Review Report

Manipulation of ovarian function in sheep and goats

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Abstract

Sheep and goats are short-day breeders. Melatonin, photoperiod, breed, nutrition, and geographical location influence seasonality. Estrus synchronization is useful in maximizing the profitability of small ruminant production. This article discusses the endocrine control of seasonality and the reproductive cycle, and manipulation of ovarian function in sheep and goats. The ovarian follicular wave pattern in these species is described to help the reader understand the mechanisms involved in the protocols for estrus synchronization and superovulation in small ruminants. The estrus synchronization protocol in these species encompasses using the male effect, photoperiod manipulation, and hormonal treatments. The male effect is an efficient synchronization technique during the transition period. Although not used routinely, photoperiod manipulation of seasonality is possible. Hormonal control of the cycle can be grouped into 2 categories; protocols used during the breeding season and protocols used for out-of-season breeding. During the breeding season, prostaglandin $F_{2\alpha}$ can be used for estrus synchronization but this protocol is not very efficient for timed artificial breeding. Progestogen-based protocols are commonly used for estrus synchronization, for both in- and out-of-season breeding. Such protocols are often combined with the use of equine chorionic gonadotropin (eCG) to improve synchrony and ovulation rate. Melatonin implants are used to induce cyclicity for out-of-season breeding and can be combined with progestogen synchronization protocols. Synchronization protocols in combination with the use of follicle stimulating hormone or eCG allow superovulation for the purpose of embryo production. The most common synchronization and superovulation protocols are described. The availability of hormones varies among countries.

Keywords: Small ruminants, estrus synchronization, superovulation, hormones, sheep, goat

Introduction

The profitability of sheep and goat production systems relies on a timely establishment of pregnancy with a goal of > 90% pregnancy rate. Estrus synchronization is a globally practiced technique in the breeding management of small ruminants.[1] Intensive production, particularly in sheep, relies on accelerated lambing systems. The consumer demand is higher during specific periods of the year. Melatonin-driven, photoperiodic seasonality limits producers from meeting this market demand. Estrus synchronization protocols for in- and out-of-season breeding management maximize profitability,[2] improve flock genetics and facilitate artificial insemination (AI) with the use of semen from superior sires. Furthermore, it is useful to match the production of a lamb or kid crop with consumer demand and availability of feed and cheaper labor, and allows efficient application of AI and embryo transfer (ET) programs.[3] In small ruminants, various synchronization methods are used, ranging from manipulation of light to a combination of hormones coupled with photoperiodic manipulation and male effect. Estrus synchronization protocols based on intravaginal delivery of progestogens followed by eCG treatment and luteolysis using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are the most commonly used.[4]

Current estrus synchronization protocols are not efficient enough to precisely synchronize time of ovulation. The protocols result in suboptimal fertility when applied to less prolific breeds and during out of season.[5] Compared to protocols involving natural mating, fertility following estrus synchronization is lesser for AI and ET programs. Genetic improvement requires the use of reproductive technologies such as AI, ET using in vivo-derived embryos, and, more recently oocyte collection for in vitro production of embryos. All these technologies require control and manipulation of ovarian follicular dynamics. The protocols used to achieve this are based on our understanding of ovarian follicular wave dynamics and hormonal mechanisms controlling them. Our aims are to: 1. describe the mechanisms controlling seasonality and estrous cycle; 2. discuss the hormonal and nonhormonal methods of induction and synchronization of cyclicity; and 3. describe common protocols for superovulation in sheep and goats.

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Seasonality of reproduction in sheep and goats

The seasonality of reproduction in ewes and does is a well-known biological phenomenon. In temperate zones, the breeding season corresponds to short days. At the end of this season, females undergo a transition period characterized by silent ovulations before entering a period of anestrus (i.e. absence of estrus and ovulation). Seasonal anestrus is followed by a transition period characterized by an increasing percentage of ovulating females but often with a short luteal phase during the first cycle. The breeding season is characterized by regular estrous cycles and an increased ovulation rate.[6–8]

The mechanisms that control the seasonality of reproduction have been studied for several years but have yet to be fully understood. The transition from seasonal anestrus to reproductive cyclicity is determined by the decreased sensitivity of the hypothalamus to estrogen-negative feedback. This change is induced by the photoperiod perceived by the retina and then transmitted by the retinohypothalamic tract to the suprachiasmatic nuclei and other structures, including the paraventricular nuclei of the hypothalamus, the mediolateral horn of the thoracic spinal cord, the superior cervical ganglia, and then the pineal gland (epiphysis).[1] The neural signal is then translated into an endocrine signal through melatonin secretion. Melatonin is produced overnight from tryptophan that is converted into serotonin and then N-acetylserotonin. Blood melatonin concentrations rise within 10 minutes after the onset of darkness and remain high throughout the night. The duration of melatonin secretion is proportional to dark hours of the night and is responsible for the seasonal variation in the photoperiod. Plasma melatonin concentrations are low during the day (< 5 pg/ml) and high at night (100-500 pg/ml in sheep and 50-150 pg/ml in goats). Melatonin is in much higher concentrations (100 times the plasma concentrations) in the cerebrospinal fluid of the 3rd ventricle.[1] Melatonin stimulates the increase in GnRH secretion that causes an increase in follicle stimulating hormone (FSH) and luteinizing hormone (LH) pulsatility, inducing follicular growth and ovulation. It is important to note that the effect of increasing melatonin on the hypothalamus, in response to decreasing daylight, takes 40-60 days.[6,7]

During seasonal anestrus, 2 mechanisms inhibit GnRH secretion. First, there is an increase in the sensitivity of the hypothalamus to the negative feedback of estradiol, involving the estradiol receptors within a population of glutamate neurons (preoptic and retrochiasmatic areas). These neurons stimulate a retrochiasmatic population of dopamine neurons, defined as the A15DA population that inhibits GnRH neurons. This inhibition occurs through a direct action at the terminals of GnRH neurons in the median eminence and through an indirect action inhibiting kisspeptin neurons in the arcuate nucleus that project directly onto GnRH neurons in the medio-basal hypothalamus. These mechanisms lead to a decrease in GnRH pulses, reducing LH and FSH secretion and thus inhibiting ovarian activity. The second mechanism involves an estradiol-independent inhibition of GnRH neurons through a melatoninergic signal of long photoperiod (summer). This signal stimulates the synthesis of thyroid stimulating hormone (TSH) in the pars tuberalis of the pituitary gland, inducing the conversion of triiodothyronine to thyroxine. This action stimulates RFRP-3 neurons and inhibits kisspeptin neurons, inhibiting GnRH secretion.[6,7] During the breeding season, the hypothalamus's sensitivity to estradiol's negative feedback is reduced, lifting the TSH inhibition pathway. This results in the activation of kisspeptin and GnRH neurons which culminate in the activation of the pituitary-gonadal axis.

The seasonality of reproduction in small ruminants is governed by a circannual biological clock, enabling endogenous reproductive seasonality to be maintained over several years without the intervention of photoperiod (constant conditions). In fact, keeping females under a short photoperiod (short days) does not extend the breeding season. A refractory period to photostimulation is necessary for the initiation and cessation of sexual activity.[6]

Seasonality is determined mainly by the photoperiod (high latitudes), but other factors can affect it, including ambient temperature, nutrition, altitude, age, and breed. In general, breeds of tropical (23°N-23°S) or subtropical (24°-34°N, 24°-34°S) origin have a longer breeding season than breeds from temperate zones (> 40°N or > 40°S). Breeds from intermediate or Mediterranean zones (35-40°N, 35-40°S) have a short anestrus period, during which a proportion of females continue to ovulate. Meat breeds are very seasonal. Prolific breeds, on the other hand, are known to have a more extended breeding season. Boer does in South Africa cycle practically all year round. This difference between breeds may be due to a difference in sensitivity to the negative feedback of estradiol.[6,7]

Estrous cycle

During the breeding season, females display regular cycles governed by the hypothalamic-pituitary-gonadal axis. Short cycles (short luteal phase) characterize the first cycles of the breeding season. The length of the cycle, follicular dynamics, and luteal activity, differ markedly between sheep and goats. Ovarian follicles develop in waves to preovulatory stage, even outside the breeding season.[8,9]

In ewes, estrous cycle length is 14-17 days. Each estrous cycle has 3 or 4 follicular waves. The number of follicular waves per estrous cycle is not affected by the prolificacy of the breed. Follicular waves emerge in response to increased FSH triggered by the pulsatile GnRH secretion from the hypothalamus. A cohort of follicles, 1-2 mm in diameter, appears every 4 days. Ovulatory follicles generally emerge ~ on day 12 of the cycle. During the luteal phase, follicles reach a maximum diameter of 4-7 mm, then regress or become atretic after 5 and 12 days, respectively. Follicular dynamics thus presents 3 phases: a growth phase of 2-4 days, a static phase of 1-4 days, and a regression phase of 1-5 days. At the end of each follicular wave, 1-4 follicles reach the preovulatory stage. Follicles that ovulate during estrus may be from the last 2 waves. Follicles from prolific breeds mature earlier and have a smaller diameter before ovulation. The antral follicles of prolific breeds have fewer granulosa cells, but their steroidogenic capacity is greater, resulting in higher serum estradiol concentrations.[10] In the absence of progesterone, estradiol-17ß induces estrus behavior that lasts an average of 36 hours (24-72 hours). Estrus is shorter in ewe-lambs and at the start of the breeding season and longer in prolific breeds. External signs of estrus are discreet in ewes, except for receptivity to the ram.[9] The cervical mucus changes from clear and runny at the beginning of estrus to highly viscous and white as ovulation approaches. The final maturation of the follicle is obtained through the increasing LH sensitivity of the granulosa cells.[9] The LH surge occurs in response to high estradiol-17ß concentrations, followed by ovulation 20-40 hours after the onset of estrus.[9] A corpus hemorrhagicum forms after ovulation and undergoes

histological and functional changes under the influence of LH. Formation of the corpus luteum (CL) takes 3-4 days. The CL diameter increases, reaching a maximum of 11-14 mm on days 9-10 of the cycle (day 0 = ovulation). The CL size is smaller in prolific than in nonprolific breeds. The increase in CL size is due to an increase in the volume of large luteal cells from day 4 until day 10 of the cycle and to the mitotic multiplication of small luteal cells on days 4-8. Progesterone concentrations rise progressively on days 3-7, reaching a plateau, then begin to decrease on days 12-14. Serum progesterone concentrations are higher in nonprolific breeds. Luteolysis is induced on days 14-16 of the cycle by $PGF_{2\alpha}$ secreted by the endometrium and transported directly by the ovarian artery (from the uterine veins and lymphatic vessels) to the ipsilateral ovary. $PGF_{2\alpha}$ causes vasoconstriction in the CL through the release of endothelin-1. Also, $PGF_{2\alpha}$ binds to the receptors of the large luteal cells, inducing a functional luteolysis by disrupting progesterone synthesis through the alteration of cholesterol transport across mitochondrial membranes. This mechanism of $PGF_{2\alpha}$ release is exacerbated by the action of oxytocin from pituitary and luteal origin.[9]

In goats, the estrous cycle length is 17-25 days. Short cycles, averaging 8 days, are common at the beginning of the breeding season. The goat estrous cycle has 2-6 follicular waves. In general, the follicles that ovulate during estrus are from the last wave. However, in some cases (prolific breeds), the ovulations are from follicles from the last 2 waves. A follicular wave begins with the recruitment of a cohort of 2-3 mm gonadotropin-sensitive antral follicles. From this cohort, 2-4 follicles grow to 4 mm and become dominant. During the luteal phase of the cycle, the dominant follicles continue to develop to reach a maximum size and then undergo atresia. In the absence of progesterone (i.e. after luteolysis), the dominant follicles continue to grow under the influence of LH and reach the preovulatory stage with a diameter of 6-9 mm. During this phase, serum estradiol concentrations rise, leading to onset of estrus. The length of estrus is 36 hours on average and ranges 22-48 hours depending on the breed, age of the female, season, and male presence. Young nulliparous females have a shorter estrus than adult does. Signs of estrus are more obvious in does than in ewes and occur in 2 phases: a proceptive phase followed by a receptive phase.[8] During the proceptive phase, the doe actively seeks the buck but does not accept mounting. During the receptive phase, the doe exhibits rapid tail movements (wagging), vocalizations, frequent urination, and accepts mounting. Estrous does may also mount each other. Estrus is often accompanied by reduced appetite and milk production, and physical changes (vulvar edema) and a characteristic vaginal cytology (> 50% eosinophilic desquamated polyhedral epithelial cells). The interval from the onset of estrus to the LH peak varies among breeds (8 hours for the Boer, 14.5 hours for the Alpine). Consequently, the interval from the onset of estrus to ovulation is also variable (9-37 hours). The ovulation rate varies from 1-5, depending on age, breed, and nutritional status. As described for the ewe, corpora hemorrhagica form after ovulation and progressively luteinize to become mature CLs by day 5 after ovulation.[8]

Methods of estrus synchronization in small ruminants

Male effect

The use of hormones in livestock is prohibited in some countries and for organic animal production systems.[11] The 'male effect' is an alternate tool for nonpharmaceutical reproductive management in small ruminants. Combined use of the male effect and hormones is emerging as a more effective estrus synchronization tool as it seems to overcome some disadvantages of hormonal methods to stimulate and synchronize ovarian activity. The use of hormones can be followed by male effect alone or male effect coupled with photoperiodic manipulation, as an alternate choice. The male effect, being more cost-effective than hormonal methods, could be very useful for developing countries; however further studies are needed as geographical location and breed influences the outcome of efforts to induce estrus and ovulation.[12]

The male effect refers to a relatively synchronized physiological response of females (induction of estrus and ovulation) after the introduction of a male into the flock. Because of the strong male effect in sheep and goats, these species are used as a model to study complex hypothalamo-hypophyseal endocrine changes in females induced by sociosexual stimuli from the male. By enhancing GnRH and thus LH, the male effect induces ovulation in cyclic and acyclic females. In acyclic animals that respond to the male effect, an LH surge is observed within 72 hours that may or may not induce ovulation. This ovulation may not be accompanied by behavioral estrus and is followed by normal or shortened luteal phases.[13] In cyclic females, luteotropic effect of the male-effect-induced LH surges occurring in the early to mid-luteal phase may lead to a prolonged estrous cycle.[14] The introduction of the male during the late luteal or follicular phase shortens the estrous cycle.[1,15]

Generally, the male effect is assumed to be effective within the same species; however, increased LH pulse frequency has been observed in ewes exposed to buck hair and in does exposed to rams.[16] Recently, the buck-to-buck effect, where sexually active male goats stimulate the endocrine and sexual activities of other males, has been reported.[17]

The male effect is thought to be mediated primarily by chemical signaling of pheromones via olfaction, along with some supplementary effects of vision and memory. The main sites of pheromone production are the sebaceous glands of the head, neck, shoulder, and rump. The 5α-reductase in these sites converts testosterone to dihydroxy testosterone, which stimulates production of pheromones that impregnate the hair or wool in these areas.

Chemically, the derivatives of 4-ethyl octanoic acid act as primary pheromones in goats and are responsible for a range of social interactions within the species, including the male effect.[1,18,19] However, the biomolecule causing the male effect is not a pheromone by definition.[20] The main pathway for the ram effect is the stimulation of the GnRH nuclei in the brain through the major and minor pathways. The pheromone mediated stimulation through the olfactory and major olfactory bulb passes through the cortical nuclei in the amygdala before reaching the hypothalamus (major pathway). The stimulation through the vomeronasal organ and accessory olfactory bulb is processed through the medial nuclei of the hypothalamus (minor pathway). Ultimately, these olfactory stimuli activate the ventromedial nucleus of the hypothalamus in the preoptic area to generate GnRH pulses. The male effect is mediated through kisspeptin and neurokinin B. The pheromone signals are conveyed from the amygdala to kisspeptin neurons in the arcuate nucleus. Neurokinin B, involved in the cellular mechanism through G- protein-coupled receptors, causes an LH surge by activating the GnRH pulse generator.[1,18,19,21]

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Factors affecting response to the male effect include the number of cyclic females, season, geographical location, breed of the female, breed of male, male's libido, depth of anestrus, body condition of female, parity, lactation, degree of anestrus of ewes or doe, male sexual activity, male novelty, and duration of contact with the male.[7,22] Male sexual behavior is also an important factor in stimulating the ovarian response and maintaining pulsatile LH secretion for ovulation.[23,24] Ewes with lower body condition scores have a lower level of LH surge in response to the male effect that delays subsequent estrus, ovulation, and progesterone concentrations.[25,26] Less seasonal breeds like the Merino ovulate within 2-3 days, whereas more seasonal breeds gradually proceed to ovulation in response to the male effect.[27] The male effect is more pronounced during the breeding season than during the nonbreeding season.[28] Response to the male effect does not seem to depend on follicular diameter at the time of male's introduction.[29] The male should be separated from the female for 15-34 days for optimum response.[30] However, as sexual activity and novelty are more crucial for efficacy of the male effect, isolation is required only for the reintroduction of familiar males.[1,31] Visual and audio-visual stimuli, including male images or male vocalization, lead to some ovarian activity and/or weaker LH secretion compared to direct contact with the male.[21,32] Socially dominant does ovulate earlier than subordinate does in response to the male effect.[33]

A 4-hour contact per day with novel rams induced ovulation in anovulatory ewes without prior separation.[34] However, ewes or does are unresponsive to the male effect if they are habitual/familiar to particular males.[31] The familiarity occurred quickly within hours and is mediated via the hippocampus, the center for learning and memory.[35] The separation of males from females diminish olfactory awareness of ewes to males, and the male can be reintroduced as a novel male. The reintroduction of familiar males after a separation period of 30 days led to a weaker LH surge than observed after the introduction of novel males.[26] The use of sexually active novel males produces a more efficient male effect than sexually inactive novel males and does not require a period of separation of the females.[26,36] Interestingly, daily rotation of photo-stimulated bucks among groups of does increased sexual activity of the buck but did not increase ovulation or pregnancy rate in the does compared to does subjected to photo-stimulated but no buck rotation.[37]

For practical application in goat breeding, 2 hours per day of contact with a photo-stimulated, sexually active male for 15 consecutive days is sufficient to induce and synchronize ovulation. One photo-stimulated sexually active male is sufficient for 9-11 does.[22] Alternatively, photoperiod-melatonin-induced, sexually activated males can be used for male effect. The sexual activation in rams in spring can be achieved by 2 months of long days (16 hours light/day) followed by 3 melatonin (18 mg each) subcutaneous implants at the end of the long days.[38]

Combined use of male effect and hormones

Application of the male effect in combination with hormonal treatments and photoperiod management offers a more predictable way to synchronize estrus. For estrus synchronization, using 2 injections (13 days apart) of $PGF_{2\alpha}$ with the introduction of the male on the day of the second $PGF_{2\alpha}$ injection increased the ovulation rate in ewes. This protocol resulted in better synchronization and ovulation than the sole introduction of males without the second injection.[39] Estrus synchronization of does in deep anestrus using artificial light (16 hours), 11-day progesterone treatment, and male effect resulted in an LH surge in 92% of the study goats within 4 days after the introduction of males, with a 98% ovulation rate.[4] Progesterone treatment coupled with the introduction of males eliminated the drawback of a short infertile estrus observed in response to using the male effect only to synchronize estrus.[4,40] However, with the increasing demand for hormone and drug-free animal products, combined use of the male effect and photoperiodic manipulation would be a natural method to synchronize estrus and ovulation. The combined use of the male effect and hormones is emerging as the most effective tool for estrus synchronization in small ruminants. The male effect is more cost effective than hormonal methods and could be very beneficial for developing countries. However, studies are needed to evaluate the effects of breed and geographical location on the outcome.[41]

Estrus synchronization using progestogens

Progestogens alter the secretion of LH by negative feedback, modifying the activity of hypothalamic GnRH. A surge in LH is observed after progesterone withdrawal.[42] The progesterone source can be natural progesterone impregnated in silicon elastomers (e.g. CIDR), sponges, or implants. Additionally, synthetic progesterone analogs such as norgestomet, fluorogestone acetate (FGA), and medroxyprogesterone acetate (MAP) can also be used. Natural progesterone is available as a controlled internal drug-releasing device (CIDR- Easi-Breed®) that is the only approved marketed progestogen treatment in the USA. Progesterone is used in CIDRs at the dose of 330 mg. FGA is used at a dose of 45 mg in goats, 30 mg in ewe lambs, and 40 mg in ewes. MAP is used in sheep for 14 days as sponges containing 40, 50, or 60 mg. Norgestomet (3 mg) is used as a subcutaneous implant. Oral megestrol acetate (MGA) is used in sheep at 0.125 mg twice daily for 8-14 days. Estrus is observed on average 48 (range: 40-60) hours after removal of the progestogen.[43]

Progesterone therapies are given for either short (5-7 days) or long (12-16 days for sheep; 14-21 days for goat) periods. Progesterone treatment for 12-14 days is the most common application.[44] The CIDRs, or progestogen-impregnated sponges, are placed intravaginally. Approximately 48 hours after the device/sponge removal, ewes exhibit estrus. Artificial insemination can be performed 48 to 54 hours after progesterone removal. In general, progesterone treatment is recommended for 16-17 days during breeding season and 14 days during nonbreeding season for goats.[45] The short-term progestogen treatment requires a luteolytic treatment because the duration of the treatment is shorter[9] than the lifespan of CL. During the nonbreeding season, hormonal induction of follicular growth at progesterone removal is required to stimulate and increase endogenous pituitary gonadotropin production. Such stimulation in cyclic animals enhances the ovulation rate.

Intravaginal progesterone delivery is associated with vaginitis with purulent vaginal discharge. Intravaginal progesterone blocks the vaginal secretions, thus affecting vaginal microflora and stimulating the proliferation of microorganisms in the vagina.[46] This vaginitis is not associated with infertility. The CIDR devices result in a lower incidence of vaginitis, vaginal discharge, and drawstring breakage compared to sponges by allowing drainage of vaginal fluids.[47–49]

The most commonly used commercial progestogen sponges are FGA (20 mg/sponge) and MAP (60 mg/sponge). The most commonly used dose of MAP is 60 mg, whereas the dose of FGA is 40 mg. Use of intravaginal sponges for either a short (5-7 days) or long (12-14 days for sheep; 14-16 for goat) period during the breeding season resulted in a similar estrous response.[48] Estrous response and pregnancy rate in Merino ewes synchronized with various amounts of MAP (40, 50, and 60 mg) were similar.[50] Recommended doses of either MAP or FGA did not affect fertility, pregnancy, or fecundity/lambing rates.[51] FGA treatment (11 versus 13 days) with 5 combinations of 10 mg of $PGF_{2\alpha}$, 330 IU of eCG, and 6 µg GnRH had no significant difference on estrus rate, sponge loss rate, vaginitis rate, total percentage of estrous ewes, conception rate, single lambing rate, twinning rate, and multiple lambing rates.[52] Vaginal sponges containing 30 or 60 mg of MAP were used for 6 or 12 days; estrus rate and pregnancy rate were higher for the 12 days treatment, independent of dose.[53]

The controlled internal drug release (CIDR), an inert silicone elastomer molded over a nylon core, was designed in New Zealand in the late 1980s.[54,55] Plasma progesterone concentrations peak 3 days after insertion and then gradually decrease.[44] Using either short (5 days) or long (14 days) estrus synchronization protocols is possible. Short protocols are gaining popularity due to reduced progesterone concentrations observed when CIDRs are placed for more than 7 days. To ensure CL absence, an initial dose of $PGF_{2\alpha}$ is recommended at CIDR insertion. In lengthy CIDR protocols, $PGF_{2\alpha}$ may be given 24-48 hours prior to the removal of CIDR or completely omitted if the duration of treatment is equal or longer than the CL lifespan. Estrous response was higher in ewes receiving CIDR treatment for 9 and 12 days than those receiving treatment for 3 and 6 days.[56]

Estrus synchronization using CIDR and MAP had no significant difference in the average time to estrus expression (23 versus 21 hours, respectively), estrus and lambing rate.[51] Also, the estrus synchronization outcome in ewes was similar for FGA and CIDR.[49] There was no significant difference in either estrus or conception rates among the ewes treated with CIDR, FGA, or MAP-based synchronization protocols.[57]

MGA is an oral progesterone with a daily dose of 0.25 mg/head/day, fed as 0.125 mg/head twice daily. The estrus induction was considerably lower (62 versus 89%) in ewes that received MGA treatment for 9 days compared to those that received MGA treatment for 12 days. However, the pregnancy rate was not different between groups (41 versus 44%).[58]

Use of gonadotropins in progestogen-based protocols

The initiation of pituitary activity after progestogen therapy can be expedited with chorionic gonadotropin (eCG), FSH or PG600® (Merck Animal Health, Summit, NJ) treatment. Gonadotropins have a crucial part in synchronization protocols by stimulating and achieving synchrony in the growth, maturity, and ovulation of follicles. The most commonly used hormone for induction of follicular growth and synchronization of ovulation in sheep and goats is eCG (Figure 1). The dose of eCG used depends on the age, breed, and season, and varies (250-750 IU).[44] Higher doses (up to 1,000 IU) might be required for goats. Several studies used 700-750 IU eCG, resulting in higher pregnancy and lambing rates, but estrus or pregnancy rates did not differ using either 300 or 600 IU eCG during the nonbreeding season.[59]

The unavailability of eCG in the USA has led to the off-label use of PG600 for estrus synchronization in small ruminants, especially ewes. PG600 is a gonadotropin preparation approved for estrus synchronization in sows. PG600 contains 80 IU/ml eCG and 40 IU/ml human chorionic gonadotropin that mimic FSH and LH, respectively. For anestrous ewes, pregnancy rates are higher for ewes that received 2.0 to 3.5 ml of intramuscular PG600 than receiving less < 2.00 ml or > 3.99 ml.[60] In cyclic ewes, the pregnancy rate was significantly lower for ewes receiving 5 ml PG600 than ewes receiving 1.5 ml PG600. Pregnancy rates were similar for ewes receiving 1.5 ml PG600 or normal saline.[61] High PG600 doses lead to overstimulation of ovaries, increased follicular size, increased estradiol-17β concentrations at the time of ovulation and reduced fertilization rate.[62] Although not commonly used, porcine FSH (pFSH) can be injected as a single injection of 68 mg (Folltropin®) 12 hours prior to progesterone removal.[63]

Prostaglandin $F_{2\alpha}$ and d-cloprostenol

Prostaglandin $F_{2\alpha}$ (dinoprost tromethamine) or its anolog (cloprostenol) (PGs) act by inducing luteolysis and thereby

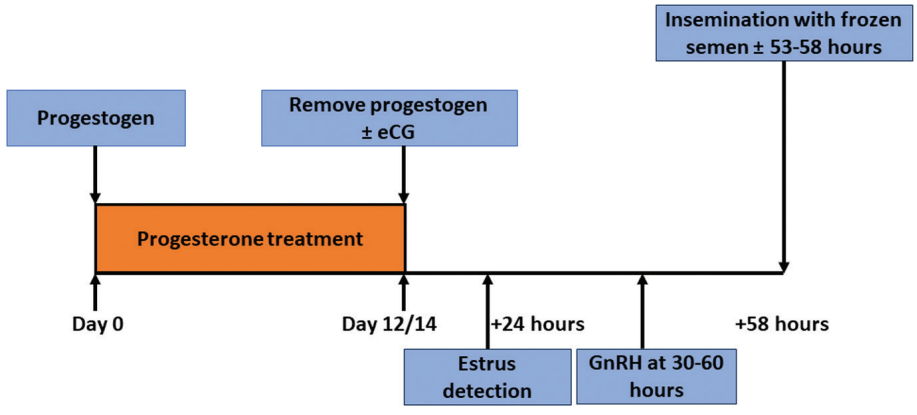

Figure 1. CIDR/progestogen bases estrus synchronization protocol for small ruminants. Artificial insemination with fresh semen can be performed 50-55 hours after CIDR removal. Laparoscopic artificial insemination should be performed 55-60 hours for frozen semen and 52-60 hours for fresh semen after CIDR removal. In the USA, eCG can be substituted with PG600.

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increasing LH pulse frequency, causing a significant increase in estradiol from the dominant follicle and induction of estrus and ovulation. PGs are only effective during the breeding season with an active CL. The sheep CL is responsive to PGs on day 3, whereas the goat CL is responsive on day 5 after ovulation (day 0). Estrus is observed 2-5 days after PGs treatment during the luteal phase.[64] The interval from PGs treatment to onset of estrus depends on the stage of the follicular wave at the time of injection.

PG treatment can result in impaired follicular function and asynchronous ovulation.[15] To better synchronize estrus and remove the variability due to the ineffectiveness of PG treatment during the early and late luteal phases, a double PG protocol has been proposed. Two PG injections are given 9-11 days apart (Figure 2). Most animals will be in the mid-luteal phase of the estrous cycle and will respond better to the second treatment. In goats, the second injection can be given 7-16 days apart.[65] The fertility following single and double-injection protocols is lower than that of progesterone-based protocols. This decrease in fertility is due to the negative influence of mid-cycle high progesterone on final follicular maturation, synchrony of ovulation after the second PG injection, and poor luteogenesis after estrus. Injecting a second dose in the early or late luteal phase results in higher estrus intensity and ovulation rate. Injecting the second dose at (hypothetical) day 5 of the induced diestrus (day 7 after first injection of PG) is recommended. This avoids animals being on the first or second day of the cycle and has a similar effect (luteolytic efficiency, percentage, and timing of estrus onset, preovulatory production of LH, ovulation, and functionality of subsequent corpora lutea) as the 3rd day of diestrus. Most ewes will be in estrus within 30-48 hours, whereas does will be in estrus within 45-48 hours after the second injection. Although timed AI is possible at 55 hours, fertility is variable.[44]

One study in tropical hair sheep had a higher response following 2 injections (9 days apart) of d-cloporstenol compared to dinoprost tromethamine (87 versus 57%); however, there was no difference in pregnancy rate.[66] Several other studies reported equal efficacy of dinoprost tromethamine and d-cloprostenol in inducing estrus in small ruminants. Giving 2 $PGF_{2\alpha}$ injections (d-cloprostenol; 0.15 mg; 10 days apart) along with GnRH (4.2 mg buserelin) before the first $PGF_{2\alpha}$ treatment resulted in a higher lambing rate.[67] The combination of $PGF_{2\alpha}$ during the early luteal phase with the male effect may be an adequate alternative for synchronizing estrus prior to artificial insemination in the absence of previous estrus detection.[44]

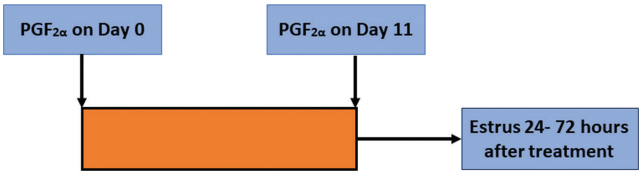

Figure 2. Protocol for estrus synchronization in small ruminants using 2 doses of $PGF_{2\alpha}$

Melatonin

Melatonin has been used in several countries since the 1980s for ovarian stimulation in small ruminants.[68] Melatonin increased ovulation rate, lambing rate, progesterone synthesis and embryo survival.[69] GnRH secretion started to increase on day 40, and GnRH and LH pulses increased from day 74 after melatonin treatment.[44,70] Melatonin enhances the release of GnRH and LH during anestrus by decreasing tyrosine hydroxylase activity and, consequently, the release of dopamine in the median eminence. The dopaminergic system has a crucial role in estradiol suppression of LH secretion during seasonal anestrus. Therefore, decreased dopamine secretion during anestrus is linked to enhanced reproductive activity.[71] Melatonin implants (Melawin®, Regulin®) release melatonin continuously throughout the day (> 100 pg/ml of plasma) without affecting the night release and thus, create a short-day-like effect. The 18 mg melatonin implant releases effective melatonin concentration for 90-100 days after the implantation. Each ram receives 3 melatonin (18 mg) implants subcutaneously at the base of the ear and is removed from the flock. Ewes receive single implants 7 days later than rams, and 40 days after the sheep implants, rams are returned to the flock. The timing of melatonin use depends on geographical location (Table 1).[44]

Melatonin also increases litter size in sheep by 15-30%; however, the mechanism is not fully understood as melatonin receptors are ubiquitous. For sheep, melatonin treatment is recommended around the spring equinox. Pretreatment with long daylight hours after the winter solstice enhances the efficacy of melatonin treatment in goats. The light treatment is recommended 06:00-09:00 and 22:00-24:00 per day.[44] Some of the commonly used protocols for estrus synchronization in sheep and goats are summarized in Table 2.

Superovulation in small ruminants

Superovulation (also known as ovarian superstimulation) involves the targeted delivery of gonadotropins at a precise time point of the estrous cycle to increase the number of follicles reaching maturation and ovulation. The primary gonadotropins used are equine chorionic hormone (eCG), FSH, or a combination of both.[79] The superovulation response is affected by the timing of the treatment, the type of hormone, breed, age, season, geographical location, and individual characteristics.[79] Multiple protocols for superovulation have been documented in sheep and goats. The most commonly used methods involve

Table 1. Recommendations on the timing of melatonin use for estrus synchronization in small ruminants

Geographical location	Effective timing of insertion of melatonin implant
More northern than N45° or more southern than S45°	Around summer solstice
More southern than N40° or more northern than S40°	Spring equinox
Mediterranean region more southern than N35° or more northern than S35°	After winter solstice
Latitudes between N20° and S20°	Limited efficacy (breeds are nonseasonal)

Table 2. Some of the commonly used protocols for estrus synchronization in sheep and goats

Protocol	Species	Synchrony and/or fertility results
GnRH and $PGF_{2\alpha}$[72]	Sheep	20% lambing rate
GnRH, P and $PGF_{2\alpha}$[72]	Sheep	47% lambing rate
MAP and eCG[41]	Sheep	32.5% lambing rate 90% estrus synchrony
FGA and eCG[73]	Sheep	80% ovulation rate
Artificial light, P_4 coupled with male effect[4]	Goat	98.7%
FGA, eCG, PG and male introduction[74]	Goat	66.7% estrus behavior
Male introduction, FGA, eCG, and PG[74]	Goat	71% estrus behavior
Long day, melatonin, and male effect[75]	Goat	100% ovulation rate 81.3% pregnancy rate
Ovsynch and timed AI[76]	Goat	58% kidding rate
P_4 sponges[77]	Goat	100% estrus behavior 87.5% pregnancy rate
FGA, PG, eCG[73]	Sheep	80% ovulation rate
PG, FGA, eCG[73]	Sheep	100% ovulation rate
PG, FGA, GnRH[73]	Sheep	60% ovulation rate
GnRH, $PGF_{2\alpha}$, eCG[73]	Sheep	80% ovulation rate
MAP (7 days) + GnRH and $PGF_{2\alpha}$[78]	Goat	88% estrus rate 84% pregnancy rate
MAP (12 days) + PMSG and GnRH[78]	Goat	80% estrus rate 72% pregnancy rate
FGA + $PGF_{2\alpha}$ + eCG + GnRH[52]	Sheep	80% conception rate 3% lambing rate

the manipulation of the estrous cycle through progestogen therapy and gonadotropins treatment at a specified time.[80] The progestogen treatments are comparable to those employed for estrus synchronization and can vary in duration. Successful superovulation requires precise control of follicular waves. Gonadotropin treatment has to be initiated before follicular deviation. Various hormonal techniques have been investigated to decrease the variability in response to ovarian stimulation by gonadotropins. These methods encompass immunization against androstenedione or inhibin, synchronization of ovulation for superovulation during first wave. Pretreatment with a GnRH antagonist from day 10 to FSH treatment to prevent follicular dominance and progesterone–estradiol cotreatment is used to synchronize follicular wave emergence.[81] Nevertheless, these techniques are hardly employed in practical applications and will not be addressed.

Superovulation using equine chorionic gonadotropin (eCG)

The hormone eCG exhibits FSH and LH activity. It is the oldest technique for inducing superovulation in ruminants. The hormone is given at a dosage ranging 750-1,500 IU in goats and 1,000-2,000 IU in sheep, commonly 1 or 2 days before progestogen removal; eCG-based superovulation protocols are no longer used due to significant fluctuations in response.[82] The poor results are likely due to the elevated LH activity of eCG and its extended half-life. The strong LH activity results in the early initiation of oocyte meiosis. The prolonged half-life causes the recruitment of follicles that are not uniform in size. Moreover, eCG-based superovulation disturbs the hormonal balance that affects the transfer of gametes and decreases the rates of ovulation and fertilization. The efficacy of eCG treatment diminishes with repeated treatment, primarily because of the development of anti-eCG antibodies.[83,84]

Superovulation using follicle stimulating hormone

Superovulation with FSH results in higher fertilization rates and embryo recovery. The FSH commonly used in practice for superovulation in ruminants is either of ovine (oFSH) or porcine (pFSH) origin. These preparations are made from pituitary gland extracts and remain costly. Recombinant FSH formulations have been studied, although they are not yet readily available. FSH has a short half-life, necessitating repeated treatment to induce follicular stimulation. FSH is given in decreasing doses every 12 hours throughout the final 3 or 4 days of the progestogen therapy. The optimal dosage of FSH for sheep and goat varies among studies. Comparison is often difficult because of the difference in measuring unit. The most reported doses are 10-14 µg of pure FSH (equivalent to 16-20 mg in Armour units, or 200 mg in NIH-FSH-P1 units). The dosage of FSH should be tailored according to the breed.[82]

Prolific breeds typically exhibit a stronger response to smaller FSH doses. For instance, in goats, an adequate dosage of FSH (1 Armour unit can contain 9.4-14.2 IU) ranges from 6-9 mg. The efficacy of porcine-derived FSH diminishes with repeated superovulation. The number of injections can be reduced by using a slow-release FSH preparation diluted in hyaluronic acid or an aluminum hydroxide gel.[85,86] Common superovulation protocols used in practice are illustrated (Figures 3, 4, 5 and 6). In goats, to prevent the effect of luteal regression on embryo yield, a progestogen sponge/CIDR must be reinserted after last AI or mating. This treatment has also been found beneficial in ewes[87]

In the short superovulation protocol, progestogen treatment lasts 6 days. The FSH treatment begins on day 4 and continues for 8 injections (36, 36/36, 34/34, 24/24, 16 mg) with $PGF_{2\alpha}$ treatment in the evening of the day of progesterone withdrawal. The females are mated or artificially inseminated after estrus detection. A dose of GnRH is given on the morning of day 8. Progestogen treatment is from day 10 until the embryos are harvested, especially in goats to counteract the regression of the CL.

Superovulation with equine chorionic gonadotropin/ follicle stimulating hormone combination

Several protocols combining FSH and eCG have been described. Typically, eCG is injected in a low dose (200 IU) at the same time as the penultimate injection of the FSH.[81] However, this is not a commonly practiced in the USA because of the unavailability of eCG.

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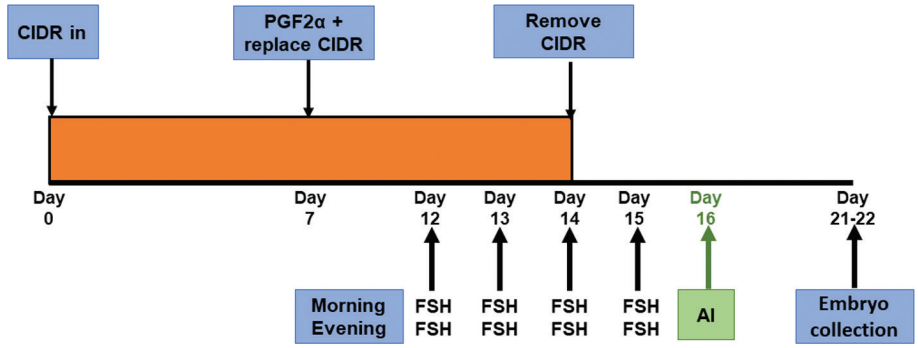

Figure 3. Long protocol for superovulation in ewes. The progestogen treatment is for 14 days but the CIDR is replaced at 7 days combined with $PGF_{2\alpha}$ treatment. The FSH treatment lasts 4 days starting on day 12. The female is either bred naturally or artificially inseminated after estrus detection. The dose of FSH varies (200-250 mg) depending on the species and breed and given in a series of injections 20/20%, 15/15%, 10/10%, 5/5%. The CIDR is removed in the evening on day 14 and an injection of the eCG (200 IU) can be given.

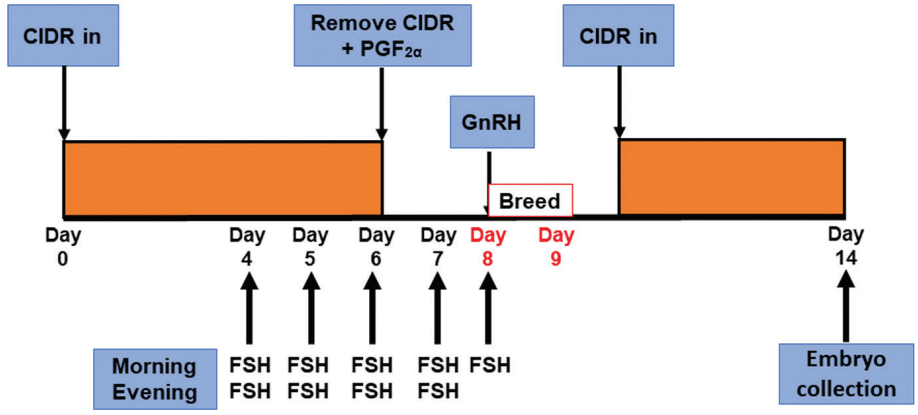

Figure 4. Short protocol for superovulation. Progestogen treatment lasts 6 days. FSH treatment begins on day 4 and 8 daily injections (36/36, 36/36, 34/34, 24/24, 16 mg) are given until $PGF_{2\alpha}$ treatment in the evening of the day of progesterone withdrawal. The females are mated or artificially inseminated after estrus detection. A dose of GnRH is given on the morning of day 8. Progestogen treatment is given from day 10 until the embryos are harvested, especially in goats to counteract the regression of the CL.

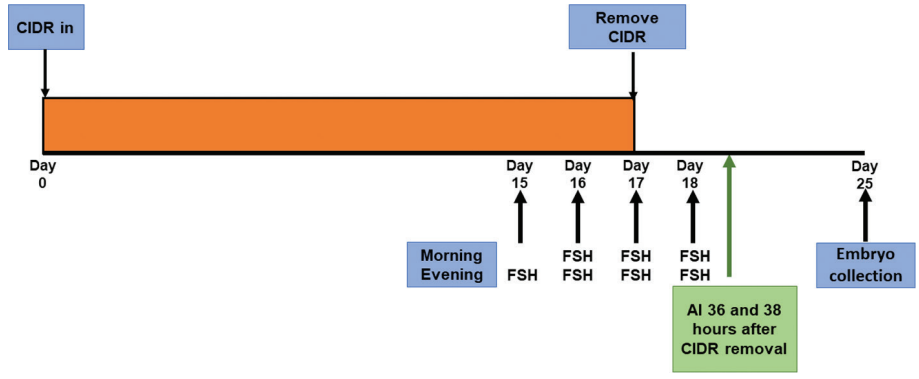

Figure 5. Long protocol for superovulation in goats. Progestogen treatment lasts 17 days. FSH treatment continues days 15-18 (50, 25/25, 25/25, 25/25 mg). AI is performed 36 and 48 hours after CIDR removal.

Prediction of superovulation response with anti-Müllerian hormone

Anti-Müllerian hormone (AMH) concentrations are indicative of ovarian follicular reserve.[88] The AMH can be a potential marker to predict the outcome of superovulatory treatment and to adjust the FSH dose before starting the FSH treatment. Serum AMH concentrations are positively correlated with the number of ovulations in the sheep and goat.[89,90] Fertilization and embryo recovery rates in ewes with

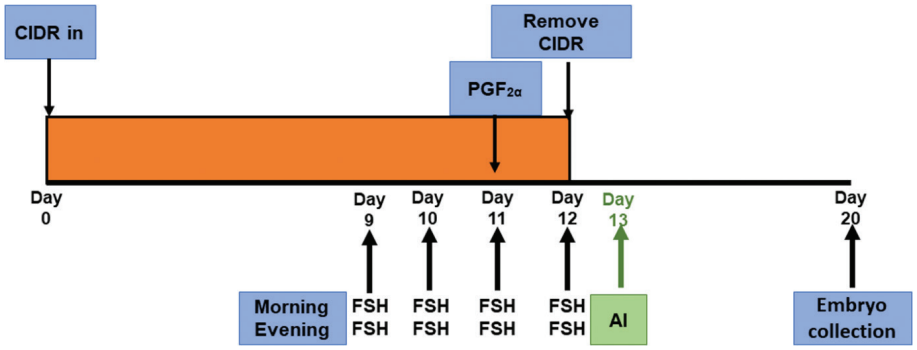

Figure 6. Long protocol for superovulation in sheep. Progestogen treatment lasts 12 days. FSH treatment continues days 9-12. The dose of FSH varies (200-250 mg) depending on the species and breed and given in a series of injections 20/20%, 15/15%, 10/10%, 5/5%.

low serum AMH can be enhanced by increasing to an 8-dose FSH treatment protocol instead of 6 doses. In ewes with high serum AMH concentrations, the embryo recovery rate is not different regardless of the number of doses of FSH treatment.[89] However, ewes with low AMH have low follicular reserve and high circulating FSH concentrations. The refractoriness of the granulosa cells to FSH is responsible for a higher FSH dose required for higher superovulatory response in the ewes with low AMH.[91]

Conclusion

Estrus synchronization in ewes can be accomplished by manipulating the timing of estrus through progesterone and/or prostaglandin treatment. These hormones are commonly coupled with gonadotropin, especially during the nonbreeding season. Initial research on synchronization protocols relied on progestogen treatment alone, but that has been refined throughout the years. Progestogens are still used as the primary method for estrus synchronization, particularly when timed AI is used. Progestogens can be used during and outside the physiological breeding season, whereas PGs are effective only for in-season estrus synchronization. The various commercial preparations of progestogens are equally effective for estrus synchronization in small ruminants.

Not all hormonal products are available in all countries. If eCG is not available, PG600 may be used; however, there is still a need for studies to evaluate proper dose for different breeds. In general, PG600 is not as efficacious as eCG. The efficiency of estrus synchronization protocols may differ based on the specific breed of sheep. Legal regulations and thus availability of certain hormonal products in various countries and consumer demand for organic products highlight the need for nonhormonal approaches (e.g. male effect). The male effect and photoperiod manipulation are a great option to avoid problems with residue and for organic farming. There is an increased demand for in vivo and in vitro production of embryos. The FSH-based superovulation protocols work well but need to be adjusted for each breed and individual. The discovery of the relationship between serum AMH concentrations and potential response to superovulation may help in refining the FSH dose for superovulation. Finally, none of these techniques for estrus synchronization or superovulation can be efficient unless other aspects of flock/herd health including nutritional flushing, trace mineral supplementation, parasite control and vaccination for common infectious diseases are also addressed.

Conflict of interest

The authors declare no conflict of interest.

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