, which normally lasts 12-14 days and is the longest component of the estrous cycle (Dutt and Casida, 1948). This ensures that any existing corpora lutea in treated ewes would have sufficient time to undergo luteolysis by the time of removal of the progestogen source. Additionally, progesterone inhibits the secretion of GnRH and LH secretion so ovulation is blocked until progesterone decreases and its inhibitory effect on the release of LH is lost (McLeod et al., 1984; Amiridis et al., 2012). In conclusion, long-term progestogen treatment serves to mimic the corpus luteum to provide an artificial luteal phase for a period of time that would allow natural luteolysis to occur until progestational treatment ceases while blocking new ovulations.

ii. Effect on Synthesis/Secretion of PGF and Duration of the Estrous Cycle

The possibility for the potential effectiveness of short-term treatments was first proposed in 1967 that adequate synchronization could be accomplished by administering progesterone for a duration shorter than the natural lifespan of the CL (Woody et al., 1967). This was based on the observation that progesterone injected during the early luteal phase would induce premature luteal regression, thereby shortening the length of the cycle (Woody et al., 1967). It has been well established that progesterone plays a significant role in determining the length of the estrous cycle through establishing the timing of the release of $PGF_{2\alpha}$ (Ottobre et al., 1980; Silvia et al., 1991; Mann et al.,

2001). It is known that progesterone is responsible for potentiating the endometrium to secrete $PGF_{2\alpha}$ and regulating the timing of initial releases of $PGF_{2\alpha}$ for luteolysis as well as modulating its episodic release (Inskeep, 2005). Decreasing concentrations of progesterone induce a greater secretion of $PGF_{2\alpha}$ by the endometrium for the completion of luteolysis to occur, and maximal secretion of $PGF_{2\alpha}$ is released after luteal regression has occurred (Griffeth et al., 2002).

Although both progesterone as well as estrogen are known to play major roles in controlling the development of endometrial oxytocin receptors and $PGF_{2\alpha}$ release, treatment with progesterone alone has been demonstrated to be sufficient to induce responsiveness to oxytocin, as measured by release of $PGF_{2\alpha}$ (Homanics et al., 1988; Vallet et al., 1990). This has been demonstrated through the use of steroid-treated ovariectomized ewes and cows (Mann et al., 2001). However, endometrial receptor numbers decline by day 6 of progesterone treatment, at which point responsiveness to oxytocin is highest, so receptor concentrations do not appear to be a limiting factor in the regulation of luteolysis by progesterone (Mann et al., 2001).

Progesterone is necessary for synthesis of $PGF_{2\alpha}$, as it induces production of prostaglandin synthetase (cyclooxygenase) and phospholipase C regulatory enzymes (Raw et al., 1988; Raw et al., 1991; Salamonson et al., 1991). It has also been determined that while estradiol 17β has no effect on endometrial epithelial prostaglandin synthase levels, progesterone stimulates its production (Salamonson et al., 1991). This is an important observation, as prostaglandin synthase is the enzyme that converts arachidonic acid to the intermediary prostaglandins G and H, which are then converted to $PGF_{2\alpha}$ and other prostaglandins (Salamonson et al., 1991). Progesterone also appears to

regulate expression of the endometrial gene encoding cyclooxygenase, which is the rate-limiting enzyme in the metabolism of arachidonic acid to $PGF_{2\alpha}$ (Eggleston et al., 1990). These observations support the concept that progesterone is an adept regulator of the onset of uterine secretion of $PGF_{2\alpha}$ in the luteolytic mechanism.

Exogenously applied progesterone can alter the length of the cycle with the degree of its effect being dependent on the stage of the estrous cycle during which treatment is initiated (Woody et al., 1967a; Lewis et al., 1968; Ginther, 1969). Additionally, its effect seems to be dose-dependent to an extent; for example, a single large dose of progesterone given on the day of or one day following estrus reduced the length of the estrous cycle by 7-10 days (Thwaites et al., 1971; Dixon et al., 1973). A number of other studies have found that exogenous treatment with progesterone shortened the length of the cycle when given early in the cycle regardless of the route of administration (Woody et al., 1967; Thwaites, 1971; Lewis et al., 1968; Dixon et al., 1973; Ottobre et al., 1980).

The degree to which progesterone shortens the cycle appears to depend upon the time that it is first administered. It has been observed that progesterone's effect on cycle length reduction diminished as progesterone treatment was delayed from around metestrus (day 0-3) to a few days later in the cycle (day 4-7) (Ginther et al., 1968; Ginther et al., 1969). Additionally, treatment with progesterone later (days 12-15) tended to lengthen the estrous cycle, presumably through inhibition of ovulation and estrus (Ginther et al., 1969). The length of the estrous cycle tended to be reduced in intact compared to hysterectomized progesterone-treated ewes (Woody et al., 1968). This

suggests that progesterone-mediated luteolysis requires uterine components, which has been abundantly established to be through endometrial release of $PGF_{2\alpha}$.

Other studies have supported the proposition that the luteolytic effect of exogenous progesterone given early in the estrous cycle is mediated by an increase either in the synthesis or secretion of prostaglandin $F_{2\alpha}$ by the uterus between days 8-11 of the cycle (Lewis et al., 1977). This has been supported by other work involving use of ovariectomized ewes with autotransplanted uteri, where treatment with progesterone for 7 or 14 days increased the mean concentration of $PGF_{2\alpha}$ (Scaramuzzi et al., 1977). Whereas progesterone by itself has been shown to increase production of $PGF_{2\alpha}$ in ovariectomized ewes, application of exogenous estrogen has not produced the same effect (Caldwell et al., 1972). However, when estrogen is administered following sufficient pretreatment with progesterone, maximal secretion of $PGF_{2\alpha}$ occurs (Caldwell et al., 1972; Louis et al., 1977; Scaramuzzi et al., 1977). This is logical, as in the natural cycle estrogen would be secreted in increasing amounts during proestrus following priming by luteal progesterone and has experimentally been shown to produce a maximal luteolytic effect when combined with $PGF_{2\alpha}$ above prostaglandin alone in hysterectomized ewes (Gengenbach et al., 1977).

It had also been proposed that progesterone's effect on inducing premature luteolysis could be due to suppression of luteotropins (Ottobre et al., 1980). In immature, ovariectomized, and mature ewes, it was observed that treatment with progesterone decreased LH secretion so this could be a contributing factor to decreasing the lifespan of these corpora lutea (Karsch et al., 1977; Foster and Karsch, 1976; Ottobre et al., 1980). Supplementation with exogenous LH or HCG, which is LH-like, early in the cycle has

been shown to partially negate the early luteolysis-inducing effect of progesterone, but additional corpora lutea were often formed with addition of these hormones (Lewis et al., 1968). Around the time of ovulation, it has been shown to be unlikely that the cycle length is decreased as a result of reduced LH surges, because cycle length was not correlated with peak levels of LH in either progesterone-treated or control ewes (Ottobre et al., 1980). This is supported by other work in which progesterone administered *after* the LH surge still shortened the length of the cycle (Dixon and Thwaites, 1973; Lewis et al., 1968; Thwaites, 1971). Nonetheless, reduced LH concentrations could be a contributing factor by increasing the sensitivity of the corpus luteum to prostaglandin (Ottobre et al., 1980). However, secretion of $PGF_{2\alpha}$ appears to have a more potent effect on luteolysis than luteotropic factors (Woody et al., 1968; Ginther, 1968; Lewis et al., 1977). These results are supportive of the concept that the initial rise in $PGF_{2\alpha}$ occurs in response to exogenous progesterone (or that secreted by the corpus luteum in the unaltered cycle).

Typically in short-term protocols a portion of ewes will not respond to treatment as a result of having corpora lutea that may not have adequate time to regress naturally during the short treatment period. The progesterone pretreatment will prevent the formation of any new luteal tissue and ensure that any resulting corpora lutea are at least 5 days old (if a 5 day progesterone pretreatment is used) so are therefore sensitive to $PGF_{2\alpha}$ (Beck et al., 1993). Exogenous $PGF_{2\alpha}$ can be included at the beginning or end of the treatment period in order to regress any existing corpora lutea that may remain, and additionally prostaglandin has been demonstrated to be capable of inducing ovulation when used with progestagen treatment with its effect likely being exerted directly on the

ovary (Davies et al., 2006). Therefore at cessation of treatment with progesterone, because all ewes should have no remaining corpora lutea, the inhibitory effect on the hypothalamus and anterior pituitary gland should be removed and ewes should enter the follicular phase in a controlled manner.

In conclusion, progesterone treatments can also contribute to synchronizing estrus by inducing an early onset of luteolysis when given early in the cycle. The maintainance of a high concentration of progesterone later in the cycle would block estrus and ovulation until cessation of progestational treatment. This occurs due to the inhibitory characteristic of progesterone on reducing the frequency of GnRH pulses, thereby preventing the LH surge required for ovulation (Inskeep, 2005). At progesterone removal, ovulation can ensue in ewes that were in the follicular phase at device insertion, and corpora lutea will have been regressed in most ewes that were in the luteal phase at CIDR insertion.

V. Effects of Application of Synthetic $PGF_{2\alpha}$ on Components of the Estrous Cycle

Single injection regimens can produce poor synchrony due to the fact that the day of the cycle in which $PGF_{2\alpha}$ is administered influences the interval to the onset of estrus. The duration to the onset of estrus has been found to be positively correlated with the stage of the estrous cycle, with ewes treated earlier showing an earlier onset and those later showing a delayed onset (Acritopoulou and Haresign, 1980; Deaver et al., 1986; Houghton et al., 1995). This is based on the variation in time necessary to reduce the elevated progesterone to its basal level, as corpora lutea gain their maximal endocrine functionality later in the luteal phase (Deaver et al., 1986; Houghton et al., 1995). These disparities in the interval to the onset of estrus could also conceivably be attributed to the

size of follicles present at luteolysis as there is a greater mixture of growing, static, and atretic follicles in the mid- to late-luteal phase (Houghton et al., 1995).

The day of cycle at which prostaglandin is administered throughout the luteal phase is also known to influence other components of the estrous cycle, including follicular function, growth, and maturation as well as the origin of the preovulatory follicle (POF) (Abecia et al., 2011; Vinoles and Rubianes, 1998). Ewes injected with $PGF_{2\alpha}$ that are in the mid-luteal phase have been observed to typically have their POF originating from the second wave (Vinoles and Rubianes, 1998). However, ewes in the early luteal phase can have their POF derived from either the first or second wave, depending on the status of the dominant follicle (growing, static, or regressing) (Vinoles and Rubianes, 1998). $PGF_{2\alpha}$ administration during the mid-luteal phase could result in compromised follicular function from the induction of lower levels of LH as a result of a higher progesterone concentration (Deaver et al., 1986; Campbell et al., 1995; Gonzalez-Bulnes et al., 2004). Additionally, injection during the endogenous FSH peak during the mid-luteal phase has been observed to result in disruption of the endogenous FSH secretion pattern and was associated with untimely follicular rupture and luteal inadequacy (Liu et al., 2006).

Induction of luteolysis during the early luteal phase has also been associated with a higher sustained concentration of plasma FSH, whereas treatment at later stages was followed by a significant decrease (Deaver et al., 1986). The possibility of compromised follicular function would be a conceivable outcome, as gonadotropins are imperative for maintaining the steroidogenic capacity of preovulatory follicles. With LH being necessary for final follicular growth and maturation, follicular functionality could

therefore be impaired (Deaver et al., 1986; Campbell et al., 1995). Therefore, these observations suggest that the early or late luteal phase would be a more ideal endocrine setting for induction of luteolysis.

Despite the varying endocrinological setting among different stages of the luteal phase, no differences have been observed between ovulation rate or duration of estrus based on stage of the estrous cycle with $PGF_{2\alpha}$ treatment (Deaver et al., 1986; Houghton et al., 1995; Barrett et al., 2002). However, greater follicular recruitment has been observed early and late in the estrous cycle, during which time progesterone is low (Brand and de Jong, 1973; Schrick et al., 1993). Additionally, ewes with low expected circulating progesterone have been observed to have an increased ovulation rate, as found in a study where ewes' endogenous progesterone was removed (through induced luteolysis) and was replaced with lower circulating concentrations with intravaginal inserts (Devonish et al., 2009). Breed differences have also been found, with more prolific Finnsheep having a lower luteal progesterone concentration and a higher ovulation rate and less prolific Western white-faced ewes having a higher progesterone concentration and lower ovulation rate (Bartlewski et al., 1999). However, this would suggest the potential for an observable improvement in lambing outcomes from induced luteolysis in ewes at the early versus mid- to late-luteal phase.

One study has investigated lambing outcomes in ewes separated into individual groups undergoing induced luteolysis between days 5-11 after ovulation and, converse to other studies, found no difference between group means for duration of time from treatment to onset of estrus (Schoombee, 1996). Additionally, similarly low conception rates were observed among all groups and no differences were detected among lambing

outcomes (Schoombee, 1996). However, the suboptimal dose of 10 mg of $PGF_{2\alpha}$ used in the experiment in combination with artificial insemination and exceedingly small samples sizes (4-14 ewes per group) allow for ample improvement in further studies to more accurately detect potential differences in lambing outcomes between treatment groups.

STATEMENT OF THE PROBLEM

Estrous synchronization is an important component of reproductive management of farm animal species. An important objective of efficient estrous synchronization in sheep operations is to provide a cost-effective mode of synchronizing parturitions in order to produce offspring at a desired timepoint, facilitate better assignment of labor, reduce neonatal mortality, and improve strategic marketing of lambs in order to increase farm production and profitability. Over the past 70 years, a number of methods have been developed which have primarily been founded upon either progestogen or prostaglandin-based treatments. Traditional progestogen-based approaches have often resulted in decreased fertility and have required a long treatment period but have typically resulted in greater estrus synchronization rates than prostaglandin-based approaches.

Short-term progestogen treatments in combination with $PGF_{2\alpha}$ have been demonstrated to be an effective method for synchronizing estrus, but present a 30% increase in treatment cost over progestogen treatment alone, and combining progesterone with $PGF_{2\alpha}$ resulted in a negative effect on ewe fertility (D'Souza et al., 2011). Results from recent studies indicate that $PGF_{2\alpha}$ inclusion may not be necessary with short-term progestogen to induce a synchronized estrus (D'Souza et al., 2011). Therefore, it is necessary to further evaluate the effect of $PGF_{2\alpha}$ on ewe fertility and to determine if the time of $PGF_{2\alpha}$ injection relative to application of progesterone significantly impacts ewe fertility in order to justify its inclusion in short-term progestogen-based protocols.

Review of existing literature provided evidence that at different stages of the estrous cycle, follicular development deviates in association with varying concentrations of circulating progesterone. Research performed on the effect of $PGF_{2\alpha}$ injected at different stages of the estrous cycle has shown variations in follicular development and origin of the preovulatory follicle; time of ovulation; estrus response, duration to onset of estrus; and concentrations of ovarian and pituitary hormones. Therefore, overall reproductive outcomes following use of $PGF_{2\alpha}$ in estrous synchronization protocols might be dependent on the day of the estrous cycle on which luteolysis is induced. However, few studies have evaluated the potential effect of $PGF_{2\alpha}$ injection at different stages of the luteal phase on final reproductive outcomes, and those that have were conducted with a number of suboptimal experimental conditions, including small sample sizes, use of a substandard dose of $PGF_{2\alpha}$, and use of artificial insemination rather than natural service. Therefore, the objective of the second study was to evaluate if day of the cycle on which $PGF_{2\alpha}$ is administered affects ewe fertility.

INTRODUCTION

Efficient methods of estrous synchronization are essential for optimizing the productivity and profitability of breeding operations by reducing the time and cost associated with lambing and producing a more uniform lamb crop that can be strategically targeted to specific markets. Over the past few decades, the sheep industry has been in a state of continuous decline, and increased reproductive efficiency could help revive the state of the industry (Knights et al., 2003). Estrus synchronization approaches have been based primarily upon their effect on manipulating the natural ovine luteal phase, which can be defined as the length of time during which one or more corpora lutea are present on the ovary (Senger, 2012). Most approaches used to synchronize estrus are based on either shortening the luteal phase through treatment with a luteolytic dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or by using progestogens to provide an artificial luteal phase for 12-14 days, during which existing corpora lutea would have sufficient time to regress through endogenously produced $PGF_{2\alpha}$ (Dutt and Casida, 1948; Robinson, 1964; Allison and Kelly, 1978). However, regimens using a single injection of $PGF_{2\alpha}$ are only effective in synchronizing estrus in the approximately 66% of ewes in a flock that are between days 4-14 of the estrous cycle and have corpora lutea that are responsive to $PGF_{2\alpha}$ (Acritopoulou and Haresign, 1980). Other prostaglandin-based regimens have been developed that use multiple injections and have had variable success (Boland et al., 1978; Allison et al., 1978).

Typical estrous synchronization rates associated with prostaglandin-based and progestogen-based protocols are approximately 70% and 80% respectively (Gonzalez-

Bulnes et al., 2002; Simonetti et al., 2000). Because the fertility obtained from a particular estrous synchronization method typically determines its feasibility, long term progestogen-based synchronization approaches have generally been preferred over $PGF_{2\alpha}$-based methods. Based on the assumption that progestogen treatment must span the length of the natural ovine luteal phase in order to ensure the absence of luteal tissue at treatment cessation, traditional progestogen-based protocols were utilized for 12-14 days or longer (Dutt and Casida, 1948). Long-term treatments have also been implicated in a reduction in the total number of follicles present as well as an impairment in the viability of resulting embryos (Gonzalez-Bulnes et al., 2005). Long-term treatments could be associated with subluteal concentrations of progesterone. Studies using progesterone containing delivery devices have demonstrated that after 72 hours circulating progesterone levels continually decrease (Wheaton, 1993). Subluteal concentrations of progesterone have been implicated with development of large (Dutt and Casida, 1948) or persistent follicles (Johnson et al., 1996). Ewes exposed to a subluteal concentration of progesterone during estrous synchronization treatment have also been observed to have decreased fertility compared to control ewes in some studies (Johnson et al., 1998; Robinson et al., 1970), whereas in other studies (Evans et al., 2001) no difference has been observed.

Short-term progestogen treatments provide a higher mean circulating concentration of progesterone over the shorter duration of treatment and recent studies have provided evidence that short-term treatments can result in higher pregnancy rates than long-term treatments (87 vs. 63%; Vinoles et al., 2001). The higher pregnancy rate obtained in ewes receiving the short-term progestogen treatment was also related to a

more rapid follicular turnover and the ovulation of more newly recruited follicles in comparison with ewes receiving a longer period of progestogen treatment (Vinoles et al., 2001).

Due to the fact that it was universally believed that it was necessary for the duration of progestogen treatment to span the entirety of the natural luteal phase, most studies utilizing short-term progestogen treatments have used them in combination with $PGF_{2\alpha}$ at progestogen withdrawal (Loubser and Van Niekerk, 1981; Fitzgerald et al., 1985; Beck et al., 1993; Dixon et al., 2006). These treatments have been effective, as the progestogen pretreatment prevents formation of corpora lutea for the duration of treatment, which ensures that any existing corpora lutea at the end of pretreatment are sensitive to an applied luteolytic dose of $PGF_{2\alpha}$ (Acritopoulou et al., 1980; Beck et al., 1993). For example, prior studies have shown an enhanced estrus response (84 vs. 62%) and first service pregnancy rates (64 vs. 44%) with combination treatments over $PGF_{2\alpha}$ alone (Dixon et al., 2006). However, despite the high degree of synchrony produced by short-term combination treatments, the inclusion of $PGF_{2\alpha}$ costs approximately 30% more, which limits the adoption of the hormonal combination for synchronization of estrus in commercial flocks.

The potential use of short-term progestogen-based treatments was first suggested in 1967 through a study where it was observed that administration of progesterone early in the estrous cycle would shorten the cycle through premature luteal regression (Woody et al., 1967). Several studies thereafter validated the original observation and demonstrated that progesterone plays a major role in establishing the timing of $PGF_{2\alpha}$ release (Thwaites, 1971; Lewis et al., 1968; Dixon et al., 1973, Ottobre et al., 1980).

However, few studies on estrous synchronization in sheep have been conducted using a short-term progestogen treatment without concomitant application of $PGF_{2\alpha}$. More recently, treatment with progesterone for 5 days alone resulted in higher conception rates (67 vs 53%) and first service pregnancy rates (58 vs. 44%) with no difference in estrous response (82%) compared to using short-term progesterone treatment with inclusion of $PGF_{2\alpha}$ (D'Souza et al., 2011).

It is also known that day of cycle on which $PGF_{2\alpha}$ is administered influences a number of variables, including the interval to the onset of estrus (Acritopoulou and Haresign, 1980; Deaver et al., 1986). Ewes injected with $PGF_{2\alpha}$ earlier in the estrous cycle have a more rapid onset to estrus (day 5, 36 hours; day 8, 41 hours; day 11, 48 hours) (Houghton et al., 1995). This is based on the variation in time necessary to reduce the elevated progesterone to its basal level, as corpora lutea gain maximal endocrine functionality as they mature (Deaver et al., 1986; Houghton et al., 1995). It has also been shown follicular dynamics vary with the day of the estrous cycle, as ewes in the mid- to late-luteal phase have a greater mixture of growing, static, and atretic follicles than those in the early luteal phase (Houghton et al., 1995). Ewes treated during the early luteal phase have a higher sustained concentration of FSH and higher concentrations of LH (Deaver et al., 1986). It has been suggested that treatments with $PGF_{2\alpha}$ later in the cycle could result in compromised follicular function as a result from greater inhibition of LH release by the higher circulating concentration of progesterone (Deaver et al., 1986; Campbell et al., 1995; Gonzalez-Bulnes et al., 2004). Therefore, the objectives of this study were 1) to evaluate the necessity and potential effect of time of application of exogenous $PGF_{2\alpha}$ relative to progesterone treatment as part of a short-term progesterone-

based estrous synchronization protocol and 2) to determine if the day of cycle at which luteolysis is induced influences reproductive outcomes.

MATERIALS AND METHODS

General

Mature, non-lactating ewes of mixed breeding were managed on mixed grass legume pastures, and were supplemented with hay as needed and provided with water *ad libitum*. Ewes were brought into holding pens for treatment and pregnancy diagnoses. Ewes were studied during the fall breeding season in August 2013 and August 2014. All ewes were separated from rams for a minimum of 30 days prior to the beginning of each experiment. All rams were sexually mature and of proven fertility, and were used at a ram:ewe ratio no greater than 1:20.

Treatments

All experimental procedures were approved by the West Virginia University Animal Care and Use Committee (protocol #10-1102).

Experiment 1

In order to evaluate the effect of time of application of $PGF_{2\alpha}$ relative to treatment with progesterone on fertility, ewes (*N*=442) from 4 farms located in WV and PA were randomly assigned to receive progesterone using a controlled internal drug-releasing device (CIDR-g, 0.3 g progesterone; Zoetis, Florham Park, NJ) for 5 days alone (*n*=123; treatment 1), in combination with 25 mg $PGF_{2\alpha}$ (5 mL Lutalyse; Dinoprost Tromethamine; Zoetis, Florham Park, NJ) via intramuscular injection at CIDR insertion (*n*=103; treatment 2) or removal (*n*=100; treatment 3), or 25 mg $PGF_{2\alpha}$ alone (*n*=116; treatment 4) prior to being joined with sexually mature rams.

Experiment 2

This study was designed to compare reproductive performance in ewes treated with $PGF_{2\alpha}$ at different stages of the estrous cycle. The estrous cycle of ewes (*N*=148) from 1 farm located in southwestern Pennsylvania were pre-synchronized using treatment with a CIDR device for 7 days. This treatment was shown to be effective in synchronizing estrus in approximately 82% of ewes within 2-3 days after removal (day 0; D'Souza et al., 2011). Ewes were randomly assigned to receive a 25 mg intramuscular injection of $PGF_{2\alpha}$ 7 (*n*=48), 10 (*n*=50), or 13 (*n*=50) days following CIDR removal which was projected to be equivalent to day 5, 8, and 11 of the estrous cycle, and sexually mature rams were introduced to all ewes at $PGF_{2\alpha}$ injection (figure 1).

Blood was collected via jugular venipuncture into heparinized tubes (EDTA; Monoject, 15% EDTA K3 liquid, Tyco Healthcare Group, Mansfield, Massachusetts, USA) two days post-CIDR removal, the day of $PGF_{2\alpha}$ injection, and the day following $PGF_{2\alpha}$ injection to determine progesterone concentration for comparison across groups as well as to verify treatment effect. Thirty-three ewes (*n*=11 per treatment) that expressed estrus after $PGF_{2\alpha}$ injection were chosen for analysis of plasma progesterone concentration. Plasma was separated via centrifugation for 15 minutes at 3500 rotations per minute and pipetting and stored at -20° C until assayed for progesterone (PROG-RIA-CT; DIAsource ImmunoAssays, Louvain-La-Neuve Belgium).

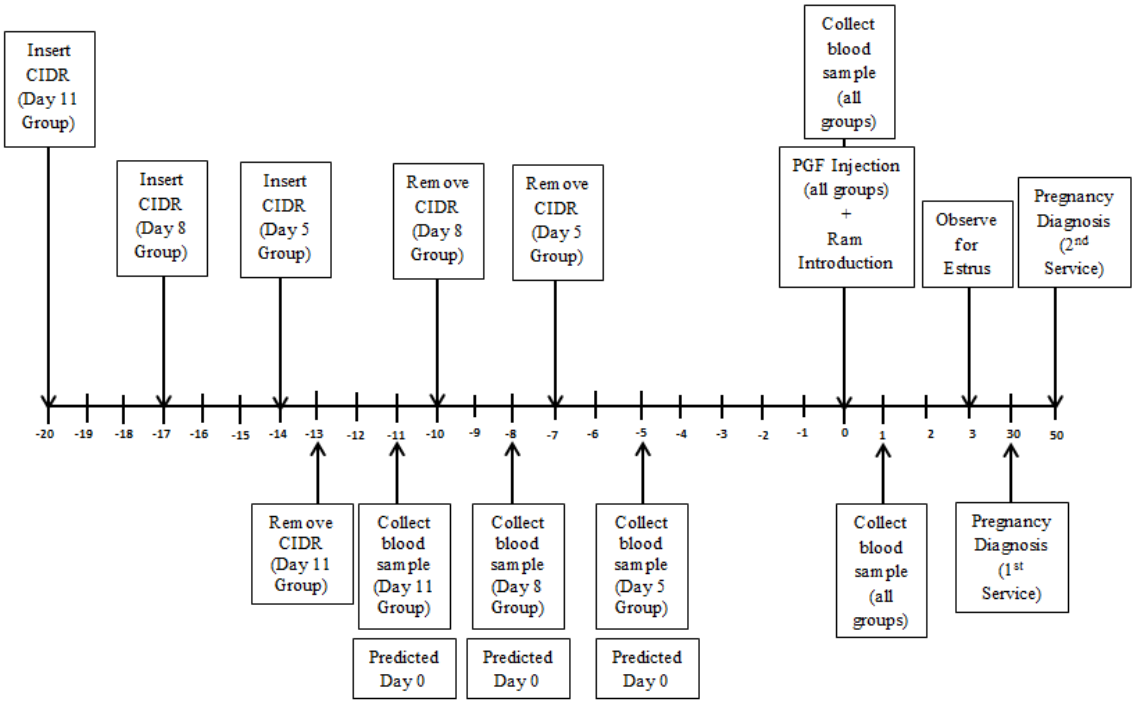

Figure 1. Logistical timeline of Experiment 2 methodology

Estrous Detection

Rams were raddled with paint crayons in harnesses on their briskets and estrus response was evaluated in both experiments through visual inspection for rump marks on ewes three days following ram introduction at all farms.

Pregnancy Diagnosis and Lambing Data

Ultrasonographic observations for pregnancy diagnoses in experiments 1 and 2 (Schrick and Inskeep, 1993), were made at 30-35 days after ram introduction to determine pregnancy to the first service and again 50-55 days post-ram introduction to determine pregnancy to the second service at all farms. Ewes were scanned via

transrectal ultrasonography using an Aloka 500 console equipped with a 7.5 mHz linear array transducer (Corometrics Medical Systems, Wallingford, CT). Lambing records, including dates and numbers of lambs born per ewe, were recorded in both experiments.

Statistical Analyses

Data were analyzed in both experiments through analysis of variance using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with the model consisting of the main effects of treatments, farms (experiment 1) and their interactions (experiment 1) and additionally, least square means for treatment effects were determined. Response variables included overall prolificacy (number of lambs born per ewe lambing), first service prolificacy, the interval from ram introduction to lambing, the mean lambing day (the day that a ewe lambed during the lambing period) and lambing rates (the number of lambs born per ewe treated). Other response variables evaluated were estrous response, conception rate, pregnancy rate to first and second services, and proportion of ewes lambing to the first service period.

RESULTS

Experiment 1

The mean response variables for ewes treated with $PGF_{2\alpha}$ at different timepoints in relation to short-term CIDR use are summarized in table 1. Estrous response did not differ among ewes receiving progesterone-based treatments but was significantly lower in ewes receiving $PGF_{2\alpha}$ only ($p=0.005$; table 1). Conception rates tended to be higher in CIDR only than ewes receiving CIDR and $PGF_{2\alpha}$ at device insertion ($p=0.07$). Pregnancy rate to first service was higher in ewes receiving CIDR devices only ($p=0.03$) than ewes receiving $PGF_{2\alpha}$ alone and at device removal (figure 2). Mean second service pregnancy rates were 82.4% and did not differ between groups (figure 2).

Overall prolificacy did not differ with treatment, but prolificacy to first service was significantly higher ($p=0.05$) in ewes receiving $PGF_{2\alpha}$ only compared to ewes receiving $PGF_{2\alpha}$ at device removal. The percent of ewes lambing to first service was greater in ewes treated with CIDR only than in both ewes treated with $PGF_{2\alpha}$ only ($p=0.05$) and in ewes treated with $PGF_{2\alpha}$ at CIDR removal. Ewes lambing to first service was also higher in ewes treated with $PGF_{2\alpha}$ at CIDR insertion than in ewes treated with $PGF_{2\alpha}$ only ($p=0.02$) and tended to be higher than ewes treated with $PGF_{2\alpha}$ at CIDR removal than ewes treated with $PGF_{2\alpha}$ only ($p=0.10$). There were no effects of treatments on mean interval from ram introduction to lambing (159 ± 3.2 days), lambing day (15.2 ± 3.2 days), and percent lambed (89.9 ± 5.5%).

Table 1. Reproductive performance of ewes in response to a 5 day progesterone treatment alone or in combination with $PGF_{2\alpha}$ at insert removal or insertion or $PGF_{2\alpha}$ alone (experiment 1).

Variable	Treatment			
	CIDR only	**CIDR + PGF at Insertion**	**CIDR + PGF at Removal**	**PGF only**
Estrous response (%)	85.6 ± 3.7^{b}	84.1 ± 4.2^{b}	83.0 ± 4.2^{b}	66.5 ± 4.0^{a}
Conception Rate (%)	73.3 ± 4.6^{a}	65.0 ± 5.3^{b}	60.7 ± 5.4^{ab}	74.5 ± 9.5^{ab}
1st Service Pregnancy Rate (%)	67.7 ± 4.4^{a}	58.3 ± 4.9^{ab}	53.8 ± 4.9^{b}	48.7 ± 4.7^{b}
2nd Service Pregnancy Rate (%)	83.4 ± 3.9	85.2 ± 4.3	80.4 ± 4.3	81.7 ± 4.2
1st Service Prolificacy	$1.45 \pm .10^{ab}$	$1.64 \pm .12^{ab}$	$1.30 \pm .17^{b}$	$1.75 \pm .15^{a}$
Overall Prolificacy	$1.56 \pm .07$	$1.59 \pm .08$	$1.51 \pm .08$	$1.64 \pm .08$
Lambing to 1st Service	54.0 ± 4.6^{a}	52.3 ± 5.2^{ab}	40.3 ± 5.3^{bc}	35.2 ± 5.0^{c}
Ram Introduction to Lambing (d)	158.4 ± 1.2	157.4 ± 1.4	160.6 ± 1.4	160.2 ± 1.3
Lambing day (d)	14.4 ± 1.2	13.4 ± 1.4	16.5 ± 1.4	16.2 ± 1.3
Lambed (%)	89.1 ± 4.0	90.4 ± 4.0	92.8 ± 4.3	86.2 ± 3.5

Values with differing superscripts across a row are significantly different ($p \leq .05$) or have a tendency to differ ($p \leq .10$).

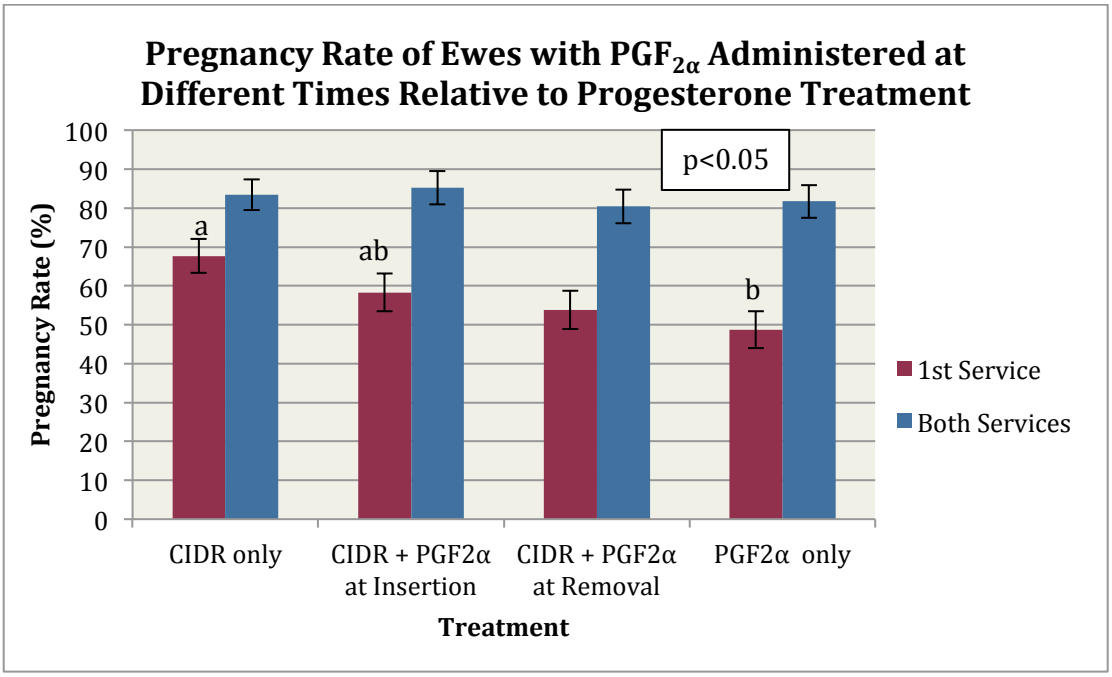

Figure 2. Pregnancy rate, determined as the percent of ewes diagnosed as pregnant of ewes exposed via transrectal ultrasonography 30 days (1st service period) or 50 days (both service periods) following ram introduction. Values with differing letters represent significant differences ($p<0.05$).

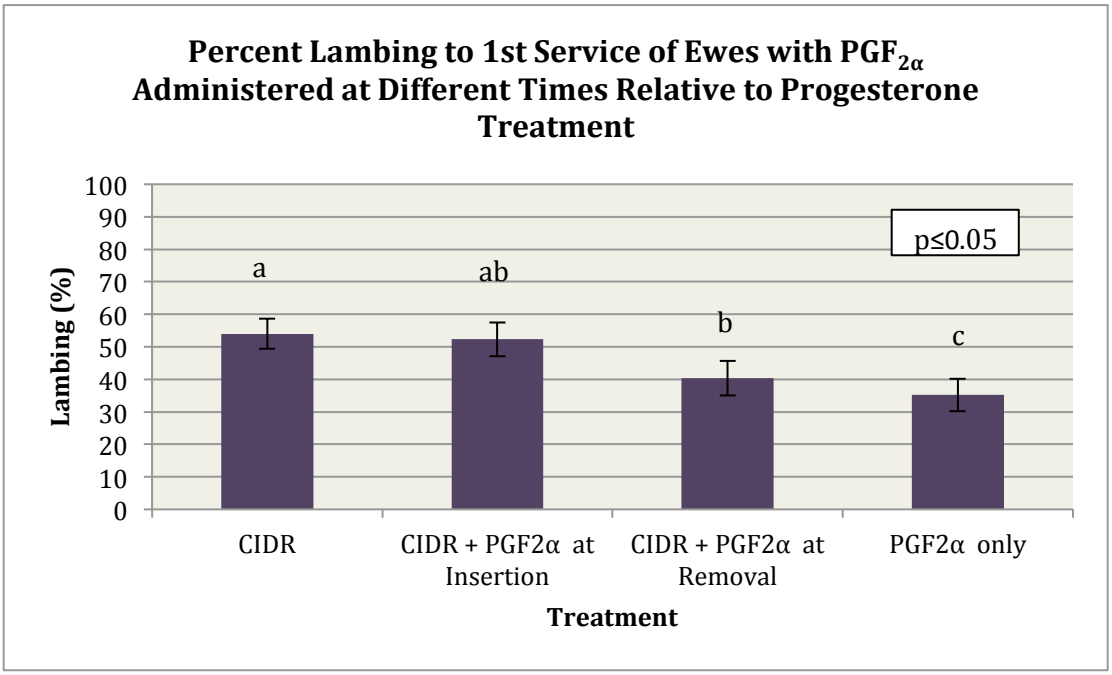

Figure 3. Lambing percent to the first service period was calculated as the number of lambs born within the first 15 days of the lambing period per ewe exposed. Values with differing letters represent significant differences ($p\leq0.05$).

Experiment 2: Reproductive Variables and Hormonal Analysis

The mean progesterone concentration measured at predicted day 0 was 3.06 ng/ml and did not differ by treatment. Ewes predicted to be on day 5 of the estrous cycle had a significantly lower mean progesterone concentration compared to ewes predicted to be on day 8 and 11 ($p<0.001$; 2.93 ± .34 vs. 4.62 ± .34, 5.16 ± .34 ng/ml, table 2). At 24 hours following induction of luteolysis, the mean progesterone concentration decreased to 2.2 ng/ml and did not differ by treatment (Table 2; figure 8).

Table 2. Mean progesterone concentrations measured at predicted day of cycle (5, 8, and 11) at different experimental timepoints

Timepoint of Measurement	**Mean Progesterone Concentration (ng/ml)**			**P-value**
	Day 5	**Day 8**	**Day 11**	
Predicted Day 0	3.07 ± .34	2.96 ± .37	3.15 ± .34	NS
Predicted Group Day	2.93 ± .34[a]	4.62 ± .34[b]	5.16 ± .34[b]	<.001
24 Hours after $PGF_{2\alpha}$ Injection	1.99 ± .34	2.26 ± .35	2.35 ± .34	NS

Values with differing superscripts across a row are significantly different ($p\leq0.05$). NS=not significantly different.

The mean values of reproductive variables for ewes treated with 25 mg $PGF_{2\alpha}$ at projected day 5, 8, and 11 of the estrous cycle are summarized in table 3. Mean estrous response was 85.1% and did not differ with treatment. Conception rate was higher in ewes injected with $PGF_{2\alpha}$ at predicted day 5 than those injected at day 11 ($p<0.05$; table 3; figure 4) and tended to be higher than ewes injected on day 8 ($p<0.1$).

Pregnancy rate to first service tended to be higher in ewes injected on day 5 than those injected on day 11 ($p=0.07$; table 3). The mean pregnancy rate to both service

periods did not differ (93.9 ± 4.9%; figure 5). Overall prolificacy was 1.54 and did not differ with treatment.

Prolificacy of ewes lambing to the first service period was greater in ewes injected on day 5 than those injected on day 11 (p=0.05; figure 6) and was intermediate in ewes injected with $PGF_{2\alpha}$ at predicted day 8 (p=0.1). More ewes lambed to first service when injected with $PGF_{2\alpha}$ at day 5 than those injected at either day 8 or 11 (p=0.05; figure 7), but the overall mean lambing percent was not affected by treatments. The mean interval from ram introduction to lambing and the lambing day were shorter in ewes injected with $PGF_{2\alpha}$ on day 5 than ewes in other treatment groups (p=0.002).

Table 3. Reproductive performance of ewes in response to $PGF_{2\alpha}$ treatment on predicted days 5, 8, and 11 of the estrous cycle (experiment 2).

Variable	**Stage of Estrous Cycle**		
	Day 5 (n=48)	**Day 8 (n=50)**	**Day 11 (n=50)**
Estrous response (%)	85.4 ± 5.2	84.0 ± 5.1	86.0 ± 5.1
Conception Rate (%)	70.7 ± 7.7[a]	52.3 ± 7.6[ab]	48.8 ± 7.5[b]
1st Service Pregnancy Rate (%)	62.5 ± 7.2[a]	48.0 ± 7.1[ab]	44.0 ± 7.1[b]
2nd Service Pregnancy Rate (%)	89.6 ± 3.5	96.0 ± 3.4	96.0 ± 3.4
1st Service Prolificacy	1.67 ± .11[a]	1.40 ± .14[ab]	1.33 ± .13[b]
Overall Prolificacy	1.56 ± .09	1.56 ± .09	1.51 ± .09
Lambing to 1st Service	57.4 ± 7.0[a]	30.0 ± 6.8[b]	36.0 ± 6.8[b]
Ram Introduction to Lambing (d)	153.7 ± 1.4[a]	160.9 ± 1.3[b]	159.7 ± 1.4[b]
Lambing day (d)	10.7 ± 1.4[a]	17.9 ± 1.3[b]	16.7 ± 1.4[b]
Lambed (%)	85.4 ± 5.1	90.0 ± 5.0	82.0 ± 5.0

Values with differing superscripts across a row are significantly different (p≤0.05) or have a tendency to differ (p≤0.10).

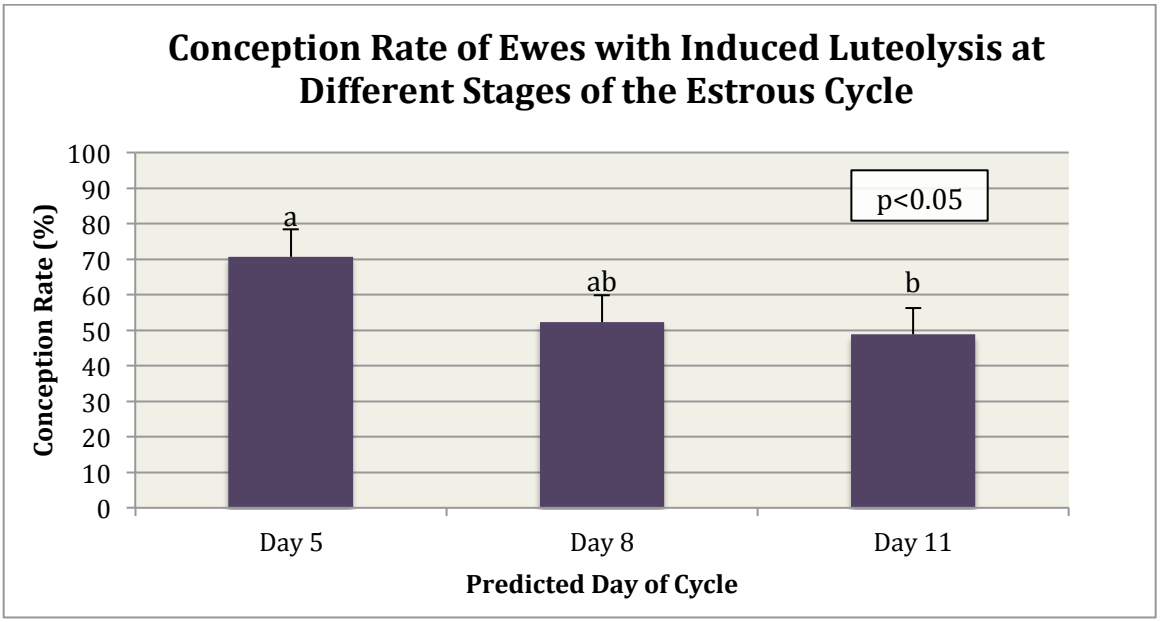

Figure 4. Conception rate was calculated as the number of ewes diagnosed as pregnant at 30 days (1st service) of the ewes that were observed to be in estrus by 72 hours following ram introduction. Values with differing letters represent significant differences ($p<0.05$).

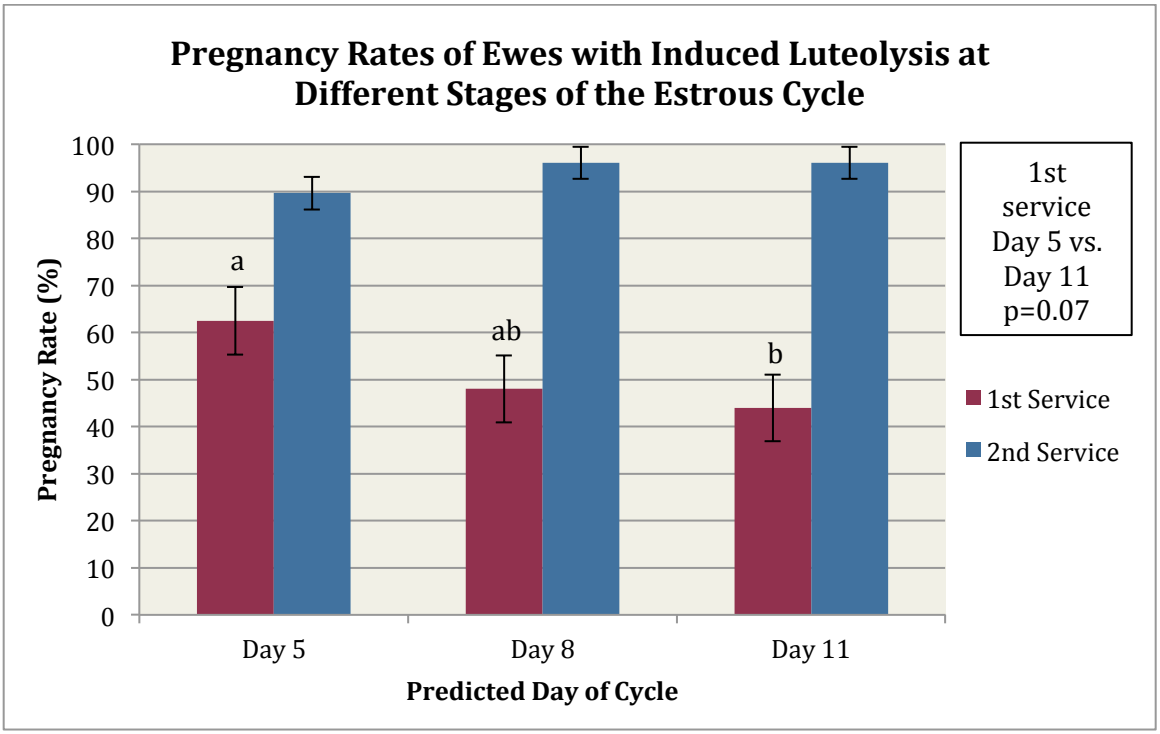

Figure 5. Pregnancy rate, determined as the percent of ewes diagnosed as pregnant of ewes exposed via transrectal ultrasonography 30 days (1st service period) or 50 days (2nd service period) following ram introduction. Values with differing letters represent a tendency to differ between treatment groups ($p=0.07$).

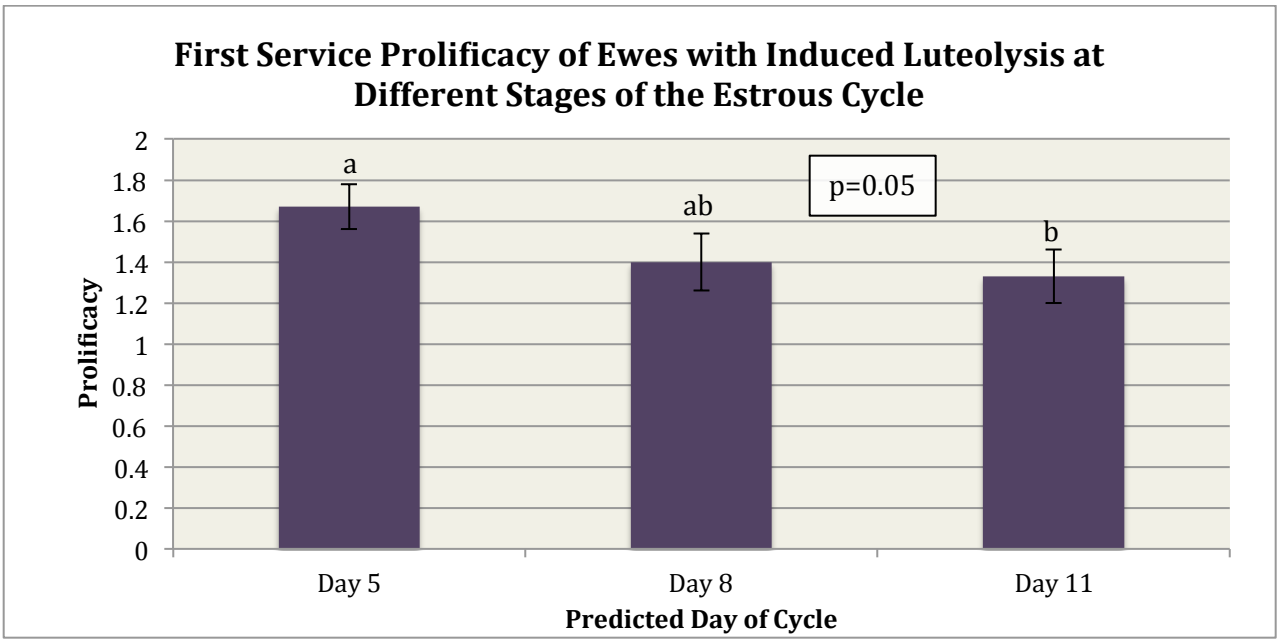

Figure 6. Prolificacy: the number of lambs born per ewe lambing to the first service period (first 15 days of the lambing period). Values with differing letters represent significant differences (p=0.05).

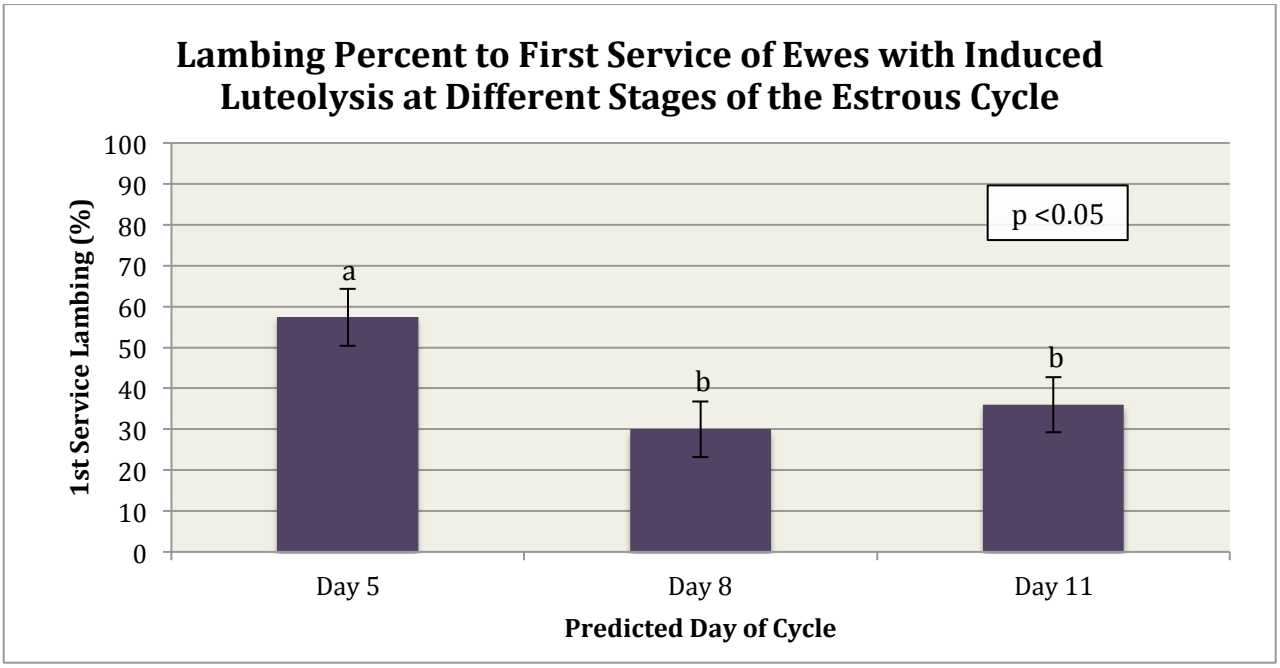

Figure 7. Lambing percent to the first service period was calculated as the number of lambs born within the first 15 days of the lambing period per ewe exposed. Values with differing letters represent significant differences (p<0.05).

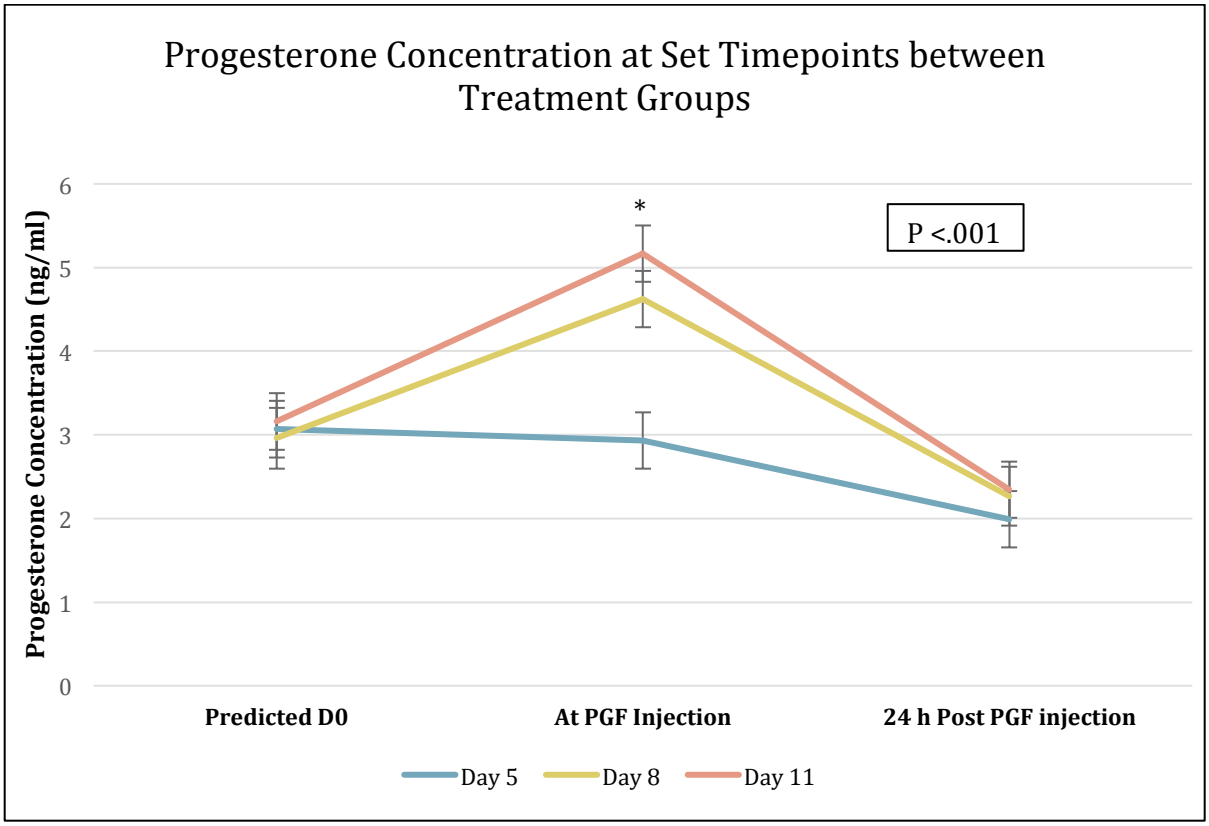

Figure 8. Mean progesterone concentration measured across groups at different predicted days of cycle at which luteolysis was induced with $PGF_{2\alpha}$, being day 5 (blue), day 8 (yellow), and day 11 (orange). Mean concentrations were obtained from plasma collected at the predicted day 0 (48 hours after CIDR removal), at $PGF_{2\alpha}$ injection, and 24 hours following application of $PGF_{2\alpha}$.

DISCUSSION

The mean estrous response of ewes treated with progesterone both alone as well as in combination with $PGF_{2\alpha}$ was approximately 84%. D'Souza et al. (2011) observed no difference between ewes receiving progesterone treatment alone versus those additionally receiving $PGF_{2\alpha}$ (82%), which is in direct agreement with outcomes of this experiment. Additionally, estrus rates of 89% (Fitzgerald et al., 1985), 84% (Dixon et al., 2006), 93% (Ozturkler et al., 2003), and 100% (Beck et al., 1993; Greyling et al., 1979) have been previously observed with the use of combination treatments. The response obtained from the current study did not differ significantly among ewes receiving progesterone pretreatment (84%), but was lower in ewes treated only with $PGF_{2\alpha}$. This is consistent with the findings of Acritopoulou and Haresign (1980), who estimated that a single injection of $PGF_{2\alpha}$ to a flock of randomly cycling ewes would synchronize 66% of treated ewes, as that would be the approximate proportion of ewes in the luteal phase with a CL sensitive to luteolysins (day 4-14) at any given time. Other studies using $PGF_{2\alpha}$ only regimens have produced similar estrus responses of 62.5% (Dixon et al., 2006) and 52.9% (Beck et al., 1993).

Few other studies have been conducted using short-term progesterone treatment alone in an attempt to synchronize estrus in ewes. Vinoles et al. (2001) obtained an estrus percentage of 95%, albeit estrus was measured at 144 hours following device removal. While the CIDR only group in this experiment had the highest first service pregnancy rate of treatment groups (67.7 ± 4.4%), the first service pregnancy response obtained by Vinoles et al. with short-term progestogen use was exceptionally high (87%).

In that study, ewes receiving short-term progestogen treatment were observed to have a more rapid follicular turnover as well as ovulation of a more newly recruited follicle (Vinoles et al., 2001). This was thought to be associated with higher progestagen levels supplied by the shorter duration of treatment (in comparison with ewes receiving progestogen for 12 days). It has also been suggested that short-term treatments providing a shorter period of exposure to progestogens could be less disruptive to ovarian function (Fitzgerald et al., 1985). These could help explain the increased pregnancy rate obtained with short-term progestogen use.

Other studies have evaluated combination of progestogen treatment with that of prostaglandin the day before (Dixon et al., 2006; Fitzgerald et al., 1985) or the day of (Ozturkler et al., 2003; Dixon et al., 2006; D'Souza et al., 2011; Loubser and Van Niekerk, 1981; Greyling et al., 1979; Beck et al., 1993) device removal. These studies did not seek to determine whether the timing of $PGF_{2\alpha}$ administration had an effect on resulting fertility, but compared the combination treatments with those of $PGF_{2\alpha}$ or progestogen alone. Results from the current study did not detect a general improvement in reproductive outcomes at either timepoint of $PGF_{2\alpha}$ injection with short-term progesterone use in comparison with progesterone treatment alone.

The conception rate resulting from progesterone treatment alone tended to be higher than ewes receiving progesterone and $PGF_{2\alpha}$ at device insertion, and has provided a value similar to that found in a similar study using 5-7 day progesterone treatment without inclusion of $PGF_{2\alpha}$ (D'Souza et al., 2011). Conception rates from other studies using combination treatments have been 77% (Dixon et al., 2006), 53% (D'Souza et al., 2011), 86% (Beck et al., 1993), and 89% (Fitzgerald et al., 1985). Ewes receiving $PGF_{2\alpha}$

only have been previously observed to have similar conception rates to that observed in the present study (74.5 ± 9.5%) of 72.0% (Dixon et al., 2006), while others have found lower rates of 44.3% (Beck et al., 1993).

In this study, progesterone-treated ewes had a higher lambing percent to first service (54%) compared with ewes treated with $PGF_{2\alpha}$ only (35%). This effectively shifted the lambing pattern to be more concentrated earlier in the season in ewes treated with progesterone than those receiving $PGF_{2\alpha}$ only (table 1). This is similar to previous data (45.8%) of ewes receiving only short-term progesterone treatment (D'Souza et al., 2011) and those receiving both progesterone and $PGF_{2\alpha}$ (56%; Dixon et al., 2006).

Pretreatment of ewes with only progesterone for 5 days was sufficient to induce fertile estrus in a high proportion of ewes, which confirms earlier results from similar studies (D'Souza et al., 2011). Additionally, progestagen treatment combined with $PGF_{2\alpha}$ generally improved reproductive outcomes in comparison with $PGF_{2\alpha}$ treatment alone, an observation which is in agreement with prior experimental results (Dixon et al., 2006). From a practical perspective these results are of benefit to sheep reproduction enterprises, as a 5 day progesterone pretreatment provides no reduction in fertility in comparison with short-term combination methods while maintaining the reduction in time required for synchronization. Additionally, facilitation of its use is less laborious and provides a 30% reduction in cost over traditional short-term approaches.

Treatment of ewes earlier in the luteal phase (day 5) confirmed our hypothesis and generally agreed with expectations from a review of the literature. The time points of day 5, 8, and 11 were chosen as representative values of the early, mid-, and late luteal phase and have been used in similar studies (Deaver et al., 1986; Houghton et al., 1995).

Also, it has been shown that the ovine corpus luteum becomes sensitive to induction of luteolysis with exogenous $PGF_{2\alpha}$ 3.5-4.0 days into the estrous cycle, with higher doses of $PGF_{2\alpha}$ producing a greater response (Pope and Cardenas, 2004; Hackett and Robertson, 1980). Although prior studies utilized vasectomized rams in order to detect estrus to determine the day of estrous cycle in ewes, this was not logistically feasible in this study. Therefore, based on the average duration of time from removal of exogenous progesterone to the onset of estrus being 31.2 (Greyling and Brink, 1987) to 48 hours (Fitzgerald et al., 1985) in ewes studied, groups were pre-treated with progesterone in order to synchronize estrus so that luteolysis could be induced using a luteolytic dose of $PGF_{2\alpha}$ on predicted days 5, 8, or 11 of the estrous cycle.

It has been established that the day of cycle at which luteolysis is induced influences the interval to the onset of estrus, with ewes treated earlier in the cycle having a shorter onset than ewes treated later in the cycle (Acritopoulou and Haresign, 1980; Deaver et al., 1986; Houghton et al., 1995). This is dependent on the amount of time required for the concentration of progesterone to return to its basal level, and therefore is the longest when progesterone concentrations are highest later in the luteal phase (Houghton et al., 1995). While estrus in this study was measured at a single time point 72 hours following $PGF_{2\alpha}$ injection and ram introduction, the expected trend was observed in both the ram introduction to lambing interval as well as in lambing day.

The difference in the interval from induced luteolysis to estrus has been attributed to differences in the capability of large follicles to ovulate (Houghton et al., 1995). Also, ewes with a lower circulating concentration of progesterone (day 5) have been shown to exhibit an earlier onset to estrus (Acritopoulou and Haresign, 1980; Deaver et al., 1986).

Therefore, they could be mated earlier and lamb earlier in the lambing period than ewes with a higher concentration of progesterone at luteolysis. In the present experiment, the lambing day was significantly earlier in ewes treated on day 5 in comparison with other groups by approximately 7 and 6 days, respectively. Additionally, in ewes treated earlier in the cycle, the ram to lamb interval was significantly shorter than that observed in day 8 and day 11. The shorter duration from ram interval to lambing and earlier lambing day are consistent with the higher pregnancy and conception rates obtained with treatment with $PGF_{2\alpha}$ earlier in the estrous cycle.

Progesterone has been shown to be a potent inhibitor of GnRH and LH secretion, and lower concentrations of progesterone appear to be stimulatory to follicular growth (Karsch et al., 1977; McLeod et al., 1984; Johnson et al., 1996). As progesterone is secreted maximally toward the end of the luteal phase, the reduction in fertility of ewes injected later in the cycle could be explained by poor final maturation of preovulatory follicles as a result of reduced LH secretion (Barrett et al., 2002). Also, ewes undergoing induced luteolysis early in the luteal phase are exposed to a higher sustained concentration of FSH in comparison with ewes treated later in the cycle (Deaver et al., 1986). Gonadotropins are necessary for maintaining steroidogenic capacity, so it is conceivable that differences in conception rate and prolificacy at the first service period could be attributed to a degree of impaired follicular functionality in ewes induced at days 8 and 11 of the estrous cycle.

Although ovulation rate was not measured in this study, a significantly greater prolificacy was obtained in ewes injected with $PGF_{2\alpha}$ on day 5 than day 11 of their estrous cycles. No differences in ovulation rate have been detected in previous studies

based on the day of cycle (Houghton et al., Deaver et al., 1986; Barrett et al., 2002) or peripheral progesterone concentration (Johnson et al., 1996), and only one study has measured lambing outcomes across treatment groups (Schoombee, 1996). In that study (Schoombee, 1996), no difference was found between group means for conception rate (56.5%) or lambing outcomes, although group sizes were exceedingly small (4-14 ewes/group) so it is likely that differences could not be detected from the limited sample sizes.

Variations in follicular dynamics have been observed between the predicted stages of the estrous cycle used in this study. For example, the ovulatory response of the largest follicles was found to be greatest in day 5 ewes (69%), least on day 8 (39%), and medial on day 11 (51%; Houghton et al., 1995). Ovulatory response is related to size, location on the ovary, presence or absence of luteal tissue on the ovary, and the relative age of the follicle at luteolysis (Houghton et al., 1995). Ewes in the mid-to late luteal phase have a greater combination of growing, static, and atretic follicles than ewes early in the luteal phase (Houghton et al., 1995). Therefore, it would be logical that ewes undergoing induced luteolysis later in the estrous cycle will ovulate a smaller proportion of large follicles and will ovulate follicles less likely of establishing pregnancy.

Ovulation rate is the primary determinant of litter size, but other factors are involved. Because no differences have been detected in previous studies in ovulation rate among ewes treated with $PGF_{2\alpha}$ during different stages of the estrous cycle, it is likely that litter size was influenced by a difference in embryonic mortality. Progesterone concentration was higher in ewes predicted to be at day 8 and 11 of the estrous cycle at induced luteolysis. It has been well established that progesterone inhibits release of

gonadotropins, which are required for the final stages of follicular development and maturation. It is conceivable that follicles induced to undergo luteolysis from periods of time in which progesterone is exceedingly high (later in the luteal phase) could result in compromised follicles that could ultimately lead to an increase in embryonic loss, which could explain the reduced prolificacy observed in ewes treated later in the estrous cycle. Further research needs to be conducted to elucidate the factors involved in the varied litter size obtained with treatment at different stages of the estrous cycle.

SUMMARY

Through estrous synchronization, producers can optimize flock productivity and profitability by reducing the time and cost associated with lambing and producing a more uniform lamb crop that can be strategically targeted to specific markets. Effective estrous synchronization methods must be economical and result in sufficiently high fertility at the synchronized estrus. While traditional approaches using short-term progestogen treatments included application of $PGF_{2\alpha}$, the present study sought to evaluate if its concomitant use was required for synchronization of estrus, and if the time of $PGF_{2\alpha}$ application relative to progesterone pre-treatment or at different predicted stages of the estrous cycle affects ewe fertility.

Data were analyzed using analysis of variance and least squares means were calculated. In experiment 1, progesterone-treated ewes had a higher estrous response than ewes receiving $PGF_{2\alpha}$ only. Ewes receiving a 5 day CIDR alone had a higher pregnancy rate to 1st service than ewes in other treatment groups, and a greater proportion of progesterone-treated ewes lambed to first service than ewes receiving $PGF_{2\alpha}$ only. Overall, no improvements in fertility were observed in ewes receiving both progesterone and $PGF_{2\alpha}$ at either timepoint over ewes receiving progesterone alone. In experiment 2, no difference in estrous response was observed between ewes given a luteolytic dose of $PGF_{2\alpha}$ at different predicted days of the estrous cycle, although ewes treated earlier (predicted day 5) had a higher conception rate than ewes treated at day 8 or 11. Ewes treated at predicted day 5 also tended to have a higher pregnancy rate to first service, had

a greater percentage of ewes lambing to first service, and had a higher 1st service prolificacy than other ewes. These results indicate that 1) $PGF_{2\alpha}$ provides no improvement in reproductive outcome when given as part of a progesterone- $PGF_{2\alpha}$ estrous synchronization protocol above treatment with short-term progesterone use alone, and 2) reproductive outcome varies with the day of the estrous cycle on which luteolysis in induced, with higher conception rates and prolificacy associated with induced luteolysis early in the cycle. However, further experiments are needed to elucidate the factors involved in the reproductive differences observed with treatment at different stages of the estrous cycle.

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