ABSTRACT

LLANES, ANNETTE. Improving Reproductive Management Strategies in Goats. (Under the direction of Dr. Char Farin.)

This thesis describes three experiments evaluating ovulation synchronization and pregnancy detection methods in goats. The objectives of the first two experiments were to examine the efficacy of progesterone-based and progesterone-free protocols for synchronizing ovulation in goats for timed-artificial insemination (TAI). The third experiment examined the relationship between pregnancy specific protein B (PSPB) and multiple births in goats.

In Experiment 1, 87 does were randomized into five treatments: (1) breed by estrus [BBE], (2) NC.Synch TAI [NCS], (3) 6-day CIDR with either a new [C6N], (4) once-used [C61], (5) or twice-used CIDR [C62]. The BBE group received two 15mg doses of prostaglandin-F2α (PGF) at a 10 days interval and bred 12 hours after the onset of estrus. The NCS group received 15mg PGF on Day 0, 50µg GnRH on Day 8, 15mg PGF on Day 15 and 50µg GnRH on Day 18, concurrently with TAI. The CIDR groups received a CIDR for a 6 day period, 15mg PGF at CIDR removal, and were given 50µg GnRH 48 hours later at TAI. All does were inseminated with a single dose of frozen semen using a non-surgical, transcervical technique. Pregnancy status was determined by ultrasonography. Daily blood samples were taken to monitor serum concentrations of progesterone until insemination. Progesterone concentrations were (Mean $\pm$ SEM) 5.3 ± 0.8^{ab}, 3.5 ± 0.8^{a}, 7.0 ± 0.8^{bc}, 7.9 ± 0.8^{c}, and 6.2 ± 0.8^{bc} ng/ml for BBE, NCS, C6N, C61 and C62, respectively ($^{abc}P<0.05$). Pregnancy rates for the BBE, NCS, C6N, C61 and C62 treatment groups were $39 \pm 11\%^{ab}$, $22 \pm 11\%^{a}$, $64 \pm 12\%^{bc}$, $77 \pm 12\%^{c}$ and $57 \pm 12\%^{bc}$, respectively ($^{abc}P<0.05$). In conclusion, reused CIDRs were as effective as new CIDRs for synchronization of ovulation in goats.

In Experiment 2, 68 does were randomized into four treatments: (1) BBE, (2) C6N, (3) NCS, (4) modified NCS [NCSM]. The NCS, BBE and C6N groups were treated as described for Experiment 1. The NCSM group received 15 mg PGF on Day 1, 50 μg GnRH on Day 8 and 15 mg PGF on Day 15.5. Does were bred via AI 60 hours after the second PGF injection and received 50 μg GnRH at timed AI. Daily blood samples were taken to monitor progesterone until insemination. After the second PGF injection in the NCS group, blood samples were drawn every 6 hours and continued until 24 hours after insemination to monitor serum concentrations of leutinizing hormone (LH). Progesterone concentrations were 1.4 ± 0.1^{a}, 3.2 ± 0.1^{b}, 1.1 ± 0.1^{a} and 1.1 ± 0.1^{a} ng/mL, for the BBE, C6N, NCS and NCSM groups, respectively ($^{ab}P<0.05$). Pregnancy rates were 53 ± 12%, 30 ± 11%, 50 ± 11% and 41 ± 12% for does in the BBE, C6N, NCS and NCSM group, respectively. In conclusion, there was no difference in mean progesterone or pregnancy rates following AI in the NCS and NCSM groups. Therefore, modification of the NCS protocol does not appear to be warranted.

In Experiment 3, 62 does were bled at Days 48 and 85 of gestation. Serum samples were assayed for PSPB. Does were grouped by number of kids born or by quartiles of total kid weight at birth. There was a tendency for an increase in PSPB between Day 48 and Day 85 of gestation (P=0.068). Pregnancy specific protein B concentrations for does with a total kid weight in the first quartile were lower compared to does in the second, third and fourth quartiles (24.5 ± 0.1^{a}, 28.4 ± 0.1^{b}, 29.0 ± 0.1^{b} and 30.7 ± 0.1^{b} ng/mL, respectively, $^{ab}P<0.05$). Pregnancy specific protein B concentration for does that had singletons was lower than for does that had twins or triplets (25.0 ± 0.1^{a}, 28.8 ± 0.1^{b} and 30.7 ± 0.1^{b} ng/mL, respectively, $^{ab}P<0.05$). In conclusion, PSPB concentration at Day 48 or Day 85 of gestation can potentially be used as a diagnostic tool to distinguish does carrying multiples.

Improving Reproductive Management Strategies in Goats

by
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BIOGRAPHY

Annette Llanes was born in Miami, Florida on March 29, 1993 and lived there until she moved to Morrisville, NC in 2007. She attended Panther Creek High School in Cary and began her academic career at North Carolina State University in 2011. Annette received her B.S in Animal Science from NCSU in 2014 and continued on to pursue an M.S. in Animal Science. Upon completion of her Masters, she will begin veterinary school at Colorado State University in Fall 2016.

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CHAPTER 1: Review of Literature

Historical Perspective

The goat was the first animal domesticated for production purposes [1]. It has followed the development of human civilizations and is regarded as a sacred religious symbol in some cultures [2]. There was a huge development in the goat industry in the 18th century when selective breeding gave rise to distinctive populations. In the 19th century the specialization of other agricultural areas gave rise to cheaper milk and meat from cows and the early 20th century actually saw a decrease in goat numbers [1]. Towards the end of the 20th century, though, there was renewed interest in goats. There was a growing sensitivity toward the environment and the goat was viewed as a more ecologically friendly livestock alternative to the cow [1] .

The past 20 years has seen an increase in goat populations around the world, specifically in developing countries that are experiencing human population booms [3]. Goat production is usually associated with poverty and; therefore, has developed a negative stigma in many developed countries [4]. This negative image has impeded the advancement of the goat industry when compared to the cattle industry. However, recently, producers have become more interested in exploiting goats for attributes such as adaptation to adverse weather and early slaughter age [1].

Global Perspective of the Goat Industry

In 2011, India produced 30% of the world's goat milk with 4.59 million tons of milk produced (FOASTAT). Developing countries primarily raise goats for meat, likely due to the

fact that goats require less capital input and space than cows [4]. Developing countries also usually lack refrigeration which can pose a problem when storing the larger quantities of milk and meat that comes from cows. There has been a rapid increase in goat numbers in developing countries to meet the demands of the rapidly rising human population [1]. It is difficult to track how much these developing countries are really producing though because most of these goat products are sold locally with only about 5% of the goods being traded [5]. Most goat producers in developing countries use the meat and milk to feed their families and local neighbors rather than marketing it to the public [4].

Even in developed countries there is little established infrastructure to market goat products. This can lead to goat-based products being more expensive than their bovine equivalents. France is the developed country with the most successful dairy goat industry, with 90% of the goat milk produced being sold as cheese [5]. There have been several attempts by other developed countries to model a dairy goat industry similar to that of France. The main objective would be to utilize the growing urban populations and demand for high value cheeses as the primary market for goat milk [4].

Other attempts to increase goat production in the developed world have been aimed at capitalizing on the good ecological image of the goat. Several developed countries are increasingly utilizing goats to positively modify vegetative cover. They are very effective at clearing out undergrowth, thick bushes and thorny vegetation to make better grazing for other livestock and also decrease the risk of forest fires [1]. Furthermore, as water shortages are projected to increase in the future, the goat may come to be regarded as a good alternative to cattle.

Benefits of Raising Goats

Along with ecological benefits, the goat also brings an array of health benefits. An 85g serving of roast goat meat provides the same amount of protein as an equivalent serving of beef but with fewer calories and grams of fat [6]. Along with making high value cheeses, goat milk can also be used as an alternative for those with allergies to cow's milk. Producers in developing countries have realized that goats are great at meeting the demands of a rising human population. However, goats can also contribute significantly to the meat, milk and fiber needs of expanding urban populations in developed countries as well.

Producers in developed countries should abandon the cliché of "goats as the poor man's cow." Prior to the 1980's the goat was regarded to be "of third world importance" only and this negative stigma has hindered its growth in the agricultural industry of developed countries [1]. Most research and improvement efforts have been focused on cattle for the past few decades. In 2013, the USDA allocated over $200 billion in funds for research involving beef cattle and $138 billion for research involving dairy cattle, while only $15 billion was given to fund research involving goats (USDA CRIS 2013). The small scale, local goat market could be beneficial in a world where economic risks are higher on the global market scale [5]. Taking into account the economic, ecological, and health benefits that goat production brings, one would expect to see continued growth in goat populations accompanied by emerging research focused on answering the needs of goat producers.

Reproduction in the Doe

Seasonality

Goats are classified as seasonally polyestrous, short-day breeders and begin cycling in the fall and winter months [7]. Fluctuations in daylight and temperature are the key regulators for the breeding season in goats [8]. As the retina of the eye is stimulated by light, neurons fire and stimulate the pineal gland of the brain. These neurons excite the cells of the pineal gland and suppress the secretion of melatonin. On the contrary, during darkness the pineal gland synthesizes and secretes melatonin which stimulates gonadotropin releasing hormone (GnRH) secretion and, therefore, supports enhanced ovarian function [7]. As day length gets shorter and the duration of melatonin secretion increases, goats reach the peak of their cyclicity. Decreasing melatonin secretion has been shown to increase the negative feedback between estrogen and gonadotropins thus decreasing ovarian function [9]. The peak of sexual activity is seen in the autumn months and the period of lowest sexual activity is seen in late spring to mid-summer [10]. Treatments involving artificial light and melatonin supplements have been used to induce out-of-season ovarian activity in goats [11, 12]. Studies have shown that by using artificial light to mimic long days (20 hours light:4 hours dark) for a minimum of 2 months in the spring, immediately followed by a month of melatonin treatment, pregnancy rates comparable to those seen during the breeding season can be achieved [11, 12].

Does bred in the fall result in kidding during the spring when pasture conditions are most favorable [13]. Nogueira et al.[14] compared follicular dynamics during the breeding and non-breeding months and found that both the growth rate and diameter of the largest

follicle were greater during the breeding months. In addition, they also found there were more co-dominant follicles during the breeding season [14]. Interestingly, a greater number of small follicles as well as total follicles were observed in the non-breeding months. It was hypothesized that this may be due to the lack of co-dominant follicles which suppress the growth of smaller follicles [14].

Follicular Waves

Goats exhibit a wave-like pattern of follicular development with an interovulatory interval of about 21 days, similar to that seen in other ruminant species [15, 16]. A follicular wave is classified as a group of small (3mm) antral follicles in which one or two are selected to grow to 6mm or larger [16]. Although the number of follicular waves can vary, the most common pattern observed among goats is four waves per estrus cycle with the fourth wave being the ovulatory wave [16]. With four waves per cycle the emergence of a dominant follicle commonly occurs on days 0, 5-6, 10-11 and 15 post ovulation for waves 1, 2, 3 and 4, respectively [17]. The number of waves per cycle does not affect the duration of the luteal phase or the interovulatory interval [18].

In recent years, the use of ultrasonagraphy has allowed several common characteristics of follicular waves to be described in goats [17]. First, the diameter of the largest follicle differs between waves, with the largest follicles emerging on the first and on the ovulatory wave [15, 16, 18]. Second, there is an average growth rate of 1mm per day from the day of emergence (detectable size of 3mm) to the day of maximum diameter [16]. Third, as the luteal phase progresses there is an increase in follicular turnover and inter-wave intervals are shorter [15, 16]. It is even suggested that dominance is not operative during the mid-luteal phase [15]. This is due to the higher levels of progesterone which attenuate the

growth of large follicles in the second and third wave and therefore promotes wave turnover [15]. Lastly, it has been observed that most ovulatory follicles are largest on the day of luteolysis [15, 16].

Endocrinology of Follicular Development and Ovulation

As stated earlier, increasing levels of melatonin stimulate GnRH secretion which is responsible for stimulating the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) [7]. Follicle stimulating hormone and LH provide the primary mechanism for follicular recruitment, selection and dominance [9]. A peak in FSH is correlated with the emergence of a new wave [19]. The number of FSH peaks are correlated to the number of waves per cycle suggesting that FSH is involved in follicular recruitment [19]. As the follicle continues to grow, the granulosa cells will begin acquiring LH receptors for further development and selection [9]. These new receptors will allow the selected follicles to grow in the presence of LH as FSH levels begin to decrease. Follicle stimulating hormone levels decrease in response to inhibin being released from the largest, dominant follicle. In this way the dominant follicle suppresses the growth of smaller follicles that have not acquired LH receptors. In general, follicular dominance is expressed weakly in goats compared to cattle. Therefore, in goats, a new wave can begin to emerge while the current largest follicle is still growing [16].This results in goats having an average of 3-4 follicular waves per cycle whereas cattle only have 1-2 waves.

The follicular phase, or the period during which the dominant follicles are growing after the corpora lutea (CL) have regressed, only compromises about 20% of the estrus cycle and it can be greatly affected by what happens in the luteal phase [7]. The average interovulatory interval for goats is about 21 days [15]. Upon luteal regression and a drop in

progesterone, estrogen levels sharply increase around day 16 after ovulation. The follicular phase of goats, approximately day 16-21, is characterized by a steady increase of estrogen secreted from growing follicles that peaks around 2 days before ovulation [15]. The smaller follicles dependent on FSH are 2-3mm in diameter; whereas, at 3-5mm in diameter, follicles will begin to acquire LH receptors [9]. The ovulatory follicle will reach a diameter of 7mm before ovulation [15].

The luteal phase is characterized as the period during which there is a functional CL secreting progesterone [7]. Progesterone has a negative feedback relationship with LH, a hormone necessary for follicular growth. The higher levels of progesterone present during the second and third follicular waves prevents follicles from reaching maximal diameter and accelerates follicular wave turnover [15]. The increasing levels of progesterone parallel the increasing size of the CL with the CL reaching its maximum diameter around day 8 after ovulation [19]. In the late luteal phase, progesterone levels drop quickly, and the drop is followed by regression of the luteal tissue mass; thus, the CL loses its functionality before it completely regresses [19]. This happens in response to the release of the luteolytic hormone prostaglandin-F2α (PGF) from the uterus [20].

Slight fluctuations in estrogen levels can be seen during the luteal phase and are associated with the growth of large follicles during each wave [19]. Once the CL has regressed, there is no longer any progesterone present, reducing the negative feedback effect on LH. The dominant follicle will continue to produce more estrogen which, in the absence of progesterone, has a positive feedback effect on LH and will eventually lead to the pre-ovulatory LH surge [21]. This LH surge causes a chain of events that will lead to the rupture of the dominant follicle and ovulation of a secondary oocyte [7].

Synchronization in the Doe

Estrus Synchronization

Estrus synchronization is a useful tool for producers because it allows them to take advantage of niche markets, forage availability, labor and rising price trends [22]. It allows greater distribution of meat and milk products throughout the year to facilitate accommodating the demands of both industry and consumers [13]. Being able to induce estrus has been an enormous advantage considering how difficult it is to detect estrus in goats due to their very social behavior. In addition, induction of estrus has led to increased use of artificial insemination [23]. Throughout the years different methods have been used to synchronize estrus in goats. These synchronization protocols attempt to either extend the luteal phase using exogenous progesterone or shorten the luteal phase using prostaglandin [24].

Progesterone Injections and Sponges

Early synchronization methods in small ruminants utilized injections of progesterone to manipulate circulating levels of progesterone. Typical protocols consisted of 14 daily, subcutaneous injections of 10 mg of progesterone in 2 mL of corn oil. Animals were bred within 1-8 days following progesterone injection [25]. The low fertility rates found associated with this method were attributed to persisting effects on the uterine tubule environment after progesterone injections had ceased [26]. Robinson et al. [26] suggested that these persisting effects needed to be addressed by utilizing a method that allowed for abrupt removal of progesterone once treatment ends. Since progesterone administered orally was shorter acting than when given subcutaneously or intramuscularly, some investigators attempted oral progesterone synchronization protocols [27]. Although better fertility rates in

sheep were obtained with oral administration, dosages as high as 1mg per kg of bodyweight were required to be effective [27].

In the early 1960s, intravaginal sponges were introduced as another method to synchronize estrus cycles in both sheep and goats [28]. The most commonly used was fluorogestone acetate-impregnated (FGA) sponges [23]. The use of a sponge was also usually accompanied in combination with an injection of pregnant mare serum gonadotropin (PMSG), which has FSH-like effects and acts to increase follicular development, improving the chances of ovulation [29]. Sponges were kept in place for 16-18 days with PMSG being administered either at sponge removal or 48 hours before spong removal. Breeding occurred at 48 or 55 hours after sponge removal, depending on the protocol [28]. Although ovulation rates were better when PMSG was given 48 hours before sponge removal, ovulation rates were still only at about 50% at the time of breeding [28]. Within 85 hours of sponge removal 94% of the does, regardless of time of PMSG administration, had ovulated. This made the timing of ovulation span a 4 day window, which is not ideal for a fixed timed artificial insemination protocol.

Along with variable ovulation time, the use of sponges was also shown to cause and even induce clinical vaginitis in goats [30]. Most of the bacteria found in cultures from vaginal swabs of progesterone sponge-treated animals were part of the staphylococcus genus and were found to be resistant to several antimicrobials [30].

Introduction of Controlled Internal Drug Release: Long and Short Term Treatment

In the 1980s, a device was designed in New Zealand known as a Controlled Internal Drug Release (CIDR) dispenser [31]. A small ruminant CIDR is a Y-shaped device made of silicone and contains 0.3g of progesterone. The impregnated progesterone is gradually

released once it is inserted into the animal's vagina [23]. Although both sponges and CIDRs are effective in synchronizing estrus, CIDRs do not impede or absorb vaginal secretions and have a higher retention rate when compared to sponges [32]. In addition, a CIDR-induced synchronization has a higher estrus response rate, a shorter time to estrus and a longer duration of estrus following CIDR removal when compared to sponges [32].

Early protocols involved having a CIDR in place for 18-21 days with most does exhibiting estrus 48 hours after CIDR removal [31].These long treatment periods were meant to mimic the 11-19 day lifespan of a CL [33]. However, it has been found that this long lasting priming can result in decreased fertility [34]. Menchacha et al. [34] claimed that with a new follicular wave emerging every 5-7 days, long term priming cannot really be justified.

Vinoles et al. [35] saw a higher pregnancy rate in ewes using a 6-day sponge treatment compared to a 12-day sponge treatment regardless of whether PMSG was administered at time of device withdrawal. In a follow up experiment, ultrasonography was used to demonstrate that the 12-day treatment of progesterone induced the ovulation of aged follicles [35]. Ovulation of aged follicles has been associated with low fertility in cattle [36] due to an early resumption of meiosis in the oocyte [37]. Menchacha and Rubianes [34] reported that if the largest follicle had emerged 4-5 days before CIDR insertion, it regressed and a new wave would emerge soon after the treatment began (unpublished). This resulted in a large, young follicle present 5 days later at the time of device withdrawal. Alternatively, if a new follicular wave was emerging just as treatment began, then the largest follicle of that wave would continue to grow and become the largest, dominant follicle present at time of device withdrawal 5 days later. In both scenarios, the 5-day treatment period ended with the presence of young, large, growing follicles [34].

Vonioles et al. [35] observed that with a 6-day or a 12-day progesterone based protocol, the timing of estrus was still variable. This was probably because luteal regression was delayed in some ewes because PGF was not used [34, 35]. This approach of combining PGF with a progesterone insert has not been studied extensively in sheep but has been shown to be successful in goats [34]. Administering PGF at the time of insertion or removal of a progesterone device has been used to ensure luteal regression [38].

With the transition to short-term instead of long-term protocols, a significant amount of progesterone still remains within the CIDR at the completion of treatment because CIDRs were originally designed to maintain elevated levels of progesterone for up to 21 days. Therefore, scientists have tested the efficacy of reused CIDRs in an attempt to drive down the costs for producers. Using a 9-day CIDR protocol comparing a new, once-used and twice-used CIDR, Nogueira et al. [32] found no significant decrease in pregnancy or increase in vaginitis rates. Only one doe came into heat before device removal and that was in the twice-used group. In cattle, reused CIDRs have been shown to be effective for synchronizing estrus and ovulation [39, 40]. Similarly, Vilarino et al. [41] reported that synchronization with reused CIDRs in goats resulted in similar follicular dynamics compared to synchronization with the new CIDRs. Pregnancy rates for goats that received a new, once-used and twice-used CIDR were 75%, 67% and 62%, respectively. The pregnancy rates reported in this experiement were acceptable for all treatment groups but those of the twice-used CIDR treatment group were significantly lower than those in the new CIDR treatment group. In sheep, a short-term (6-day) CIDR protocol involving new, once-used or twice-used CIDRs demonstrated that reused CIDRs were as effective at synchronizing estrus and obtaining good pregnancy rates as new CIDRs [42]. In cattle, sheep, and goats reusing

CIDRs has been proven effective and acceptable pregnancy rates were obtained. Therefore, it appears that, with proper hygeine, producers can reuse a CIDR to succesfully synchronize estrus and ovulation and achieve satisfactory pregnancy rates.

Prostaglandin

Prostaglandin-$F_2\alpha$ (PGF) alone has also been used in synchronization protocols in goats since PGF has been identified as the uterine luteolytic agent in ruminants [43, 44]. Prostaglandin-$F_2\alpha$ is marketed as the product dinoprost, or Lutylase (Zoetis, Durham NC) [45]. Administering PGF induces luteal regression resulting in a subsequent follicular phase and return to estrus [46]. Due to differences in ovarian status at the time of injection there are varying intervals between treatment and onset of estrus and ovulation [47]. Prostaglandin-$F_2\alpha$ given early in the estrus cycle has a shorter interval to estrus, while PGF given later in the cycle results in a longer interval to estrus. This is due to the longer time needed to decrease circulating progesterone levels as the CL acquires its full endocrine functionality later in the luteal phase [47]. Follicular status has also been found to affect the response to PGF [48]. A large follicle present at the time of treatment will continue to grow and ovulate shortly after PGF administration. If treatment occurs as the largest follicle is regressing, a new follicle still needs to emerge and grow so ovulation will occur later [48]. In sheep, a recently formed CL has also been shown to have a refractory period during the first two days after ovulation [49].Luteolysis occurred in all ewes treated on Day 3 of the estrus cycle compared to only 1 out of 8 ewes treated on Day 2, showing that the refractory period is slightly longer in sheep than in cattle [49].

Menchacha et al. [34] proposed an ovine timed artificial insemination (TAI) program, Synchrovine, which uses two PGF injections 7 days apart to induce and synchronize ovulation. Traditional treatments consist of two PGF doses 9-14 days apart [33]. This has most ewes showing estrus after the second dose of PGF over a four day period. However, estrus detection is necessary, rendering this impractical for TAI. The hypothesis behind the Synchrovine protocol was that if the second dose is given 7 days after the first, this coincides with days 3-5 post ovulation when the largest follicle is still growing and the young CL has just become responsive to PGF [34]. With TAI using fresh, undiluted semen at 42 hours after the second injection of PGF, a 50% pregnancy rate was seen using Synchrovine. Synchrovine has not been used to synchronize estrus in goats. In goats, giving two PGF injections 10 days apart with AI upon estrus detection has been used succesfully in an experiment utilizing boer-cross does. Bowridge et al. [50] observed a pregnancy rate of 53% (35/66) when using this treatment in goats.

Gonadotropins

Many of the above mentioned protocols involve the use of a gonadotropin, usually in the form of PMSG. The FSH-like activity of PMSG ensures that sufficient gonadotropin is available to initiate pre-ovulatory events [23]. In recent years, the use of PMSG has gone down drastically since animals previously given PMSG develop antibodies against it [51]. In a longitudinal study, two dairy goat herds were followed for 4 consecutive years [52]. Animals in these herds had not been exposed to PMSG before the experiment. Annual estrus synchronization treatment consisted of a vaginal fluorogestone acetate (FGA) sponge in place for 11 days with 50 μg of Cloroprestonol (PGF analogue) and either 250 or 400 IU of PMSG given 48 hours before sponge removal followed by AI 43-45 hours after sponge

removal. Results of this experiment agreed with earlier observations [51, 53] that repeated use of the PMSG was followed by an increase in antibody production to PMSG. This was accompanied by a delayed estrus and LH surge as well as decreased fertility [52].

In an attempt to eliminate the use of PMSG, Holtz et al. [54] utilized a non-steroidal approach to synchronize ovulation in goats in the form of gonadotropin releasing hormone (GnRH). By administering 1mL of Recaptal (containing 0.004mg GnRH analog Buserelin) they found a tightly synchronized LH surge within 2 hours of GnRH injection in most does [54].

Ovulation Synchronization

Cattle Protocols

Due to the fact that estrus synchronization still had a high degree of variability with most cows showing estrus within a 5-day period [55], researchers began looking at methods to synchronize animals more precisely. One way to accomplish this is to synchronize ovulation rather than estrus. The first protocols developed for ovulation synchronization involved giving GnRH at an unknown stage in the cycle, followed by PGF 7 days later, with GnRH 2 days after that and AI 24 hours after the second GnRH injection [56]. Pursley et. al [56] used transrectal ultrasonography to monitor follicular development in response to this treatment protocol. The first GnRH injection initiated or coincided with a new follicular wave in all cyclic cows (n=20). The PGF injection regressed the CL of all cows and the second GnRH injection caused ovulation in all cows within 24-32 hours [56]. A later study using a larger sample size (n=156) showed that 64% of cows ovulated in response to the first GnRH injection and 87% ovulated to the second [57]. With breeding occurring 16 hours after

the second GnRH injection, it was found that this ovulation synchronization protocol significantly decreased the days between calving and conception in dairy cows when compared to estrus synchronization using PGF [58]. This protocol was designated by Pursley et al. as 'OvSynch' [56].

The effectiveness of the OvSynch protocol has been evaluated when initiated at different times of the estrus cycle. Vascancello et al. [57] used transrectal ultrasonography and serum progesterone concentrations to monitor follicular response to OvSynch initiated in the early, middle and late estrus cycle. It was found that when treatment began in the middle of the estrus cycle, cows had greater pregnancy rates [57]. The same study revealed that cows ovulating to the first GnRH injection had a much higher synchronization and pregnancy rate compared to cows that did not ovulate to the first GnRH injection [57].

Moirera et al. [59] developed a Pre-Synch protocol that involved giving 2 PGF injections 7 days apart and then initiating OvSynch 12 days later. This yielded better pregnancy rates when compared to starting treatment at random times in the estrus cycle. The success was attributed to beginning the OvSycnh treatment period at a more favorable, earlier stage of the estrus cycle [59].

Small Ruminant Protocols

Similar to its use in cattle, fixed timed artificial insemination (TAI) has a number of benefits in goats. It can speed up the progression of genetic merit, allow gestating females and resulting kids to be managed in a group, and it is useful when estrus detection is not efficient [54]. A good TAI program has high pregnancy rates with low variability [34]. Traditional progesterone and PMSG protocols resulted in high but variable pregnancy rates [34]. Estrus synchronization protocols involving only PGF are not recommended for fixed

TAI since PGF synchronization results in variable timing of ovulation [46]. Light supplementation and the buck effect have been suggested as other alternatives for synchronization but they require repeated AI after detected estrus and are, therefore, not useful in goats for a fixed TAI protocol [54].

It has been shown that using Pursley's OvSynch protocol in sheep yielded promising results as a fixed TAI protocol [60]. Holtz et al. [54] examined the efficacy of this protocol in goats compared to the conventional progestagen-sponge treatment+PMSG protocol to synchronize ovulation. Does in the OvSynch treatment group had a pregnancy rate of 58% and 46% in the sponge + PMSG treatment group. Mean serum progesterone concentrations at time of PGF analogue (Luprostiol) injection were 15.6 ng/mL, indicative of the presence of a CL, and dropped down to 0.9ng/mL within 3 days after injection, indicative of luteal regression. Holtz et al. [54] found a tightly synchronized LH surge within 2 hours of GnRH injection in most does in the OvSynch group, while does in the sponge treatment group had a much more variable interval to LH surge 30-55 hours after PGF injection. It has been reported that ovulation in goats occurs about 22 hours after LH surge [61]. Holtz attributed suboptimal fertility rates in the OvSynch group to the post-treatment short cycles. It was hypothesized that this was due to the pre-mature ovulation of young follicles [54]. Conclusions from this experiment suggested allowing more time between PGF and the second GnRH injection, currently 48 hours, and testing for progesterone before treatment to confirm cyclicity.

A later experiment in goats attempted to reduce the incidence of these short cycles by providing more sustained stimulation of the CL in the form of longer acting human chorionic gonadotropin (hCG) instead of GnRH [62]. It was hypothesized that the longer acting hCG

would make the CL less prone to premature regression. Yacoub et al. [62] had three treatment groups that consisted of (1) GnRH 48 hours after PGF, (2) hCG 48 hours after PGF and (3) no injection control with breeding 16 hours after estrus detection. Serum progesterone levels were monitored and animals did not receive a PGF injection until levels were above 5 ng/mL. Results did not yield improved pregnancy rates for either group and actually had increased incidences of CL insufficiency when compared to the control group. The conclusions of this experiment advised not using PGF as an ovulation synchronization method for TAI [62].

Recently, a new synchronization protocol, NC.Synch, was proposed to address the issue of short cycles and premature ovulation by utilizing a pre-synchronization PGF injection. The NC.Synch protocol involves giving a pre-synchronization PGF shot on day 1, GnRH on day 8 to ovulate any follicles present and PGF again on day 15 to induce luteolysis [50]. Does are bred via AI 72 hours after the second PGF injection and given a final GnRH injection at time of breeding. When compared to an estrus synchronization protocol involving two PGF injections administered 10 days apart, with breeding based on estrus behavior, there was no difference in pregnancy rates between the NC.Synch and control groups, 68% versus 53% ($P>0.05$), respectively [50]. Only one doe was observed to have had a short cycle. In cattle, it has been hypothesized that short cycles following treatments are due to exogenous GnRH not creating an adequate LH surge [63]. However, based on data in goats [54] the magnitude of an LH surge induced with the GnRH analogue, Buserelin, was similar to that of a normal LH surge. Thus, it is not likely that inadequate LH surges are as large of an issue in goats as in cattle. Overall, the improved pregnancy rates seen with the

NC.Synch protocol demonstrate an advancement in the efforts to synchronize ovulation in goats.

Pregnancy Detection in the Doe

A reliable technique for early pregnancy detection in goats is necessary to determine culling and re-breeding decisions [64]. The inability to detect early pregnancy can result in economic losses in milk or meat production due to a longer kidding interval [64]. The traditional method for pregnancy detection in goats is estrus detection 21 days post-breeding. Does that fail to conceive will return to estrus. Therefore, a non-return to estrus would indicate pregnancy [65]. However, a non-return to estrus can also be observed in goats that have long cycles or become anestrus, making estrus detection an unreliable method of pregnancy detection in goats [64, 65]. Clinical methods and hormonal assays are also available to diagnose pregnancy about 3 weeks after breeding [65].

Ultrasonography

The first attempts at diagnosing pregnancy in small ruminants via ultrasonagraphy were done between 60-80 days of gestation [64]. Later on, studies were done to define criteria for early pregnancy diagnosis in goats using ultrasonagraphy. Martinez et al. [66] performed transrectal ultrasonographic examinations on does from days 13-40 post-mating. Pregnancy viability was confirmed with a heartbeat as early as day 21. It was also found that between days 19-38 crown-rump length was best represented by a linear regression, making crown-rump length a useful tool in aging embryos.

Suguna et al. [67] conducted a similar experiment in which does were subjected to both transrectal and transabdominal ultrasonography up to day 120 of gestation. An embryo was detectable on day 21 and day 28, and a heartbeat on day 21 and day 35 for transrectal and transabdominal methods, respectively. Using the transrectal probe, placentomes were identified by day 42 and singles versus twins were identified by day 35. In contrast, using the transabdominal probe the same features were seen at day 50 and day 42, respectively.

Ultrasonography, transabdominal and transrectal, has been shown to be a very useful tool in pregnancy detection in goats, but it does have its drawbacks. An extended urinary bladder, hydrometra and pyometra can result in false positives [64]. It is recommended that food and water be withheld for 12 hours to minimize the risk of these false positives, but that can prove difficult in certain production settings [68]. Older, multiparous animals also have a greater risk of generating a false positive due to more folds in the uterus that make it more difficult to see the embryo clearly [68]. When utilizing transrectal ultrasonography it requires extraction of the feces from the rectum along with complete immobilization of the animal to avoid rectal damage [69]. For these reasons, this method may not be the best suited for field conditions [65]. Transabdominal ultrasonography is a less invasive procedure and has been shown to be accurate in early pregnancy detection [67].

Progesterone and Pregnancy Associated Glycoproteins (PAGs)

In addition to ultrasonography, serological methods provide another way to determine pregnancy. Examples of serological methods include progesterone (P4) or pregnancy associated glycoprotein (PAG) assay. Whereas, ultrasonography gives an immediate diagnosis of pregnancy, P4 or PAG assay requires laboratory analysis and preclude

immediate results [65]. However, when working large numbers of animals, utilizing the P4 or PAG assay may be more efficient than ultrasonography [65]. Furthermore, ultrasonography requires expertise to obtain a reliable diagnosis early in pregnancy, making it less suitable for producers with limited resources and training [70].

Milk P4 concentrations measured by radioimmunoassay (RIA) have been used to detect pregnancy 20-22 days after mating with an accuracy of 80-98% [71]. An on-farm ELISA kit developed for cattle has also been used to measure P4 concentration in the milk of dairy goats with a pregnancy detection accuracy of 83-88% [72]. Along with milk, blood serum P4 concentrations can also be used to detect pregnancy. Gonzalez et al. [65] found that serum P4 concentrations were 100% accurate for detecting pregnancy on day 22 post-breeding and 82.8% accurate for detecting non-pregnancies. The decreased accuracy for detecting non-pregnancies was attributed to the fact that high serum P4 concentrations on day 22, indicating a functional CL, is not always indicative of pregnancy, particularly in does with long cycles. Elevated levels of P4 on day 22 can also be associated with early embryonic death, an extended luteal lifespan, hydrometra, or luteal cysts [73]; ultimately resulting in a false positive pregnancy diagnosis.

Pregnancy associated glycoproteins are proteins secreted from the placenta that are released into the peripheral circulation of the mother and are, therefore, reliable indicators of pregnancy [70]. In 1991, Zoli et al. [74] purified one of these PAGs (later designated as boPAG-1) from bovine fetal cotyledons. Antisera was raised in rabbits against this bovine PAG which allowed for the development of a radioimmuno assay (RIA) for detecting pregnancy in cattle as early as 28-30 days post breeding [75]. In 1998, Garbayo et al. [76] purified PAGs from caprine placenta. Gonzalez et al. [70] found that using the anti-caprine

PAG_{55+59} antisera on a day 21 sample resulted in 99% accuracy of pregnancy diagnosis. In a study done with ewes, it was also found that PAGs were significantly more accurate than P4 assays at identifying non-pregnant animals [73]. This was attributed to the fact that PAGs are produced by the trophoblast binucleate cells and are a strong indication of viable trophoblast tissue. In contrast, high P4 due to a functional CL may not always be associated with pregnancy as discussed above [73].

Pregnancy Specific Protein B

Research regarding pregnancy specific protein B (PSPB) first began in cattle. It was known that implantation of the embryo did not occur until day 30, yet maternal recognition or pregnancy occurred by day 17. This led researchers to believe that the conceptus was secreting some sort of substance responsible for maintenance of the CL. Reports of such pregnancy associated proteins had appeared in early scientific findings [77, 78]. Butler et al. [79] wanted to develop an antisera to detect these pregnancy specific proteins in order to isolate and characterize them. Using bovine extraembryonic membranes, an antisera was developed and two pregnancy specific proteins were successfully isolated, pregnancy specific protein A (PSPA) and PSPB. The antisera also reacted with endometrial extracts and extraembryonic fluids of pregnant cows. However, it did not react with extracts of somatic tissues from pregnant cows or endometrial extracts from non-pregnant cows. This demonstrated that presence of PSPB was indicative of pregnancy and it had placental origins. Pregnancy specific protein A was later found to be alpha-fetoprotein but PSPB was a novel protein. This novel protein, PSPB, had a molecular weight of 47,000-53,000 daltons and an

isoelectric point of 4.0-4.4, similar to pregnancy specific proteins reported in earlier experiments [79].

After isolation and characterization of this novel protein, Sasser et al. [80] developed a double antibody RIA for detection of PSPB in the serum of cattle. This test provided a serological method for detecting pregnancy in cattle, in contrast to the conventional method of transrectal palpation. In testing the validity of this RIA, Sasser et al. [80] used a commercial herd of 102 beef cows and found that the PSPB RIA could detect pregnancy earlier and more accurately than rectal palpation. The bovine PSPB (bPSPB) antibodies in the RIA developed by Sasser et al. [80] were also used to detect pregnancy in sheep. However, this was only used as a qualitative test since ovine PSPB (oPSPB) antigens cross reacted incompletely with the bPSPB antibodies. Willard et al. [81] developed a quantitative RIA for PSPB in the serum of sheep by developing an antisera specific to oPSPB. Pregnancy specific protein B has also been found in the peripheral circulation of other ruminants such as goats [82] and deer [83].

The oPSPB RIA can also be used to quantify PSPB levels in goats and was used by Humblot et al. [84] to characterize a PSPB curve throughout gestation in French alpine dairy goats. Animals were bled daily from days 16-27 post-AI and pregnant animals were bled until day 30. Of the pregnant does, 9 of them were bled every 2 weeks until parturition. It was found found that day 24 was the first day at which PSPB concentrations were significantly different in pregnant versus non-pregnant does, compared to day 21 in sheep [81]. Goats with 2 or more conceptuses expressed an earlier rise in PSPB and had a higher mean concentration than those with singletons but it was concluded that individual variation was too high to use this as a diagnostic tool for predicting multiple births.

A similar study was done to characterize PSPB profiles in cows with singletons or twins [85]. Beef cows with pregnancy resulting from the transfer of in vitro fertilized (IVF) single or twin embryos and singleton pregnancies resulting from AI were sampled monthly starting at 40 days of pregnancy. Vaques et al. [85] found that cows carrying twin IVF fetuses had significantly higher PSPB concentrations at days 40 and 240 of gestation compared to those carrying single IVF pregnancies (3.85 ng/mL and 715 ng/mL compared to 2.04 ng/mL and 415 ng/mL, respectively). It was also found that PSPB concentrations were accurate in detecting retarded embryo growth up to 70 days of gestation. These results suggested that there was a correlation between PSPB concentrations and fetal growth rates in cattle. It was suggested that sampling at day 40 and 70 may be useful diagnostic tools for identifying embryonic loss occurring later than 21 days of gestation.

Patel et al. [86] also concluded that PSPB could possibly be used to gauge bovine feto-placental viability. In their study, singleton and twin-bearing pregnancies resulting from embryo transfer were sampled every third day from day of standing estrus (day 0) to parturition. There was a significant difference observed between PSPB concentrations of singletons versus twins from day 50 onwards and by mid-gestation (day 140) there was a thirty-fold increase in the PSPB concentration of singleton bearing cows compared to a ten-fold increase in twin bearing cows.

A later study investigated whether PSPB profiles are predictive of pregnancy outcomes in adolescent ewes in which growth of the placenta has been compromised by competing nutrient demands of maternal tissue synthesis [87]. Adolescent ewes were fed either a high or low proportion of a complete diet to promote rapid (n=8) or normal (n=11) maternal growth (MG) rates and blood was sampled three times a week throughout gestation.

A biphasic pattern of PSPB was observed with peaks around day 60 (40 ng/mL) and 120 (80 ng/mL) in the RMG and NMG ewes, respectively. Peaks at day 120 were followed by a prepartum decline in PSPB levels. Overall, mean PSPB concentrations were lower in RMG ewes compared to NMG ewes, but individual variation was very high. Along with lower PSPB levels, the RMG ewes experienced more abortions, lower placental mass and live lamb birth weight. In the incidence of abortion (4 RMG ewes and 1 NMG ewe), the mass of the fetus was highly correlated with PSPB concentration up until Day 120. A correlation was also found between the PSPB samples taken from Days 50 to 100 and the placental mass at term. However, the same correlation was not found between PSPB concentrations and lamb birthweight. The results of this experiment suggest that rapid maternal growth in adolescent ewes throughout gestation may compromise placental growth and sequential measurements of PSPB throughout gestation may provide a reliable indicator of fetal distress and possibly adverse pregnancy outcomes [87].

BioPryn ELISA

BioTracking, LLC was established in 1992 to develop and market PSPB testing for pregnancy in cattle. This test came to be called BioPryn, where PRYN stands for 'Pregnant Ruminant Yes/No.' The technology was licensed by BioTracking from the University of Idaho in 1993 and in 2002 the test was converted from an RIA to an ELISA. Establishment of the BioPryn ELISA resulted in an affordable, blood based pregnancy test for cattle. In 2004, a test for sheep and goats was established and within the past year a plate capable of testing cattle, sheep and goats has been released.

The BioPryn ELISA has an assay accuracy of 100% when it comes to detecting pregnant cows [88]. Because of this high accuracy rate, non- pregnant cows can safely be designated as non-pregnant and re-synchronized without concerns for inducing abortions.

Statement of the Problem

With goat populations around the world increasing, more research efforts are needed to provide producers with practical information to increase the efficiency and productivity of their animals. Improving reproductive strategies is a proven way for producers to increase overall production while also increasing efficiency and decreasing costs.

Timed artificial insemination programs allow producers to take advantage of the benefits of utilizing artificial insemination while also eliminating the need for the increased labor of estrus detection. A popular method for synchronizing ovulation in goats for TAI involves using a CIDR but these devices can be very costly to producers. Therefore, the objective of the first experiment was to assess the effectiveness of reused CIDRs in synchronizing ovulation to achieve satisfactory pregnancy rates in does. Being able to reuse a CIDR will greatly drive down the per doe cost from $6/head to as low as $2/head.

The objective of the second experiment in this thesis is to improve the pregnancy rates achieved when using the NC.Synch TAI protocol. Although CIDRs have been shown to be effective, they pose potential health and environmental risks. If not handled properly, progesterone stored in a CIDR can leach into the skin and disrupt normal progesterone levels in humans. This is of special concern in developing countries, where goat numbers are increasing the most, and where producers lacking education may not adhere as strictly to proper handling procedures. Any excess progesterone left in a CIDR could also leach out into

the environment when it is disposed of, affecting even larger numbers of people. Finally, although there are ways to minimize risk with proper washing and storage, reusing a CIDR could lead to the spread of disease and infection amongst animals within a herd. Therefore, establishing an effective, non-progesterone, non-CIDR-based protocol would be optimal.

In the NC.Synch (NCS) protocol, PGF is given on Day 0, GnRH on Day 7, PGF on Day 14 and then GnRH at TAI on Day 17. This current protocol involves administering the second GnRH injection approximately 72 hours after the second prostaglandin injection. An alternative protocol has been proposed, NCS Modified (NCSM), in which the timing of the second GnRH injection is changed to 60 hours after the second prostaglandin injection instead of 72 hours. This protocol has been proposed to take into consideration the hypothesis of Holtz et al. [54] that the timing of 48 hours between PGF and the second GnRH injection needs to be extended. Mucus consistency at the time of breeding can also give insight as to what point in the estrus cycle the doe is in. Ideal mucus at the time of breeding is thick, stringy and slightly cloudy, while late estrus mucus has a more opaque and cottage cheese-like consistency. Based on unpublished observations, late estrus mucus has been commonly observed when NC.Synch protocol is used but it is not observed when does are bred by the AM/PM rule based on first standing estrus. Thus, a decrease time interval from the second PGF to TAI in the NC.Synch protocol may improve the timing of TAI relative to estrus onset resulting in improved pregnancy rates.

The third experiment explores the possibility of using serum PSPB concentrations from samples taken throughout gestation to predict multiple births or abnormal fetal development in does. The ability to predict which does in a herd are carrying multiple fetuses

would allow them to be managed more appropriately to maximize animal welfare and productivity.

CHAPTER II: Comparison of Ovulation Synchronization Protocols in Goats

Introduction

Similar to its use in cattle, fixed TAI has a number of benefits in goats. It can speed up the progression of genetic merit, allow gestating females and resulting kids to be managed in a group, and it is useful when estrus detection is not efficient[54]. A good TAI program has high pregnancy rates with low variability [34]. Traditional progesterone-based protocols to synchronize estrus resulted in high but variable pregnancy rates [34]. Estrus synchronization protocols involving only PGF are not recommended for fixed TAI since PGF synchronization results in variable timing of ovulation [46]. Light supplementation and the buck effect have been suggested as other alternatives for synchronization but they require repeated AI after detected estrus and are, therefore, not useful in goats for a fixed TAI protocol [54].

A short-term CIDR protocol combined with PGF to ensure luteolysis can be used to successfully synchronize ovulation for TAI. With the transition to short-term (6 day) instead of long-term (12-18 days) protocols, a significant amount of progesterone still remains within the CIDR at the completion of treatment. Scientists have tested the efficacy of reusing CIDRs in an attempt to reduce costs for producers [32, 41, 89]. Using a 9-day CIDR protocol comparing a new, once-used and twice-used CIDR, Nogueira et al. [32] found no significant drop in pregnancy rate (93%, 73% and 80%, respectively) or increase in vaginitis with the used CIDRs. Only one doe came into heat before device removal and that was in the twice-used group. In cattle, reused CIDRs have been shown to be effective for synchronizing estrus and ovulation [39, 40]. Similarly, Vilarino et al. [41] reported that synchronization with reused CIDRs in goats resulted in similar follicular dynamics compared to

synchronization with the new CIDRs. In sheep, a short-term (6-day) CIDR protocol involving new, once-used or twice-used CIDRs demonstrated that reused CIDRs were as effective at synchronizing estrus and obtaining good pregnancy rates as new CIDRs [42]. Overall, it appears that, with proper precautions, producers can reuse a CIDR to succesfully synchronize ovulation and achieve satisfactory pregnancy rates.

In sheep, ovulation synchronization with the OvSynch [56] protocol yielded promising results as a protocol for fixed TAI [60]. Holtz et al. [54] examined the efficacy of this protocol in goats compared to the conventional progestagen-sponge treatment supplemented with PMSG to synchronize ovulation. In their study does in the OvSynch treatment group had a pregnancy rate of 58% and a tightly synchronized LH surge within 2 hours of the second GnRH injection in most does. Does in the sponge control group had a pregnancy rate of 46% and the LH surges were not as tightly synchronized. Holtz observed that several does in the OvSynch group experienced pre-mature CL regression following treatment. It was hypothesized that this was due to the pre-mature ovulation of young follicles [54]. These authors suggested that allowing more time between the PGF injection and the second GnRH injection, currently set at 48 hours, would result in a more optimal time of ovulation. They also suggested testing for progesterone levels in the blood before treatment to confirm cyclicity among the herd.

Recently, a new synchronization protocol, NC.Synch, was proposed that addresses the issue of short cycles and premature ovulation observed with the traditional OvSynch protocol when applied in goats. In the NC.Synch protocol, a pre-synchronization injection of PGF is given on Day 1, GnRH is given on Day 8 to ovulate any follicles present and PGF is administered again on Day 15 to induce luteolysis [50]. At 72 hours after the second PGF

injection, does are bred by AI and given a final GnRH injection at time of breeding. Pregnancy rates in does synchronized with the NC.Synch protocol were 68% and only one doe was observed to have had a short cycle. The improved pregnancy rates seen with the NC.Synch protocol demonstrate an advancement in the efforts to synchronize ovulation in goats.

The objectives of the following experiments were to examine the efficacy of progesterone-based and progesterone-free protocols in synchronizing ovulation in goats for TAI. In Experiment 1, the efficacy of re-used CIDRs that have been used to their full 21-day lifespan for synchronizing ovulation for TAI was evaluated. In Experiment 2 a modification to improve the viability of ovulated follicles in the NC.Synch protocol was assessed in an attempt to improve pregnancy rates.

Materials and Methods

Experiment 1

Experimental Design

A total of 87 Boer-cross does were blocked by age, breed and parity and randomley assigned to one of the following five protocols: (1) traditional Breed by Estrus [BBE] , (2) NC.Synch-Timed Artifical Insemination [NCS], (3-5) a 6-day CIDR protocol with either (3) a new CIDR [C6N], (4) a once used CIDR [C61] or (5) a twice used CIDR [C62]. The experiment was performed in two replicates with 8-9 does per treatment per replicate.

Treatment Groups

Does in the BBE treatment were given two 15 mg PGF (Lutylase) injections administered i.m. 10 days apart to synchronize estrus. Animals were observed for estrus twice daily after the second prostaglandin injection and bred 12 hours after onset of estrus (Figure 1).

Does in the NC.Synch treatment received one 15 mg dose of PGF administered i.m. on Day 1. On Day 8 does received 50 µg GnRH (Cystorelin; Merial, Duluth GA) to initiate a new follicular wave. On Day 15, does received a second 15 mg dose of PGF to lyse any corpora lutea present. Does were bred via artificial insemination at 72 hours after the second prostaglandin-F2α injection and recieved a second 50 µg dose of GnRH at the time of breeding (Figure 1).

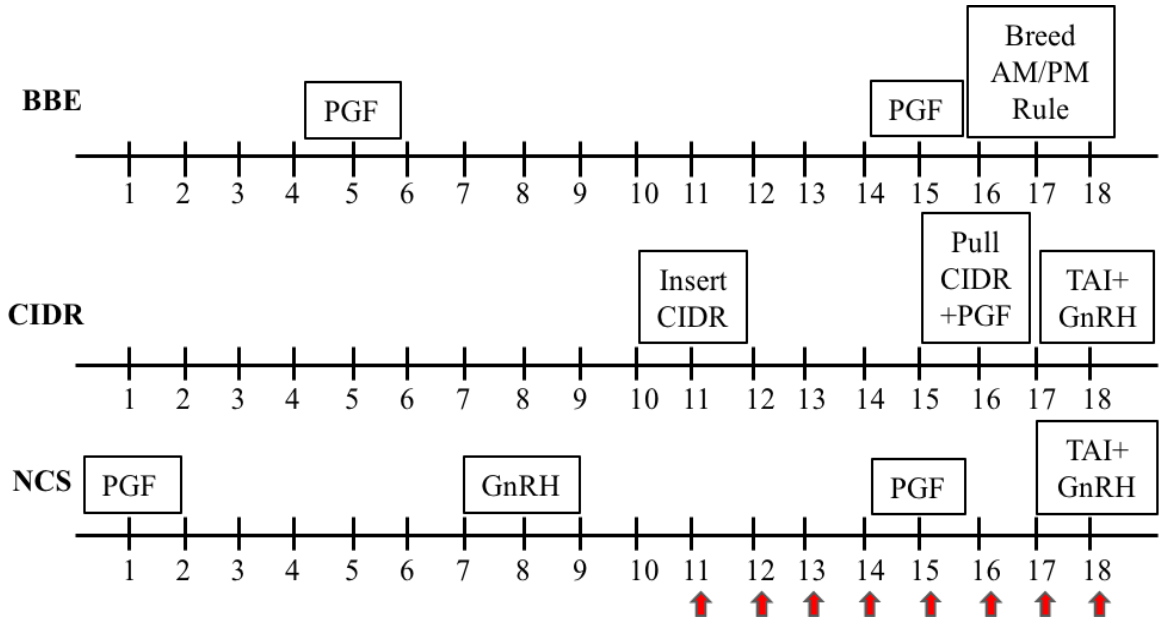

Figure 1 Timeline for treatment groups in Experiment 1. ⬆ represents days where blood was drawn for progesterone assay from a subset of does in each treatment group.

Does in the CIDR groups received a CIDR (Zoetis, Durham NC) on day 0 of treatment. Six days later the CIDR was removed and does were given 15 mg PGF. Does were bred via artificial insemination 48 hours after CIDR removal and received 50 μg of GnRH administered at the time of breeding (Figure 1).

Preperation of Used CIDRs

For the C61 group, the CIDRs had been in place, in a doe not used in the experiement, for a 10 day period before being used in this experiment. For the C62 group, the CIDRs had been in place for a 10 day period, removed from the animal, cleaned and then put in place for an additional 5 day period before being used in this experiment. This meant that the twice-used CIDRs were being used through their full 21 day life span. All reused CIDRs were disinfected prior to use with a dilute Novalsan (chlorohexidine diacetate) solution, rinsed in clean water, thoroughly dried and stored in a refrigerator until time of use.

Experiment 2

Experimental Design

A total of 68 Boer-cross does were randomly blocked by breed, age and parity and then randomley assigned to one of the following four treatment groups: (1) traditional breed by estrus [BBE], (2) 6-day CIDR protocol with a new CIDR [C6N], (3) NC.Synch-Timed AI protocol [NCS], (4) a modified NCS protocol [NCSM]. The experiment was performed in 4 replicates with 5-6 does per treament per replicate.

Treatment Groups

Does in the NCS, BBE and C6N groups were treated as described for Experiment 1 (Fig. 2). Does in the NCSM treatment group received 15 mg PGF on Day 1, 50 μg GnRH on Day

8 and 15 mg PGF on Day 15.5. Does were bred at 60 hours after the second PGF injection and received 50 μg GnRH at timed AI (Figure 2).

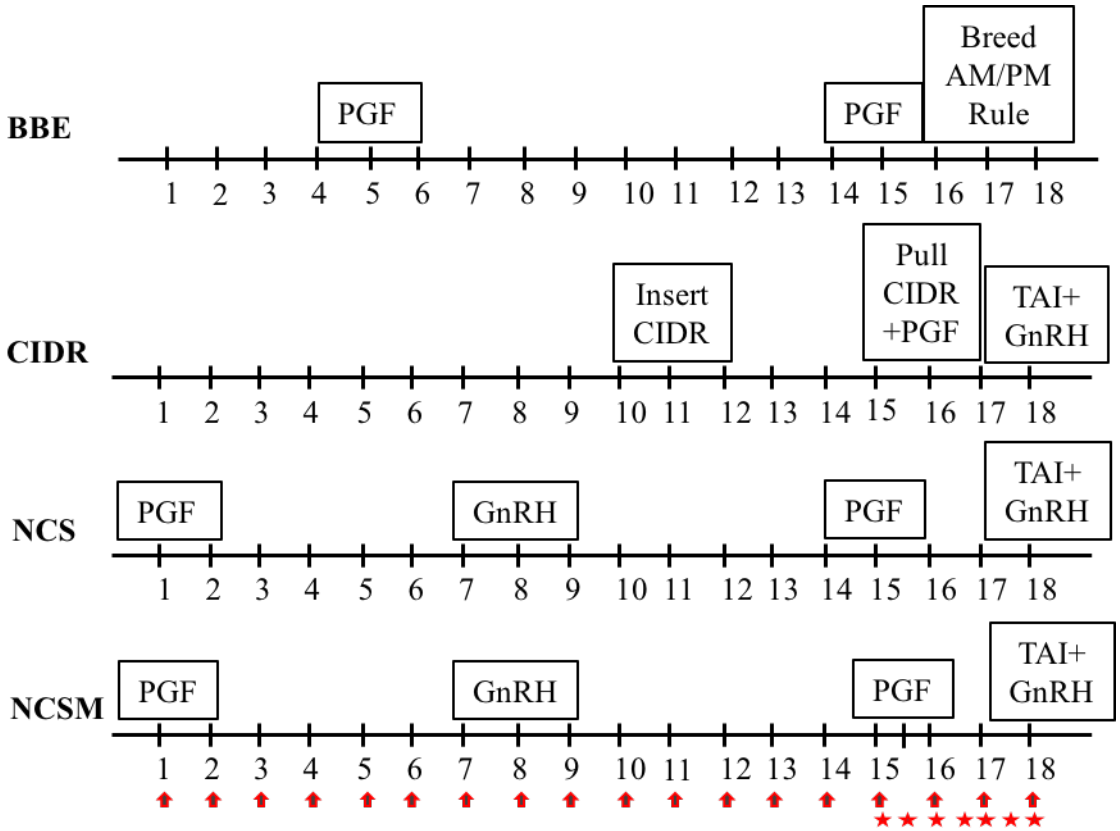

Figure 2 Timeline for treatment groups in Experiment 2. ⬆ represents days where blood was drawn for progesterone assay from a subset of does in each treatment group. ★ represents days where blood was drawn for LH assay from a subset of does in each treatment group.

Artificial Insemination

Animals in both experiments were bred with a single dose of frozen semen using a non-surgical, transcervical insemination technique by experienced inseminators. All treatment groups were coordinated such that all inseminations occured during the same four day period within each experimental replicate. At time of breeding the condition of cervical mucus was documented and classified as either clear and stringy, thick and cloudy, or cottage

cheese-like. These desginations correlate roughly with the onset of estrus, mid-estrus, and late estrus. Following insemination, post-thaw motility from a drop of semen remaining in the AI catheter was observed and recorded. After the artificial insemination breeding period, all animals were returned to the flock and managed according to farm procedures. Animals were grazing a fescue and orchardgrass mixed pasture and supplemented with 0.5-1.0 lb of a 12% crude protein grain mix per head per day. Animals were examined several times throughout the experiment and those with a Famacha score above 3 were dewormed with an oral drench of Cydectin (Boehringer Ingelheim, Ingelheim am Rhein Germany) and Prohibit (Agri Laboratories LTD, St. Joseph MO) per farm protocol.

Blood Samples

In Experiment 1, a random subset of four does from each treatment group within each replicate were bled from the time of CIDR insertion to the time of insemination to monitor serum progesterone concentrations (Figure 1).

In Experiment 2, a subset of three does from each treatment group within each replicate were bled daily beginning at the time of the first prostaglandin injection in the NCS group until the time of breeding to monitor progesterone concentrations (Figure 2). After the second PGF injection in the NCS group, blood samples were drawn from the subsets of does from each treatment group every 6 hours from 72 hours before TAI and continuing until 24 hours after breeding to monitor serum concentrations of LH.

All blood samples were drawn from the jugular vein into a glass, silicone coated, vacutainer tube. Samples were refrigerated for 12 hours and then centrifuged at 877 g for 20 minutes at 4°C. Serum was removed and stored in a -20°C freezer until analysis.

Determination of Pregnancy

At approximately 50 and 85 days after artificial insemination, animals were monitored for pregnancy status using transabdominal ultrasonography. Data on kidding including the number of females kidding to AI, kidding date, number of kids born, number of kids born alive and total kid weight were recorded.

Radioimmunoassay

A Coat-A-Count® radioimmunoassay kit (Siemens Medical, Los Angeles, CA) was used to determine the concentration of progesterone in serum. Assays were performed as described previously [90]. Progesterone samples were run in one assay in 2013. In 2015 progesterone samples were run in a total of 2 assays with an inter-assay COV of 14.1% and an intra-assay COV 3.6% based on duplicate high pooled samples.

An Abnova (Taipei City, Taiwan) enzyme-linked immunoassay was used to determine serum concentrations of LH as previously described [91]. Samples were run in a total of 10 assays. The intra-assay coefficient of variation was 4.38% in the "high" pooled samples and 6.85% in the "low" pooled samples. The inter-assay COV was 44% in the "high" pools and 56% in the "low" pools. All samples representing the entire LH profile for one animal from each treatment group was represented on each plate. Since the assays were only used to detect the time of the LH surge and the LH peak for each doe sampled and not the magnitude of the LH peak, the high inter-assay COV was of no consequence because the intra-assay COV was still within an acceptable range.

Statistical Analysis

Data for pregnancy rates (Experiment 1 and 2) were analyzed by two-way ANOVA with a model that included effects of treatment, replicate and their interaction. Means were

separated by Duncan's test. Data for serum progesterone concentrations were analyzed with an ANOVA model that included the effects of treatment, replicate, day and their interactions. Means were separated by Duncan's test. The number of hours from the last PGF injection to the peak of the LH surge was calculated (Experiment 2) and data were analyzed with a one-way ANOVA that included the effect of treatment. The number of hours from the LH surge to breeding were also calculated and does were grouped into three classifications: breeding occurred (1) -6-0 hours, (2) 6-12 hours or (3) 18-24 hours after the LH surge. Preganancy data was analyzed with an ANOVA that included the effects of classification and treatment in the model.

Results

Experiment 1

Twenty-eight of thirty-nine (71%) does had endogenous progesterone at the beginning of sampling period, demonstrating that the herd was cycling at the initiation of treatments (Figure 3a). As expected, there was a sharp increase of progesterone upon CIDR insertion and then a sharp decline in progesterone after CIDR removal and PGF injection (Figure 3a). Does in the NCS and BBE groups also demonstrated an expected drop in progesterone after the second PGF injection on Day15. There was no significant treatment by day interaction for progesterone concentrations in the treatment period prior to AI, indicating that the patterns of progesterone during this period did not differ between treatments ($P>0.05$).

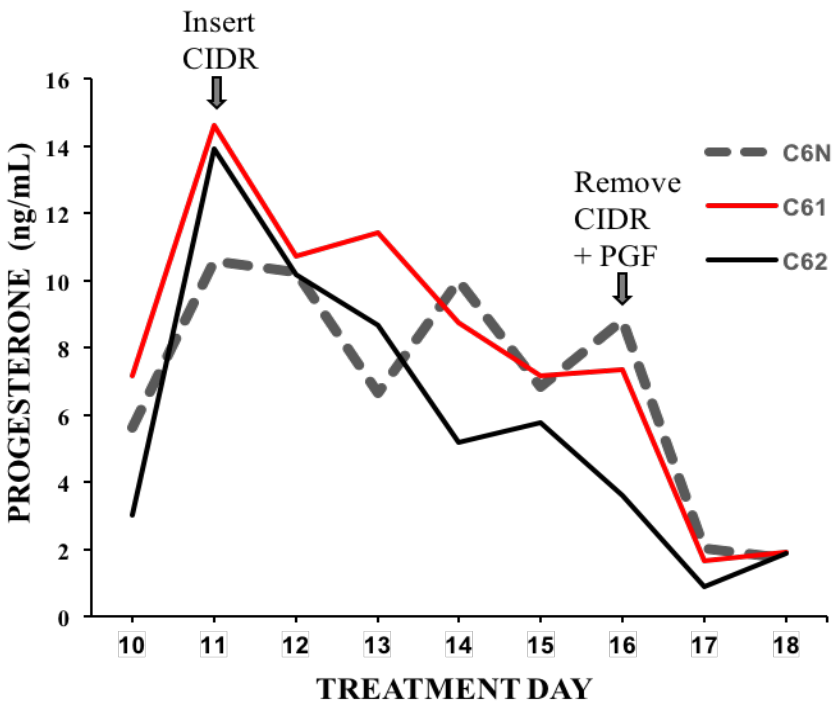

Figure 3a. *Experiment 1* Progesterone profiles for does in the CIDR treatment groups.

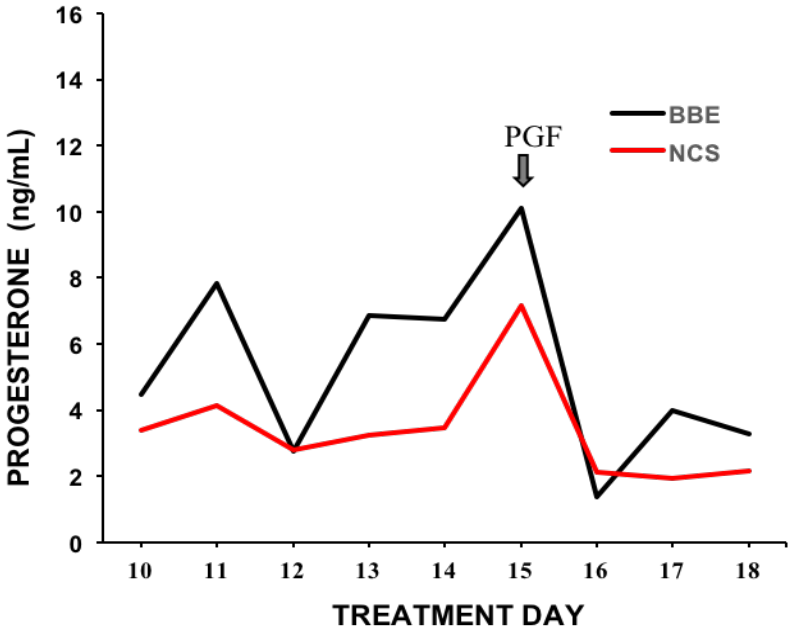

Figure 3b. *Experiment 1* Progesterone profiles for does in the control treatment groups.

Progesterone concentrations were also averaged across the synchronization period prior to AI for each treatment group (Figure 4). The mean progesterone concentration during the treatment period prior to AI was 6.9±0.8 ng/mL, 7.8±0.8 ng/mL and 6.1±0.8 ng/mL, for the new once, once-used and twice-used CIDR groups, respectively. There was no significant difference in mean progesterone concentration amongst does in the CIDR treatment groups. The mean progesterone concentrations during the treatment period prior to AI for does in the BBE and NCS group were 5.3±0.8 ng/mL and 3.5±0.8 ng/mL respectively. There was no difference in mean progesterone concentrations amongst does in the BBE and NCS groups ($P>0.05$). However, all of the CIDR treatment groups had higher ($P<0.05$) mean progesterone concentrations compared to the NCS group. In addition, the once-used CIDR group had higher ($P<0.05$) mean progesterone levels than both the NCS and BBE treatment groups (Figure 4).

Based on ultrasonography at approximately 48 days post-AI, 64±12%, 76±12% and 56±12% of does in the new, once-used and twice-used CIDR groups, respectively, were pregnant to AI (Figure 4). No difference ($P>0.05$) in pregnancy rates was observed amongst does in the CIDR treatment groups. In the BBE and NCS treatment groups 39±11% and 22±11% of does were found pregnant to AI (Figure 4). There was no difference in pregnancy rates amongst does in the BBE and NCS treatment groups. Treatment with a once-used CIDR achieved higher ($P<0.05$) pregnancy rates than either the BBE or NCS treatment groups. Interestingly, pregnancy rates for each group corresponded to the mean levels of progesterone in the treatment period prior to AI.

There was no effect of AI technician observed on pregnancy rates achieved (Appendix C). In addition, there was no difference in total kids born or total kid weight

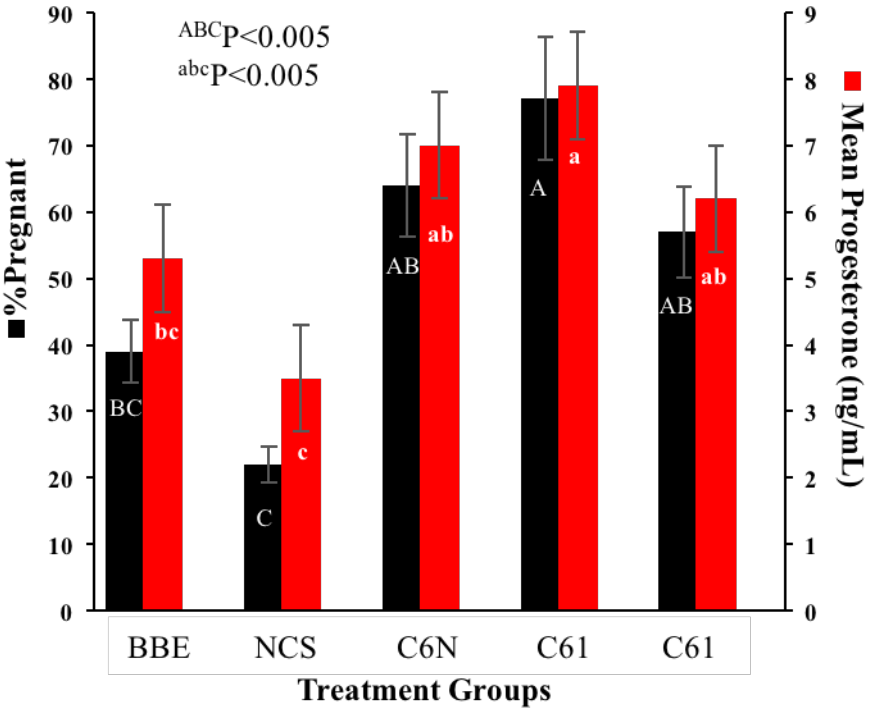

Figure 4. *Experiment 1.* Mean progesterone concentrations and pregnancy rates among breed by estrus (BBE), NC.Synch (NCS), new CIDR (C6N), once-used CIDR (C61) and twice-used CIDR (C62) treatment groups

amongst all treatment groups. There was also no effect of treatment on mucus consistency at time of breeding (P>0.05).

Experiment 2

Progesterone profiles for does sampled in the NCS and NCSM groups are presented in Figure 5a. A drop in progesterone was seen after the first PGF injection on Day 1 and again after the second PGF injection on Day 15 (NCS) or Day 15.5 (NCSM). Progesterone levels began to rise around Day 7, just before GnRH was given on Day 8.

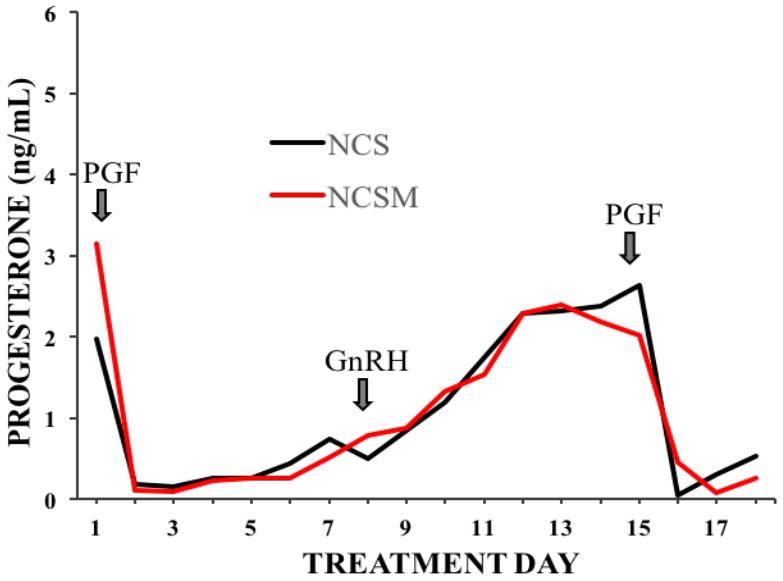

Figure 5a. *Experiment 2* Progesterone profiles for does in the NCS and NCSM treatment groups.

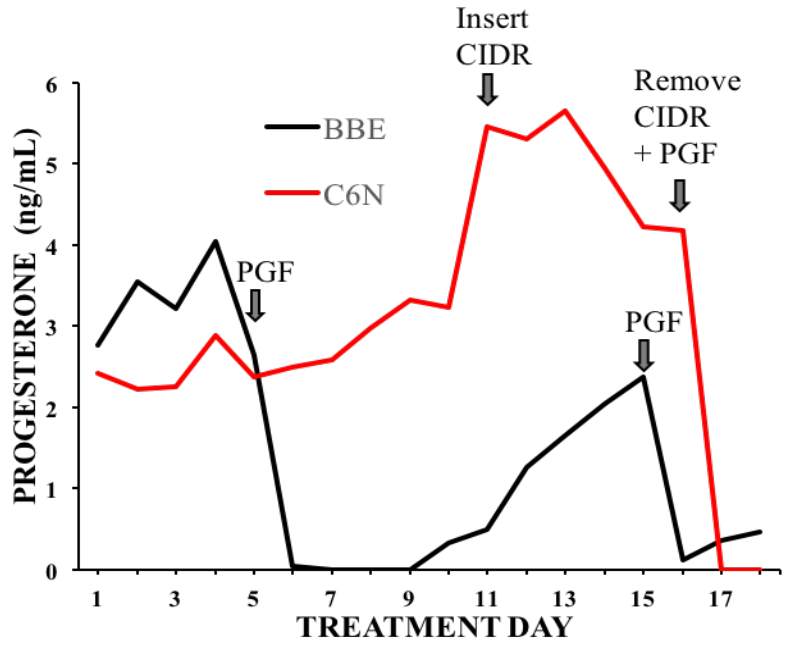

Figure 5b. *Experiment 2* Progesterone profiles for does in the BBE and C6N treatment groups.

Profiles for does sampled in the BBE and C6N groups are presented in Figure 5b. Does in the BBE treatment group had drops in progesterone at Days 5 and 15 when PGF was given. Does in the CIDR group had a dramatic increase in progesterone when CIDRs were inserted followed by a sharp decline after CIDR removal and PGF injection. Of the does sampled, only twenty of thirty-eight (53%) animals had endogenous progesterone at the beginning of treatment, demonstrating that all of the animals in the herd may not have been cycling at that time.

The mean progesterone concentration during the synchronization period prior to AI was 1.4±0.1 ng/mL, 3.2±0.1 ng/mL, 1.1±0.1 ng/mL and 1.1±0.1ng/mL, for the BBE, C6N, NCS and NCSM groups, respectively. There was no difference in mean progesterone concentration among the BBE, NCS and NCSM treatments compared to the CIDR treatment group which had a higher mean progesterone level ($P<0.05$).

The number of hours from final PGF injection to the LH surge in does sampled from each treatment group is shown in Table 1. Does in the CIDR group surged approximately 46 hours after PGF injection, very close to the time of AI which occurred at 48 hours after PGF treatment. In contrast, does in the NCS and NCSM treatment groups surged about 10 hours prior to time of breeding.

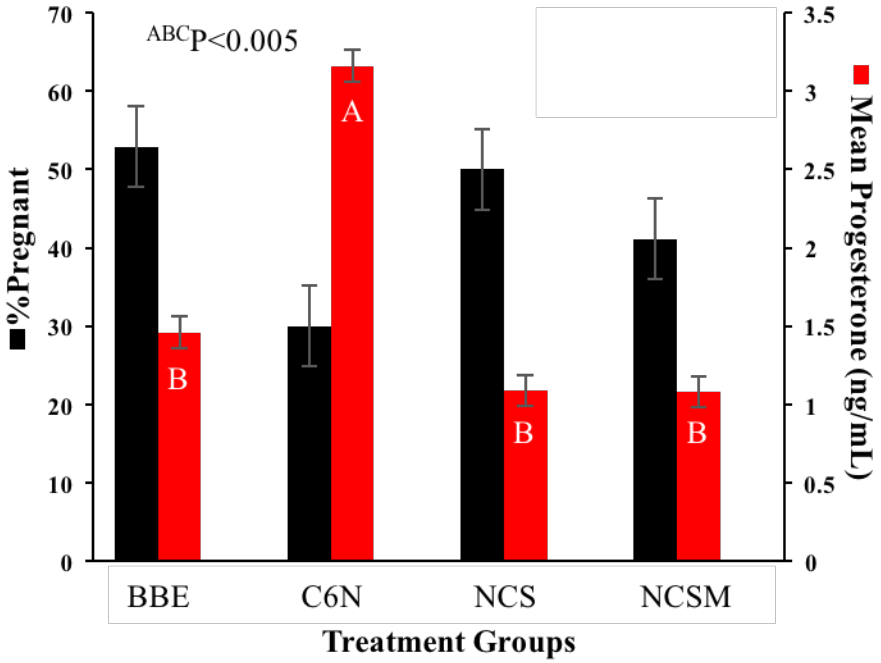

Figure 6. *Experiment 2.* Mean progesterone concentrations and pregnancy rates among breed by estrus (BBE), CIDR (C6N), original NC.Synch (NCS) and modified NC.Synch (NCSM) treatment groups

There was no difference in the number of hours from PGF to the LH surge between the NCS and NCSM groups. Thus, by moving the second PGF injection 12 hours later, we were unable to influence the timing of the LH surge in does of the NCSM treatment group.

The number of hours from the peak of the LH surge to breeding was calculated (Table 2). On average, the LH surge lasted 6-12 hours. The timing of the LH surge varied from 6 hours before to 24 hours after breeding. Animals were grouped so there were equal numbers of does within each classification (Table 2). There was also an equal distribution of treatment groups among these classifications (Appendix C). When does were bred 6 hours before or at the time of the LH surge (n=9) the resulting pregnancy rate achieved was 33%. Similarly (P>0.05), does bred 6-12 hours after the surge (n=10), achieved a pregnancy rate of

20%. In contrast (P<0.05), does bred 18-24 hours after the LH surge (n=9) achieved a pregnancy rate of 77%.

Table 1. *Experiment 2.* Hours (ls mean ± SEM) from last PGF injection to LH peak in all treatment groups

TREATMENT GROUP	MEAN HOURS FROM PGF TO LH SURGE
BBE	53.0 ± 3.9^{AB}
C6N	46.3 ± 3.6^{B}
NCS	60.0 ± 3.6^{A}
NCSM	50.6 ± 3.6^{AB}
^{A,B}P=0.08	

Table 2. *Experiment 2.* Hours from LH peak to breeding with corresponding pregnancy rates (ls mean ± SEM)

HOURS FROM LH PEAK TO BREEDING	PREGNANCY RATE (%)
-6-0hrs (n=9)	33.3 ± 0.1^{A}
6-12hrs (n=10)	20.0 ± 0.1^{A}
18-24hrs (n=9)	77.8 ± 0.2^{B}
^{A,B}P<0.05	

Pregnancy status was determined by ultrasonography at 50 days post-AI. A total of 53±12%, 30±11%, 50±11% and 41±12% of does in the BBE, C6N, NCS and NCSM groups, respectively, became pregnant following AI (Figure 6). There was no difference in pregnancy rates observed among does in all treatment groups. In contrast to experiment 1, we did not observe a concordance between progesterone concentrations in the period prior to AI and pregnancy rates.

There was no difference in either the number of kids born or total kid weight amongst all treatment groups ($P>0.05$, Appendix C). Also, there was no effect of AI technician observed on pregnancy rates achieved ($P>0.05$, Appendix C). There was no effect of treatment on mucus consistency at time of breeding ($P>0.05$, Appendix C). However, we did observe that of the five does that had late estrus, cottage cheese-like mucus, 2 were in the NCS group, 2 were in the NCSM group and 1 was in the C61 group.

Discussion

Use of ovulation synchronization and fixed timed artificial insemination (TAI) has a number of benefits to goat producers. It can speed the progression of genetic merit, allow gestating females be managed as a group, and improve breeding efficiency. There are a number of methods available to producers to synchronize ovulation in goats including short-term CIDR protocols combined with prostaglandin treatment [34] and progestagen-free protocols such as NC.Synch [50]. The present experiments were designed to examine the efficacy of progesterone-based and progesterone-free protocols for synchronizing ovulation in goats for TAI.

In Experiment 1, for all does synchronized with a CIDR, whether it was new, once-used or twice-used, progesterone concentrations increased immediately after CIDR insertion and remained elevated until time of device removal. Furthermore, even with the twice-used CIDRs having been used for a total of 21 days, there was no difference in mean concentration of progesterone found in does from any of the CIDR treatments. Comparable pregnancy rates ranging from 56% to 76% were observed for all of the CIDR treatment groups, therefore, it is clear that CIDRs can be reused to their full 21-day lifespan to successfully synchronize ovulation for TAI. With reuse of CIDRs, however, the risk for disease transmission remains. Therefore, if producers choose to reuse CIDRs it is recommended that precautions including proper cleaning and storage, be taken to minimize this risk.

In cattle, exposure to insufficient concentrations of progesterone during the period of growth of the ovulatory follicle is an important factor that adversely affects fertility [92-94]. In Experiment 1, our observation that the mean concentrations of progesterone during the synchronization period prior to AI paralleled pregnancy rates following AI is consistent with this conclusion. Unfortunately, this was not observed in Experiment 2. There was no difference in pregnancy rates or mean progesterone concentration in the period before AI between the BBE, NCS or NCSM treatment groups. Although mean progesterone was higher in does in the CIDR group it was not associated with a higher pregnancy rate.

Based on individual progesterone profiles of does in Experiment 2 only 50% of the animals sampled had endogenous progesterone at the beginning of the experiment compared to 100% of the animals sampled in Experiment 1. A fully cycling herd was defined as at least 75% of the animals having a CL, or endogenous progesterone, at any given time. It was

hypothesized that the does were not fully cycling at the start of Experiment 2 which may be the reason why pregnancy rates were significantly lower than in Experiment 1 ($P<0.05$). Thus, to maximize fertility post-AI, it is suggested that does be cycling well before implementing an ovulation synchronization protocol in a herd.

Experiment 2 tested the hypothesis that changing the timing of the second prostaglandin injection would improve pregnancy rates. However, no difference in pregnancy rates achieved between the modified protocol and the standard NCS protocol was found. Thus, modification of the NCS protocol was not recommended.

When compared to other ovulation synchronization protocols used in small ruminants, the NCS protocol combines simplicity and efficacy. Data from previous years shows NCS having a pregnancy rate of 60% [45]. The Synchrovine protocol used in sheep is 2 prostaglandin injections 7 days apart followed by TAI 42 hours later. Although this protocol is simple, a low conception rate of 38% was obtained [34]. When the OvSynch protocol was used in goats [54], a pregnancy rate of 58% was achieved. This was comparable to pregnancy rates achieved with NCS, however, the OvSynch protocol requires more instances of animal handling than the NCS protocol.

Ovulation synchronization experiments in sheep have also evaluated variations in the timing between the second prostaglandin injection and administration of GnRH at the time of insemination in order to improve ovulatory synchrony [54, 95, 96]. Oliviera et al. [34] attempted several experiments in sheep involving the Synchrovine protocol and giving GnRH at TAI at 24, 36 and 44 hours after the last prostaglandin injection. The resulting pregnancy rates were 10%, 33% and 38%, respectively, with no difference in pregnancy rates among ewes inseminated at 36 and 44 hours [95, 96]. Although these protocols resulted in

good synchrony of estrus and ovulation in sheep, they resulted in low pregnancy rates after cervical or intrauterine insemination despite the fact that fresh semen was used for insemination.

According to the data, the optimal time for TAI in does was 18 to 24 hours after the LH surge. Does will ovulate about 24 hours after the onset of the LH surge [97]. This was also true when does are synchronized by the buck effect or synchronized with progesterone sponges [98]. By breeding 18 to 24 hours after the LH surge, insemination likely occurred concurrently with ovulation. Interestingly in the current study, LH surges occurred between 50 and 60 hours after the final prostaglandin injection in both the NCS and NCSM groups. This was actually before the time of the final GnRH injection in the NCS and NCSM treatments. Thus, animals in these groups most likely had a spontaneous LH surge, not an LH surge induced in response to the final GnRH injection. Therefore, it is possible that the final GnRH injection of the NCS protocol may not be necessary.

Conclusions

Several conclusions can be drawn from these experiments. First, reused CIDRs are equally as effective at maintaining elevated progesterone concentrations during insertion and in achieving satisfactory pregnancy rates as new CIDRs. Second, levels of progesterone in the treatment period prior to AI can affect fertility in the subsequent estrus cycle in goats. Third, the current timing of injections in the NC.Synch protocol is effective at synchronizing ovulation. Finally, highest pregnancy rates are achieved when insemination occurs at 18 to 24 hours after the LH surge.

CHAPTER III: PSPB as a Diagnostic Tool for Predicting Multiple Fetuses

Introduction

A reliable technique for early detection of pregnancy in goats is necessary to determine culling and re-breeding decisions [64]. The inability to detect pregnancy early in gestation can result in economic losses in milk or meat production due to a longer kidding interval [64]. The traditional method for pregnancy detection in goats is estrus detection 21 days post-breeding. Animals will return to estrus if there was a fertilization failure; therefore, a non-return to estrus would indicate pregnancy [65]. However, a non-return to estrus can also be observed in goats that have long cycles or become anestrous, making estrus detection an unreliable method for pregnancy detection [64, 65].

Ultrasonography has been shown to be a very useful tool for pregnancy detection in goats. Limitations of ultrasound include an extended urinary bladder, hydrometra and pyometra which can all result in false positives [64]. Older, multiparous animals also have a greater risk of generating a false positive due to more folds in the uterus that make it more difficult to see the embryo clearly [68]. When utilizing transrectal ultrasonography it requires extraction of the feces from the rectum along with complete immobilization of the animal to avoid rectal damage [69]. For these reason, this method may not be the best suited for field conditions [65]. Transabdominal ultrasonography is a less invasive procedure and has been shown to be accurate in early pregnancy detection [67]. However, ultrasonography requires expertise to obtain a reliable diagnosis early in pregnancy, making it less suitable for producers with limited resources and training [70].

In addition to ultrasonography, serological assessments provide another method to determine pregnancy. Examples of serological methods include progesterone (P4), pregnancy

associated glycoprotein (PAG), or Pregnancy Specific Protein B (PSPB) assay. Whereas, ultrasonography gives an immediate diagnosis of pregnancy, serological methods require laboratory analysis which precludes immediate results [65]. However, when working large numbers of animals, utilizing serological methods may be more efficient than ultrasonography [65].

Humblot et al. [84] characterized circulating concentrations of PSPB throughout gestation in French alpine dairy goats. Goats with two or more conceptuses expressed an earlier rise in PSPB and had a higher mean concentration than those with singletons. However, it was concluded that individual variation was too high to use this as a diagnostic tool for predicting multiple births [84]. A similar study was done to characterize PSPB profiles in cows with singletons or twins [85]. Cows carrying twin IVF fetuses had significantly higher PSPB concentrations at Days 40 and 240 of gestation compared to those carrying single IVF pregnancies [85].

BioTracking (Moscow, ID) has developed a PSPB ELISA to test for pregnancy in cattle. This test is called BioPryn, where PRYN stands for 'Pregnant Ruminant Yes/No.' Establishment of the BioPryn ELISA resulted in an affordable, blood-based pregnancy test for cattle. In 2004, a test for sheep and goats was established. In 2015 a plate capable of testing cattle, sheep and goats simultaneously was developed.

The objective of this experiment was to assess the efficacy of using serum PSPB concentrations measured using the BioPryn ELISA from samples taken throughout gestation to predict multiple births in does. The ability to predict which females in a herd are carrying multiple fetuses would allow them to be managed more appropriately to maximize animal productivity.

Materials and Methods

Experimental Design

A total of 62 Boer-cross does were used in this experiment which was conducted over a 2 year period. Animals were grazing a fescue and orchardgrass mixed pasture and supplemented with 0.5-1.0 lb of 12% crude protein grain mix per head per day. Animals were examined several times throughout the experiment and those with a Famacha score above 3 were dewormed with an oral drench of Cydectin and Prohibit per farm protocol. At approximatly 48 and 85 days after artificial insemination, pregnancy was determined via transabdonimal ultrasonagraphy. Blood samples were drawn from the jugular vein into a glass, silicone coated, vacutainer tube. Serum was harvested and stored at -20°C. For each doe the number of kids born and total kid weight at kidding was recorded.

Pregnancy Specific Protein B (PSPB) Analysis

Serum samples were assayed for PSPB using the BioPryn ELISA kit (Biotracking LLC; Moscow ID). A standard curve was included to allow for quantification of PSPB levels [99]. Assays were run as per manufacturer's instructions. A total of 6 assays were run and both a high and a low PSPB pool sample was included in each assay. The interassay COV based on duplicate pool samples was 6.60% and 9.83%, and the intrassay COV was 1.98% and 0.95% for the high and low pool samples, respectivley.

Statistical Analysis

Does were grouped by number of kids born (singleton, n = 19; twins, n = 28; triplets, n = 15) or by quartiles of total kid weight at birth (1^{st} quartile [0-9 lb], n = 16; 2^{nd} quartile [9.1-13.9 lb], n = 15; 3^{rd} quartile [14-16.9 lb], n = 18; 4^{th} quartile [17-25 lb], n = 13). Overall, serum PSPB concentrations were analyzed using a two-way ANOVA that included year

sample was taken and day of gestation. Additional two-way ANOVA models examined the effect of kid number and year sample was taken on serum PSPB concentrations at Day 48 or Day 85. Two-way ANOVA models were also used to examine the effect of kid weight quartile and year samples were taken on serum PSPB concentrations at Day 48 or 85. All means were seperated by a Duncan's test.

Results

There was a tendency for an increase in PSPB between Day 48 and Day 80 of gestation (P=0.079). The mean PSPB level at Day 48 was 26.8±1 ng/mL and the mean PSPB level at Day 80 was 28.6±0.1 ng/mL. There was also an effect of the year samples were taken on the level of PSPB measured. The mean PSPB concentrations from samples taken in year 1 were higher (P<0.05) than in year 2, 28.8 ± 0.1 ng/mL and 26.7 ± 0.1 ng/mL, respectively.

Of the 62 does sampled over a two-year period, the population was divided into quartiles based on total kid weight at birth. There were 16 does with a total birth weight in the first quartile (0-9lb), 15 does in the second quartile (9.1-14lb), 18 does in the third quartile (14.1-16.9lb) and 13 does in the fourth quartile (17-21lb). For this same population of pregnant animals, 19 had singletons, 28 had twins and 15 had triplets.

At Day 48 of gestation mean PSPB concentrations for does with a total kid weight in the first quartile were lower (P<0.005) compared to does in the second, third and fourth quartile (21.6±0.1 ng/mL, 28.6 ± 0.2 ng/mL, 29.7 ± 0.2 ng/mL and 30.5 ± 0.2 ng/mL, respectively, Figure 1). There was no difference in PSPB concentrations amongst does with a total kid weight in the second, third and fourth quartiles (Figure 1). At Day 48 mean PSPB

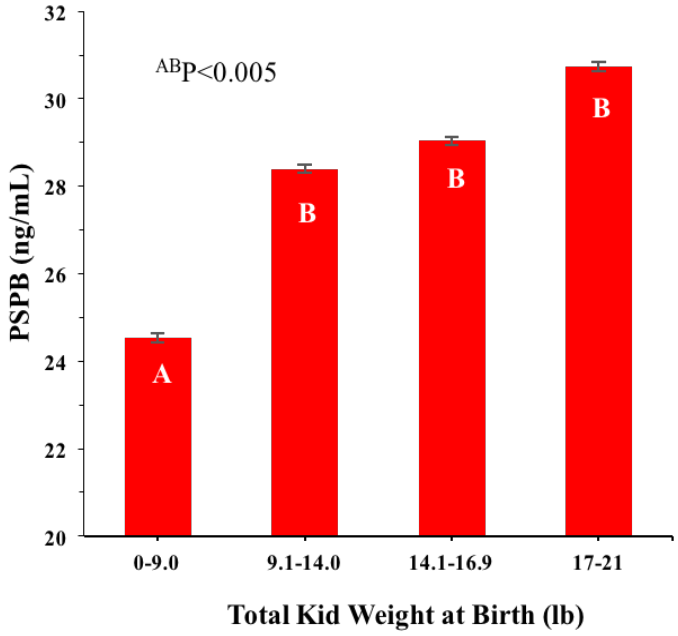

Figure 1. Mean (ls mean ± SEM) PSPB concentrations at Day 48 for kid weight quartiles.

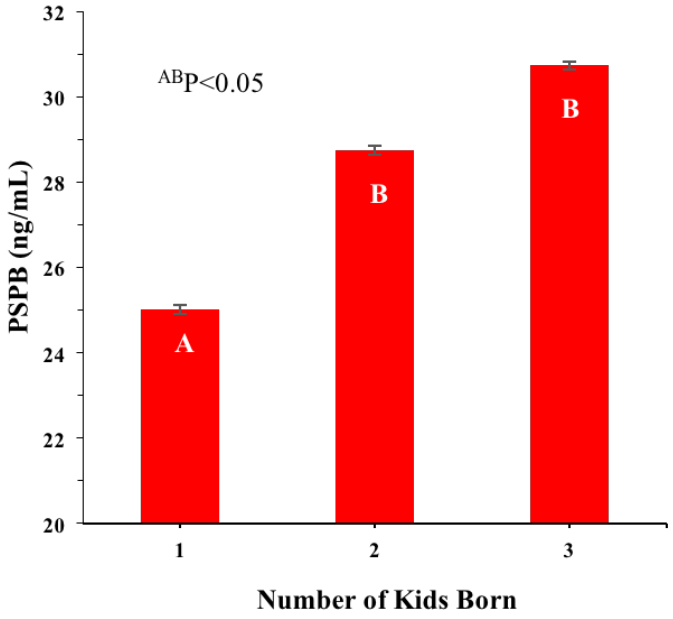

Figure 2. Mean (ls mean ± SEM) PSPB concentrations at Day 48 for total number of kids born

concentration for does that had singletons were significantly lower ($P<0.005$) than for does that had twins or triplets (23.0 ± 0.2 ng/mL, 28.9 ± 0.1 ng/mL and 29.9 ± 0.2 ng/mL, respectively, Figure 2). There was no difference in PSPB concentrations among does that had twins or triplets (Figure 2).

Mean PSPB concentrations at Day 85 of gestation tended to be lower ($P=0.1$) for does in the first and second quartile compared to does in the third and fourth quartile (27.4 ± 0.1 ng/mL, 28.2 ± 0.1 ng/mL, 28.3 ± 0.1 ng/mL and 30.9 ± 0.1 ng/mL, respectively, Figure 3). At Day 85 mean PSPB concentrations were different ($P<0.05$) in does carrying singletons, twins or triplets (27.0 ± 0.1 ng/mL, 28.5 ± 0.1 ng/mL and 31.6 ± 0.1 ng/mL, respectively, Figure 4).

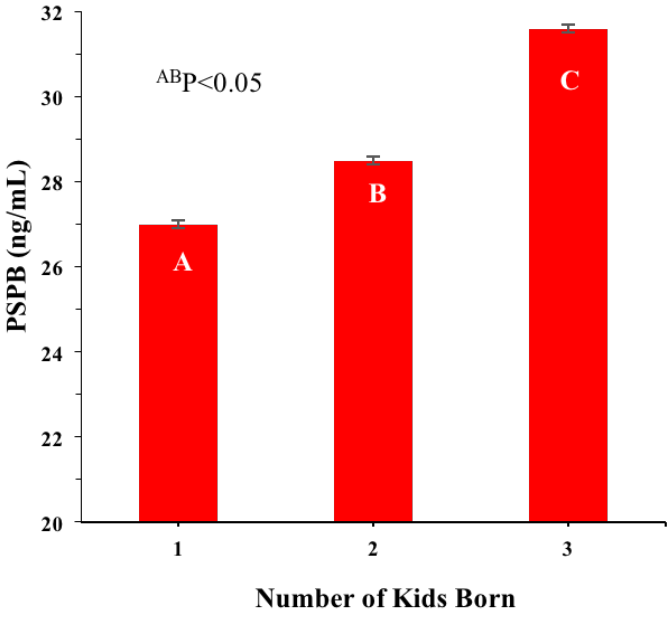

Figure 3. Mean (ls mean ± SEM) PSPB concentrations at Day 85 for total number of kids born

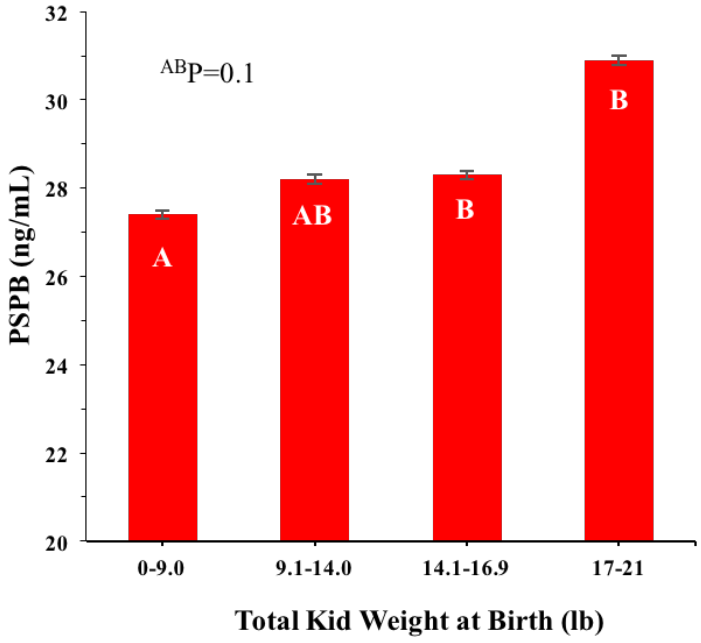

Figure 4. Mean (ls mean ± SEM) PSPB concentrations at Day 85 for kid weight quartiles.

Discussion

When working large numbers of animals, utilizing serological methods for pregnancy detection may be more efficient than ultrasonography. There are currently affordable blood pregnancy tests available to goat producers on a commercial scale where farmers simply mail a blood sample to a lab and get results back in a couple of days. Blood tests measuring PSPB have proven to be more reliable than pregnancy tests that rely on other hormones such as progesterone [65]. It has been hypothesized that PSPB concentrations throughout gestation can also be used to predict multiple births in small ruminants. The ability to predict which females in a herd are carrying multiple fetuses would allow them to be managed more appropriately to maximize animal welfare and productivity. Most, if not all, of the previous experiments examining the efficacy of PSPB as a diagnostic tool to predict multiples have used an RIA to measure PSPB concentrations in the blood. This experiment utilized an ELISA to measure PSPB concentrations and examined the relationship between PSPB concentrations during gestation and the incidence of multiple births in goats.

When blood was drawn at Day 48 or 85 of gestation, there was a significant difference in serum PSPB concentrations between does carrying singletons or multiples. There was also a significant difference between does with a lighter fetal mass (0-9lb) or heavier fetal mass (9.1-21lb) at birth. Between Days 48 and 85 of gestation the range of mean serum PSPB concentration was 27-29 ng/mL. This is similar to what has been previously reported at this stage of gestation [84, 100].

Humblot et al. [84] monitored PSPB concentrations throughout gestation for 25 goats that were synchronized and artificially inseminated. They found that PSPB concentrations were higher in goats carrying twin fetuses than those carrying singletons. However, it was

concluded that individual variation of PSPB concentration was too high to make PSPB an effective diagnostic tool for predicting multiples.

A later study done by Willard et al. [100] measured PSPB concentrations throughout gestation in 53 ewes that were synchronized and bred by a ram. Between days 60 and 120 of gestation, PSPB concentration was 78% accurate in identifying ewes that were carrying twins. The main factors that affected PSPB concentration were breed, fetal number, and day of gestation. They recommended that commercial producers take breed, body condition score, and previous history of twinning into consideration when using PSPB as a diagnostic tool for predicting multiples.

Both of these studies utilized an RIA to measure PSPB concentrations. The use of an RIA requires the disposal of radioactive material, thus, making an RIA more expensive and difficult to use on a commercial scale. As stated earlier, there is a commercially available, affordable, ELISA-based blood pregnancy test available to goat producers. This study was able to use an ELISA to measure serum concentrations of PSPB in 70 does and discern between singleton bearing and twin bearing females. Further studies need to be done to determine viability across different breeds of goats, different management conditions, different regions and different laboratories running the ELISA.

The use of an ELISA to quantify PSPB in the blood presents producers with an economically feasible option for diagnosing pregnancy and a potential diagnostic tool for predicting multiple births in their herd. This test would only require producers to draw a blood sample from the animal and, therefore, could be easily implemented into the reproductive strategies of a farm. This experiment took blood samples at Day 45 and Day 85 of gestation, but the ELISA can also be used as a method for early pregnancy detection

beginning at Day 30. Future studies would need to determine whether PSPB concentrations at Day 30 of gestation are also correlated with the total number of kids born. Being able to diagnose pregnancy and the number of fetuses the does is carrying from a single blood sample at Day 30 would allow the producer to make more informed management and culling decisions.

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Appendices

Appendix A

List of Acronyms

BBE, breed by estrus

CIDR, controlled internal drug release

C61, once-used CIDR treatment group

C62, twice-used CIDR treatment group

C6N, new CIDR treatment group

LH, luteinizing hormone

NCS, NC.Synch (a progesterone-free ovulation synchronization protocol)

NCSM, NC.Synch modified (modification of the original NC.Synch protocol)

PAG, pregnancy associated glycoprotein

PGF, prostaglandin-F2α

PMSG, pregnant mare's serum gonadotropin

PSPB, pregnancy specific protein B

TAI, timed artificial insemination

Appendix B

Individual Animal Hormone Profiles for Experiments 1 and 2

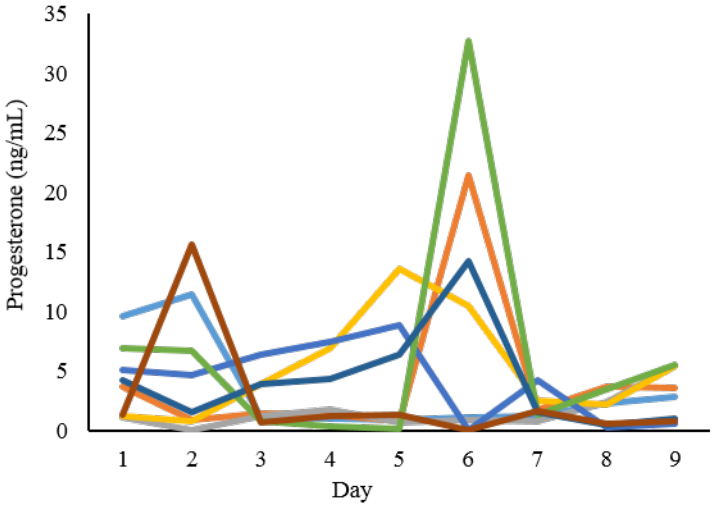

Figure B1. *Experiment 1.* Individual progesterone profiles for does in the BBE group

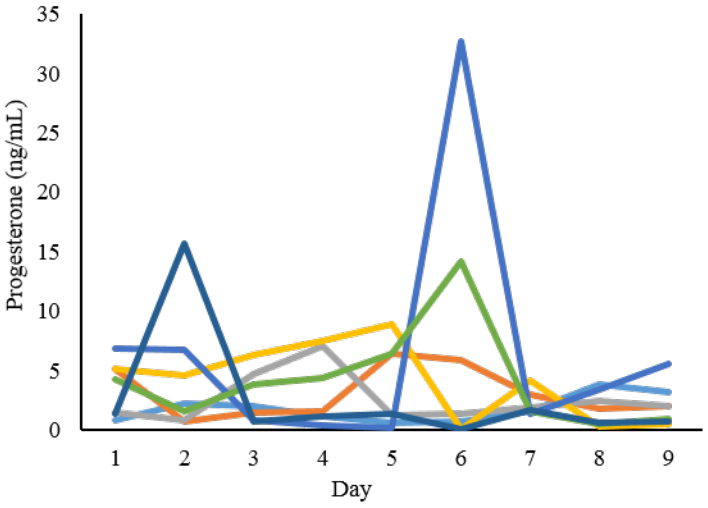

Figure B2. *Experiment 1.* Individual progesterone profiles for does in the NCS group

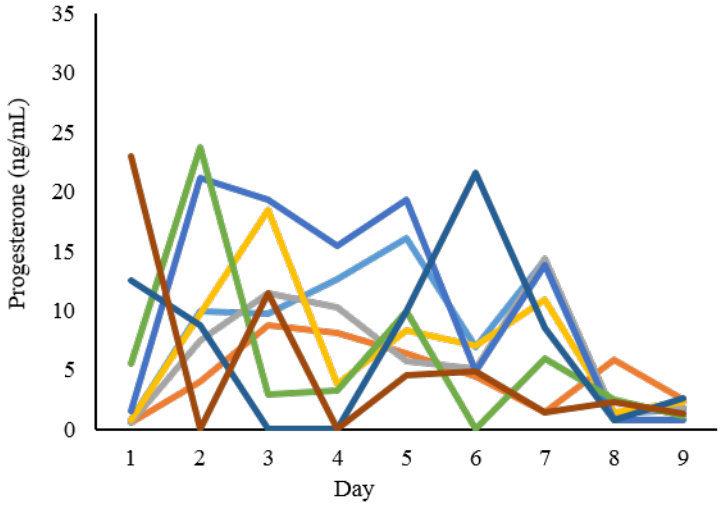

Figure B3. *Experiment 1.* Individual progesterone profiles for does in the C6N group

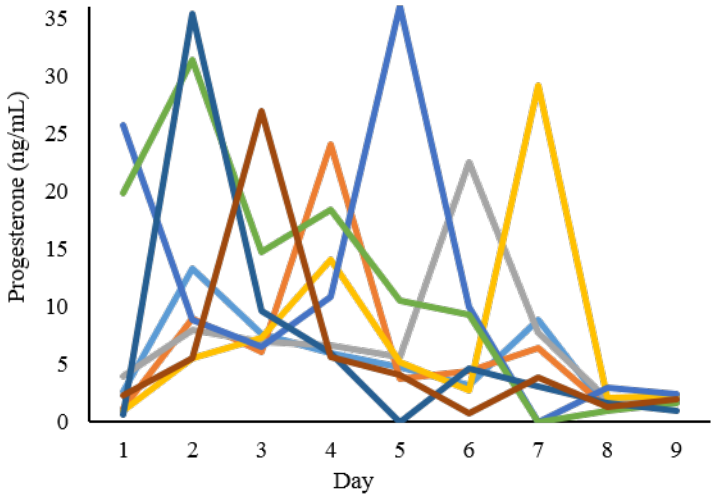

Figure B4. *Experiment 1.* Individual progesterone profiles for does in the C61 group

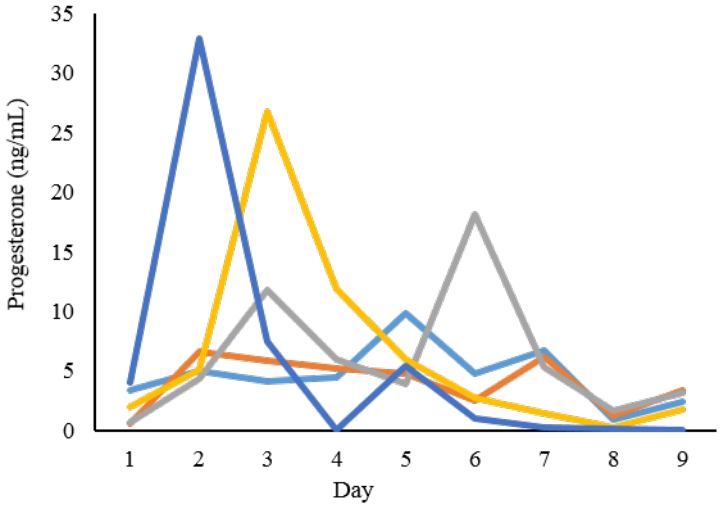

Figure B5. *Experiment 1.* Individual progesterone profiles for does in the C62 group

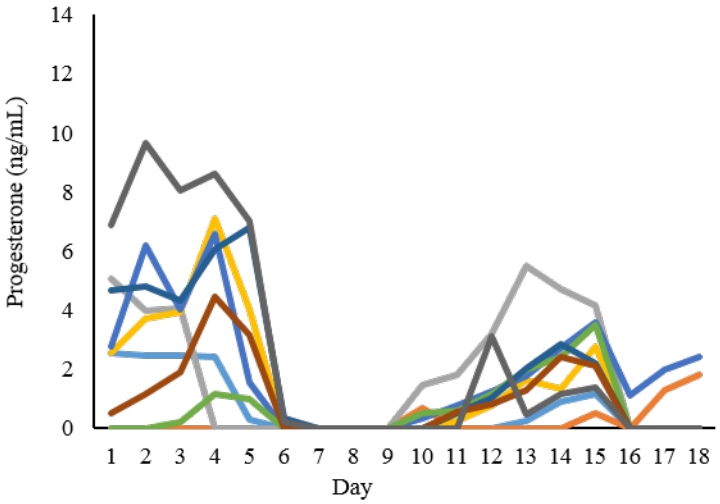

Figure B6. *Experiment 2.* Individual progesterone profiles for does in the BBE group

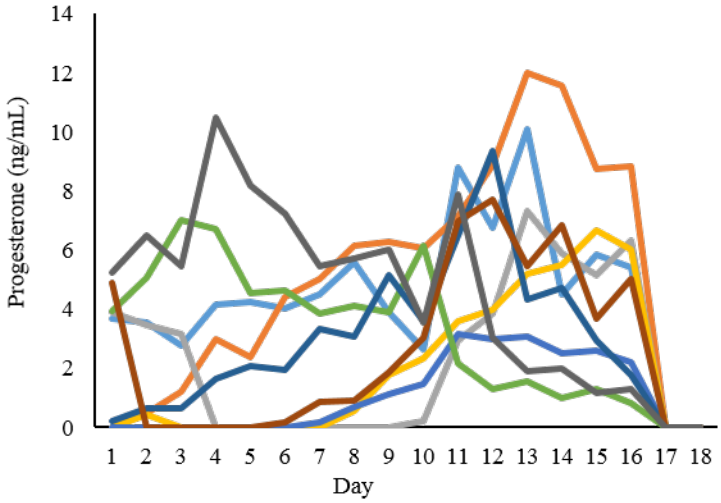

Figure B7. *Experiment 2.* Individual progesterone profiles for does in the C6N group

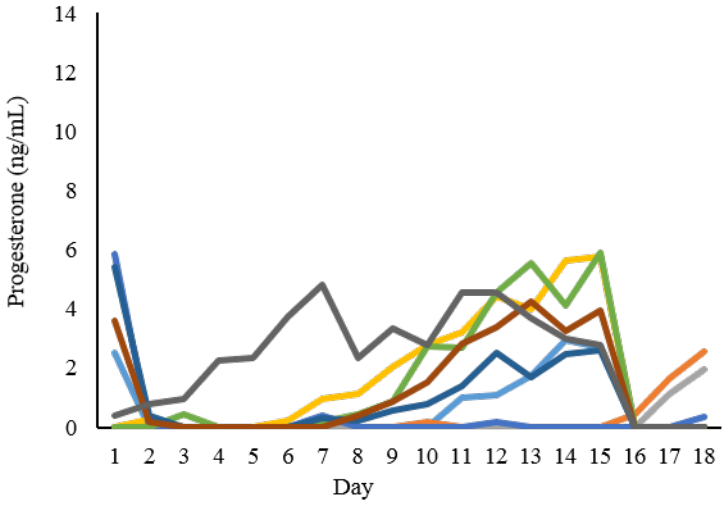

Figure B8. *Experiment 2.* Individual progesterone profiles for does in the NCS group

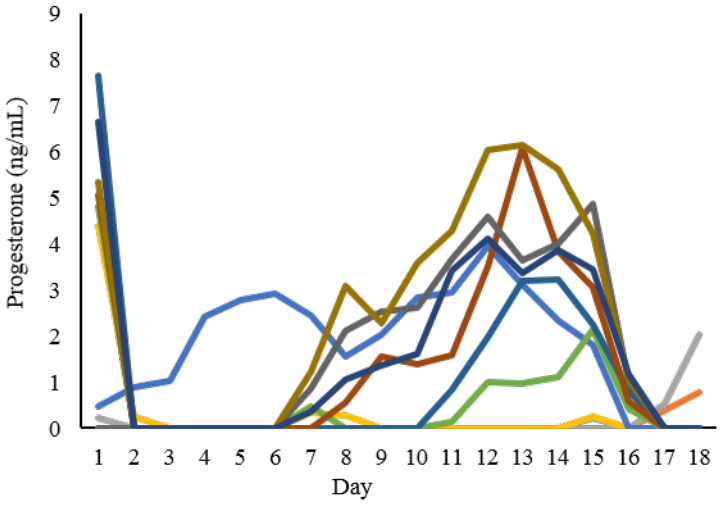

Figure B9. *Experiment 2.* Individual progesterone profiles for does in the NCSM group

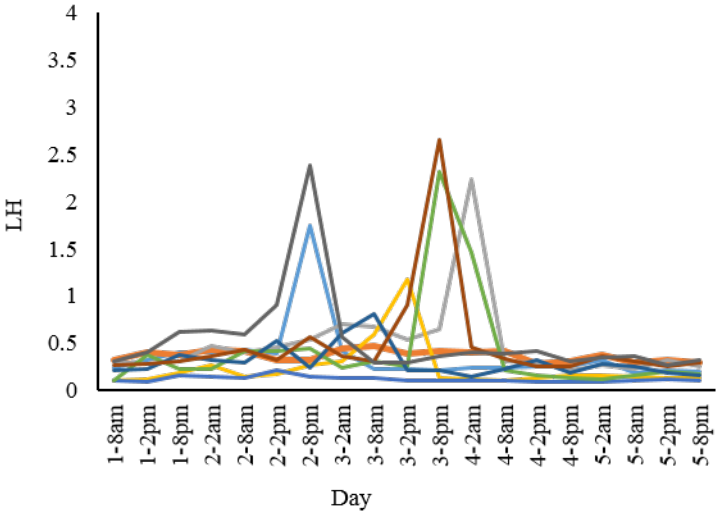

Figure B10. *Experiment 2.* Individual LH profiles for does in the BBE group

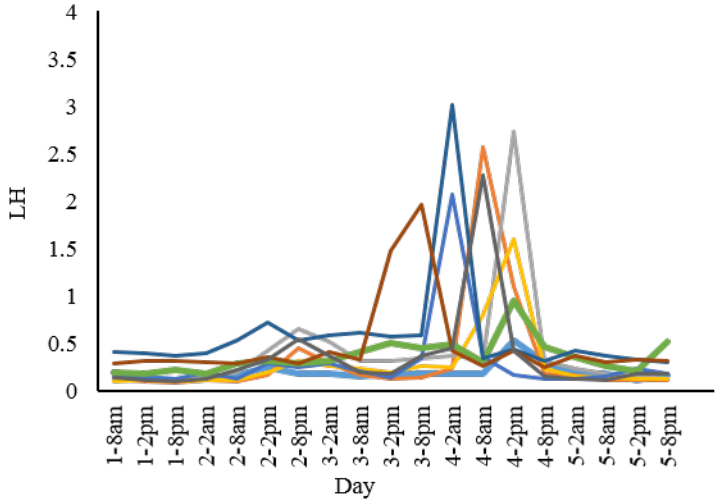

Figure B11. *Experiment 2.* Individual LH profiles for does in the C6N group

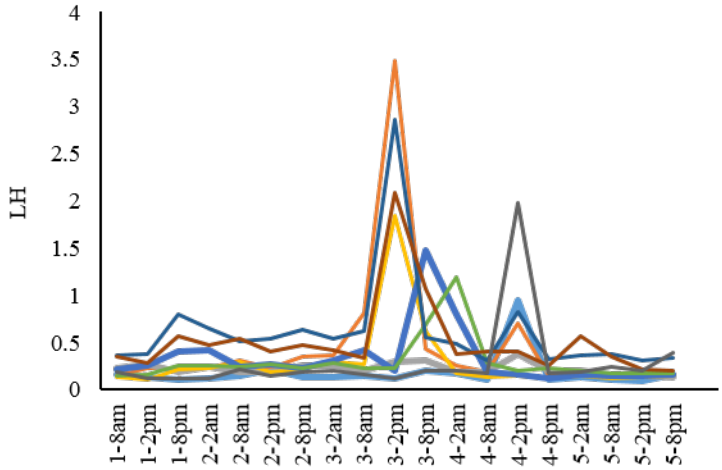

Figure B12. *Experiment 2.* Individual LH profiles for does in the NCS group

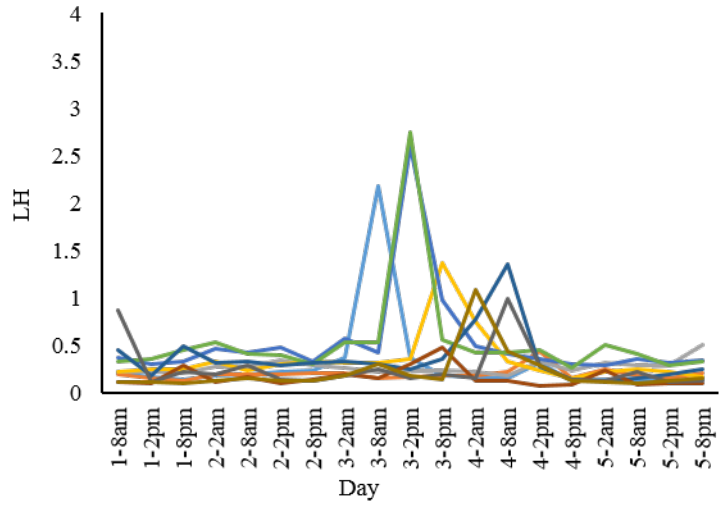

Figure B13. *Experiment 2.* Individual LH profiles for does in the NCSM group

Appendix C

ANOVA Tables for all Experiments

Table C1 – ANOVA table for progesterone values data analysis in Experiment 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	89	6476.56397	72.77038	1.74	0.0004
Trt	4	803.116153	200.779038	4.81	0.0009
Rep	1	309.108802	309.108802	7.40	0.0070
Day	8	1732.300799	216.537600	5.18	<0.0001
Trt*Rep	4	50.000663	12.500166	0.30	0.8783
Trt*Day	32	1569.462649	49.045708	1.17	0.2462
Rep*Day	8	962.787759	120.348470	2.88	0.0043
Trt*Rep*Day	32	1142.616618	35.706769	0.85	0.6946
Error	260	10859.45958	41.76715		
Total	349	17336.02356			

Table C2 – ANOVA table for pregnancy rates data analysis in Experiment 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	4.33559432	0.48173270	2.13	0.0365
Trt	4	3.17225020	0.79306255	3.51	0.0110
Rep	1	0.12053571	0.12053571	0.53	0.4671
Trt*Rep	4	0.97925172	0.24481293	1.08	0.3701
Error	76	17.15277778	0.22569444		
Total	85	21.48837209			

Table C3 – ANOVA table for total number of kids born data analysis in Experiment 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	6.25647841	0.69516427	1.32	0.2641
Trt	4	0.33182540	0.08295635	0.16	0.9582
Rep	1	1.53491012	1.53491012	2.92	0.0971
Trt*Rep	4	4.64433951	1.16108488	2.21	0.0899
Error	33	17.37142857	0.52640693		
Total	42	23.62790698			

Table C4 – ANOVA table for AI technician data analysis in Experiment 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	63.28638988	0.36515443	1.53	0.1526
Trt	4	2.97788356	0.74447089	3.13	0.0198
Tech	1	0.00062409	0.00062409	0.00	0.9593
Trt*Tech	4	0.33469120	0.08367280	0.35	0.8423
Error	73	17.38830891	0.23819601		
Total	82	20.67469880			

Technician	Pregnancy Rate (%)*
1	54.1 ± 0.1
2	53.5 ± 0.1
*ls means ± SEM	

Table C5 – ANOVA table for mucus discharge data analysis in Experiment 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.66060986	0.16515246	1.69	0.1769
Trt	4	0.66060986	0.16515246	1.69	0.1769
Error	33	3.23412698	0.09800385		
Total	37	3.89473684			

Table C6 – ANOVA table for sire data analysis in Experiment 1

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	49	13.00803213	0.26547004	1.14	0.3470
Trt	4	2.38800194	0.59700049	2.57	0.0561
Sire	12	3.34031239	0.27835937	1.20	0.3248
Trt*Sire	33	7.46350615	0.22616685	0.97	0.5305
Error	33	7.66666667	0.23232323		
Total	82	20.67469880			

Table C7 – ANOVA table for progesterone values data analysis in Experiment 2

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	71	1450.444666	20.428798	6.34	<0.0001
Trt	3	488.7590293	162.9196764	50.59	<0.0001
Day	17	474.934457	27.9373145	8.68	<0.0001
Trt*Day	51	486.8909714	9.5468818	2.96	<0.0001
Error	608	1957.911732	3.220250		
Total	679	3408.356399			

Table C8 – ANOVA table for pregnancy rate data analysis in Experiment 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.60922099	0.20307366	0.81	0.4927
Trt	3	0.60922099	0.20307366	0.81	0.4927
Error	70	17.55294118	0.25075630		
Total	73	18.16216216			

Table C9.1 – ANOVA table for hours from LH Surge to breeding data analysis in Experiment 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	3.27380952	0.32738095	1.55	0.2040
Surge	2	1.39687011	0.69843505	3.31	0.0609
Trt	3	0.21450895	0.07150298	0.34	0.7972
Surge*Trt	5	1.49909910	0.29981982	1.42	0.2663
Error	17	3.58333333	0.21078431		
Total	27	6.85714286			

Table C9.2 – ANOVA table for hours from LH Surge to breeding data analysis in Experiment 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.70158730	0.85079365	4.13	0.0283
Surge	2	1.70158730	0.85079365	4.13	0.0283
Error	25	5.15555556	0.20622222		
Total	27	6.85714286			

Hour Classification	Proportion of Animals			
	BBE	C6N	NCS	NCSM
1 (-6-0)	2/9	4/9	1/9	2/11
2 (6-12)	2/9	3/9	2/9	3/11
3 (18-24)	2/9	0/9	4/9	3/11

Table C10 – ANOVA table for Hours from PGF to LH Peak data analysis in Experiment 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	691.523810	230.507937	2.54	0.0814
Trt	3	691.5238095	230.5079365	2.54	0.0814
Error	23	2087.142857	90.745342		
Total	26	2778.666667			

Table C11 – ANOVA table for AI technician data analysis in Experiment 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2.72032193	0.24730199	0.98	0.4728
Trt	3	1.21040775	0.40346925	1.60	0.1985
Tech	2	0.01939021	0.00969510	0.04	0.9623
Trt*Tech	6	1.60972735	0.26828789	1.07	0.3935
Error	59	14.85714286	0.25181598		
Total	70	17.57746479			

AI technician	Pregnancy Rate (%) *
1	47.5 ± 0.1
2	43.4 ± 0.1
3	45.0 ± 0.1

*ls mean ± SEM

Table C12 – ANOVA table for mucus discharge data analysis in Experiment 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.56862745	0.18954248	0.54	0.6598
Trt	3	0.56862745	0.18954248	0.54	0.6598
Error	64	22.66666667	0.35416667		
Total	67	23.23529412			

Table C13 – ANOVA table for sire data analysis in Experiment 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	8.37746479	0.29919517	1.37	0.1767
Trt	3	0.36378206	0.12126069	0.55	0.6486
Sire	9	2.82697155	0.31410795	1.43	0.2049
Trt*Sire	16	4.71429072	0.29464317	1.35	0.2165
Error	42	9.20000000	0.21904762		
Total	70	17.57746479			

Table C14 – ANOVA table for number of kids born (Kn) and Day 48 PSPB data analysis for Experiment 3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4.37235545	0.87447109	2.13	0.0747
Kn	2	4.19416873	2.09708436	5.12	0.0091
Yr	1	1.49378518	1.49378518	3.64	0.0614
Kn*Yr	2	0.15192812	0.07596406	0.19	0.8313
Error	56	22.95438810	0.40989979		
Total	61	27.32674355			

KN	PSPB (ng/mL)*
1	23.0 ± 0.16^{A}
2	28.9 ± 0.14^{B}
3	29.9 ± 0.21^{B}
*ls mean $\pm$ SEM $^{A,B}P<0.05$	

Yr	PSPB (ng/mL)*
1	25.4 ± 0.1^{A}
2	29.1 ± 0.2^{A}
*ls mean $\pm$ SEM P=0.0614	

Table C15 – ANOVA table for total kid weight (Kw) and Day 48 PSPB Data Analysis for Experiment 3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	8.22211055	1.17458722	3.36	0.0047
Kw	3	7.57130791	2.52376930	7.22	0.0004
Yr	1	1.05518481	1.05518481	3.02	0.0879
Kw*Yr	3	0.69165459	0.23055153	0.66	0.5804
Error	55	19.22198786	0.34949069		
Total	62	27.44409841			

Kw	PSPB (ng/mL)*
1	21.56 ± 0.15^{A}
2	28.6 ± 0.16^{B}
3	29.7 ± 0.17^{B}
4	30.5 ± 0.17^{B}
*ls mean ± SEM $^{A,B}P<0.05$	

Yr	PSPB (ng/mL)*
1	26.2 ± 0.1^{A}
2	29.0 ± 0.1^{A}
*ls mean ± SEM P=0.0879	

Table C16 – ANOVA table for number of kids born and Day 85 PSPB data analysis for Experiment 3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	5.13563883	1.02712777	7.72	<0.0001
Kn	2	1.418674747	0.70933737	5.33	0.0076
Yr	1	1.48893083	1.48893083	11.19	0.0015
Kn*Yr	2	0.09936316	0.04968158	0.37	0.6901
Error	56	7.45209666	0.13307315		
Total	61	12.58773548			

Kn	PSPB (ng/mL)*
1	27.0 ± 0.1^{A}
2	28.5 ± 0.1^{B}
3	31.6 ± 0.1^{C}
*ls mean $\pm$ SEM $^{A,B,C}P<0.05$	

Yr	PSPB (ng/mL)*
1	30.9 ± 0.1^{A}
2	27.3 ± 0.1^{A}
*ls mean $\pm$ SEM $^{A,B}P<0.05$	

Table C17 – ANOVA table for total kid weight and Day 85 PSPB data analysis for Experiment 3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	4.67643198	0.66806171	4.56	0.0005
Kw	3	0.89759519	0.29919840	2.04	0.1188
Yr	1	2.57038047	2.57038047	17.54	0.0001
Kw*Yr	3	0.22230901	0.07410300	0.51	0.6799
Error	54	7.91130350	0.14650562		
Total	61	12.58773548			

Kw	PSPB (ng/mL)*
1	27.4 ± 0.11^{A}
2	28.2 ± 0.11^{AB}
3	28.3 ± 0.11^{B}
4	30.9 ± 0.11^{B}
*ls mean $\pm$ sem $^{A,B}P=0.12$	

Yr	PSPB (ng/mL)*
1	30.9 ± 0.1^{A}
2	26.5 ± 0.1^{B}
*ls mean $\pm$ sem $^{A,B}P<0.005$	