EFFECTS OF GNRH AND PROSTAGLANDIN COMBINED WITH A SHORT PROGESTIN REGIMEN ON THE SYNCHRONY OF ESTRUS AND OVULATION IN EWES DURING THE BREEDING SEASON

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ABSTRACT

The lack of effective, consistent synchronization protocols for ewes is a barrier to the use of artificial insemination in sheep. This study compared the estrus and ovulation percentage and window of synchrony of estrus and ovulation for ewes synchronized with three experimental protocols. The industry's current standard protocol using PG600, an 11 d CIDR and PGF2a was compared to two alternative protocols utilizing GnRH, a 7 d CIDR and PGF2a. Forty Suffolk ewes were divided into 5 groups and each group was placed on a different protocol. Blood sampling began 18 h following CIDR removal and samples were collected every 2 h for 19 consecutively collections. Mean serum concentrations of LH differed between groups ($P<0.05$) from 22-42 h following CIDR removal. The two protocols using the shorter 7 d progestin regimen and GnRH to control follicular dynamics resulted in higher estrus and ovulation rates and an acceptable window of synchrony.

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INTRODUCTION

Artificial insemination (AI) has been a vital tool for the genetic improvement of livestock for many years. The cattle industry, especially dairy operations, have utilized AI to a greater extent than any other livestock species. There are many factors that contribute to their wide use of AI, but one of the greatest contributors is the application of effective synchronization protocols. The sheep industry has much to gain from the increased use of AI, but there are several obstacles impeding the use of AI within this industry.

Several problems that make AI less profitable and efficient are the lack of effective, consistent synchronization protocols and the difficulty of estrus detection. These problems could be minimized by the development of a timed artificial insemination (TAI) protocol that yields consistently higher conception rates and eliminates the need of estrus detection. The generally accepted method of estrus synchronization for TAI in sheep uses a 11-19 d regiment of progestin administered via a controlled internal drug release (CIDR) device, an injection of prostaglandin F2a (PGF2a) at CIDR insertion, and in some cases an injection of equine chorionic gonadotropin (eCG) in the form of pregnant mare serum gonadotropin (PMSG) at CIDR removal. There are three negative components to this protocol, an excessive exposure to progesterone, inconsistent concentrations of eCG from the available sources, and a wide window of time of ovulation. Recent research findings show a synchronization protocol which uses a 7 d CIDR, gonadotropin releasing hormone (GnRH), and PGF2a may be an effective way to synchronize ewes for TAI. These protocols use GnRH

to dictate follicular wave development on the ovaries and to cause ovulation of dominant follicles after CIDR removal. Dickison et al. (2010) reported ewes synchronized with this protocol reached estrus from 34-40 h after CIDR removal compared to ewes synchronized with the current industry standard protocol which reached estrus from 25-68 h. This tighter window of ovulatory synchrony is very desirable for ewes to be inseminated at a fixed time. Therefore the objective of this study is to determine the time of ovulation of three experimental groups and two control groups of ewes synchronized using different protocols. Time of ovulation will be determined by the level of luteinizing hormone (LH) found in blood samples collected from the experimental ewes.

OBJECTIVES

1. Determine the time of ovulation and window of synchrony of three experimental groups and two control groups of ewes synchronized using different protocols.
2. Determine if the second GnRH injection given to Group 3 at 38 h will result in tightly synchronized ovulation without inducing ovulation too early as reported when administering the second injection at 30 h.

LITERATURE REVIEW

Estrus synchronization is very important to producers implementing AI in their sheep breeding program. Estrus detection is one of the most limiting factors of AI in sheep because it is problematic and often unsuccessful due to the number of ewes that may be inseminated at once and the difficulty of identifying ewes in estrus. Using one or more vacetamized rams or acquiring labor to watch for signs of estrus in ewes are the primary methods for estrus detection. Each of these methods can be very costly. It is possible to avoid these problems by using a TAI instead of AI following signs of estrus. Choosing a synchronization protocol that prepares ewes for TAI alleviate the need for estrus detection and helps to more easily achieve acceptable conception rates. Menchaca and Rubianes (2004) showed pregnancy rates and the synchronization of ovulation to be closely related. This means manipulation of ovarian function is necessary for a protocol to yield acceptable conception rates using TAI. Rajakoski (1960) was the first to discover ovarian follicles grow in waves during the estrous cycle. Growth of these follicular waves is stimulated by the gonadotropins follicle stimulating hormone (FSH) and LH. The release of GnRH from the hypothalamus dictates the release of FSH and especially LH (Herbison, 1997). The decapeptide hormone, GnRH, targets receptors on the anterior pituitary where it binds and stimulates gonadotropin release. The release of LH follows the release of GnRH very specifically. The levels of FSH follow GnRH in a much more general way. LH in low levels in a pulsatile pattern is caused by the pulsatile release of GnRH during the luteal phase when P4 levels are high (Karsch et al., 1997). This pulsatile release of LH supports follicular development from the recruitment stage to the dominant stage. A single spike in LH levels is the natural cause of ovulation of

dominant follicles in the estrous cycle. This spike in LH is driven by elevated estrogen (E2) levels from the dominant follicle during estrus. E2 stimulates the hypothalamus to release large amounts of GnRH causing the pre-ovulatory spike in LH. Since LH levels mimic GnRH so closely, an exogenous injection of GnRH is an adequate surge to cause this pre-ovulatory spike in LH. Therefore an injection of GnRH at the beginning of a synchronization protocol for TAI will cause developing follicles to ovulate or undergo atresia, thus resetting the follicular wave. Additionally an injection of GnRH can also be given at the end of a protocol to induce ovulation of any dominant follicles present. According to Dickison (2010), an injection of GnRH 30 hrs after CIDR removal caused ovulation to occur at 30-34 hrs.

The industry's current standard protocol uses PMSG or PG600® which acts like FSH and LH in the ewe's endocrine system. This property causes it to narrow the window of ovulation time when PMSG is used in synchronization protocols (Evans, 1988; Menchaca and Rubianes, 2004). A recently discovered drawback to the use of PMSG is the immune response of ewes after PMSG is administered multiple times. Maurel et al. (2003) showed that PMSG causes an immunogenic response in ewes. The immune response to PMSG causes decreased conception rates as it is used to synchronize ewes from year to year. The effectiveness protocols utilizing PMSG can also be very inconsistent. This is probably due in part to the immune response some ewes have to its effects. PMSG acts similarly to LH and FSH, but does not have a well known and defined affect on the endocrine system. The functions of the PMSG alternative, GnRH, in ruminant animals are better understood. The lack of consistent results from exogenous PMSG in AI protocols may be due to additional affects it has on the reproductive system. There may also be other factors contributing to its

unpredictability which could be discovered through further research. These negative aspects of PMSG point out the need to find an effective protocol that uses GnRH in the place of PMSG.

Many cattle synchronization protocols effectively use GnRH to harmonize the ovarian function of cows at the beginning of the protocol and to induce ovulation of mature follicles at the end of the protocol. GnRH used alone will cause an LH surge 2 h following intramuscular injection (Rubianes et al., 1997). Dickison et al. (2010) demonstrated that a 7 d CIDR in conjunction with PGF2a on d 6.5 and a GnRH injection at CIDR insertion and 30 h following CIDR removal yielded a narrow window of estrus and ovulation. Ewes treated with this protocol showed signs of estrus and peak LH levels within a 4 h time frame. Such a narrow window of synchrony is very desirable for TAI. The problem Dickison et al. reported was a lower conception rate following natural service for this treatment group than another group following the same protocol without the GnRH injection 30 h after CIDR removal. This indicates the induction of ovulation compromised conception rates. Ovulation may have been induced too soon causing ewes to have silent heats leading to them not being serviced by the rams. The group of ewes that was not induced into ovulation began estrus behavior from 36-56 h. More research is needed to determine if the second GnRH injection is delayed would that protocol yield similar ovulation synchrony and higher conception rates.

Progestin treatment is commonly utilized in synchronization protocols because of the negative feedback it causes on the hypothalamus. In a ewe's natural estrous cycle, a functional corpus luteum secretes progesterone (P4) causing GnRH levels to remain low which causes gonadotropin levels to also remain low. If gonadotropin levels remain low,

there will not be a LH surge to cause dominant follicles to ovulate. Thus, P4 treatment can be used in synchronization protocols to prevent ovulation of mature follicles. Increased progestin levels over extended periods of time is known to compromise oocyte quality, leading to lower pregnancy rates than are desirable. Though the use of progestin is effective in synchronizing estrus, long P4 treatments result in aged follicles which have been linked to low fertility in cattle (Austin et al., 1999). Subluteal P4 levels have also been shown to cause unnatural growth and prolonged existence of dominant follicles according to Vinoles et al. (1999). Therefore, these older follicles result in lower fertility rates as stated by Austin et al. (1999). Short duration progestin treatment of 6 d resulted in 20% higher conception as compared to treatment for 12 d (Vinoles et al., 2001). Also, higher levels of P4 treatment have yielded a shorter duration of follicular waves causing younger follicles which should result in higher conception rates (Menchaca and Rubianes, 2002). These findings reveal another component that could be improved within the sheep industry's current standard TAI synchronization protocol. The use of a short-term high-level progestin treatment could yield equal or enhanced synchrony with improved conception rates.

Prostaglandin is another hormone that is commonly used in estrus synchronization for TAI. It causes regression of the CL in the normal estrous cycle of ewes. It is often utilized in synchrony protocols which use P4 treatment. Prostiglandin F2a (PGF2a) is secreted by the endometrium of the uterus in a ewe's natural estrous cycle. In dairy cattle, PGF2a was shown to cause lyses of the CL if administered by intramuscular injection (Thatcher and Chenault, 1976). This causes a natural change in hormones leading to estrus behavior and eventually ovulation of mature follicles. The CL is not responsive to prostaglandin's luteolitic effects

immediately following ovulation. Wiltbank and Niswender (1992) showed the prostaglandin resistance of the CL to only be present for two days following lutenization of a dominant follicle. For ewes treated with a CIDR for several days, a single injection of PGF2a will be effective in causing luteolysis of the CL preceding ovulation.

MATERIALS AND METHODS

Forty primiparous and multiparous Suffolk ewes were randomly assigned to one of five treatment groups. The ewes used in this experiment will be managed according to the guidelines of the Angelo State University Institutional Animal Care and Use Committee. Each group was synchronized using a different protocol. All protocols utilized a CIDR. Each protocol was started at respective times so that CIDR removal was simultaneous for each group. Blood collections via jugular veinapuncture began 40 h following CIDR removal and were repeated every 2 h for 38 h consecutively. Approximately 5 mL of blood was collected from each ewe and placed directly on ice. The blood samples were then centrifuged and frozen at -80°C until the time of the LH assay. Ewes were observed for signs of estrus from the time of CIDR removal to the end of the bleeding period. Mark times were recorded for each ewe as soon as they were in standing estrus and the ewes had a definite mark from the rams equipped with breeding harnesses.

Concentrations of LH in the samples were determined by a double antibody radioimmunoassay (RIA) as described by Recabarren et al. (1996) analyzed for LH concentration to estimate time of ovulation. On d 1, 500 uL of 1% phosphate buffered saline (PBS) and egg white (PBS-EW) were added to the non-specific binding (NBS) and the 0 standard tubes. Two-hundred microliters of standard and 300 uL of 1% PBS-EW were added to each standard tube. Three-hundred microliters of 1% PBS-EW and 200 uL of each sample were put into each unknown tube. The reference preparation tubes contained 300 uL of 1% PBS-EW and 200 uL of reference preparation. The primary antibody anti-oLH was diluted with PBS-EDTA and normal rabbit serum (NRS) at a 1:400 ratio. Two hundred microliters

of the antibody was then added to all tubes except the NSB and total count tubes. A trace consisting of 100 uL of ^{125}I-oLH (20,000 CPM/100 uL diluted in 0.1% PBS-EW) was added to all tubes. Then the tubes were vortexed and incubated for 24 h at 4°C for 48 to 72 h. On d 4, 3.0 mL of ice cold PBS (0.01 M; pH 7.0) was added to all tubes except the total count tubes. The samples and reagents were then centrifuged at 3000 x G for 1 h at 4°C. Once centrifugation was complete, the tubes were decanted, and the supernatant was discarded. The tubes were then counted in a gamma counter. The intra- and inter-assay coefficients of variation for the controls for LH were 15% and between 5 and 20% (n=2 assays), respectively.

For the statistical analysis, effects of time, treatment, and treatment*time on serum LH concentrations were analyzed. Concentration of P4 were analyzed for comparison during the estrous cycle for the effects of time, treatment, and treatment*time. The data was analyzed by Proc GLM of SAS (SAS; Cary, NC). Data was considered statistically different if $p \leq 0.05$.

The first group (GnRH1) was synchronized using the following protocol: on d 0 a progestin implant (CIDR containing 0.3 g progesterone) was inserted intravaginally and a GnRH injection (Cystorelin® 50 ug/mL) was administered IM, on d 6.5 an IM injection of prostaglandin (Lutalyse® 5 mg/mL) was given and on d 7 the CIDR was removed. The second group (GnRH2 was synchronized using the same protocol as Group 2 with an additional GnRH injection 38 h following CIDR removal. The third group (PMSG) was synchronized using the industry's current standard protocol which is: on d 0 a progestin implant (CIDR containing 0.3 g progesterone) was inserted intravaginally and an

intramuscular (IM) injection of prostaglandin (Lutalyse 5 mg/mL) was given and on d 11 the CIDR was removed and an injection of PG600 was administered. The fourth and fifth groups (CIDR7 and CIDR11, respectively) served as control groups for the experimental protocols using 7 and 11 d CIDRs. CIDR7 had CIDRs inserted on d 0, prostaglandin injections was given on d 6.5 and the CIDRs were removed on day 7. CIDR11 received CIDRs on day 0 which were removed on d 11. CIDR11 also received an injection of prostaglandin on d 10.5. Ewes were fed 2 lbs per hd per day of a balanced ration for sheep and had ad libitum access to hay and water.

RESULTS

All ewes retained their CIDR for the duration of the protocols except for one ewe in GnRH2. Her CIDR was absent 20 hrs before the designated CIDR removal time. This resulted in her estrus and ovulation being observed before the other ewes in GnRH2. Two ewes and their data that were intended to be included in this study were removed. One ewe from CIDR7 was removed during the bleeding period due to complications encountered with the breeding process. One ewe was removed from GnRH1 because it was realized that she was exposed to a ram before the trial began and could have been pregnant during the experiment. The Group 2 ewe that lost her CIDR before designated CIDR pull was included in the data. This is a practical problem that could arise in any of the protocols being observed, so her data was not removed from the results.

Mean serum concentrations of LH, shown in Table 1, differed between groups ($P<0.05$) from 22-42 h following CIDR removal. As displayed in Table 1 and Figure 1, the CIDR11 protocol yielded the earliest rise in LH following CIDR removal. The GnRH2, PG600 and CIDR7 groups simultaneously followed with their LH rise about 8 h later. GnRH1 was the last group to show a peak in LH at 30 h. The GnRH2 group shows an early rise in LH as a result of 2 ewes that peaked 12 h earlier than the predominant peak for the group. One of those 2 ewes was the individual that lost her CIDR approximately 20 h early. The GnRH2 protocol produced the highest and most defined LH peak at 42 h post CIDR removal (Figure 1). The GnRH1 group showed a defined spike at h 32. The remaining 3 groups show times of increased serum LH levels, but individual animal peaks are not as harmonious within these 3 treatments.

Table 1. Mean serum concentrations ng/ml of LH for each group. Time shown in h after CIDR removal.

Time	GnRH1	GnRH2	PG600	CIDR7	CIDR11	Pr > |t|
18	1.64	2.29	3.43	1.80	8.04	0.2216
20	1.83	2.55	3.34	1.87	13.11	0.1096
22	1.70	3.15	4.15	2.20	14.81	0.0681
24	1.85	6.13	4.80	6.09	17.58	0.0137
26	1.74	11.64	10.84	9.10	28.16	0.0001
28	3.74	18.22	15.35	20.26	29.69	0.0001
30	14.46	18.28	14.91	19.21	28.11	0.0001
32	25.16	12.69	14.80	20.57	22.03	0.0001
34	21.11	13.60	12.38	16.11	13.43	0.0001
36	13.70	16.56	7.03	7.01	7.81	0.0006
38	6.81	20.46	4.23	3.06	3.75	0.0089
40	4.01	27.03	4.18	1.90	2.50	0.0070
42	2.88	32.69	3.10	2.06	2.25	0.0037
44	4.61	12.84	2.34	1.33	2.38	0.0979
46	7.60	5.54	2.73	1.33	1.73	0.1796
48	6.09	3.39	2.21	1.20	1.84	0.2936
50	3.25	2.35	2.14	1.06	2.06	0.4370
52	0.06	1.80	2.34	1.51	1.51	0.5084
54	1.76	1.76	1.88	1.29	1.66	0.5494

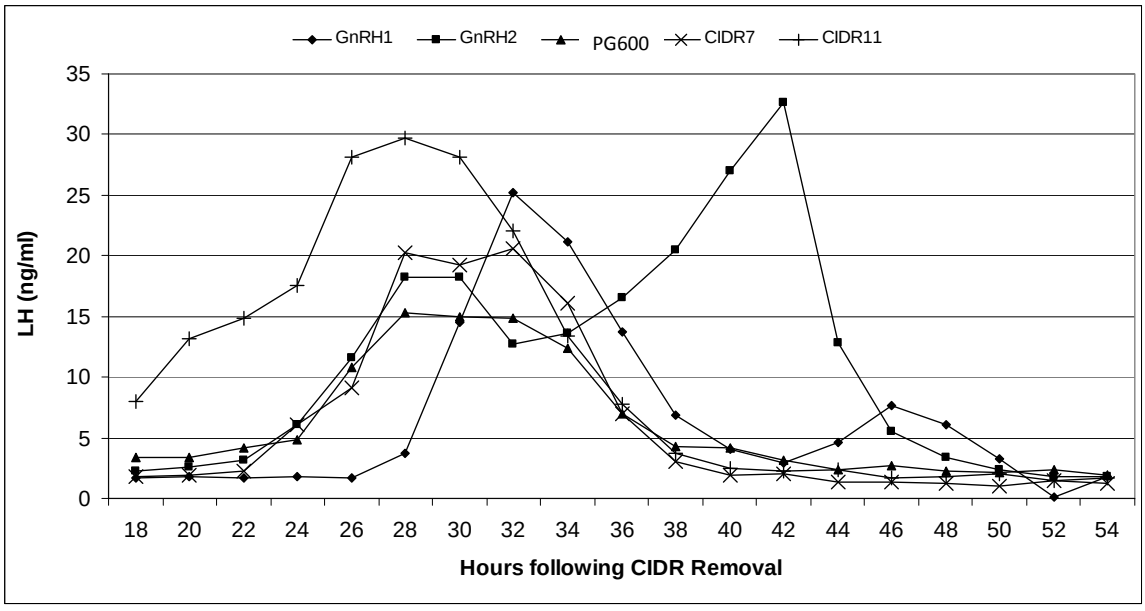

Figure 2. Mean serum concentrations of LH by treatment from 18 to 54 h following CIDR removal.

Ewes were observed for signs of estrus from the time of CIDR removal to the end of the bleeding period. Mark times were recorded for each ewe as soon as they were in standing estrus and the ewes had a definite mark from the rams equipped with breeding harnesses. In GnRH2 and CIDR11 all ewes were marked by the rams. The GnRH2 group had the next highest mark rate of 85.71%. The PG600 and CIDR7 treatments followed with 50% and 62.5%, respectively. In GnRH2, 6 of the 8 ewes came in estrus in a 3 h window. One of the 2 ewes that were outside the 3 h window for GnRH2 was the ewe that lost her CIDR approximately 20 h before the set CIDR removal time. It is suspected that the loss of her CIDR was responsible for her much earlier entry to estrus time compared to other ewes in GnRH2.

In the ovulation results, GnRH2 stands out as the most successful protocol at achieving ovulation during the bleeding period of the trial because all ewes in the treatment ovulated. Treatments for GnRH1 and CIDR11 caused half or more of the ewes to ovulate in a 3 h window (57.14% and 50%, respectively). The GnRH2 protocol brought 6 of 8 ewes in estrus in a 3 h window, but only resulted in 3 of 8 ewes ovulating in a 3 h window. The CIDR7 group had the narrowest window of ovulation at 6 h, but only 4 of 7 ewes in the group showed an ovulatory spike in LH. The PG600 treatment resulted in the lowest ovulation rate and the fewest ovulations within a 3 hour window. It produced the second narrowest range of ovulation times, but the range was for only 3 ewes because the other 5 in the group did not show an ovulatory spike in serum LH concentrations.

Table 2. Number of ewes, percentage of ewes that marked, percentage of ewes that marked in a 3 h window and range of mark time.

Group	n	% Marked	% in 3 h window	Range (h after CIDR removal)
GnRH1	7	85.71	42.86	28-34
GnRH2	8	100.00	75.00	22-39
PG600	8	50.00	25.00	5.5-38
CIDR7	7	62.50	37.50	24-32
CIDR11	8	100.00	50.00	5-34

Table 3. Number of ewes, percentage of ewes that ovulated, percentage of ewes that ovulated in a 3 h window and range of ovulation time.

Group	n	% Ovulated	% in 3 h window	Range (h after CIDR removal)
GnRH1	7	71.43	57.14	30-46
GnRH2	8	100.00	37.50	26-42
PG600	8	37.50	25.00	26-34
CIDR7	7	57.14	28.57	26-32
CIDR11	8	87.50	50.00	20-32

DISCUSSION

The goal of this study was to determine which of the protocols being examined is expected to yield the best results when used to synchronize ewes for TAI. The three experimental protocols have been used in the industry to successfully prepare ewes for TAI. The results help to determine which protocol will achieve the narrowest window of ovulation and the highest ovulation rates which should return the highest conception rates. The results of this experiment can also be used to estimate which protocol would be the most useful when natural service is the method of breeding. Serum LH concentrations from the blood sample data showed treatment to have an affect on serum LH concentrations from 24-42 h following CIDR removal. Table 1 and Figure 1 show the different times that each group begins to rise in their LH concentrations. The GnRH2 protocol resulted in the highest LH concentration at a single time and the most defined peak. Most ewes in this treatment were already beginning to show elevated levels of LH before the second GnRH injection was given at 38 h, but the injection seemed to cause the defined peak that is seen at 42 h post CIDR pull. The GnRH1 treatment produces the next most defined LH spike. The spike for GnRH1 occurs at approximately 32 h after CIDR removal. The peaks of ewes in GnRH1 and GnRH2 were farther apart than expected. The protocols are identical out to 38 h after CIDR removal, so it was expected that their peaks would occur at very similar times. Figure 1 shows GnRH2 to have a small rise within 4 h of GnRH1's LH rise. This was due to 2 ewes showing an ovulatory rise in LH at h 28 and 30, but all other ewes in GnRH 2 showed their rise about 8 h later. The PG600 protocol, considered to the be industry's most commonly

used TAI protocol, gave the lowest LH peak of any protocol. This is due to the small number of ewes that ovulated from this protocol.

The defined LH peaks of GnRH1 and GnRH2 support the idea that these protocols are controlling follicular dynamics at the ovaries. This also supports the need for a TAI protocol to not only manipulate the function and life of CL, but also to program follicular waves and ovulation. The aspect unique to these two protocols is the intent of the first GnRH injection. It is intended to cause ovulation or atresia of any developing follicles on the ovaries at the present time. Since these two protocols produced the most defined LH spikes, the proposal and intent of the GnRH injection is supported. The second GnRH injection in GnRH2 seemed to produce a higher, longer lasting LH surge in the ewes. Experiments where GnRH was used to synchronize estrus in cattle have shown similar results. According to Mee et al. (1993), serum LH concentrations were observed to be higher 2 h following a GnRH injection and stayed high for a 6 h period. Data from this study shows LH levels to be rising at 34 and 36 h following CIDR removal, and then a greater rise from 38 to 40 and 42 h. This rise can be attributed to the GnRH injection given at 38 h which caused LH to spike for 4 h before falling. This LH surge from the time of GnRH injection to 4 h following the injection will help to insure ovulation in ewes in this group. It can be assumed that any ewes that had not already ovulated before the injection would ovulate due to the spike following the injection.

Another objective of this study was to determine if the second GnRH injection being placed at 38 h would tighten ovulation without causing silent heats from premature ovulation in GnRH2. Serum LH concentrations from the blood samples showed the GnRH2 protocol to

be effective as reported by Dickison et al. (2010) at synchronizing ovulation. With the second GNRH injection being administered at 38 h, it did not induce ovulation of premature dominant follicles. All ewes in GnRH2 came into estrus before the second injection was given. Dickison et al. (2010) reported a narrow window of synchrony and low conception rates with a similar protocol with the second GnRH shot given at 30 h post CIDR removal. Poor conception rates with natural service were thought to be a result of inducing ovulation before the follicles produced enough E2 to cause estrus. Some ewes in the protocol used by Dickison et al. (2010). ovulated but never came in heat, so they were not bred by the rams. In GnRH2, most ewes had already showed peaked or rising LH levels before h 38, but the GNRH injection expedited ovulation in the ewes lagging behind the majority. With the second GnRH injection given at 38 h after CIDR pull, all GnRH2 ewes showed signs of estrus and were marked by the rams. Though GnRH2 had fewer ovulations within the 3 h window, it produced the highest ovulation percentage and the most predominant spike in LH of any group.

The occurrence and time of ovulation was determined to be the time at which serum LH concentration reached 45 ng/ml. For this experiment, this was assumed to be the ovulatory LH spike. The GnRH2 protocol achieved the highest ovulation percentage of any group. The CIDR11 group followed closely, having only one ewe that didn't show an ovulatory LH peak. The ewe from CIDR11 that did not have an LH spike came in estrus only 5.5 h after CIDR removal. It is possible that she ovulated before the bleeding period began at 18 h. Whether the CIDR11 treatment resulted in a 100% or 87.5% ovulation rate, it showed to be reliable protocol at synchronizing ovulation. The downfall of this protocol is the wider

window of ovulation, especially if one ewe ovulated before the bleeding period began. An aspect added to this protocol to control follicular dynamics may offer a narrower, improved range of ovulation time. One ewe per group, with the exception of GnRH2 marked as showing standing estrus, but did not show an ovulatory LH spike. This is likely due to those ewes mounting other ewes and being mounted by the rams during estrus activity of other ewes in the same pen. One possible exception to this explanation is in the CIDR11 group where one of the ewes may have ovulated before the first bleeding time at 18 h after CIDR pull, as stated earlier.

The reaction of ewes to the PG600 protocol was less than desirable in terms of the ovulation percentage. The ewes also displayed the widest window of estrus synchrony. This group also recorded the lowest ovulation and estrus percentage. One ewe that came in estrus only 5.5 h following CIDR removal may have ovulated before the bleeding period began, but only 3 ovulatory peaks of LH were observed in this group. This data is supported by the findings of Dickison et al. (2010). Titi et al. (2010) reported that ewes given a long term progestin source and a PG600 injection at progestin removal showed very different times of LH surge. Data from this experiment supports the idea that a short P4 treatment is more effective than a longer P4 treatment when using other exogenous hormones to synchronizes ewes. The results also infer that a short CIDR coupled with GnRH and PGF2a produces a narrower window of synchrony of ovulation than protocols using PG600 instead of GnRH. This data agrees with findings by Titi et al. (2010) and Jabbour and Evans, (1991).

Though natural service or AI following estrus detection was not the focus of this study, the data obtained for mark times of the ewes is helpful when considering these

protocols for those service types. In the GnRH2 and CIDR11 groups, all ewes came into estrus and stood to be mounted and marked by the vasectomized rams they were with. Only 1 of 7 ewes in GnRH1 did not show signs of estrus or mark to the rams. These three protocols show good potential for being used to synchronize ewes for breeding by natural service or AI following estrus detection. In scenarios were ewes are synchronized for natural service, the CIDR11 protocol caused all ewes to mark within a 29 h window. For natural service, having ewes bred in a 29 h time frame would be desirable for most cases. The two CIDR protocols are also the cheapest and least labor intensive. This coupled with the data, suggests the CIDR11 protocol would be the best suited protocol for synchronizing ewes for natural service.

The GnRH1, GnRH2 and CIDR11 groups all hold potential to be used for synchronizing ewes for AI with estrus detection. This method of AI is not as commonly used in the sheep industry because estrus detection is labor intensive, expensive and often inaccurate. For breeders that choose to AI ewes following estrus detection, these protocols are viable synchronization options. These three treatments yielded the highest percentages for marking and ovulation data. If cost is not a very influential factor on the operation, the GnRH2 protocol showed to have the best estrus and ovulation rates and a tight window of synchrony, especially estrus synchrony. With the second GnRH injection at 38 hours following CIDR removal, ewes on this protocol are allowed time to come into estrus naturally before ovulation is induced. If the time window of heat or ovulation needs to be narrow, but cost and labor are of some concern, the GnRH1 protocol offers a slightly cheaper option that requires one less injection. It still showed to achieve a smaller range of estrus

times, but did that with one less injection. If the window of estrus synchrony is not an important issue and financial input is important, the CIDR11 protocol is a viable, easy to use option.

In terms of using one of these protocols for a TAI, the most important fact is that GnRH2 showed such a high estrus and ovulation rate with an acceptable window of synchrony for both. It is to be expected that the GnRH2 protocol will produce the highest conception rates following a TAI because it caused the highest ovulation rate within an acceptable window that all ewes could be inseminated at the same set time. Though some of the other protocols had a tighter window of ovulation, the ewes that do not ovulate will not produce a pregnancy after a TAI. Sperm have a window of life in the reproductive tract of a ewe, so a successful synchronization protocol needs to provide the highest amount of oocytes ready for fertilization within that time period.

Several conclusions can be drawn from the data obtained during this project. Primarily, this data demonstrates the potential for a TAI protocol utilizing a short duration P4 implant coupled with one or two injections of GnRH to be a viable synchronization option. In comparison to the standard protocol that is currently being utilized in the industry, the GnRH1 and GnRH2 protocols offer a higher rate of estrus and ovulation and a tighter window of synchrony. Research publications of synchronization protocols for ewes is somewhat limited at this point, but further research in this area will be beneficial to the sheep industry. Comparing the proposed GnRH1 and GnRH2 protocols to the PG600 protocol in an experiment where the ewes are TAI should be the next step in determining the efficacy of the protocols. A project designed to compare pregnancy rates, lambing rates and number of

lambs born per ewe between these protocols will help determine if profit of an operation can be increased through better results from the GnRH protocols. If higher conception rates can be achieved through more effective synchronization protocols, it would help to increase profit of producers already using AI and it could make AI more economically feasible for producers not currently implementing the procedure into their breeding program. This would also aid the industry in expediting genetic progress because elite stud rams would be able to sire more offspring in their lifetime and after their death.

SUMMARY

This study compared the reproductive responses to protocols using GnRH coupled with a 7 d CIDR treatment and PGF2a to the industry's standard protocol using PG600 instead of GnRH. The objective was to gain more insight into which of these protocols would be the most useful in preparing ewes for a TAI. Compared to the PG600 protocol, the two GnRH treatments yielded higher ovulation rates and acceptable synchrony windows. Though a TAI was not performed in this study, the data presented gives producers and researchers enough information to predict the best time for insemination to be performed following each protocol.

As shown in this study, GnRH, PGF2a and a short 7 day CIDR regimen can effectively prepare ewes for a TAI by synchronizing estrus and ovulation. Dickison et al. (2010) demonstrated that acceptable pregnancy rates could be achieved using natural service following similar protocols. Further research is necessary to determine if these protocols will produce desirable pregnancy rates following TAI, but results from this project support that idea. The shorter CIDR treatment appears to allow for a narrow window of synchrony and potentially ovulation of higher quality oocytes. Treatment with GnRH in place of PG600 resulted in higher ovulation response rates. This is likely due to greater consistency of the GnRH product and elimination of the immune response to PG600. Further research is required to prove the efficacy of these protocols producing increased conception rates to TAI compared to current protocols being utilized in the industry.

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