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Recent Advances in Estrous Synchronisation in Small Ruminants

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Introduction:

Sheep and goats are seasonally polyestrous, meaning they naturally experience estrous and ovulation during short daylight periods (the breeding season) and not during long daylight periods (out of season). Since their gestation period lasts five months, ewes and does typically produce only one set of offspring per year without intervention, which limits their productivity and profitability. However, it is possible to increase the number of offspring to three sets every two years or five sets every three years if the females can be made to conceive outside of their normal breeding season. This would also provide a year-round supply of lamb, kid meat, and milk. To induce estrus and ovulation outside of the breeding season, breeders can use estrus synchronization. However, it is essential to note that both ovulation and pregnancy rates tend to decline outside the normal breeding season.

Principle:

Estrus synchronization focuses on the manipulation of either the luteal or the follicular phase of the estrous cycle. In does and ewes, the opportunity for control is greater during the luteal phase, which is of longer duration and more responsive to manipulation. Strategies can be employed to extend the luteal phase by supplying exogenous progesterone or to shorten this phase by prematurely regressing existing corpora lutea (CL). Successful techniques must not only establish tight synchrony, but also provide an acceptable level of fertility upon artificial insemination or natural mating. The latter is commonly accomplished through co-treatments using gonadotropin. After these conditions are met, estrus synchronisation becomes the basis for successful AI and embryo transfer programs.

Considerations for Small Ruminants:

Estrus synchronization is affected by the seasonal breeding patterns in most temperate breeds of goats and sheep. In anovular does and ewes, estrus may not only have to be synchronized, but also initiated. Systems requiring the regression of an active CL will not be effective under these conditions. However, after cyclic activity can be induced in anovular goats and sheep, seasonal breeding can be manipulated and the production cycle can be shortened. A second opportunity in small ruminants is the propensity of many breeds to carry and raise multiple offspring, which can often be controlled by adjustments in dosage levels and nutritional manipulations as part of the ES regimen.

Advantages of Estrus Synchronization in Small Ruminants:

1. Improved Reproductive Efficiency

- Concentrated Breeding Season: Estrus synchronization allows for the concentration of breeding within a short period, which can result in a more uniform lambing or kidding season. This uniformity simplifies management practices such as feeding, health monitoring, and birthing assistance.
- Increased Conception Rates: By controlling the timing of estrus, synchronization ensures that all females are in heat simultaneously, making it easier to plan and optimize the use of artificial insemination (AI) or natural mating. This can lead to higher conception rates and more efficient use of superior genetics.

2. Optimized Use of Artificial Insemination (AI)

- Facilitated AI Scheduling: Estrus synchronization is particularly beneficial when using AI, as it allows for precise timing of insemination, which is critical for success. Synchronization ensures that all females are inseminated at the optimal time for fertilization, reducing the need for repeated AI attempts and lowering costs.
- Access to Superior Genetics: By synchronizing estrus, farmers can better utilize semen from genetically superior sires, leading to improved genetic quality in the offspring. This is particularly important in programs focused on genetic improvement or specific breeding objectives.

3. Enhanced Flock or Herd Management

- Labor Efficiency: Synchronization reduces the labour required for estrus detection, which can be time-consuming and challenging in extensive systems. By controlling the timing of estrus, farmers can plan and allocate labour more effectively during the breeding season.
- Predictable Birthing Season: A synchronized estrus leads to a more predictable and concentrated birthing season. This predictability allows for better planning in terms of housing, feeding, and veterinary care, reducing the risks associated with parturition and improving overall animal welfare.

4. Economic Benefits

- Increased Productivity: By improving reproductive efficiency and optimizing the use of AI, estrus synchronization can lead to higher lamb or kid production per breeding season. This increased productivity directly translates to higher profitability for the farmer.
- Better Market Timing: A synchronized breeding and birthing season can be timed to align with market demands, allowing farmers to produce and sell their products when prices are highest. This strategic timing can enhance the economic returns from small ruminant production. (Abecia et al., 2012)

Basic approaches for estrous synchronization:

The basic approaches for controlling estrous cycle length are: administration of prostaglandin for the corpus luteum (CL) regression before natural luteolysis in animals; administration of progesterone or, more commonly, synthetic progestins to temporarily suppress ovarian activity in animals; and another way of creating estrous synchrony is by using gonadotropinreleasing hormone (GnRH) or an analogue, either alone or in combination with other hormones, which causes the ovulation of a large follicle.

An estrous synchronization protocol has several advantages, such as a short calving interval allows females (especially heifers) to conceive earlier; artificial insemination and embryo transfer technique to reduce time and labour detecting estrous; and uniform calf crop production, i.e., of a similar age group.

The reproductive season of ewes:

Sheep are generally classified as seasonal polyestrus breeding animals; the breeding season begins as early as April and lasts until September. The natural breeding season for ewes is concentrated between late June and early September, which allows ewes to give birth between late November and early February (Weems et la., 2015). Sheep are animals that depend on the photoperiod through the secretion of the melatonin hormone and other environmental factors such as temperature and nutrition (Chemineau et al., 2008). The reproductive performance of ewes during the year shows a period of sexual dormancy followed by a period of sexual activity during the reproductive season. Several estrus cycles appear until the end of the season, or the female becomes pregnant (Abecia et al., 2017). The reproductive season of females is regulated by the presence of photosensitizers in the retina of the eye; impulses are sent by the optic nerve and other nerve impulses to the sheep's pineal gland to detect changes in daylight by the biological clock in the hypothalamus, which in turn sends signals by the axons of the gonads through the pineal gland (Malpaux et al., 1997). Ewes' reproductive season appears when the day's length is shortened (Mura et al., 2017).

Asadi-Fozi et al. (2020) pointed out that most species of animals that live in areas confined between 35 north and 35 south latitudes have different animals showing reproductive activity depending on the seasons. The reason is due to the different hours of light and the intensity of illumination, and there is no doubt that light plays an important role in the reproductive season in animals that are active in certain seasons and are sexually inactive in the rest of the seasons (Goodman et al., 2010). The melatonin hormone is secreted from the pineal gland which plays an important role in the occurrence of these significant changes in the functions of the gonads of animals that are affected by the season. The evidence indicates that the action of the melatonin hormone and its effect on the gonads occurs at the level of the brain, hypothalamus, pituitary and epithelial tissues as the pineal gland plays a pivotal role in for the hormone GnRH from the hypothalamus (Batailler et al., 2018). Thus, the melatonin hormone indirectly regulates the reproductive season by controlling the secretion of gonads hormones from the anterior lobe of the pituitary gland and their activity is regulated in the reproductive season (Kopycińska et al., 2022).

Hormonal regulation of the oestrous cycle of the ewes

The Hypothalamus -Pituitary -Ovaries Axis is the main axis that controls the regulation of the reproductive cycle in sheep (Zhang et al., 2019). The regulation of the estrus cycle requires mutual interference of the endocrine and neuroendocrine mechanism represented by reproductive hormones of the hypothalamus and anterior lobe of the pituitary gland and steroid hormones of the ovaries, as well as interference between the uterus and ovaries (Handa and Weiser, 2014). During the period of the end of estrus, progesterone concentrations are at high levels, as it leads to blocking the release of the hormones GnRH, FSH, and LH through the negative feedback mechanism of the hypothalamus and the anterior lobe of the pituitary gland and in case pregnancy does not occur (Moenter et al., 2020). The prostaglandin hormone (PGF2α) is released from the uterus and is released to the ovaries through the uterineovarian vein to the ovarian artery, as it leads to luteolytic of the corpus luteum and a decrease in the level of progesterone concentration (Kim et al., 2015). As the decrease in the concentration of progesterone works to remove the negative feedback of the hypothalamus and the anterior lobe of the pituitary gland, and then the impulses of GnRH, FSH and LH begin to be released and then stimulate the growth of ovarian follicles and increase the secretion of estrogen, and after 2 or 3 days of a decrease in the level of progesterone concentration, the level of estrogen concentration will reach the concentrations that stimulate the secretion of sufficient levels of gonadal inducers GnRH, FSH and LH before ovulation by positive feedback mechanism for the hypothalamus (Misztal et al., 2008). The FSH hormone works on the growth and development of the ovarian follicles until they reach maturity, and then it is called the mature follicle; the inhibin hormone is secreted from the mature follicle, as it regulates the release of the FSH hormone during estrus and then prevents excessive stimulation of the follicles. The secretion of the LH hormone before ovulation occurs in the form of waves. The wave that occurs before ovulation is responsible for stimulating the final maturation of the ovum and breaking the wall of the mature follicle and ovulation events (Goodman et al., 2019). The secretion of the LH hormone occurs before ovulation at the beginning of estrus and lasts for 6-10 hours in most animal species, and the ovulation process occurs after the secretion of the LH hormone for about 24-30 hours in cows and ewes and goats 30-36 hours, and after ovulation, a corpus luteum is formed at the site of ovulation and begins to secrete progesterone 2-4 days after ovulation (Bartlewski et al., 2011). FSH, in synergy with estrogen, helps regulate the receptors of the hormone LH on granulosa cells; the secretion of the prolactin hormone Prolactin at the end of estrus also helps to perpetuate the LH hormone receptors on Granulosa cells, and when ovulation begins, the interaction of the LH hormone receptors with granulosa cells, which leads to their conversion into a corpus luteum (Fabre et al., 2006). The LH hormone works in perpetuating the function of the corpus luteum by increasing the rate of blood flow to the corpus luteum (Wiltbank et al., 2012).

Estrous synchronization in sheep

In sheep, estrous synchronization is done by various means, such as manipulating photoperiod, ram effect (Evans et al., 2004), and various hormonal regimens (Biehl et al 2019). The exogenous hormones used for synchronization protocol include equine chorionic gonadotropin (eCG) (Abdullah et al 2002), progesterone (Emsen et al 2011), prostaglandins (Fierro et al., 2013), melatonin (Mura et al 2019), and human chorionic gonadotropin (hCG) (Dias et al 2020). Estrous synchronisation in sheep can be accomplished by using sexually active rams during breeding and non-breeding seasons (Nakafeero et al 2020) and inducing estrous by altering photoperiod during the non-breeding season (Fleisch et al 2015).

Hormones used for estrous synchronization

Progesterone

Treatment using progesterone (P4) includes injectables, intravaginal sponges, and controlled internal drug-releasing (CIDR) devices. In conjunction with gonadotropins, progesterone therapies are administered for both short (5–9 days) and long (14 days) periods (Martinez-Ros et al 2019). Sponges containing various fluorogestone acetate (FGA) concentrations and medroxyprogesterone acetate (MPA) have also been employed. However, prolonged use of intravaginal sponges is frequently linked to vaginitis and discharge that is purulent in nature (Martinez-Ros et al 2018). Intravaginal sponges block the vaginal secretions (Al Hamedawi et al 2003) that stimulate the proliferation of micro-organisms like Salmonella spp. and Staphylococcus aureus (Swartz et al 2014). Antibiotics (locally or systemically) were given during sponge implantation to combat all these undesirable effects (Gatti et al 2011).

Intravaginal sponges

A similar estrous response was produced when intravaginal sponges were inserted for either a short (7 days) or long (12 days) period during the breeding season. Intravaginal sponges of different MPA concentrations (40, 50, and 60 mg) were compared for estrous response and pregnancy rate in Merino ewes; As low as 40 mg of MPA was found to be beneficial for estrous synchronisation, indicating that MPA concentrations did not affect the results (Simonetti et al 2000).

Controlled internal drug-releasing device (CIDR)

Besides different CIDR treatment lengths, the ewes also got 300 IU of eCG at the time of CIDR removal and were inseminated 48 hours later. Results indicated that estrous response was significantly greater ($P < 0.05$) for ewes treated with CIDR for 9 and 12 days than those treated for 3 and 6 days.

Melengestrol acetate (MGA) and norgestomet

The 0.22 mg concentration of MGA led to higher rates of conception and estrous response (Salas-Razo et al 2014). When norgestomet implants were used, ewes underwent further treatment with 400 IU of eCG, 25 g of GnRH, or 400 IU eCG+25 g GnRH at the time of implant removal after 14 days, which enhanced estrous response and pregnancy rate. Compared to therapy with eCG alone, oral administration of MPA (60 mg MPA per ewe per day for 20 days) and eCG (30 mg per ewe per day for 16 days) to anestrous ewes enhanced fertility after estrous induction. (Brunner et al 1964).

Prostaglandins

Prostaglandin is only effective during the breeding season due to its active corpus luteum (CL) presence. Further, ovine CL is responsive to PGF2alpha on day 3 following ovulation (Day 0) (Fierro et al 2013). Therefore, the timing of PG administration is crucial for the estrous response, and double PG injections are frequently advised for synchronisation. The variation in the time between two PG injections in the literature ranges from 7 to 16 days (Souza-Fabjan et al 2018). A comparison of PGF2α (dinoprost tromethamine vs. d-cloprostenol) was made for estrous synchronization during the breeding season by treating ewes with two doses of PG, 9 days apart. Despite greater estrous response (87% vs. 57%) in ewes treated with d-cloprostenol than dinoprost tromethamine ($P < 0.05$), the pregnancy rates were similar ($P > 0.05$) between the two treatments (Ramírez et al 2018). Using Prostaglandin F2alpha: Due to the absence of luteal tissue during anestrous, PGF2alpha alone cannot induce estrous. (Miguel-Cruz et al 2019). However, giving out-of-season ewes two PGF2alpha injections (D-Cloprostenol; 0.15 mg; 10 days apart), together with GnRH (4.2 mg buserelin) before the first PGF2alpha treatment, led to a greater lambing rate (Mirzaei et al 2017).

Gonadotropins

The researchers have employed a wide variety of gonadotropins, including GnRH (Titi et al 2010), follicle-stimulating hormone (FSH) (Forcada et al 2011), and eCG (Macías-Cruz et al 2013). In synchronisation protocols, gonadotropins promote and synchronise follicular growth, maturation, and ovulation. A single injection of FSH (Follitropin 68 mg) 12 hours before progesterone withdrawal (0.3 g progesterone [CIDR-G]) and the introduction of ram during the management of out-of-season breeding led to only a slight increase in the ovulation rate in comparison to a single injection of FSH (Follitropin 68 mg) 36 hours before progesterone withdrawal and introduction of ram in out-of-season ewes (Knights et al 2003).

Ram effect

In an attempt to decrease the usage of exogenous hormones in animal production globally due to growing concerns about residues in food products (meat, milk). Altering LH secretion due to the ram effect causes anovulatory ewes to ovulate (Evans et al 2004). The sudden introduction of a ram causes fertile estrous in ewes even outside the breeding season (Delgadillo et al 2009). The role of the ram effect in estrous synchronization regimens involving PGF2α, progesterone, and eCG has also been evaluated in sheep by various researchers. Recently, Nakafeero et al (2020) found that using progestin-based protocols with or without an eCG and the ram effect produced similar ($P > 0.05$) estrous response, pregnancy, and lambing rates in Merino ewes during the out-of-breeding season

Estrous synchronization in goats

Various estrous cycle synchronization techniques can be used depending on the season and its relationship to the doe's natural breeding season. Dairy goat owners are interested in out-of-season breeding because it lessens the seasonal variation in the herd's milk production. The sudden introduction of an odoriferous buck during the transitional phase often advances the start of the cycle by a few weeks, and the does may also exhibit some synchronisation.

Prostaglandin and its analogue

Using prostaglandins to promote luteolysis and induce the follicular phase of the estrous cycle in goats is a simple approach to synchronising estrous. In small ruminants, prostaglandin F2alpha is the primary luteolytic agent (McCracken et al 1970). Administration of a synthetic PGF2alpha analogue in does with a functional corpus luteum will result in luteolysis and bring does into heat in around 2–5 days. Using prostaglandin is ineffective during anestrous phase as there is the absence of ovulation and corpus luteum. There have been reports of impaired follicular function after prostaglandin treatment, which causes ovulation to occur at variable times (Hawken et al 2007). By doing this, two prostaglandin F2alpha injections must be given at intervals of 9 to 11 days. Most animals would be in the middle of the luteal phase of the oestrous cycle and would respond better to the second treatment (Amiridis and Cseh 2012).

Progesterone and its analogues:

Another way to synchronise estrous is to utilise natural progesterone that has been impregnated in silicon elastomers, sponges, or implants, and you can also use synthetic progesterone analogues such as norgestomet, fluorogestone acetate (FGA), methyacetoxy progesterone (MAP), and medroxyprogesterone acetate (MPA) (Ungerfeld and Rubianes, 2002). Progesterone modifies pituitary LH secretion, causing negative feedback, altering hypothalamic GnRH activity (Hansel and Convey, 1983), and causing a preovulatory LH surge after device withdrawal, if eCG is given to anoestrous females to promote the development of follicles, including the preovulatory follicles. Natural progesterone is available in the market as SilOestrous® implant and Eazi-Breed® controlled internal drug release devices™ (CIDR). Traditionally, intravaginal sponges were implanted for 9 to 21 days, and in most cases, an eCG or PGF2 was given 2 days before the removal of the pessaries.

Progestagen devices

May use short (~5 days) or long (~14 days) estrous synchronization protocols. Short protocols are becoming popular because of declining progesterone concentrations when CIDRs placed for >7 days. An initial dose of PGF2α is recommended at the start of the short protocol (i.e., at CIDR insertion) to ensure the absence of the corpus luteum at the time of removal of CIDR. When using long protocols using CIDR, PGF2α may be administered before 24-48 hours of CIDR removal or entirely omitted because there are chances of corpus luteum lifespan to be exceeded by the time of removal. After progestagen therapy, the initiation of estrous activity will be accelerated by administering a gonadotropinreleasing hormone agonist, pregnant mare serum gonadotropin (PMSG), or follicle-stimulating hormone (FSH). Also, typically given at the end of progestagen therapy is commercially marketed for use in pigs that contains both PMSG and human chorionic gonadotropin. Due to their super-ovulatory effects, FSH and PMSG can be administered to boost ovulation rate and litter size. This technique can produce good conception rates and allow for fixed-time insemination during or outside the breeding season.

Gonadotropins

For many years, sheep and goat farmers have used PG-600 to induce estrous during out-of-season estrous. The dosage that is most frequently mentioned in the literature, 5 mL per ewe, has been observed to overstimulate the ovarian tissue (Habeeb et al 2019), resulting in unusually big mature, large follicles and higher estradiol-17b concentrations at the time of ovulation (Safranski et al 1992).

Protocols for estrus synchronization in small ruminants

1. G-P-G / OvSynch protocol

Administration of first injection of gonadotrophin-releasing hormone (GnRH) on day 0 causes an LH surge that ovulates or luteinizes most large follicles present in the ovaries (Farooqi et al., 2021; Holtz et al., 2008). A new follicular wave then begins 1 to 2 days later. First GnRH injection is followed by a PGF2α injection seven days

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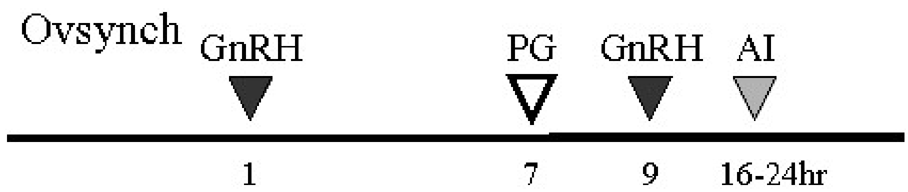
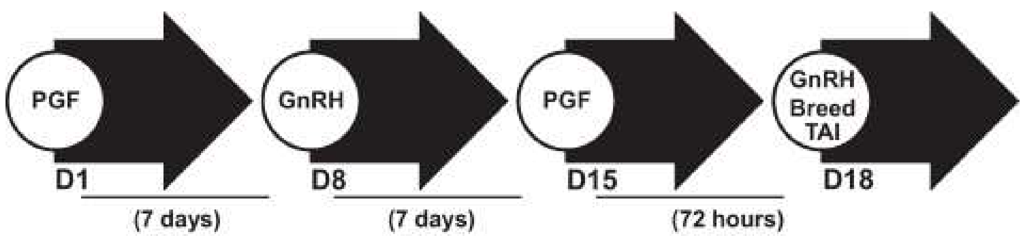
later, most animals will possess mature dominant follicle of similar size at CL regression, resulting in a more synchronous ovulation with second GnRH injection on day 9. Second GnRH injection induces ovulation of the dominant follicle recruited after the first GnRH injection. Animals are inseminated at 12-14 hours after the second GnRH injection. Additionally, the GnRH induced luteinization of dominant follicles will induce cyclicity in many anestrus animals. There are several variations of GnRHPGF2α based breeding programme used in small ruminants. Conception rate with AI is 58 – 70%. OvSynch protocol with natural service heat (estrus) detection is required. For natural service 1 buck is required per 6 females in one pen. Conception rate in natural service is higher (66 – 80 %) as compared to A.I. Method.



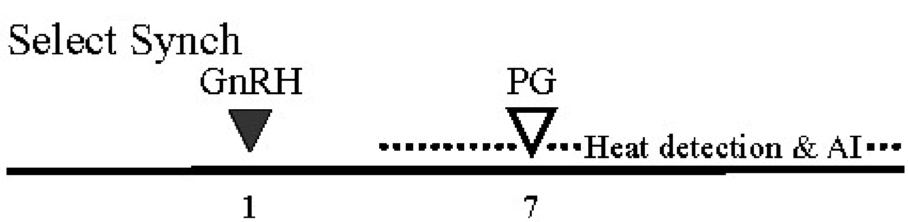
2. NCSynch protocol

NCSynch – TAI (PGF2α -Gn RH- PGF2α - GnRH) protocol is like OvSynch type but pre-synchronization with PGF2α was used. Prostaglandin was given on day 1 as pre-synchronization treatment. On day 8, GnRH was administered to ovulate or luteinize the present dominant follicles. Seven days later, on day 15, a second dose of PGF2α injection was administered to induce the luteolysis. Does were artificially inseminated after 72 h of the second dose of PGF2α injection at which time a second injection of GnRH was administered to induce the LH surge and ovulation. Conception rate is about 68% (Bowdridge et al., 2013).



3. Select Synch protocol

This protocol is similar to OvSynch protocol but 2nd GnRH is not given. In select synch protocol first GnRH was given on day 0 then after 7 days PGF2α was given then go for natural mating. This protocol reduces the cost of 2nd dose of GnRH injection and also save the time with this protocol the conception rate was 66.66% (Pujar et al., 2016).



4. Two PGF2 alpha protocols

Two PGF2 alpha injections were given in ewes at 11 days intervals. 85-90% conception rate was obtained (Habeeb and Anne Kutzler, 2021; Anggraeni et al., 2021)

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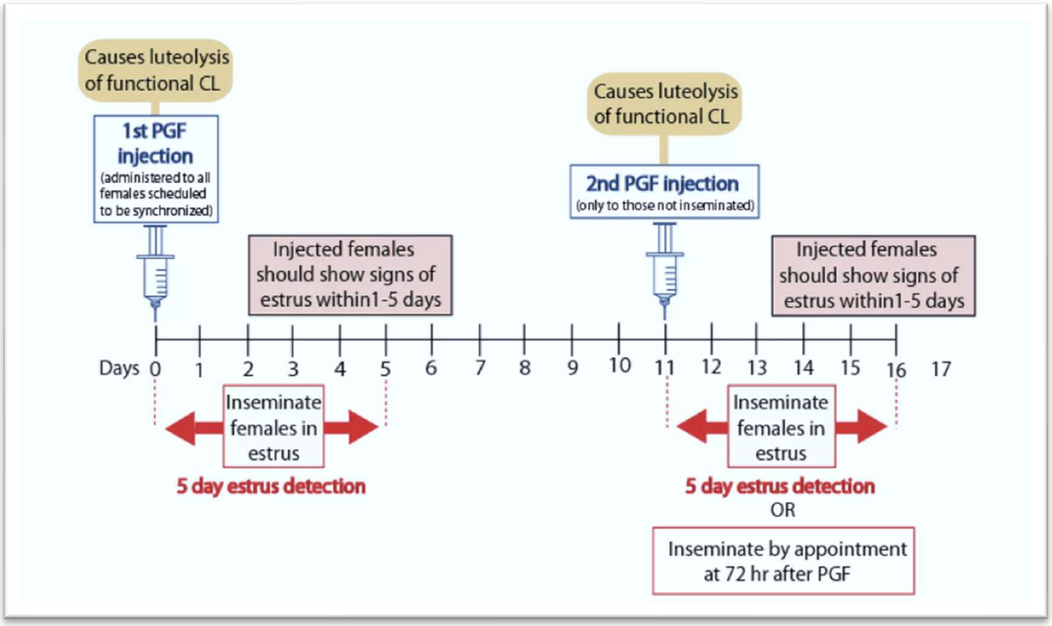


5. Intravaginal devices

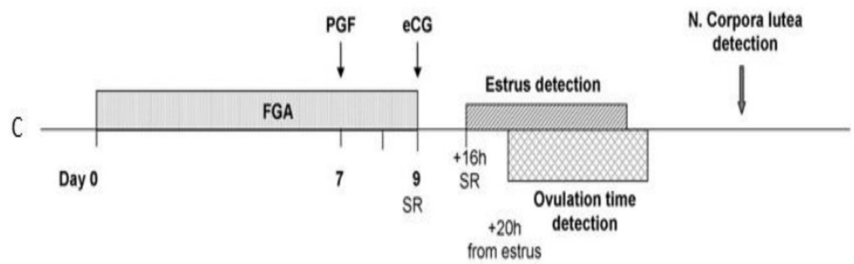
Progestogen/progesterone impregnated intravaginal device contain synthetic P4 (Norgestomate). It slowly releases progesterone. Plasma progesterone levels increase rapidly after insertion of intravaginal device, reach highest concentration on day 3 and then gradually deceases intravaginal device modulate the pituitary LH secretion, inducing negative feedback, modifying the hypothalamic GnRH activity, followed by a preovulatory LH surge after intravaginal device removal in order to support the development of follicles, including the preovulatory follicle(s). The intra vaginal devices mimic CL function and provides possibilities to control the preovulatory development and controlling either luteolysis and follicular development to obtain more precise synchronization of estrus and ovulation.

a) CIDR + eCG Protocol

CIDR is control internal drug release device which contain 9-12% of synthetic P4. This device is made up of silicon elastomer moulded over a nylon core. CIDR devices offer low natural dose of progesterone, induce earlier compact synchronization and do not absorb or obstruct drainage of vaginal secretions (Nogueira et al., 2011). In this protocol the females were treated with intravaginal devices containing 0.3 g progesterone (CIDR), inserted for a 12-day period. At insert withdrawal, they were treated with eCG I/m. In anestrous goats, the eCG or a similar effect (such as male effect) is absolutely necessary for preovulatory follicles development. Then go for natural service or AI. AI is done after 50 hr of eCG injection. Conception rate is 85-90 % (Habeeb and Anne Kutzler, 2021). In another protocol CIDR was inserted for 9 days, post-insertion on day 7th PGF2-alpha was given then CIDR was removed and injection of PMSG was given. AI was performed after 24 or 48 hr and found with 28 or 42 % of pregnancy rate, respectively (Kim et al., 2021).

b) FPE Protocol

FPE protocol (fluorogestone acetate (FGA) – PGF2α – eCG), in the long-term method the FGA is introduced for 11 days and PGF2 alfa is given on 9th day and at insert withdrawal on 11th day, ewes were treated with eCG injection. In short term protocol FGA is insert only for 5 days. At insert with drawl PGF2α and eCG is given together (Fig.3.B). AI is performed after 48 hr. The fact that progesterone prevents the new corpus luteum formation and safeguards that 5day old or persistent CL are subjected to luteolysis by PGF2α (Martemucci and Alessandro, 2011). In short day protocol FGA is inserted for 9 days and PGF2α given on the 7th day then eCG at sponge removal.



6. OvSynch with intravaginal device

The classical hormonal protocol is based on a seven-day intravaginal sponge with an I/m injection of GnRH on day 0 of synchronization of ovulation, PGF2α at the time of sponge removal followed by a second GnRH injection at 36-48 hr after the PGF2 alpha administered. Natural service or AI was done at 12-14 hr after the second GnRH injection for timed breeding (Senthilkumar et al., 2016).

Conclusion

Sheep and goats are seasonally polyestrous, breeding primarily during short daylight periods. To enhance productivity and provide a year-round supply of meat and milk, estrus synchronization can be used to induce estrus outside the natural breeding season. This involves manipulating the estrous cycle through hormonal treatments, such as progesterone and prostaglandins, to control and synchronize ovulation. This technique offers several advantages, including improved reproductive efficiency, optimized use of artificial insemination, enhanced flock management, and economic benefits. However, the effectiveness of these protocols can vary depending on seasonal factors and the specific breed of the animals.

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