EVALUATION OF THE EFFECT OF PROGESTERONE CIDR DEVICES ON CIRCULATING LEVELS OF PROGESTERONE IN CYCLIC EWES

A Thesis

by

MICHAEL CAREY SATTERFIELD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2004

Major Subject: Physiology of Reproduction

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Approved as to style and content by:

W. Shawn Ramsey (Co-Chair of Committee)	Thomas E. Spencer (Co-Chair of Committee)
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ABSTRACT

Evaluation of the Effect of Progesterone CIDR Devices on Circulating Levels of Progesterone in Cyclic Ewes. (December 2004)

Michael Carey Satterfield, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. W. Shawn Ramsey
Dr. Thomas E. Spencer

A homogeneous group of thirty-one crossbred ewes was used to determine the effect of administering a progesterone Controlled Intravaginal Drug Releasing Device (CIDR) on circulating levels of progesterone in the subsequent cycle following CIDR removal. Circulating progesterone levels were determined for each ewe through daily blood collection via jugular venipuncture. Each ewe underwent a pretreatment 25 day sampling period (Period 1), a 12 day treatment period characterized by the presence of the CIDR (Period 2), and another 25 day sampling period following CIDR removal (Period 3). During the initial period of the study, progesterone levels in peripheral circulation changed ($P < 0.0001$, effect of day) in accordance with stage of the estrous cycle and were elevated during the luteal phase. In the second period of the study, progesterone levels were elevated ($P < 0.0001$) in ewes due to exogenous progesterone from the CIDR device (Period 1 versus Period 2: 1.3 ± 0.1 ng/ml versus 2.4 ± 0.1 ng/ml, respectively). After withdrawal of the CIDR in the third period of the study, circulating progesterone levels were not ($P > 0.10$) different from those observed in the initial period of the study (Period 1 versus

Period 3: 1.3 ± 0.1 ng/ml versus 1.4 ± 0.1 ng/ml, respectively). Data collected in this study revealed that treatment with exogenous progesterone via CIDR for a 12-day treatment period does not influence circulating levels of progesterone in subsequent estrous cycles.

DEDICATION

To my wife and family who have given me the love and support needed to reach this accomplishment and for their continued support in my next endeavor.

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INTRODUCTION

The lack of availability of effective and efficient methods for estrus synchronization in sheep and goats is a critical detriment to the industry. The ability to rapidly improve genetics, and thus increase profits, is centered around the ability to use artificial insemination and embryo transfer. Both of these advanced reproductive techniques require the ability to effectively synchronize estrus. Unlike other domesticated species the ability to traverse the cervix of a ewe is extremely difficult, therefore the use of transcervical artificial insemination (AI) is limited in the sheep industry. The predominant method for AI in the United States is through laparoscopy. The expense of equipment and the relatively high skill level required to inseminate ewes in this manner adds greater constraints to estrus synchronization protocols. Acceptable protocols must allow for the ability to breed females off of a fixed time regimen not daily heat detection as can be done in other species due to the ease of insemination. This constraint severely limits the ability to use prostaglandin regimens to synchronize estrus. Currently the only other method of synchronization that yields an acceptable window for timed AI is through the use of long-term progesterone treatment. Daily injections of progesterone are however very labor intensive and relatively unfeasible. The use of subcutaneous ear implants containing a progesterone analog was effective, however, they have recently become unavailable in the United States. Progesterone soaked sponges are currently

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This thesis follows the style and format of Biology of Reproduction.

available however there are hygienic concerns about this method related to infections of the reproductive tract. The development of a new product, a Controlled Intravaginal Drug Releasing Device (CIDR) has recently come to the forefront in various countries throughout the world for the synchronization of sheep, goats, and cattle. The objective of this study was to determine if a twelve-day progesterone CIDR device treatment significantly increased daily progesterone levels in the estrous cycle succeeding the treatment versus the cycle immediately prior to the CIDR treatment.

LITERATURE REVIEW

The administration of exogenous progesterone as a means to manipulate the estrous cycle has been widely evaluated throughout the research community. The first attempts to use progesterone as a synchronization device were in cattle in the 1960's and 1970's (1, 2). Animals were subjected to daily injections of progesterone for a maximum of 20 days. The results of this treatment yielded acceptable levels of synchrony, although fertility at the synchronized estrus was low. Future research focused on other modes of progestin administration due to the impracticality of a daily injection protocol. Oral, transdermal, and eventually intravaginal methods of administration were developed and used in estrous synchronization protocols (1). The transitions in the mode of administration stemmed from a need to decrease time and energy associated with both daily injections and daily oral administration, as well as a need for consistent dosage of the hormone which can be difficult when feeding oral progesterone. Unfortunately, the available options are limited for the synchronization of estrous using exogenous progesterone in the United States.

Currently, estrous synchronization in most ruminant species involves one of two basic strategies. These strategies involve either luteolysis of the corpus luteum (CL) through administration of prostaglandin F2 alpha (PGF2α) or one of its analogs or prolonged treatment with native or synthetic progestins (1, 3, 4). Prostaglandin F2α and its analogues induce estrus through rapid luteal regression of a functional CL and the resultant ovulation of a dominant follicle. However, the use of luteolytic agents has restrictions that limit its effectiveness as indicated by the necessity for the

presence of a functional CL. Long-term progestin treatment synchronizes estrus by suppressing folliculogenesis through inhibition of hypothalamic function. Cessation of progestin treatment allows folliculogenesis to resume and is followed by ovulation (3). The use of either hormone yields acceptable synchrony in cyclic cattle, although PGF2α administration is ineffective during the postpartum interval. In sheep, seasonal considerations, associated with the role of the photoperiod in controlling estrous, are critical in determining the efficacy of each regimen. In addition, Godfrey et al. (5) found that when using these two strategies in a timed AI protocol in hair sheep, PGF2α administration yielded significantly lower conception rates versus long-term progesterone treatment with a CIDR. The differences observed with timed AI are most likely due to the fact that long-term progesterone treatment yields a more narrow range of synchrony and thus prediction of the time of ovulation is more reliable, resulting in higher conception rates. However, there were no differences between the two protocols when natural service was used instead of timed AI.

Regardless of the synchronization protocol, the sheep industry is in desperate need of an acceptable method of controlling estrus. The seasonal breeding exhibited by most breeds of sheep has resulted in a highly variable supply of lamb (6, 7). The variability in supply has been detrimental to the sheep industry due to inefficiencies associated with lamb feeders and packers in regards to labor and capital utilization. In addition, the laws of supply and demand coupled with seasonal breeding has resulted in lower prices for the majority of producers who are unable to take

measures to alter their production systems (7). By implementing synchronization technologies, producers can better manage time, labor, pasture and feed resources. In addition to increasing efficiency, producers have the opportunity to increase revenues by marketing their product at a more favorable time of year or by accelerating the lambing rate of the flock (6, 8).

Synchronization of ewes during their normal breeding season can be performed with relative ease using either progestin treatment or PGF2α administration. However, in the non-cycling ewe, administration of PGF2α is ineffective due to the lack of a functional CL to undergo luteolysis. Therefore, the control of estrus in the non-cycling ewe is limited to progesterone treatment. Due to the aforementioned impracticality associated with daily injections of progesterone, most current research has focused on the intravaginal administration of this steroid. The first intravaginal administration of progestins came with the development of the intravaginal sponge. Next, the intravaginal CIDR was developed, which was constructed with a silicone elastomer containing exogenous progesterone (6, 9). The CIDR replaced the sponge primarily due to aesthetic reasons. Sponges absorb vaginal secretions and are thus less desirable to handle upon removal compared to the non-absorbent silicone covering of the CIDR (6). Regardless of the route of administration of the long term progesterone treatment, the accompaniment of this treatment with an injection of pregnant mare serum gonadotropin (PMSG) upon cessation of progesterone treatment is beneficial when breeding out-of-season. The additional administration of PMSG stimulates the preovulatory follicular development leading to an increased

ovulation rate (6, 7). Hamra et al. (10) and Wheaton et al. (11) found that CIDR treatment alone was ineffective in inducing estrus in ewes during late spring. It was however shown by Wheaton et al. (11) that in late July CIDR treatment alone was effective in inducing estrus and indicates an increase in receptiveness to progesterone by ewes following the summer solstice. CIDR treatment alone in the late winter months was also sufficient in inducing estrus and yielded a pregnancy rate of 94.5% following natural service (9).

Attempts have been made to achieve acceptable levels of estrus synchrony and ovulation rates without PMSG administration. Omitting PMSG from the synchronization protocol would reduce the cost associated with the procedure (9). The use of the ram effect immediately following CIDR removal to induce estrus has proven to be beneficial. Welch et al. (12) reported that removal of PMSG from the synchronization protocol made little difference in the number of ewes exhibiting estrus when rams were introduced immediately following CIDR removal. Further research by Wheaton et al. (8) showed that CIDR removal followed by immediate introduction of rams successfully stimulated breeding in both late spring and early summer. Research on Elds deer (*Cervus eldi thamin*) observed a similar trend in that stag exposure advances the LH surge and the onset of behavioral estrus over that of isolated females (13). In the sable antelope however, male presence had no effect on the timing of ovulation when does were synchronized with CIDR devices (3).

Early research involved in the development of the CIDR focused on determining the optimal dosage level. CIDRs containing 3, 6, 9, and 12% progesterone were

designed and tested. CIDRs containing 3% progesterone were ineffective in inhibiting estrus and were thus discontinued. Subsequent research revealed higher plasma progesterone concentrations and equal or greater fertility in the 9% and 12% CIDRs over that induced by the 6% devices (9). Further research comparing the 9% and 12% devices revealed comparable circulating progesterone levels and thus 9% (0.37g) devices were chosen for mass production due to a reduction in cost resulting from decreased progesterone requirements (9). Treatment with these CIDR devices is most commonly over a range of 12 to 16 days (1, 3, 6, 10, 14, 15).

Although it seems that the use of CIDRs to control estrus in sheep would be beneficial to the sheep industry, research into the effects of long-term progesterone treatment on circulating progesterone levels and subsequent fertility, sperm transport, endocrine function, and embryonic growth and development must be evaluated. Treatment of livestock with long-term progesterone and its synthetic analogues has been known to have various effects on circulating progesterone levels and subsequent follicular activity and fertility. Prior research has shown that in a normal cyclic ewe plasma progesterone levels will reach a maximum level of approximately 2-3 ng/ml between days 8 and 12 of the estrous cycle (16). Herriman et al. (17) observed much higher mean circulating plasma progesterone concentrations of up to 6 ng/ml on day 11 in normal cyclic ewes. These differences could potentially be breed related. The lowest plasma progesterone concentrations are observed at the time of estrus and reach levels as low as .12 ng/ml as detected by Thorburn et al (16). This lowest value is similar to levels found in anestrous and ovariectomized ewes as

well as in wethers. Schrick et al. (18) evaluated serum progesterone profiles in both cyclic and pregnant ewes and observed maximal concentrations of approximately 3 ng/ml in cyclic ewes and between 3-4 ng/ml in pregnant ewes with maximal values observed between day 12 and 13 of the estrous cycle.

In cattle, peripheral plasma progesterone concentrations reach levels of 6 to 8 ng/ml between days 9 and 16 of the estrous cycle before falling to their lowest concentrations of approximately 0.25 ng/ml at the time of estrus (17, 19). These lowest concentrations at the time of estrus were similar to those observed by Nation et al. (20) in normal anestrous dairy cows. Again, variations do exist as shown by Herriman et al. (17) and Roberson et al. (21) who observed plasma progesterone concentrations of around 10 ng/ml in cyclic dairy heifers and beef cows, respectively. In heifers where serum progesterone concentrations were measured, Burke et al. (22) found maximal levels of 3 ng/ml around day 14 and basal levels of less than 0.25 ng/ml at the time of estrus.

Evaluations of preliminary research in sheep on plasma progesterone concentrations following estrous synchronization with CIDRs have shown variable results. In an initial study by Hamra et al. (15) it was shown that within 24 hours of CIDR insertion, progesterone levels had risen to near maximal levels of 2.1 ng/ml, reached peak levels of 2.4 ng/ml on day 4, and then declined steadily to a concentration of 1.5 ng/ml on day 13. When compared to progesterone levels observed during treatment with progesterone sponges, which yielded mean concentrations of 1.0 ng/ml over the 13 day treatment period, CIDRs produced

significantly higher mean levels of circulating progesterone, especially evident in the latter portion of the treatment period. Later research by Ainsworth and Downey (23) using 12% CIDRs in ovariectomized ewes showed peak concentrations of 5.5 ng/ml within 2 hours of insertion with a subsequent rapid decrease following a curvilinear pattern to a low of 1.7 ng/ml on day 13.

In a study by Wheaton et al. (9) to reveal the effects of CIDRs on plasma progesterone concentrations over an extended period of time, cyclic ewes were treated with a CIDR for a period of 45 days. Insertion of CIDRs during the luteal phase of these cyclic ewes resulted in maximal progesterone levels of 4.5 ng/ml and fell to 0.5 ng/ml by day 27 to day 31. During this period of treatment, estrous was not exhibited at the first expected date. However by day 31, all ewes had ovulated as shown by a subsequent rise in progesterone levels. The occurrence of an ovulation as revealed by the progesterone concentrations was however, not accompanied by a behavioral estrous (9).

Interestingly, Haresign (24) evaluated the rate of decline of plasma progesterone concentrations following progesterone implant removal compared to the rate of decline during luteolysis in a natural cycle. The resulting research showed an increase in the mean rate of decline of plasma progesterone levels by 9-fold following implant removal compared to natural luteolysis. A similar dramatic increase in the secretion of tonic LH was observed and substantiates the role of progesterone in regulation of LH. Godfrey et al. (5) compared estrous synchronization protocols using PGF2α, progesterone sponges, and progesterone

CIDRs. They found that progesterone concentrations in ewes at the time of ram introduction were higher in PGF2α treated ewes than those of sponge and CIDR treatments. No differences in rate of decline in progesterone levels prior to estrus were observed between the three protocols. Additionally, evaluation of a period of 16 days following the synchronized estrus revealed similar progesterone concentrations between the three treatments and concentrations were similar to those seen in normal cyclic ewes with normal corpora luteal formation (5).

In cattle, progesterone treatment yields similar results to those seen in sheep. Macmillan and Peterson (1) analyzed plasma progesterone concentrations in ovariectomized cows treated with a CIDR-B device for 12 days. The study revealed mean plasma progesterone concentrations of 6.7 ng/ml on day 1 following device insertion and falling to a mean concentration of 2.3 ng/ml 3 days following device removal. Burke et al. (25) evaluated plasma progesterone concentrations during treatment with a CIDR alone and accompanied with estradiol benzoate. CIDRs were inserted on day 13 of a natural estrous cycle and subsequent plasma progesterone concentrations increased from 6.7 to 10 ng/ml within 2 hours of CIDR insertion. However, on day 3 and 5 post treatment no significant differences in progesterone concentrations were observed between treatment and control groups. Previous research by Burke et al. (26) showed that CIDR device insertion maintained circulating progesterone levels above 2 ng/ml for at least 7 days in ovariectomized cows. Burke et al. (25) have thus postulated that either endogenous progesterone secretion may decline in cyclic cattle and/or progesterone metabolism may increase

in response to higher circulating progesterone concentrations due to progesterone treatment.

Research done by Munro was the first to compare trends in plasma progesterone profiles between CIDR devices and progesterine releasing intravaginal devices (PRIDs) containing two different dosage levels. He found that regardless of treatment type, mean progesterone levels prior to treatment (0.6 ng/ml) were not significantly different than concentrations observed 24 and 48 hours post treatment (0.7 and 0.6 ng/ml respectively). Upon initiation of the treatments, concentrations rose rapidly to levels of about 11 ng/ml on day 1 and declined to lows of about 4 ng/ml on day 14 of treatment. Upon statistical analysis, no significant differences between treatments were observed (27). Similar profiles following PRID insertion were observed again by Munro one year later (28).

Munro and Moore (29) also compared plasma progesterone concentrations in ovariectomized and prepubertal heifers upon treatment with intramuscular injections and intravaginal administration of progesterone. Results of this experiment indicated that progesterone concentrations following intravaginal administration more closely resembled those of a normal luteal phase than concentrations observed during daily injection treatment. This may be due to rapid spikes in concentrations followed by return to near basal levels prior to the next injection resulting in an unphysiologic pattern as seen in rats and mice (30). Evaluation of differences between ovariectomized heifers and prepubertal heifers in regards to progesterone concentrations following PRID insertion were also observed. In ovariectomized

heifers, PRIDs maintained progesterone concentrations of 6-8 ng/ml for 6 to 7 days while in prepubertal animals these concentration levels were maintained for over 14 days (29). This observation suggests that factors such as prior stimulus to sex hormones before ovariectomy could have effects upon treatments following ovary removal.

Research has shown that long-term progesterone treatment elicits effects on fertility and endocrine function. Preliminary research using exogenous progesterone to synchronize estrous cycles showed effective control of both estrus and ovulation, however fertility of controlled estrus was reduced following long-term (17-21 day) progestin treatment (31, 32, 33). Similarly, Robinson (34) and Robinson et al. (35) found that long-term progestin treatment in sheep decreased fertility at the synchronized estrus. Later research showed that if progestin treatment could be reduced to 10-12 days by accompanying this treatment with a luteolytic agent, conception rates were normal (4, 36, 37, 38, 39). In a long versus short-term intravaginal treatment of progestagens in heifers, it was revealed that decreasing length of progestagen administration from 20 days to 10 days resulted in an increase of about 12% in conception rate. An 18% reduction in conception rate was observed between heifers treated for 20 days versus untreated control heifers (40). In a study by MacMillan and Peterson (1), a reduction in the length of progesterone treatment via CIDR from 21 to 14 to 7 days + PGF2α revealed a subsequent increase in percent calving rate to 1st service from 39.8 to 45.8 to 60.5% respectively. As in cattle, a reduction in the number of days of progesterone treatment in sheep has

shown increases in conception rates over those first observed in preliminary research, to levels comparable to normal untreated ewes (41, 42).

In a recent study by Wehrman et al. (43) conception rates were compared between cows receiving a 10-day exogenous progesterone treatment with either 1 or 2 PRIDs. Results showed an increase in conception rate following AI in cows receiving 2 PRIDs over those only receiving one. These researchers hypothesized that the decreased fertility associated with the lower progesterone concentrations observed in single PRID treated cows were characteristic of a luteal phase deficiency and was the result of an extended period of exposure to 17β estradiol. This reduction in fertility due to luteal function deficiencies has been previously characterized in cattle (44, 45, 46, 47).

Johnson et al. (48) observed similar reductions in fertility in ewes due to treatment with progesterone and attributed it to an increase in circulating estradiol produced by older ovulatory follicles. Conception rates in ewes with higher concentrations of estradiol prior to mating were lower than conception rates in ewes with low concentrations of estradiol. A dose dependant response on fertility has been observed in sheep. Robinson et al. (35) found that fertility increased as dosage levels increased over a given treatment time period. This inverse relationship between progesterone and estradiol concentrations is interesting for synchronization programs when estrus detection is required. The conception rates associated with the relative concentrations of these hormones have been characterized. However, there is also an interesting inverse relationship between these two hormones on estrus

behavior. Davidge et al. (49) found that an increase in the dosage and resultant concentrations of progesterone yielded a dramatic decrease in mounts received and stands observed in these cows. This could potentially have dramatic effects on research if estrus detection is less than optimal. This also bodes well for the use of timed AI when estrus synchronization is achieved using protocols that incorporate progesterone into the regimen.

The observation of reduced fertility in long-term progesterone treated animals stimulated research into the cause of this decline. It has been speculated that the relative concentrations of progesterone and estradiol could potentially affect sperm transport in the female reproductive tract, follicular development, and early embryonic development. Early research focused on the effects of progestin treatment on sperm transport. Allison and Robinson (50) evaluated three different dosage levels of progestagens and found that as dosage increased the number of tubal spermatozoa and lambing percentage both rose. Other research showed that 17β-estradiol plays an inhibitory role in the movement of sperm through the female reproductive tract (51, 52). These findings are consistent with early research that showed that insufficient dosages of progestins along with a corresponding increase in 17β-estradiol concentrations were characteristic of a luteal deficiency and yielded reduced fertility. Later research by Pearce and Robinson (53) illustrated similar results showing an increase in the number of spermatozoa located within the cervix as dose levels of progesterone increased from 200 to 400 to 600 mg. However, progesterone concentration profiles from the three treatments were not significantly

different and thus the observation of reduced spermatozoa could not be associated with insufficient plasma progesterone concentrations. Instead, these researchers postulated that the reduction in spermatozoa was due to the rapid decline in progesterone concentrations following sponge removal and the resulting prolonged interval between progesterone treatment and an observed estrus and subsequent insemination.

Research attempting to reveal more insight into reasons for reduced fertility focused on the prolonged exposure to 17β-estradiol and its association with luteal deficiencies. This research postulated that altered follicular development could result in improper oocyte development, or an improper uterine environment could develop which is not conducive to early embryonic growth (43). Changes in steroid concentrations have been shown to be associated with structural changes during oocyte maturation and are thought to prevent premature nuclear maturation (54). In addition, low doses of progesterone have been shown to extend the life span of some dominant ovarian follicles if progesterone treatment is initiated just prior to or at the normal time of luteolysis (55, 56, 57). This prolonged dominance may lead to the development of an abnormal oocyte, which upon ovulation has reduced fertility. Furthermore, formation of a CL from this abnormal follicle may result in an irregular luteal phase and thus early embryonic mortality (55). Future research will undoubtedly reveal the mechanism associated with reduced fertility associated with long-term progesterone treatment, although the addition of a luteolytic factor with

slightly shorter treatment duration can be used to reduce detrimental effects of progesterone administration.

Specifically, in regards to progesterone treatment via CIDR device, the analysis of the progesterone concentration profiles is the first step needed for determination of the efficacy of this synchronization tool. From this point, investigation into achieving optimal fertility and the development of a comprehensive regimen that is effective and economically viable for the sheep industry can be undertaken.

MATERIALS AND METHODS

A long-term progesterone treatment via vaginal delivery system trial was conducted at the Sheep and Goat Center in the Animal Science Teaching Reasearch Extension Complex (ASTREC) of Texas A & M University, College Station, Texas. The trial began on October 4, 2000 and concluded on December 7, 2000. All procedures used in this study were approved by the Institutional Animal Care and Use Committee at Texas A & M University. A homogenous group of thirty-one cycling, multiperous crossbred ewes were evaluated for differences in circulating levels of progesterone before, during, and after implantation with a progesterone CIDR. A twenty-five day sampling period was observed prior to CIDR implantation to ensure that each ewe displayed a complete estrous cycle. A twelve-day sampling period then ensued during which time exogenous progesterone was administered to each ewe via the CIDR. Following removal on day twelve of treatment, these ewes were placed with either intact or vasectomized rams to determine if there was an induction of estrus and an addition twenty-five day sampling period was undertaken.

Ewe Preparation

Prior to initiation of the study, ewes were sonogrammed to determine reproductive status. Ewes were randomly confined to two equal sized bermuda grass paddocks 15 m wide by 86 m long. Ewes were supplemented with free choice alfalfa hay and 2.2 kg of whole corn daily to maintain an acceptable body condition. Shearing of the neck was performed prior to initiation of the study as well as once during the study to facilitate jugular venipuncture.

Blood Collection and Handling

Blood samples were collected at 0700 daily via jugular venipuncture into Vacutainer Evacuated Blood Collection Tubes (Becton-Dickinson, Franklin Lake, NJ). The blood samples were allowed to coagulate at room temperature for one hour prior to serum extraction. Following the coagulation period, samples were spun at 1500 rpm for 15 minutes at room temperature to separate the various blood components. The serum was then removed from the blood collection tubes and stored in individually labeled plastic tubes. The serum was stored at -20°C until analyzed. Concentration of progesterone in serum samples were later determined using an Active Progesterone Radioimmunoassay kit (Diagnostic Systems Laboratories, Inc., Webster, TX). Assay results were calculated using the AssayZap program (Biosoft, Ferguson, CA). Assay sensitivity was 0.10 ng/ml. The intra- and inter- assay variations were 17.37 and 15.96%, respectively, which fall within the acceptable limits of the test.

Statistical Analysis

Data was analyzed by least squares analysis of variance (ANOVA) using the general linear models procedure of the Statistical Analysis System (Cary, NC) (58). Least square means were generated from ANOVA and are presented with standard errors.

RESULTS AND DISCUSSION

During the course of the study it was determined that three of the ewes were in fact pregnant and therefore they and their previously collected blood samples were removed from the study.

Pre-implantation Progesterone Levels

The pre-implantation or control period of the study was analyzed to determine two characteristics: cyclicity and control serum progesterone levels. During this initial period, it was determined that progesterone levels in peripheral circulation changed ($P < 0.0001$, effect of day). Changes in peripheral circulating levels of progesterone were in accordance with the stage of the estrous cycle and were elevated during the luteal phase. Mean circulating progesterone concentration during the pretreatment estrous cycle was 1.3 ± 0.1 ng/ml. This is similar to that observed by Oladimeji et al. (59) of 1.57 ng/ml observed in Yankasa ewes during the late wet season. Mean peak serum progesterone level during this stage of the experiment was 4.71 ± 0.46 ng/ml with peak values observed on day 11 of the estrous cycle with a range from day 7 to day 15-post estrus, determined by day of lowest serum progesterone concentration. Bartlewski et al. (60) observed peak serum progesterone levels of around 4 ng/ml on two representative Western white-faced ewes, however when they analyzed serum progesterone concentrations in two representative Finn ewes they observed peak serum concentrations of around 3 ng/ml. Differences in this study between breeds were statistically significant ($P < 0.05$) with Western-

white faced ewes having higher peak serum progesterone levels from Day 4 to Day 14 of the estrous cycle than Finn ewes.

Schrick et al. (18) observed peak serum progesterone concentrations of slightly over 3.0 ng/ml in a group of 36 mature ewes of predominately Suffolk cross descent. Peak serum progesterone concentrations observed in our study are slightly higher than those seen by other researchers, however are similar to those observed by Bartlewski et al. (60) in Western white-faced ewes. Animals used in this study are a cross between Suffolk and Western white-faced sheep and thus could potentially validate the slightly higher levels observed in our study based upon variability between breeds.

Mean minimum circulating levels of serum progesterone in our study were observed at the time of estrus, as expected, and were below the sensitivity of the assay which was 0.10 ng/ml. These levels are similar to those seen by Bartlewski et al. (60) of 0.12 ng/ml at this stage of the estrous cycle.

Effect of CIDR Treatment on Progesterone Levels

In comparison to the pre-implantation period it was found, as expected, that circulating serum progesterone levels were elevated ($P < 0.0001$; 1.3ng/ml vs. 2.4 ng/ml, respectively) in ewes following insertion of the CIDR device. Mean peak levels of serum progesterone were observed on Day 5 of CIDR treatment with a mean peak concentration of 3.27 ng/ml. The concentration fell to its lowest level on the final day of treatment to a mean level of 1.26 ng/ml (Figure 1). This pattern of increase to maximum and then decline is similar to that seen by Hamra et al. (15).

These researchers observed peak plasma progesterone levels of 2.2 ± 0.3 ng/ml on day four of treatment using a 9% CIDR device with declining levels to 1.4 ± 0.2 ng/ml on day thirteen of treatment. Wheaton et al. (9) observed almost identical results such that peak plasma progesterone concentrations of 2.4 ng/ml were observed on day four of treatment (Day 0 characterized by day of CIDR insertion) and declined in a gradual manner until day thirteen where levels were down to 1.5 ng/ml.

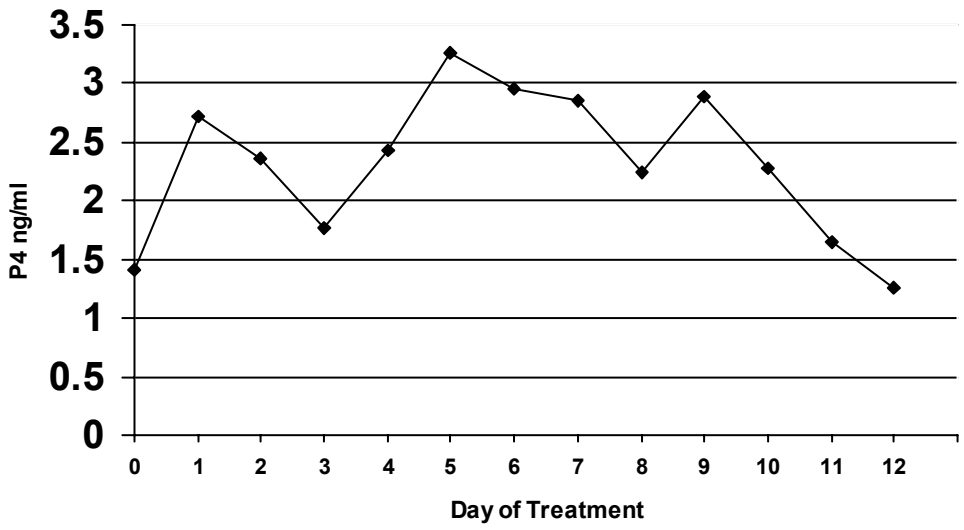

Figure 1. Mean progesterone concentrations during treatment with progesterone CIDR device.

Pre-implantation Versus Post-implantation Levels

The final comparison of this study was a characterization of the estrous cycle immediately following the removal of the CIDR compared to the estrous cycle

immediately prior to CIDR insertion. Only, the first fifteen days of each respective estrous cycle were analyzed for mean circulating serum progesterone concentrations due to inadvertant exposure to intact rams during the time of estrus.

As previously stated we observed a pre-implantation mean progesterone concentration of 1.3 ± 0.1 ng/ml versus a 1.4 ± 0.1 ng/ml mean circulating progesterone concentration in the estrous cycle immediately following CIDR removal. Mean values of circulating progesterone levels were not statistically significantly different ($P > 0.10$) between period 1 and period 3. A comparison of representative estrous cycles for ewe number twelve pre- and post-implantation can be found below (Figure 2).

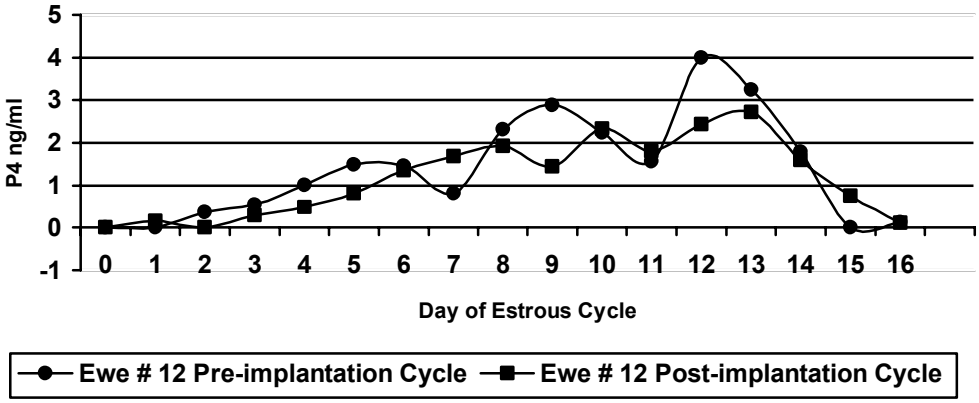

Figure 2. A representative comparison of the estrous cycles of ewe #12 before and after treatment via progesterone CIDR device.

In an evaluation of the estrous cycle following CIDR treatment, Godfrey et al. (5) found that progesterone profiles were characteristic of normal luteal formation

and function. In our study, mean peak progesterone levels in the post-implantation estrous cycle were 4.23 ± 0.46 ng/ml. The peak concentration in this study in the post-implantation period was slightly lower however not significantly different (P = 0.4605) than peak concentrations observed in the pre-implantation estrous cycle (Figure 3).

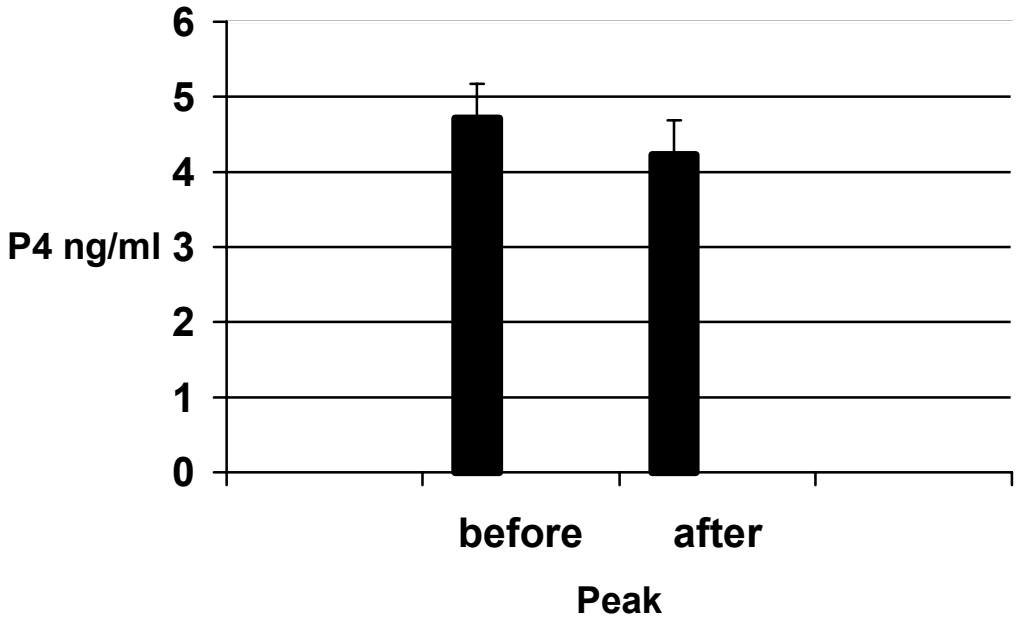

Figure 3. A comparison of the mean peak progesterone levels in the estrous cycle immediately prior to and the estrous cycle immediately following a twelve-day treatment via progesterone CIDR device.

SUMMARY

Results of this study were in line with our expectations and paralleled the findings of other researchers. As expected, the insertion of a progesterone CIDR device increased circulating serum progesterone levels to a level higher than mean progesterone concentrations during the pre-treatment cycle. These progesterone concentrations were, however, characteristic of levels that could be observed during the luteal phase of any untreated ewe.

When comparing the pre- and post-implantation estrous cycles there were no differences in mean or peak circulating serum progesterone concentrations. This indicates that upon CIDR removal circulating serum progesterone is quickly removed from the blood stream, which allows a relatively rapid induction into estrus and a resulting seemingly normal and characteristic estrous cycle.

One concern with using implants such as a progesterone CIDR device is the ability of the body to store those compounds in various tissues. It is known that adipose tissue stores progesterone, however at least with this form of natural progesterone there is seemingly no slow release from these tissues following CIDR removal, which is evidenced by the seemingly normal circulating progesterone concentrations in the estrous cycle following treatment.

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