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

A Dissertation

by

JAMES WILLIAM DICKISON

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

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Animal Science

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

Effects of GnRH and Prostaglandin Combined with a Short Progestin Regimen on the Synchrony of Estrus and Ovulation in Ewes During the Breeding Season.

(December 2010)

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Two trials were conducted to quantify the effects of GnRH and prostaglandin in conjunction with a 7-d CIDR on estrus and on pregnancy rate in comparison with a traditional synchronization protocol. In trial 1, ewes (n=12) were randomly allotted to one of three treatments: CIDR (7 d) with administration of GnRH (Cystorelin®, 50µg, im) at CIDR insertion and PGF2α (Lutalyse®, 20 mg, im) on d 6.5 (GnRH1); the GnRH1 protocol with a second injection of GnRH 30 h after CIDR removal (GnRH2); and CIDR (11 d) with administration of PGF2α at CIDR insertion and PMSG (400 iu) at CIDR removal (PMSG). A blood sample was obtained every 2 h for 42 h after CIDR removal for serum LH analysis. On d 8 after CIDR removal, blood samples were obtained at 12 h intervals for 36 h for serum P4 analysis. One ewe in the GnRH1 group did not retain the CIDR device and was excluded from the analysis. Mean LH concentration did not differ ($P = 0.48$) among groups. Time and time x treatment affected ($P < 0.001$) mean LH concentration. Mean P4 concentration was not affected ($P = 0.26$) by time, treatment or their interaction. In trial 2, ewes (n=72) were randomly allotted to one of the three

treatments described in trial 1. At CIDR removal, three ewes per treatment were joined with a single ram fitted with a marking harness in each of 8 pens. Ewes were monitored every hour for estrus activity and ultrasounded transabdominally 60 d after CIDR removal for pregnancy. Estrus activity did not differ ($P > 0.05$) among the groups. Marking frequency was 92%, 75%, and 88% for GnRH1, GnRH2, and PMSG groups, respectively. Mean interval to estrus was shorter ($P < 0.05$) for the GnRH2 than for the PMSG group and tended to be reduced ($P < 0.10$) compared with the GnRH1 group. Pregnancy rate differed ($P < 0.05$) among treatments (79%, 58% and 38% for GnRH1, GnRH2, and PMSG groups, respectively). These results indicate that synchrony of estrus and pregnancy rate to natural service can be increased in response to a CIDR protocol when combined with administration of GnRH rather than PMSG.

DEDICATION

To my Family

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I have always thought of myself as someone who could do anything that I put my mind to and do it without the help of others. I have found with this particular endeavor that this was not the case in the least. I have many people to thank for not only their guidance and assistance, but for their unwavering drive to get me through this process and on to the next step in my life. First of all, I have to thank my partner in life, my beautiful and loving wife, Brook. Brook, without your patience and encouragement to stay the course and finish this endeavor, I know that I would not have accomplished all that has been achieved this far. You have given me the two greatest things a person could give another, your unending love and a son that gives me so much joy every day. Rett thank you for all the laughs and smiles you give me every day, as well as your love, even when daddy has been gone for weeks at a time.

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CHAPTER I

INTRODUCTION

Timed artificial insemination (TAI) is a crucial reproductive management tool utilized by producers of all species of domestic meat animals. It is even more important in small ruminants due to the nature of the techniques that are used to artificially inseminate females. Specifically the use of abdominal laparoscopic artificial insemination (LAI) in sheep requires the ability to manipulate the hormonal and ovarian dynamic in order to tighten the window of synchrony in females. Thus, allowing for the highest percentage of successful pregnancies possible utilizing these methods of reproductive technology. This particular need for TAI is warranted when detection of estrus is unfeasible due to the number of females put into a synchronization program.

The use of TAI is being implemented into more management practices with every passing breeding season. Current protocols allow acceptable conception rates but there is much room for improvement with our ever growing knowledge of ovarian dynamics. In order to optimize the conception rates in sheep, we must test new ideas to help the producer optimize these reproductive management techniques. Synchronization of the estrous cycle and manipulation of the ovarian dynamic has aided producers with reproductive management and facilitated scientific study of reproductive endocrine events.

This dissertation follows the style and format of the Journal of Animal Science.

An efficient TAI program requires the use of protocols that ensure acceptable pregnancy rates (% of pregnant animals among treated females) with a very low variation in the response between flocks. Pregnancy rates are closely linked to the synchronization of ovulations obtained in treated females (Menchaca and Rubianes, 2004). Most traditional TAI protocols involve the use of a progestin treatment between 11-19 days, as well as the utilization of a prostaglandin with or without an eCG (PMSG or PG-600). The justification for the many variations of the TAI protocol is that most small ruminants are put into a minor livestock category and most pharmaceuticals utilized in synchronization protocols are not approved for use in small ruminants. The use of products not labeled or approved for minor livestock species, therefore must then be used. As a result of extra-label use, standardized protocols and dosages does not exist. A variety of synchronization protocols and product combinations have been used to synchronize females of these species.

CHAPTER II

REVIEW OF LITERATURE

Synchronization of the estrous cycle and manipulation of the ovarian dynamic has aided producers with reproductive management and facilitated scientific study of reproductive endocrine events. Estrus synchronization, by definition, is the manipulation of the estrous cycle in order to bring a large group of females at different stages of the estrous cycle into estrus at a precise time. Females may then be inseminated according to estrus or standing heat. In large species such as cattle, this is usually 12 h after estrus behavior is observed. In small ruminant species such as sheep, a fixed-time insemination method is necessary due to the physiological size of the animal and the nature of the procedure which is used to inseminate. An efficient TAI program requires the use of protocols that ensure acceptable pregnancy rates (% of pregnant animals among treated females) with a very low variation in the response between flocks. Pregnancy rates are closely linked to the synchronization of ovulations obtained in treated females (Menchaca and Rubianes, 2004). Most traditional TAI protocols in small ruminant species consist of a progestin treatment anywhere from 11-19 d, as well as the utilization of a prostaglandin with or without an eCG (PMSG or PG-600).

Follicular growth

Oogonia population of the ovary and growth of the follicles occur in the female fetus before parturition. During the second trimester of fetal life, the fetal ovary bears a primordial follicular pool which contains oogonia. A ewe is born with a complete, non-recyclable pool of oogonia in primordial follicles that are made up of only a single flat cell layer (Erickson, 1966). The ovaries of young ewes contain between 40,000 and 300,000 primordial follicles (Cahill et al., 1979; Mariana et al., 1991). This pool of primordial follicles represents the entirety of the females reproductive life, in such, it cannot be replenished or recycled and the majority of these primordial follicles will never mature or will undergo atresia during the growth phase. Ovarian follicles undergo many transformations with each stage of follicular growth. Initially, primordial follicles are transformed into primary follicles. The first follicles to form and to leave the primordial pool are those in the innermost regions of the ovarian cortex (Smith et al., 1993). Once follicles are committed to growth, this process is irreversible and can no longer return to their quiescent state. Primary follicles are characterized by the surrounding cells becoming cuboidal and proliferating, known as granulosa cells. These granulosa cells proliferate many times allowing many cell layers to surround the oocyte, this follicle is known as a secondary follicle. During this time, cavities begin to form within the follicles and become filled with follicular fluid. These cavities converge and make one large cavity inside the follicles known as the follicular antrum. At this stage, the follicle is known as an antral follicle or tertiary follicle. Fully matured follicles are

known as Graafian follicles and are preovulatory after the first preovulatory gonadotropin surge and before the first ovulation (onset of puberty). A very small number of follicles will ovulate in the life span of a female, most will become atretic.

Folliculogenesis is thought to take an estimated 6 mo, with most of this time being devoted to the growth of primary follicles to a diameter of 2.5 mm (Souza et al., 1997). Growth of follicles to this particular size is seemingly independent of gonadotropin support and involves no significant secretion of estradiol (McNatty et al., 1982). However, there is evidence that follicle stimulating hormone (FSH) receptors are functionally active during preantral development; granulosa cells increased in number and there was more thymidine uptake after being stimulated with FSH in serum-free cultures of bovine oocytes (McNatty et al., 1999). The consensus is that primary follicles can continue to grow independently of pituitary gonadotropins despite gonadotropin receptor expression, but their growth rate may be altered by FSH and/or LH (Hirschfield, 1985; Peluso et al., 1991). The growth of follicles from 2.5 to 5 mm occurs very rapidly in a few days, and this step in the selection process of a follicle to a "dominant" or estrogenic stage is dependent on the hormonal environment (Souza et al., 1997).

The hypothesis that growth of ovarian follicles occurs in a wave-like fashion was first observed by Rajakoski. Rajakoski (1960) uses the term "follicle wave" in order to describe the pattern of distribution of medium and large follicles on the ovaries of heifers collected at slaughter. It was observed that follicles of ≥ 5 mm in diameter were uniformly organized into two distinct growth periods. This observation was termed

"waves" of growth. This suggestion was controversial with studies supporting or refuting the idea in cattle until 1988 (Evans, 2003). Pierson and Ginther (1988), Savio (1988), and Sirois and Fortune (1988) utilizing ultrasonography verified the wave-like pattern of follicular growth in cattle. Evidence for and against wave-like growth in the sheep ovary has been studied and argued for many years. However, most of the recent studies favor the description of the pattern of follicle development as being wave-like during the estrous cycle (Evans, 2003). Utilizing transrectal ultrasonography, Lopez-Sebastian et al. (1997), noted patterns of growth and regression of individual follicles indicated a relatively constant number of follicles available for ovulation in each ewe. Therefore, follicular wave-like pattern could not be determined in these studies. Ginther et al. (1995) found that follicles in cyclic polypay ewes which reached only 3 or 4 mm in diameter did not exhibit an organized pattern of growth and atresia. A follicle wave is the organized development of a cohort of gonadotropin-dependent follicles all of which initially increase in size. The number of remaining (dominant) follicles is specific to the species and is indicative of litter size (Evans, 2003). Apparent waves of follicular growth were observed in ewes when only follicles of ≥ 5 mm in diameter were considered. In ewes, a follicular wave will generally consist of 1 to 3 follicles growing from 2 to 3 mm to a maximum size of 4 to 7 mm in diameter before regression or ovulation (Duggavathi et al., 2003) with follicular emergence restricted to a 24 to 48 h period.

There are three characterized and accepted stages of follicular growth. Recruitment utilizes gonadotropin support to stimulate a growing pool of follicles. The

next defined stage is selection, a recruited follicle is favored by hormonal support to grow into a dominant follicle thus exerting a negative feedback and suppressing its subordinate follicles. This is the final stage of follicular growth, dominance. Utilizing ultrasonography, the emergence of a follicular wave can be detected with follicles of 4 or 5 mm in diameter that are increasing in number. After the corpus luteum (CL) regresses, the dominant follicle of the final wave will become the ovulatory follicle. Although in sheep, the ovulatory follicle can also derive from the penultimate follicular wave (Bartlewski et al., 1999; Gibbons et al., 1999).

Hormonal control of the estrous cycle

The estrous cycle is one of massive complexity. Hormonal secretions effect the physiological changes that take place, and in turn, the physiological changes affect how the hormonal secretions are released. The hormonal aspect of the estrous cycle is governed by the hypothalamic-hypophyseal-gonadal axis. Gonadotropin releasing hormone (GnRH) is a decapeptide produced by neurons in the pre-optic area of the hypothalamus and released in pulses into the portal blood system which directly connects the hypothalamus to the anterior portion of the pituitary gland. GnRH dictates the synthesis and release of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior portion of the pituitary (Herbison, 1997). These 2 hormones are much similar in that they are glycoproteins in nature, then synthesized and released from gonadotroph cells which are specialized cells in the anterior pituitary. GnRH is released in a pulsatile fashion this is most necessary to prevent the down-regulation of the GnRH receptors due to long term exposure (Roche and Diskin, 1996).

It also determines the pulsatile pattern of LH release by the pituitary. Much different than LH, FSH is more passive and only partly controlled by GnRH, keeping FSH from being ultimately pulsatile in nature. Although, during the luteal phase of the cycle ewes show waves in their concentrations of FSH with peaks occurring about 6 d apart (Bister et al., 1991). These are probably associated with the development and regression of large follicles in the ovary, as has been reported in cattle (Fortune et al., 1991). These peaks are associated with an increase of inhibin at the beginning of each follicular wave, and estradiol increases during the first and last follicular waves to regulate FSH. Although GnRH regulates the gonadotrophs, GnRH itself is regulated by progesterone, the hormone of pregnancy, in turn regulating the length of the estrous cycle. Progesterone (P4) is a steroid hormone in nature which is derived from cholesterol. P4 concentrations in the peripheral blood increase approxiamtely d 3-4 of the estrous cycle, while maximum concentrations are achieved by d 10-12 and stay high until luteolysis around d 14-15 in the ewe. Once luteolysis begins, progesterone concentrations in the blood begin to decline and within 24 h reach the lowest values during the cycle. Concentrations remain low throughout the follicular phase until ovulation 2-3 d later (Scaramuzzi et al., 1993). Late in the luteal phase of the estrous cycle prostaglandin F2α is secreted from the uterus this causes lysis of the CL and is the cause for the drop in P4 levels and allows an increase in GnRH pulsatility and an increase in concentration and pulsatility of LH. An increase of estradiol also at this time begins estrus behavior in the female. This increase in estradiol also further increases the GnRH pulses and leads to a surge of GnRH and ultimately a peak in LH concentrations (Bryner et al., 1990), causing

ovulation of the next ovulatory follicle. An FSH surge is concurrent with the LH peak, this is considered the first FSH surge (Bergfelt et al., 1997).

Controlling the estrous cycle

The sheep estrous cycle can be manipulated by the use of exongenous hormones such as progestins and prostaglandins or gonadotrophins such as pregnant mare serum gonadotropin (PMSG) or GnRH which mimic physiological events in the cycle. The use of either progestins or prostaglandins such as PGF2α yields acceptable synchrony of cyclic cattle, although PGF2α is ineffective during the postpartum interval. In sheep, seasonal considerations are critical in determining the efficacy of each synchrony regimen. Godfrey et al. (1999) found that when utilizing both of these strategies in a TAI protocol in hair sheep, PGF2α usage yielded much lower conception rates versus long-term progesterone treatment with a controlled internal drug release (CIDR) device. This discrepancy with TAI is due to the fact that long-term P4 treatment yields a tighter range of synchrony and the time of ovulation is more accurate, allowing for higher conception rates. Although there were no differences seen when the 2 protocols were utilized for natural service. Gonadotrophins have been incorporated with progestin treatment to stimulate ovarian activity in sheep (Menchaca and Rubianes, 2004).

The use of these gonadotrophins both PMSG and GnRH have proven to offer a more compact ovulation time in ewes (Evans, 1988; Menchaca and Rubianes, 2004; Zeleke et al., 2005), and in turn offering the potential for increased pregnancy rates after TAI.

Progestin usage

Progesterone is the dominant ovarian hormone present in the circulation during metestrus and diestrus of the estrous cycle and is secreted from the CL. As stated previously, this stage of the estrous cycle is known as the luteal phase and lasts from the development of a functional CL 2-3 d post ovulation until luteolysis occurs around d 14-15 of the cycle. The use of progestins in artificial insemination protocols and the control of the estrous cycle have been widely researched and utilized in cattle and in sheep. Progestin treatment synchronizes estrous by suppressing folliculogenesis by inhibiting hypothalamic function. Cessation of progestin treatment allows folliculogenesis to resume and is followed by ovulation (Thompson and Monfort, 1999). Studies during the 1940's revealed that estrus could be delayed and therefore, synchronized by utilizing the administration of exogenous progestins to cattle and sheep. Although, the first attempts to utilize progestins as a synchronization tool weren't done until the 1960's and 1970's in cattle (Macmillan and Peterson, 1993). Animals were given injections of P4 daily for 20 d. These studies yielded acceptable levels of synchrony but fertility with the induced estrus was low. Melengestrol acetate (MGA) was the next step in exogenous progestins. Melengestrol acetate (MGA) could be fed to cattle at a rate of 0.5 mg/hd/d and effectively suppress estrus. Although, long-term feeding of MGA effectively synchronized estrous, fertility was compromised (Zimbelman and Smith, 1966). Melengestrol acetate (MGA) has also been utilized to synchronize estrous in sheep with variable results not only in lambing rates (Powell et al., 1996), 25% to 85% respectively, but also with differences reported in length of feeding treatment and breeding length

(Powell et al., 1996). The most recent research into the administration of progestins has utilized intravaginal administration. Intravaginal sponges impregnated with either medroxyprogesterone acetate (MAP) or fluorogestone acetate (FGA). These impregnated sponges are effective in synchronizing the estrous cycles of all treated females whether used in the breeding or the non-breeding season. Devices are effective but don't offer the convenience of the alternative. Controlled internal drug release (CIDR) device was the next step in the application of exogenous progestins. The CIDR is constructed with a silicone elastomer containing exogenous progesterone. Controlled internal drug realeas (CIDR) devices are much more convenient and offer a higher degree of sanitation than the impregnated sponge devices.

At the present time, most synchronization protocols utilize a very long progestin treatment, of 10-19 d. As a result of this treatment, a high percentage of ewes show estrus, but fertility is much lower than with a natural estrus (Robinson et al., 1970). Consequently, this low fertility rate has been attributed to changes in the hormonal milieu that results in an asynchrony between estrus and ovulation (Scaramuzzi et al., 1988). An alteration of subsequent sperm transport was also observed (Pearce and Robinson, 1985). Investigators have proposed this length of time to have adverse effects to the overall fertility of the population being synchronized. Recent studies have paid particular attention to the effects of subluteal P4 concentrations on follicular health. In ewes, subluteal P4 levels promoted excessive growth and persistence of the largest follicle (Vinoles et al., 1999), increasing the age of the ovulatory follicles (Johnson et al., 1996). Exposure to long progesterone treatments adversely causes ovulation of aged

follicles in small ruminants. In cattle, the ovulation of an aged follicle is followed by low fertility (Austin et al., 1999; Savio et al., 1993). A similar detrimental effect of long exposure to a P4 treatment has been observed on conception rates in the ewe (Menchaca et al., 2004; Vinoles et al., 2001).

High P4 concentrations, in contrast, have a positive effect on follicular turnover increasing the number of young large follicles with the potential to ovulate. Supraluteal P4 levels affect the dominance of the largest follicle of Wave 1, inducing early regression and accelerating the emergence of the next follicular wave, which results in the ovulation of a healthy young follicle (Menchaca and Rubianes, 2002; Rubianes et al., 1996). Recently, studies have shown that short term treatment of progestin devices during the non-breeding season were as effective as long term treatment to induce estrus, and the following fertility rates were also higher (Ungerfeld and Rubianes, 1999). Vinoles et al. (2001) reported higher pregnancy rates after a short term treatment (6 d, 87%) compared to the traditional 12 d treatment either with (67%) or without (63%) PMSG.

Ultimately the concept that a high-level short-term progestin treatment could possibly be more effective at controlling follicular dynamics and improving conception rates when compared to a long term progestin treatment.

Prostaglandin (PGF2α)

A P4 treatment alone will not effectively synchronize estrus for TAI. The use of other hormones must be utilized to ensure the least possible dispersion of ovulation time among ewes. Prostaglandins are lipids consisting of a 20-carbon unsaturated hydroxy

fatty acid chain that is derived from arachidonic acid. Prostaglandin F2α is produced by the uterine endometrium and is the hormone that is solely responsible for luteolysis, or degradation of the CL, in ruminants. Prostaglandin F2α is the most potent luteolytic agent in sheep (Mccracken et al., 1972). The discovery of this luteolytic agent was the topic of choice for many researchers in the 1970's. Thatcher and Chenault (1976) reported that an intramuscular injection of PGF2α caused a rapid regression of the CL which initiated a normal transition of hormonal patterns resulting in ovulation in estrous in cycling dairy heifers. Prostaglandin F2α has similar effects in sheep as in cattle, therefore is a popular method of estrous synchronization. Although the ability of PGF2α is day, dose, frequency of exposure and route of administration dependent. Prostaglandin F2α offers a very high variability of response depending on the ovarian status of each ewe (Menchaca and Rubianes, 2004). When incorporating a TAI protocol all ewes are synchronized at the same time not taking their individual cycles into account. This poses a problem when synchronizing ewes due to the fact that a newly formed ovine corpus luteum is considered to be refractory to the effects of PGF2α. Such refractoriness has been shown to be restricted to the first 2 d after ovulation (Acritopoulou and Haresign, 1980; Wiltbank and Niswender, 1992). Thus, ewes treated with prostaglandin shortly after they ovulate will not synchronize as tightly as those who immediately undergo luteolysis after prostaglandin administration.

Prostaglandin F2α treatment alone has proven to be an effective method to synchronizing estrus in not only cattle but sheep. Although it is effective at

synchronizing females, due to its high variability amongst females in a herd, PGF2α alone does not prove to be useful in a TAI situation.

Gonadotropins in synchronization - PMSG

As stated previously, most synchronization protocols utilize a gonadotropin such as PMSG. Pregnant mare serum gonadotropin is a glycoprotein secreted from the endometrial cups of pregnant mares. It is utilized because of its long half-life and the fact that it carries both FSH and LH like patterns. This injection of PMSG is most commonly given at the time the progestin device is removed, although alternative timing has been evaluated. Eppleston et al. (1991) reported that PMSG administered at 2 different time points (24 h before or at time of progesterone insert removal), produced no significant difference in timing of ovulation. Zeleke et al. (2005) also reported no significant difference between time and route of administration of PMSG and that the type of progestin it was used with had no difference. The use of PMSG has been shown to aid in a more compact instance of synchrony (Evans, 1988; Menchaca and Rubianes, 2004a; Zeleke et al., 2005), and consequently reporting potentially higher pregnancy rates when utilized with TAI. Although there has been recent evidence that the use of this hormone could be associated with problems with subsequent breeding seasons, the use of such hormones have been associated with negative effects on pregnancy rates (Baril et al., 1996; Drion et al., 2001) it has also been reported that PMSG is immunogenic when used in ewes (Maurel et al., 2003; Roy et al., 1999). In some cases in sheep, the use of PMSG has been associated with the development of follicular cysts followed by low pregnancy rates (Vinoles et al., 2001).

GnRH

There have been countless studies and it is widely accepted that GnRH release from the hypothalamus is the mediator of the preovulatory surge of LH in ewes. As stated by Karsch et al. (1997), GnRH is secreted as low-frequency pulses during the luteal phase of the estrous cycle when circulating concentrations of P4 are high and estradiol is relatively low. Pulse frequency will then increase and the amplitude of the pulses will decrease during the midfollicular phase when P4 is declining as estradiol levels increase. This happens with onset of the preovulatory LH surge, the high-frequency, low amplitude pulse pattern gives way to an unambiguous GnRH surge. This surge of GnRH begins at the same time as the LH surge and continues long after the LH surge has ended. Numerous studies have looked into the use of GnRH as an alternative to other gonadotropins in sheep as well as in cattle. Gonadotropin releasing hormone utilized by itself will induce a synchronized LH surge 2 h after intramuscular injection during the breeding and non-breeding seasons (Rubianes et al., 1997). Kohram (1998) reports that GnRH has had significant effects on follicular dynamics, a GnRH injection increases the number of medium sized follicles within 3 d of treatment, eliminates the large follicles by means of ovulation or atresia at any stage of the estrous cycle and most importantly induces the emergence of a new follicular wave therefore allowing for follicular turnover. Although there are some reports that GnRH when given without PMSG had decreased the estrous response, when given 36 h after CIDR was removed (Luther et al., 2007). In contrast to PMSG, GnRH has had no reported negative

consequences on subsequent breeding yr or any immunological effects that may hinder the females ability to rebreed in later breeding seasons.

Synchronization of estrous for artificial insemination

More recently, research on controlling the length of the estrous cycle has led to a greater understanding of follicular control. Consequently, this improved understanding of folliculogenesis has allowed for better methods to control and manipulate follicular development. These ideas have been joined with traditional methods to control estrous length to target the timing of estrus and the timing of ovulation. Many methods have been developed for synchronization of estrous in sheep (Maxwell and Butler, 1984), although the most successful attempts have been those which utilize suppression of the estrous cycle by way of progestin (Gourley and Riese, 1990; Maxwell and Barnes, 1986). While incorporating gonadotropin support to stimulate ovarian activity, the most commonly utilized is PMSG. As researchers, our ultimate and primary goal should be to devise a treatment that will facilitate the use of timed insemination without the use of estrus detection. As stated previously, in small ruminant species such as sheep, a fixed-time insemination method is necessary due to the physiological size of the animal and the nature of the procedure which is used to inseminate. The "industry standard" for TAI in sheep is direct deposition of semen into the uterus with the aid of a laparoscope (Gourley and Riese, 1990). Therefore, more so in sheep than any other species, TAI is a good technique for improving reproductive efficiency and a way to introduce new genetics, but it is also a necessity.

As stated previously, there have been methods developed to synchronize the estrous cycle and to control ovarian events in order to gain greater success when AI is utilized in sheep as well as other species. The most widely utilized is the use of a progestin for 11-19 d coupled with PMSG. This technique synchronizes estrous of a majority of the females, Luther et al. (2007) reported that progestin for 14 d with PMSG at the end of treatment gave a 90.6% synchrony of females and a 62.5% pregnancy rate following TAI. Eppleston et al. (1991) reported the same 90% rate of synchrony utilizing a different avenue of administration of progestin but with the same dosage of PMSG and a lower pregnancy rate of 51% with a much larger number of females utilized. Similar and acceptable pregnancy rates have been reported for TAI using a laparoscope 40-62% when utilizing frozen-thawed semen (Eppleston and Roberts, 1986). Researchers have begun utilizing a short term progestin treatment and are reporting similar and in some instances higher success rates than with a traditional long term progestin. Utilizing a 6 d MAP impregnated sponge, Ungerfeld and Rubianes (1999) reported a pregnancy rate of 75% after TAI. Vinoles et al. (1999) reported a much higher pregnancy rate utilizing a short MAP treatment length of 6 d when compared to a traditional 12 d sponge length of 87% and 67% respectively.

Although the use of a progestin coupled with PMSG seems to be the industry standard there is other work utilizing different means of estrous synchronization. In cattle, Pursley et al. (1995) reported that timing of ovulation following PGF2α injection in the GnRH-PGF2α treatment ranged from 84 to 120 h. Therefore, to increase the synchrony of ovulation, researchers added an additional injection of GnRH 48 h after the

PGF2α injection. Ovulation was then synchronized within an 8 h window; this protocol of a GnRH-PGF2α-GnRH treatment was termed Ovsynch, due to the fact that it synchronized not only follicular development but estrus and ovulation as well. This approach has been studied in the synchronization of sheep to some degree of success when coupled with TAI. Deligiannis et al. (2005), utilized a similar protocol to the one developed by Pursley et al. (1995). A pregnancy rate of 50% among females subjected to TAI was reported (Deligiannis et al., 2005).

In a study conducted by Titi et al. (2010), investigators utilized numerous protocols to determine the effects of combinations of different hormonal treatments. A traditional FGA impregnated sponge for 14 d coupled with an injection of PMSG, a different group was administered GnRH and PGF2α, while a final group of females was administered an FGA impregnated sponge and injection of GnRH simultaneously with an injection of PGF2α at sponge removal. Results reported after TAI were as follows 67%, 60% and 87% respectively for each of the groups in the study.

CHAPTER III

EFFECTS OF GnRH AND PROSTAGLANDIN COMBINED WITH A SHORT PROGESTIN REGIMEN AND ITS IMPACT ON SYNCHRONY OF ESTRUS AND OVULATION IN EWES EXHIBITING SEASONAL ESTRUS

Introduction

Estrus synchronization in timed artificial insemination (TAI) is very critical for the success or failure of the procedure that is utilized. This process of estrus synchronization uses the manipulation of either the luteal or follicular phase of the estrous cycle. In small ruminants, such as sheep, the luteal phase is somewhat more accessible to manipulation due to its length and responsiveness to exogenous hormones. One principal that is universal for all TAI protocols is the use of exogenous hormones to lengthen this phase to more tightly synchronize all females. No matter what technique is utilized to synchronize estrus for TAI, the outcome must be two-fold; one to establish a uniformly tight level of synchrony across females and second to allow for an acceptable level of pregnancy with TAI or natural mating. Timed artificial insemination is not widely utilized commercially in the sheep industry partly due to the differences in opinions as to what synchronization protocols are the most effective. Over the last 2 decades, a considerable amount of research has been conducted to identify a universally accepted method for synchrony. The majority of work that has been conducted has put more emphasis on what exogenous hormones should accompany a progestin regimen and not the length in which the progestin treatment should persist. Thus, the objectives

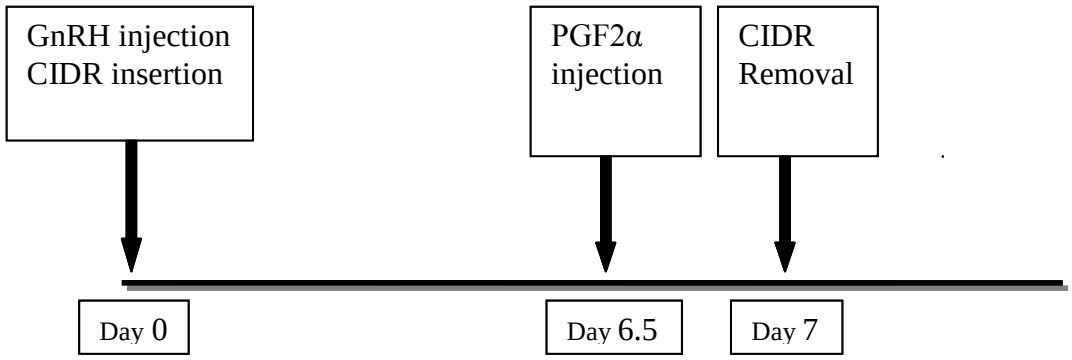
of this study were to evaluate the circulating LH, P4 and pregnancy rates for TAI in response to a novel, short duration progestin treatment coupled with exogenous GnRH and prostaglandin in comparison with a "traditional" synchronization protocol.

Materials and methods

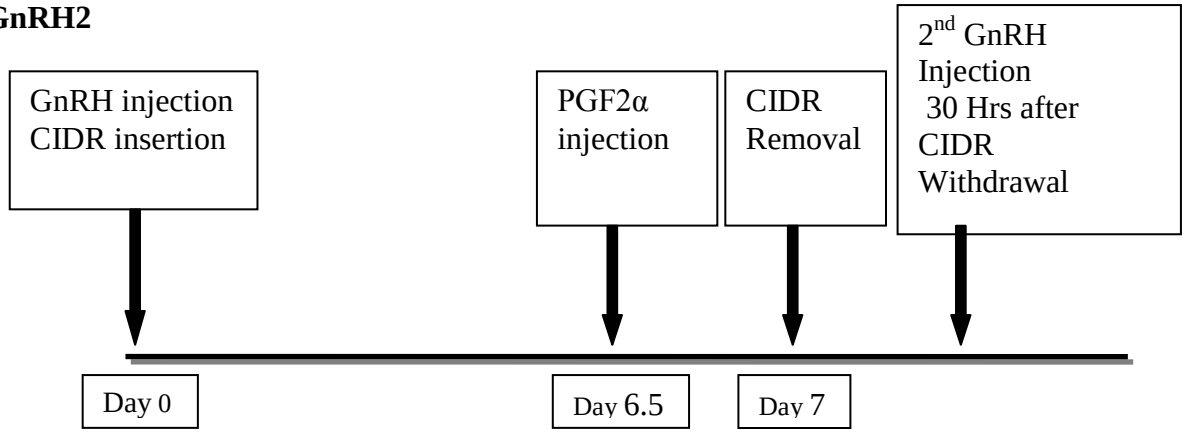
A study was conducted utilizing sheep from the research flock located at the San Angelo research and extension station. Ewes used in this study were maintained under the approval of the Texas A&M University Institutional Agricultural Animal Care and Use Committee using guidelines set forth by the Federation of Animal Science Societies (1999). Ninety multi-parous ewes ranging in age from 3 to 5 y with an average body condition score of 3-3.5 and in good health were utilized for the studies conducted. Ewes were fed a 12% crude protein, pelleted concentrate at a rate of 0.4kg/d/hd and had access to hay ad libitum.

Trial 1. Ewes (n=12) were randomly divided into 3 treatment groups. Group 1 (GnRH1; Figure 1) received the following treatment: on d 0 a progestin releasing device (CIDR-G® containing 0.3 g progesterone; Interag, Hamilton, New Zealand) was inserted intravaginally and a GnRH injection (Cystorelin® 50 µg/mL; Merial Limited, Athens, GA) was administered intramuscularly, on d 6 ½ ewes were given an injection (im) of prostaglandin (Lutalyse® 5 mg/ml, 4 ml; Pharmacia & Upjohn, Pfizer Inc.) and on d 7 the device was removed. Treatment group 2 (GnRH2; Figure 1) underwent the same protocol as group 1 with an additional injection of GnRH 30 h after device was removed. Group 3 (PMSG; Figure 1) was the control, and underwent the "industry standard" protocol. On d 0 a progestin releasing device (CIDR) was inserted and an

GnRH1



GnRH2



PMSG

Figure 1. Schematic diagrams of estrus synchronization protocols for GnRH1, GnRH2, and PMSG for trial 1 and trial 2.

injection of prostaglandin (lutalyse 5 mg/ml, 4 ml) was administered. On d 11, the CIDR device was removed and an injection (im) of PMSG (400 iu; Folligon, Intervet Limited, Whitby, Canada) was administered. Ewes were monitored to insure CIDR remained in place for duration of trial. Blood sampling to determine LH levels began at device removal every 2 h for 42 h for serum LH analysis to characterize the ovulatory LH surge. A second bleeding period beginning eight days after device removal with blood sampling occurring at 12, 24, and 36 h for a day and a half (3 samples) for P4 analysis to confirm CL function.

All blood samples were taken via jugular venipuncture. Samples were taken every 2 h, beginning 2 h after CIDR was removed, over a 42 h time period. Collections were accomplished during no more than a 15 min time frame at each collection to standardize samples. Approximately 5 mL of blood were collected and placed directly on ice. Once all were collected, samples were allowed to clot for approximately 30 min at room temperature and then centrifuged in a refrigerated centrifuge for 60 min at 3000 x g. Following centrifugation, serum was transferred to microcentrifuge tubes and stored at -20°C until time of assay.

LH hormone concentrations were evaluated by double antibody radioimmunoassay (RIA) described previously by Recabarren et al. (1996) over a 4-day period. On d 1, 500 µL of 1% phosphate buffered saline (PBS) with egg white (PBS-EW) were added to the non-specific binding (NSB) and the 0 standard tubes. Two-hundred microliters of standard and 300 µL of 1% PBS-EW were added to each standard tube. Three-hundred microliters of 1% PBS-EW along with 200 µL of each sample

were put into each unknown tube. The reference preparation tubes contained 300 µL of 1% PBS-EW and 200 µL of reference preparation. The primary antibody was anti-oLH, which was diluted with PBS-EDTA and normal rabbit serum (NRS) in a 1:400 ratio. Two hundred microliters of the antibody was then added into all tubes with the exception of the NSB and total count tubes. A tracer consisting of 100 µL of ^{125}I-oLH (20,000 CPM/100 µL diluted in 0.1% PBS-EW) was added to all tubes and then vortexed and allowed to incubate for 24 h at 4°C. On d 2, 200 µL of sheep-anti-rabbit gamma globulin diluted in PBS-EDTA without NRS was added to all tubes except the total count tubes. Tubes were once again incubated 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 for the total count tubes. The samples and reagents were then centrifuged at 3000 x G for 1 h while maintained at 4°C. Once centrifugation was complete the tubes were decanted and supernatant discarded. 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.

Serum P4 was analyzed using single-antibody RIA kits. (Coat-A-Count®, Diagnostic Products Corp., Los Angeles, CA). The kit contained all required reagents including antibody-coated polypropylene tubes, iodinated progesterone and standards. A sample volume of 100 µL was used for each assay with a sensitivity of the progesterone assay equaling 0.1 ng/ml.

The effects of treatment, time and time * treatment on serum LH concentration were analyzed. Serum concentrations for P4 were analyzed for comparison during the estrous cycle, for the effects of treatment, time and time * treatment. Data were analyzed by Proc GLM of SAS (SAS; Cary, NC, USA). All data was considered significantly different if $P \leq 0.05$.

Trial 2. Seventy-two ewes were randomly divided into 3 groups. Groups one, two and three (GnRH1, GnRH2 and PMSG respectively; Figure 1) represented the same treatment groups applied in experiment one. CIDR removal was staggered so that only 3 ewes were introduced to a ram at a time. Introduction of ewes to the ram was staggered in order to allow bucks time to mark each female and not have 12 ewes coming into heat at approximately the same time. The PMSG group, was the first to be introduced. The 24 ewes in this group were randomly allotted into one of the 8 pens. The next group to be introduced to the rams, were the GnRH1 treated ewes. These females were randomly allotted into the 8 pens.

This was done 12 h after the first group was introduced to allow rams to adjust. Twelve hours later, treatment GnRH2 ewes were randomly allotted into the 8 pens. The females were monitored every h for breeding marks. One h after the initial breeding mark was applied the ewes were separated from the rams for a period of 2 wk to allow for pregnancy determination via ultrasound and lambing data by date of lambing.

Eight rams were utilized for this study and chosen from a group of 14. Selection was determined by 2 factors, scrotal circumference and motility. Rams chosen all had a scrotal circumference of 34 cm or larger and exhibited 90% motility when semen was evaluated under a microscope.

Number of ewes marked by a ram, marktime, pregnancy, and lambing data were recorded. Treatment effects on interval from CIDR removal to onset of estrus (marktime) were analyzed by Proc GLM of SAS (SAS; Cary, NC, USA). Treatment effects on marking, pregnancy, lambing rate and twinning rate were analyzed by chi-square test of SAS (SAS, Cary, NC, USA). All data were considered significantly different if $P \leq 0.05$.

Results

Trial 1. All of the ewes in this trial, with the exception of one (not included in the analysis), retained CIDR device for the entire treatment period; (GnRH1 n = 3, GnRH2 n = 4, PMSG n = 4; Total n = 11). Mean serum concentrations of LH increased in the GnRH2 group after second GnRH injection was administered. This increase occurred earlier in the sampling time frame than in the other two groups (Figure 2). Thus, a difference in time of LH concentration increase between groups was reported (P<0.0001) also there was an interaction between time x treatment (P < 0.0001) as shown in Table 1. Analyses of serum LH concentrations following CIDR removal indicate that there was no difference between the mean concentrations among groups (P = 0.48).

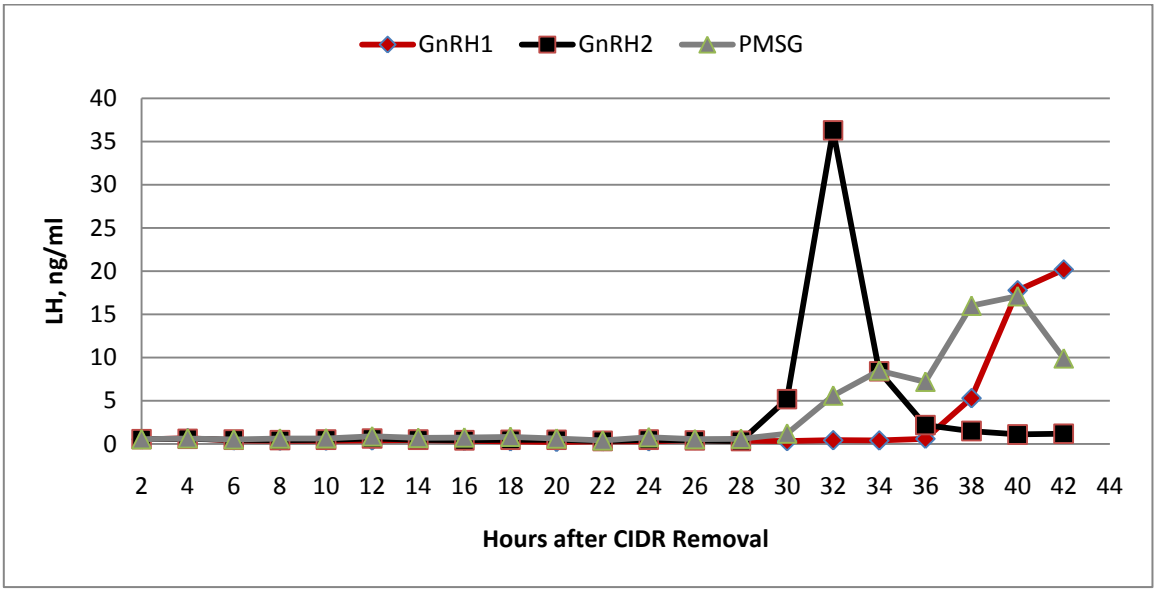


Figure 2. Mean serum concentrations of LH by treatment, from 2 h to 42 h after CIDR removal. Time x treament effect was observed from 32 to 42 h after CIDR removal (P < 0.05).

Table 1. ANOVA table for mean serum concentrations of LH for ewes in each of the three treament groups from CIDR removal to the end of the sampling period.

Source	DF	SS	Mean Square	F Value	Pr > F
Trt	2	47.8	23.9	0.73	0.4819
Time	20	4061.5	203.1	6.24	<.0001
Time*trt	40	4790.6	119.8	3.68	<.0001
Error	167	5438.8	32.6		
Total	229	14543.2			

The pattern and concentrations of serum P4 indicate the treatments did not alter post ovulatory CL function (Figure 3). Mean serum concentrations were not different between treatment groups, and there was no interaction between groups ($P > 0.05$) as shown in Table 2.

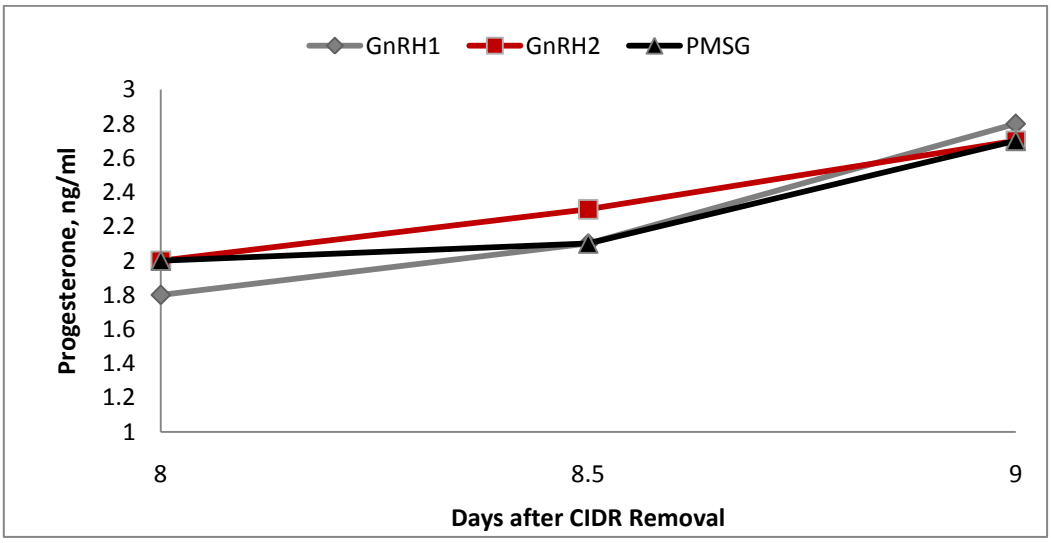


Figure 3. Mean serum concentrations of P4, beginning 8 d after CIDR removal every 12 h (3 samples).

Table 2. ANOVA table for mean serum concentrations of P4 for ewes in each of the three treatment groups.

Source	DF	SS	Mean Square	F Value	Pr > F
Trt	2	0.1	0.04	0.03	0.9719
Time	2	3.8	1.9	1.4	0.2646
Time*trt	4	0.2	0.04	0.03	0.998
Error	27	36.6	1.4		
Total	35	40.6			

Trial 2. Ewes were closely monitored for breeding marks once exposed to fertile rams. Mean interval to estrus was shorter ($P < 0.05$) for ewes in the GnRH2 group when compared to ewes in the PMSG group (Table 3). Initiation of estrus was influenced by treatment ($P < 0.10$) among all groups (Table 3).

Table 3. Mean (±SE) interval from CIDR removal to onset of estrus, as well as range of mark times between females in each treatment group.

Group	n	Marktime	Range
GnRH1	24	41.5±1.76[a,b]	36-56h
GnRH2	24	36.8±1.95[b]	34-40h
PMSG	24	42.4±1.81[a]	25-68h

[a,b]Means with unlike superscripts differ $P < 0.05$.
[b]Means with like superscripts tend to differ $P < 0.10$.

Ewes were monitored for estrus over a 72 h time frame following CIDR removal. Estrus activity within each of the 3 groups was not significant ($P > 0.05$). Marking

frequency observed for the 3 treatments were 92%, 75%, and 88% for GnRH1, GnRH2, and PMSG respectively (Figure 4). Ewes were evaluated to determine pregnancy 60 d following placement with rams via transabdominal ultrasonography. At this time it was determined if pregnancy was established following the experimental induced estrus. Reported number of females becoming pregnant to induced estrus was significantly different between treatment groups ($P < 0.05$). Percentages among treatment were 79%, 58% and 38% for GnRH1, GnRH2, and PMSG groups respectively (Figure 4). To verify and strengthen ultrasound data, lambing data was recorded at time of parturition. The number of ewes lambing on appropriate dates confirming ultrasound and mark data was also significant ($P < 0.05$); percentages among treatments were 75%, 58%, and 38% for GnRH1, GnRH2, and PMSG groups respectively (Figure 4). The numerical discrepancy between ewes confirmed pregnant and ewes that lambed were different because one ewe, within the GnRH1 group, was confirmed pregnant and never lambed.

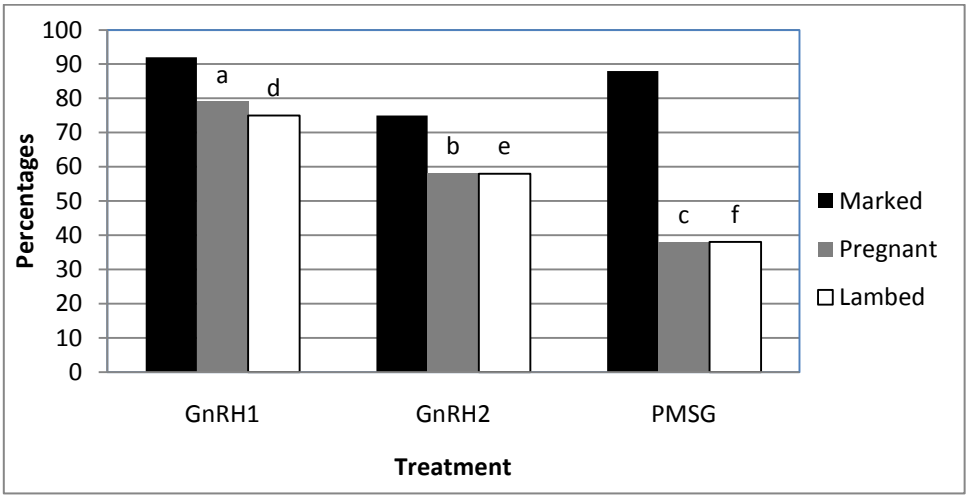


Figure 4. Effect of treatment on percentages of ewes marked, pregnant and lambed.
Means within columns with no superscripts do not differ $P > 0.05$.
[abc]Means within columns differ $P < 0.05$
[def]Means within columns differ $P < 0.05$

Effect of treatment on instance of twinning among groups was not significantly different ($P > 0.05$). Percentages of females having twins for each of the 3 treatments were 13%, 21% and 4% for GnRH1, GnrH2 and PMSG groups respectively (Figure 5).

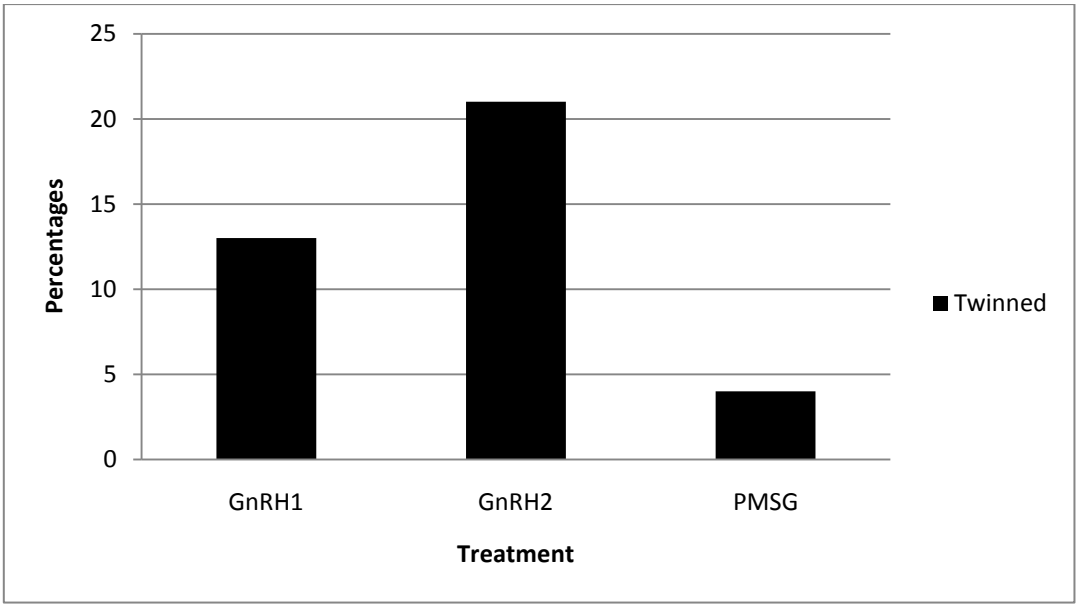


Figure 5. Effect of treatment on instance of twinning.
Means within columns do not differ $P > 0.05$

Discussion

The breeding protocols utilized in these trials were designed to facilitate the fixed time or TAI of ewes and were compared to a protocol that is considered an industry standard. Methods were designed to evaluate not only if the tested protocols would allow for a high percentage of ewes to breed to induced estrus, but also to allow for high percentage of lambing. Blood samples for trial 1 were needed to strengthen mark data and not only determine how treatments would tighten synchrony, but to also determine when to artificially inseminate females when protocols were applied in a TAI management program.

Serum LH concentrations were evaluated in this study in order to hormonally characterize the pre-ovulatory surge of LH and determine if treatments effectively allow for ovulation. Additionally, LH concentrations indicate how synchronous the evaluated treatments were among females in each group. In the present study, a mean concentration difference between treatments was not seen; however, an effect of timing of the LH surge was seen between groups ($P < 0.05$).

A time specific LH surge was observed in all 4 ewes in the GnRH2 group. This increase in LH concentrations, was 5 fold above baseline (0.8 ng/mL). This was observed in all ewes in the GnRH2 group 30 h after CIDR removal and approximately 15 min after the second injection of GnRH was administered. Similar results were also reported by Rippel et al. (1974). In which females given a 25 µg dose of GnRH intramuscularly showed a marked increase in LH concentrations 15 to 20 min after

injection. Ewes in the present study sustained an increase in LH over a period of 6 to 8 h, after which concentrations dropped back to baseline (0.08 ng/mL).

Studies conducted in cattle with GnRH induced LH response post CIDR removal have shown similar responses. Mee et al. (1993) found that serum LH levels were markedly higher 2 h after injection of GnRH and stayed elevated over a 6 h period. The GnRH1 group allowed for a more natural LH surge with the deletion of the second injection of GnRH. Moreover, the GnRH1 group displayed synchrony in its LH surge although it began 8 h later than the GnRH2 group. Two of the 3 ewes in this group began an LH surge at 38 h after CIDR removal and continued until sampling was discontinued although levels were still very much above baseline. The third ewe was just entering the LH surge when sampling ended marked by an 8 fold increase of LH concentration levels on the last sample. It can be assumed that these ewes LH levels would have stayed increased above baseline for at least one more sampling if not more, although an assumption made by investigators due to the ending of the sampling period was found to be too soon to characterize LH surge for all ewes in the trial.

The control or PMSG group was similar in that we only see a LH surge for 3 of the 4 ewes in the group, however their LH surge patterns were more varied and began at different time points in the sampling period. The first began 32 h after CIDR removal, a second ewes surge began at 36 h after CIDR removal, and the last ewes surge was just beginning as sampling period ended. This illustrates a similar outcome found by Titi et al. (2010) in that ewes treated with a long term progestin device coupled with an injection of PMSG at CIDR removal effectively induces ovulation and an estrus

response, but LH surge tends to be very different among ewes. Data allow us to see the difference in utilization of a short CIDR or a longer duration of progestin treatment coupled with a different regimen of exogenous hormones. It also infers that use of a short CIDR coupled with GnRH and prostaglandin allows for a tighter range of LH surge synchrony which is consistent with other similar studies (Jabbour and Evans, 1991; Titi et al., 2010).

Serum P4 concentrations were evaluated to determine if treaments affected CL function and P4 secretion. No difference in serum concentrations of P4 between any of the groups in the study was indicated. All concentrations were consistent with most previously reported data. Thorburn et al. (1969) found that a normal ewe will reach a maximum plasma progesterone level of approximately 2-3 ng/ml between d 8 and 12 of the estrous cycle. Although there has been some conflicting data reported by Herriman et al. (1979) which stated that investigators observed much higher P4 concentrations of up to 6ng/ml between d 8 and 11 of the estrous cycle, this difference could possibly be attributed to breed differences. The P4 data contained within this study also allows us to infer that all but 1 of the females in trial 1 ovulated following synchronization protocol due to levels of circulating progesterone even though sampling period for LH was apparently not long enough to characterize all ewes LH surge.

Estrus response and time to estrus response was recorded to determine the treatment affect on breeding activity. A previous study conducted by Husein and Kridil (2003) and Husein et al. (2005), demonstrated an advance of estrus response when GnRH was utilized before prostaglandin injection when primed with a short CIDR as

compared to a short CIDR use with PMSG. Similar results were recorded in the present study.The GnRH2 group had a significant difference ($P < 0.05$) in time to estrus compared to the traditional PMSG group (36.8±1.95; 42.4±1.81) respectively. There also tended to be a difference ($P < 0.10$) between all treatment groups (41.5±1.76) GnRH1 respectively. The mean interval to estrus for the PMSG group was similar to that reported by Ustuner et al. (2007). In this study, mean interval to estrus for a group treated with an 11 d CIDR and PMSG following removal of CIDR was 38.18±4.7.

Range of marktime between treatment groups was much different. The GnRH2 group recorded the shortest range of marktime between ewes (34-40 h after CIDR removal). The range of the GnRH1 group (36-56 h after CIDR removal) was longer but, it began approximately the same time as the GnRH2 group. The PMSG groups range (24-68 h after CIDR removal) was again varied and much longer than either of the 2 GnRH groups, but it was similar to previous studies. Ungerfield and Rubianes (1999) and Simonetti et al. (2000) both reported a range of estrus response among ewes with a similar CIDR-PMSG treatment being from 24-84 h after CIDR removal. Ustuner et al. (2007) reported a range from 12-78 h in females treated with the same 11 d CIDR and PMSG at removal. This information combined with the serum LH concentration data allows the conclusion to be drawn that both of the GnRH groups more effectively synchronize estrus when utilized at the beginning of CIDR treatment and in combination with prostaglandin.

Mark percentage data showed no significant difference between groups (92%, 75% and 88% for GnRH1, GnRH2 and PMSG groups, respectively). The GnRH1

groups percentages differ somewhat from percentages of ewes showing estrus response in a study conducted with a norgestomet implant for 14 d and GnRH injection occurring 36 h after implant removal where 76% of ewes exhibited an estrus response (Luther et al., 2007), although this percentage correlates with percentages seen in the GnRH2 groups from the present study. Luther et al. (2007) also reported that ewes being administered a CIDR for a 12 d period with an injection of PMSG coming at CIDR removal showed a 90% estrus response to treatment which is very similar to the 88% reported in this study. This data shows that the treatment utilized on the GnRH1 group in this study allows for a higher percentage of females showing estrus, although not significantly different.

Ultrasound examination allowed for pregnancy determination at d 60 after mating. The GnRH1 group had a much higher percentage of ewes becoming pregnant to induced estrus, 79%, compared to 58% for the GnRH2 group, and 38% for the PMSG group. These percentages were significantly different allowing for the assumption that the GnRH1 protocol is the most effective when utilized in a natural breeding situation. The percentage of ewes becoming pregnant to treatments differs to those seen in similar studies. Titi et al. (2010) utilized a similar protocol to the GnRH1 treatment, utilizing a 5 d CIDR coupled with an injection of GnRH at CIDR insertion and an injection of prostaglandin at CIDR removal. A 47% pregnancy rate to induced estrus was reported. This difference could be attributed to the difference in CIDR priming. The same study showed a very low percentage of ewes becoming pregnant to the PMSG group of 0% compared to 38% in the present study. Luther et al. (2007) reported a pregnancy rate of

80% when a 12 d CIDR and PMSG was utilized to synchronize ewes when laparoscopic artificial insemination (LAI) was incorporated instead of natural breeding. A much larger percentage was reported becoming pregnant when natural bred after utilization of a 12 d CIDR with no associated treatment in the breeding season of 72% (Godfrey et al., 1997). Ustuner et al. (2007) reported similar pregnancy percentages (32%) when LAI was utilized to ewes subjected to the same protocol as the PMSG group.

These differences could be due to breed differences and type of PMSG utilized within studies being of different origin. Similar pregnancy percentages have also been reported in ewes subjected to a similar protocol as the GnRH2 group in the present study. Luther et al. (2007) observed a 51.5% pregnancy rate with ewes subjected to a norgestomet implant for 14 d with injection of GnRH 36 h after removal of implant. The discrepancy between pregnancy and lambing percentages for the GnRH1 group is due to that one ewe that was ultrasounded pregnant never lambed any time during the lambing season.

Implications

These results indicate that GnRH and prostaglandin coupled with a short 7 day CIDR regimen can effectively synchronize estrus and ovulation and allow for acceptable pregnancy rates. The GnRH2 group synchronized the estrus response and the LH surge more effectively than the PMSG group, and it also allowed for higher pregnancy rates when ewes were introduced to fertile rams. The GnRH1 group was not as effective at synchronizing estrus response or LH surge as the GnRH2 treatment. Although treatment groups were not significantly different, the GnRH1 protocol did allow for a higher pregnancy and lambing rates than either the GnrH2 or PMSG treatments. Although the PMSG treatment does synchronize estrus, the range of ewes entering estrus is much larger than for either of the other 2 treatment groups. It also did not allow for a pregnancy rate which has been seen by other investigators who have tested a similar treatment. Additional research is warranted to determine if the 2 GnRH treatments would be as effective in allowing similar pregnancy rates when utilized in a TAI situation.

CHAPTER IV

SUMMARY

In this study, the analyses of reproductive parameters between groups treated with a GnRH injection at CIDR insertion, prostaglandin injection 12 h before CIDR removal, and CIDR removal on day 7 with or without an injection of GnRH at 30 h after CIDR removal, allows for a much tighter instance of synchrony as indicated by LH analysis and the range of marks between groups. When compared to the "industry standard" of a long term CIDR coupled with an injection of PMSG following CIDR removal, this should allow GnRH treated females to have a higher opportunity to become pregnant when TAI is incorporated. Although TAI was not performed in the present study, times at which to inseminate ewes can be inferred utilizing the data that was collected.

Ewes receiving the GnRH1 treatment would be possibly best to AI at 52-56 h after CIDR removal. Luteinizing hormone analysis of this group compared to the PMSG group showed a similarity to when females undergo a surge of LH and ovulation which would allow you to infer that breeding these females at the same time as one would to the PMSG group. The GnRH2 group allows for the most freedom in which to incorporate TAI. Ewes could be inseminated at time of GnRH injection, although this could possibly be too soon. Females could also be inseminated 12 h or 24 h after the second injection of GnRH seen to be successful in cattle (Bo et al., 2006). As shown in the present study, GnRH and prostaglandin coupled with a short 7 day CIDR regimen

can effectively synchronize estrus and ovulation and allow for acceptable pregnancy rates. There were, however, problems with a lower conception rate within the PMSG group versus what has been reported in other studies using LAI or natural breeding in oestrus ewes. The shorter CIDR length as shown in the two GnRH treatments could be a more practical tool in TAI because it allows for a tighter instance of synchrony and a smaller range of time to estrus from CIDR removal. Further research is needed to prove these treatment groups effectiveness in a TAI management situation in order to allow for the highest instance of pregnancy possible.

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