Hormonal Control of Estrus in the Non-Breeding Season of Turcana Breed Sheep - A Review

Nicolae Adrian Giurginca[1], Marioara Nicoleta Caraba[2,3*], Gabi Dumitrescu[3,4], Ioan Pet[4], Catalin Bogdan Sirbu[4], Daniela Puscasiu[2,3], Adrian Sinitean[5], Ion Valeriu Caraba[3,4]

[1] Doctoral School "Engineering of Vegetable and Animal Resources", University of Life Sciences "King Mihai I" from Timişoara, Calea Aradului 119, 300645-Timişoara, Romania
[2] Department of Cellular and Molecular Biology, Faculty of Medicine, "Victor Babes" University of Medicine and Pharmacy of Timisoara, E. Murgu, 2, Timisoara, 300041 Romania
[3] ANAPATMOL Research Center, "Victor Babes" University of Medicine and Pharmacy of Timisoara, E. Murgu, 2, Timisoara, 300041 Romania
[4] Faculty of Bioengineering of Animal Resources, University of Life Sciences "King Mihai I of Romania" from Timisoara, Calea Aradului 119, RO 300645, Timisoara, Romania
[5] Department Biology-Chemistry, Faculty of Chemistry-Biology-Geography, West University of Timisoara, Pestalozzi, 16, 300315 Timisoara, Romania

Abstract
Reproduction management in sheep microfarms is a key element in increasing fertility, birth rate and economic and financial impact at the farm level. Fertility in microfarms is influenced by the ability to induce estrus and ovulation in non-breeding season, as sheep are acyclic animals. Factors that influence estrus induction and ovulation can be represented by: breed, season, photoperiod, duration of the lactation period, nutritional and health status of the sheep, duration of the postpartum period, the ratio between the number of rams and ewes, the time of introduction of the ram into the flock, administration of hormonal preparations, type of hormonal preparation and administration schedule. The administration of hormonal preparations in sheep plays a major role in the synchronization of estrus and the control of reproduction in non-breeding season: among the hormones most frequently used for this purpose we mention: progestogens, PGF2α and its analogues, pregnant mare serum gonadotropin (PMSG or eCG), gonadotropin-releasing hormone (GnRH), melatonin which is administered alone or in combination with other hormones. Among these, progestogens are the most frequently used during but also in non-breeding season, PGF2α and its analogues are used during the breeding season, and melatonin is usually used in non-breeding season. The current concerns of many groups of researchers are related to studies on the mode of action of hormonal preparations for inducing and synchronizing estrus, respectively stimulating ovulation in sheep in the non-breeding period to ensure increased birth rates in microfarms.

Keywords: fertility, birth rate, progestogens, melatonin

1. Introduction - General aspects of sheep reproduction

Sheep reproduction management involves the use of biotechnological methods aimed at increasing the reproductive potential of the animals. For this, it is important to have a deep knowledge of the biological reproductive patterns of sheep, the application of biotechnological methods should be carried out only on healthy animals with optimal physical condition [1]. To optimize the management of the reproductive function in sheep, a series of zootechnical and organizational measures are currently implemented, but biotechnological processes are also applied: induction and synchronization of estrus, respectively of ovulation, induction of

* Corresponding author: Caraba Marioara Nicoleta, Tel, 0744477087, nicoleta.caraba@umft.ro

superovulation, artificial insemination or embryo transfer [2].

During the non-breeding season (spring and summer), as well as in animals that do not show signs of estrus (for 20 days or more from the beginning of the breeding season or remain non-pregnant after the first mating/sowing), it is necessary to stimulate sexual behavior and promote gestation as soon as possible. Since after calving in some breeds of sheep a refractory period sets in, it is known that the stage of sexual arousal manifests itself only with the onset of autumn, when daylight hours shorten [3]. As a result, inducing estrus in sheep outside the breeding season is a necessity in order to increase fertility and birth rates.

Synchronization of ovine estrus is a very important reproductive technique because it can effectively create the reproductive performance of ewes, shorten the estrus interval and increase the gestation rate and birth rate, and shorten the time between generations [4]. In sheep farms where increasing lamb production is the main objective, estrus synchronization becomes an important factor because it can minimize the effects of seasonal factors on ewes' reproduction, extend the breeding season of ewes, adjust the time of lamb delivery, shorten the lambing cycle, achieve two to three thousand-year deliveries in which to increase the number of lambs in an economic period and implicitly obtain economic benefits [5]. Therefore, inducing estrus during the breeding season and increasing the fecundity rate in sheep offers the best opportunity to increase the efficiency of lamb production.

In sheep during the breeding season, prostaglandins can be used to induce estrus, since a functional corpus luteum must be present in the ovaries. In contrast, in the non-breeding season, females should be treated with progesterone-based hormonal treatments, accompanied by equine chorionic gonadotropin (eCG), releasing hormones and other compounds. Induction and synchronization of estrus in the non-breeding season in sheep has been achieved by using intravaginal sponges impregnated with synthetic progesterone analogues. However, the effect of hormonal treatments on the female reproductive system manifests different modes of action depending on the functional state of the reproductive organs, but also on the hormonal status. There may be situations in which the drugs are administered incorrectly and then the consequences may occur.

Also, there is insufficient knowledge about the patterns of change in reactivity of the sexual nerve centers and the corresponding reactions of the body to the administration of hormones, which may reduce their effectiveness, which limits their widespread use. In general, the induction and synchronization of estrus in sheep currently represents an important direction of scientific research and a problem of interest for sheep breeding [6, 7, 8].

2. Hormonal treatments for induction and synchronization of estrus in sheep

2.1. Hormonal treatments with progesterone

Hormonal treatments with progesterone are frequently used in the induction and synchronization of estrus in sheep farms, the administration period can be short or long. Since in recent years the interest in artificial insemination in small ruminants has increased greatly, there is an increased interest in the control of ovulation and then in estrous behavior at the level of sheep microfarms.

The most frequently used method of hormonal, short-term treatment with progesterone in sheep involves the intravaginal administration of this hormone using silicone devices or polyurethane devices (intravaginal sponges) that are maintained for periods of time between 12 and 14 days, followed by the administration of a single dose of eCG at the end of the treatment. The principle of the method takes into account the inhibitory role of progestogens on the hypothalamic-pituitary axis, thus acting as an exogenous corpus luteum. In sheep, the insertion of intravaginal devices containing 0.3 g of progesterone results in a rapid increase in blood concentrations of this hormone (>5 ng/mL) over approximately 4-5 days of treatment [9]. If these are maintained intravaginally, it is found that after 6 or 7 days, blood concentrations of progesterone decrease to subluteal levels (<2 ng/mL). These decreases are intense enough to block the ovulation process, but cause a persistent growth in the dominant follicle.

If intravaginal progesterone devices are maintained for 12-14 days, the decrease in progesterone concentration may affect fertility. In this regard, a method of intravaginal use of hormonal preparations with progesterone for 5-7 days has been proposed, associated with the

administration of a dose of 200 to 400 IU of eCG to induce ovulation, so that at the end, when the intravaginal device is removed, a luteolytic dose of PGF2α is administered. Numerous studies conducted over time have presented this method of inducing and synchronizing estrus in sheep both during the breeding season and in the non-breeding season. The use of intravaginal progesterone devices or different analogues of it, followed by doses of eCG and doses of PGF2alpha, is recommended, subsequently monitoring the establishment of estrus, followed by natural mating and/or insemination [10].

Hormonal treatments with progesterone or its analogues began in the late 1990s and have shown that prolonged exposure to progestogens, for 12 days, is not necessary to induce estrus in ewes during the non-breeding season, so a long period of exposure to progesterone is not justified.

In this regard, it has been demonstrated that 3-day administration of medroxyprogesterone acetate (MPA) was as effective as 6 or 12 days administration to induce estrus in anestrous ewes (Ungerfeld and Rubianes, 1999). Following MPA administration, natural mating rate and pregnancy establishment rate were monitored, and the values recorded for these parameters were similar for 3, 6, and 12 day administrations.

Studies on the timing of administration of hormonal preparations in sheep, on different administration schedules, continued, thus comparing a short-term administration protocol, for 6 days, with a long-term administration of progesterone in silicone intravaginal devices for 14 days.

In one of these studies conducted on 1750 multiparous sheep that were inseminated intrauterinely by laparoscopy, it was found that the pregnancy rate was significantly higher in the case of administering the short-term treatment regimen than in the long-term administration, the recorded pregnancy rates being 43.5% and 37.8%, respectively [11].

In another study conducted on 922 sheep that followed an intravaginal hormonal treatment for 6 days and 14 days, the pregnancy rates were also different, registering values of 41.2% and 29.1%, respectively, the pregnancy rate also being higher when applying the short-term protocol. Based on the studies conducted, it can be concluded that as progesterone levels decrease as a result of the use of intravaginal devices for long periods, a decrease in fertility also occurs [10].

Intravaginal devices with progesterone analogues are always used in hormonal treatment regimens, there is a multitude of such devices on the market, their effects on ovulation induction and pregnancy establishment being different and associated with the exposure time. The silicone device DICO (Dispositivo Intravaginal Caprino y Ovino, Syntex) determined results very similar to those obtained when applying the CIDR-G device (Zoetis), for the 2 parameters analyzed. Both intravaginal devices contain 0.3 g of progesterone in a silicone matrix, and the values of the analyzed parameters did not show differences between serum progesterone levels, follicular dynamics, ovulation time and pregnancy rate [12, 13]. However, when comparing the values of the parameters of interest when using intravaginal devices containing progesterone with those of intravaginal sponges containing MPA in short-term treatment regimens, the pregnancy rate was lower when using vaginal sponges [13]. The type of hormonal preparation progesterone / MPA, the type of silicone device / sponge are factors that can affect fertility.

In another study, it was found that the application of an intravaginal sponge with a placebo effect (i.e. without hormonal preparation - MPA) for 13 days before the installation of spontaneous estrus, reduced the conception rate compared to the other sheep that did not benefit from an intravaginal sponge. It can be highlighted that the local effect of the intravaginal sponges, in the above case, negatively affected fertility, when applied over a long treatment [14]. It is also worth mentioning the risk of vaginitis due to the increase in the number of bacteria at the time of removal of the device, compared to CIDR-G [15], highlighting a negative effect on the vaginal environment and fertility.

Another study used a different implant and a different progesterone analogue, for example - subcutaneous ear implants containing norgestomet (Crestar, Intervet/MSD) and found that the onset of estrus was delayed and conception rates were lower with the 5-day treatment variant (48%) compared to the 14-day treatment variant (83%) [16]. The device used - the Crestar implant originally designed for cows contains 3 mg norgestomet and was cut in half before use in cyclic ewes, resulting in high serum progestogen

levels and excessive blockade of LH synthesis and follicular growth. Thus, a longer period of time was required to return to adequate progesterone levels. Therefore, the results obtained indicate that fertility is influenced by a number of factors: the type of device, the route of administration, the pharmacokinetics and metabolism of the hormone preparation, the amount of hormone contained and the release rate from the device, the period of exposure to progestogen.

2.2. Possibility reuse of intravaginal devices

The progesterone content of some hormonal devices indicates the possibility of their reuse. Thus, there are intravaginal devices that contain 0.3 g of progesterone (CIDR-G® and DICO®), but they were designed to be used for 14 days. In this regard, experiments were carried out in which they were reused 2 or 3 times. The results obtained indicate that the establishment of estrus and the induction of ovulation were not affected, but the pregnancy rate recorded significant decreases after the second and third application [17, 13]. It is worth noting that the reuse of devices did not affect follicular turnover, but it did not have the ability to determine a new follicular wave in 20% of the females, in which ovulation occurred from a dominant follicle. This may explain the lower pregnancy rate in ewes treated with reused devices [17].

The results of studies in which progesterone vaginal devices were used for 12-14 days did not determine high fertility. The question of shortening the application period of progesterone devices to 5-7 days and their association with other hormonal preparations PGF2alpha and eCG upon removal of the device is raised. The conclusions of the studies suggest that these hormonal treatments applied for short periods determine a series of beneficial effects on the reproductive function in sheep: better control of follicular stimulation and ovulation, higher pregnancy rates, sometimes reuse of intravaginal devices [11].

2.3. Different methods of applying hormonal treatments for induction and synchronization estrus

Identifying the optimal moment of ovulation is an important element in the establishment of gestation both in the reproductive period and in the non-breeding season, both in the case of natural breeding and in the case of artificial insemination. The timing of ovulation after short-term treatment regimens with intravaginal devices for 5–7 days with progestogens, followed by PGF2alpha and 250–300 IU eCG at device removal is assumed to occur approximately 60 hours after intravaginal device removal in sheep [17]. Therefore, it is recommended that mating should occur a few hours earlier.

Hormonal treatments in ewes that are prepared for artificial insemination but also for natural mating involve the use of devices that release progesterone, followed by the administration of equine chorionic gonadotropin (eCG) on the day of device removal. Studies conducted to date on ovarian physiology support the idea of shortening exposure to progesterone released by intravaginal devices from 14 days to 5-7 days. These administration schedules are considered to provide adequate progesterone concentrations to induce follicular stimulation and ovulation. These treatment schedules also involve the intramuscular administration of a single dose of PGF2alpha at the time of device removal and with the administration of eCG. It is assumed that ovulation would occur approximately 60 hours later, and mating can be achieved within 48-54 hours. Over time, several ovulation induction protocols have been proposed, the one presented above is supposed to determine a higher pregnancy rate, compared to classic protocols lasting 14 days, and the progesterone remaining in the intravaginal devices can be in sufficient quantity to be reused. Stimulation of follicular function, stimulation of ovulation, administration of hormonal preparations, choosing the optimal time for mating are the key elements that must be taken into account to increase birth rates on sheep farms [10].

The aim of another study was to induce estrus in the non-breeding season, and to compare the efficiency of using intravaginal sponges with FGA in combination with PMSG or cloprostenol in inducing and synchronizing estrus in Awasi ewes (Iraqi sheep). In this regard, all ewes were treated with vaginal sponge containing 20 mg fluorogestone acetate (FGA) for 14 days. Sheep in group 1, considered the control group, received: FGA + normal saline, sheep in group 2 received: FGA + PGF2α, sheep in group 3 received: FGA + PMSG, and sheep in group 4 received FGA + PMSG + PGF2α. The results obtained and the statistical analysis of the data highlight statistically significant differences ($p<0.05$)

between sheep in the control group and those in the treated groups. Also, the onset of gestation, lambing rate and lamb size did not show statistically significant differences between the ewes in the experimental and control groups ($p<0.05$). Concluding based on the results obtained regardless of the treatment regimen applied FGA +PGF2α; FGA +PMSG; FGA+PMSG+PGF2α, the induction and synchronization of estrus recorded better values in the ewes in the experimental groups compared to the ewes in the control group, even if no significant differences were evident for the other parameters analyzed [18].

The synchronization of estrus in sheep can be influenced and stimulated by the application of hormonal treatments that act on the corpus luteum, follicular activity and ovulation. Synchronization of estrus in sheep in the non-breeding season is very important for improving the management of sheep reproduction, since sheep are seasonal polyestrous animals dependent on the seasons in terms of ensuring reproduction. Also, the reproductive season of sheep varies depending on the region, many studies have shown that most sheep breeds are in the non-breeding season during spring and early summer [19, 20, 21].

Numerous studies have highlighted the importance of hormonal treatments to induce and synchronize estrus in the non-breeding season in order to reduce the period of sexual inactivity, through the use of intravaginal sponges impregnated with progesterone. Intravaginal sponges containing progesterone are one of the most frequently applied treatments for estrus synchronization in sheep both during the breeding season and in the non-breeding season. Intravaginal sponges are used for periods of 12-14 days, either alone or in association with PMSG, generally in the non-breeding season, with PMSG administration being done either on the day of sponge removal or 48 hours before sponge removal. The results of the studies indicate that PMSG can increase pregnancy and twinning rates in lambs. However, it must be taken into account that the effect of PMSG is influenced by a series of factors, the most important of which is the season [22, 23].

Intravaginal sponges impregnated with progesterone or its synthetic analogues: medroxy progesterone acetate (MAP) and fluorogestone acetate (FGA) are usually maintained in the vagina for periods of time ranging from 6 to 14 days, administering PMSG (in non-breeding season) and sometimes prostaglandins injected intramuscularly upon removal of the sponge or 48 hours before removal of the sponge. Prostaglandin and its synthetic analogues represent the luteolytic factor in sheep, and prostaglandin F2α or its analogues cause luteolysis in sheep that have a functional corpus luteum at the time of treatment. Synchronization and induction of estrus outside the breeding season determine positive effects on the management of reproductive function with the aim of increasing economic productivity [24, 25, 26].

Another study compared the effect of five hormonal treatment regimens for estrous synchronization in Hu sheep. An attempt was made to establish the most efficient and economical protocol for administering hormonal preparations in large-scale sheep farms. The 5 experimental protocols use intravaginal sponges impregnated with fluorogestone acetate (FGA, 45 mg) in association with intramuscular administration of PGF2a and pregnant mare serum gonadotropin (PMSG). The reproductive parameters analyzed were: estrus detection, sponge loss rate and the possibility of vaginitis, pregnancy rate, lambing rate, respectively, calculations were performed to highlight the costs for each of the 5 experimental protocols applied. The results obtained showed that the percentage of ewes in which estrus was induced was higher in hourly intervals 24-36 hours, respectively 36-48 hours after vaginal sponge removal. Also, the analysis of the results showed no statistically significant difference in the percentage of ewes that were induced and established estrus (regardless of the hormonal treatment application scheme), the rate of sponge loss, the rate of vaginitis installation, the total percentage of ewes in estrus, the pregnancy rate, the single calving rate, the twinning rate and the multiple calving rate. Regarding costs, differences were highlighted in terms of the type of hormonal treatment applied, the cost of drugs for estrus synchronization in ewes in experimental group II proved to be the most effective in the macrofarm where hormonal treatments were applied [27].

Hormonal control of estrus and ovulation in sheep with the use of progesterone and its analogues is widely applied in sheep breeding programs, taking into account the breed characteristics and climatic conditions of sheep breeding. In the Jammu and

Kashmir regions, the sheep breeding season is between April and September, as a result of which methods of inducing ovulation are constantly being studied [28]. The methods of inducing estrus in sheep during the breeding season involve the use of progesterone or PMSG injections, the administration of progestogens can be done differently in the form of daily intramuscular injections, orally, with intravaginal devices such as CIDR, PRID or subcutaneous ear implants such as Crestar.

In general, progestogens or their analogues, administered together with gonadotropins, are frequently used to induce estrus in ewes in the non-breeding season, although pregnancy rates in ewes synchronized with progestogens are lower in the non-breeding season than during the breeding season. Intravaginal sponges are very commonly used, which are kept in place until the end of the breeding season, usually for periods of 6–14 days and 1 day, and at the time of sponge removal or before, the ewes are given an injection of PMSG [29, 30, 31].

Many of the studies conducted to ensure reproduction in the non-breeding season have associated norgestomet implants with PMSG - pregnant mare serum gonadotropin. The ram effect is also applied in sheep farms to induce estrus outside the breeding season, ewes in anestrus are isolated from rams before the start of the breeding season. Along with the induction of estrus in the non-breeding season, the ram effect can also determine a certain synchronization of estrus within the flock [32, 33].

Regarding the rate of estrus induction and pregnancy rate, multiple studies have been conducted, with results varying depending on the estrus induction protocol, breed, and climatic conditions. In this regard, studies have been conducted on the efficacy of using gonadotropin-releasing hormone (GnRH) instead of eCG in Merino ewes undergoing intravaginal sponge treatment with medroxyprogesterone acetate. Administration of GnRH at a dose of 100 pg 12 hours after removal of the sponge resulted in a reduction in the time to estrus onset and a higher incidence of heats was observed [27].

Another study recommends the use of surfagon (a GnRH analogue) at a dose of 15 µg (i.m.) in combination with other biologically active substances. The results obtained revealed an increase in prolificacy, but also a simultaneous increase in calving weight. The joint use of GnRH and biologically active substances during the breeding season increases fertilization of Merino sheep breeds. In another study, different methods of stimulation and induction of estrus were considered, as well as schemes for introducing complexes of biologically active substances for different sheep breeds. The general scheme of stimulation of estrus induction involved the administration of vaginal sponges with progesterone for 14 days, in combination with vitamin supplements. The authors showed that the use of their improved scheme gives positive results in all genotypes studied and provides a high level of fertilization. The use of a complex of biologically active substances and a GnRH analogue when the dose of eCG was reduced by 30%, increases the fertility of some breeds of sheep [34].

2.4. The "ram effect" in estrus induction and association with hormonal treatments

Introducing rams into sheep flocks in the non-breeding season can be a cheap and easy alternative, which partially replaces hormonal treatments for estrus induction and increases the chance of pregnancy after natural mating, even if heat is not visible. The effects of introducing rams into sheep flocks cause an increase in the concentration of luteinizing hormone (LH), which ends with the so-called "silent" ovulation, without signs of heat. The corpus luteum may regress after the first ovulation, but another ovulation accompanied by heat may occur 17-19 days after the introduction of rams into the sheep flock. However, there are also situations in which the corpus luteum regresses after 4-5 days, but another "silent" ovulation occurs. If the ewes have been subjected to hormonal treatments (at least 6 days with progestogens) prior to the introduction of the ram into the flock, heat may occur associated with the first ovulation [35, 36, 37, 38].

The introduction of rams into sheep flocks can be carried out in a variety of other situations, for example in the case of females in certain physiological conditions, to ensure postpartum re-breeding, in order to associate with hormonal treatments, in flocks of ewes to enter estrus and produce ovulation. Regardless of the situation, the effect of introducing a ram into the flock is to cause an increase in LH secretion that ends with ovulation [39, 40,41].

Although the rates of induction of estrus, ovulation and conception are lower in ewes than in adult sheep, inducing cyclic activity in ewes would be an easy technique to apply. Ewes reach normal body weight thresholds for reproduction during the spring, as a result, those ewes that respond to the ram effect would gain 6 months in their reproductive life. However, there are ewes that do not respond to this technique and are then bred and bred in the next breeding season. Rams should induce ovulation in ewes that have not reached puberty, which should be done in the non-breeding season, but the response to this technique is limited, also due to the lack of sexual experience of the ewes [42, 33, 41].

In one study, it was highlighted that although the ability of sheep to respond to the ram effect is minimal in the middle of the breeding season, the overall response of sheep to this technique was quite high. The response was different depending on the age of the sheep, in adult sheep the effects being more evident compared to lambs, but it can also be attributed to the action of rams on adult sheep, respectively to the observance of an optimal ratio between the number of rams and the number of sheep in the flock when applying this technique [43, 41].

Also, ewes were highlighted that ovulated without heat being detected, even if they were previously treated with progestogens for 6 days. These silent ovulations are more evident in ewes compared to mature ewes during the normal onset of the breeding season, but there is also a lower sensitivity of the hypothalamic-pituitary connection in ewes compared to mature ewes. Lower sensitivity may explain the delayed onset of estrus in ewes, but it is recommended to analyze if after a period of 17-20 days ovulation does not occur, increasing the chances of pregnancy establishment after mating [43].

The introduction of rams into sheep flocks in the non-breeding season to induce ovulation and to compare the reproductive response of mature ewes to that of 1-year-old ewes to the ram effect was carried out in another study, on Merilin sheep in the middle of the breeding season. Mature ewes and 1 year old ewes were pretreated with intravaginal sponges for 6 days before the introduction of rams into the flock. Mature ewes showed higher values compared to 1-year-old ewes for the parameters estrus induction (87.5% and 66.7%, respectively), estrus (66.1 and 30.3%), ovulation (1.27 ± 0.05 and 1.10 ± 0.04, respectively), and pregnancy rate (39.3 and 16.7%, respectively). Although yearling ewes respond to the ram effect in terms of estrus and ovulation in the non-breeding season, the reproductive response is still lower compared to that of mature ewes [44].

The aim of a study conducted in the above-mentioned areas was to evaluate the effect of intravaginal progesterone sponges, in combination with the ram effect or equine chorionic gonadotropin (eCG) on the reproductive efficiency of ewes in the non-breeding season. The ewes were grouped into 5 experimental groups and hormonal preparations were applied to them according to different treatment schemes: group I - Norgestomet 1/3 3 mg + PMSG 200 IU; group II - Norgestomet 1/3 3 mg + "ram effect"; group III - PSG P4 + PMSG 200 IU; group IV - P4 sponge + "ram effect", and group V, the control group of ewes that did not benefit from hormonal treatment. The reproductive parameters analyzed in the ewes from the 5 experimental groups were represented by: response to estrus induction, onset of estrus, duration of estrus, intensity of estrus, conception rate and calving rate. The ewes subjected to hormonal treatments showed estrus with weak, normal, intense intensities, respectively the pregnancy rate was reported in 50% of the ewes in the experimental groups except the ewes in group IV in which the pregnancy rate was lower. As expected, since the ewes were in the non-breeding season, the ewes in the control group did not show estrus and could not be subjected to mating. The calving rate in the ewes in the experimental groups was similar with percentages of 100% [45].

Regarding the intensity of estrus, it was described as intense, good and weak, all ewes in the experimental groups responded to the treatment and showed estrus, while the ewes in the control group did not show estrus during the study period. The results of the study are consistent with the results obtained by other groups of researchers by inducing estrus in the non-breeding season, by treating ewes with exogenous progestogen for a certain period of time or by combining progestogen and gonadotropins [46, 47, 48, 49]. Low rates of estrus induction in non-breeding season in sheep have been reported in other studies conducted on different breeds of sheep and

under different climatic conditions [50, 51, 52, 53, 54].

Results on the induction of estrus in non-breeding season sheep by introducing rams into the flock, the ram effect is reported to be as high as 90-100% [55, 45], in addition this aspect also contributed to the synchronization of the estrous cycle within the sheep in the flock [33]. The increased rates of induction of estrus in non-breeding season sheep by the ram effect can be associated with the following parameters: good physiological condition of the sheep, good ram to ewe ratio, efficient management of sheep feeding and maintaining health.

Other studies conducted on sheep in non-breeding season on the ram effect have highlighted lower rates of induction of estrus [36, 56, 57]. Variations in estrus induction in ewes in the non-breeding season through the ram effect can be attributed to other factors: the age of the ewe, the breed, the interval from calving to estrus induction, the breed and number of rams introduced into the flock, the intensity of anestrus, the geographical location of the farms.

The onset of estrus in non-breeding season ewes treated with hormonal preparations may occur earlier, especially in ewes treated with PMSG or eCG compared to those not treated with such preparations [51, 58, 59]. In contrast, other studies report longer intervals until the onset of estrus [60]. These results may be due to a low level of circulating testosterone in the ram used in the study, which could have delayed the rise in blood LH levels, and therefore delayed the onset of estrus and ovulation. Factors that affect circulating testosterone levels in the ram will in turn affect the quality and efficacy of the ram stimulus. Also, other factors such as the quality, type and duration of the ram stimulus are considered essential for the magnitude of the ovulatory response to the ram effect, which can lead to late onset of estrus and subsequent ovulation.

The duration of estrus differs depending on the moment of introduction of the ram into the sheep flock, if the ram is introduced into the flock close to the beginning of the breeding season, an increased response of the sheep and a prolonged duration of estrus are observed [45]. However, there are studies that expose the opposite regarding the short duration of estrus when applying the ram effect [61, 54]. The intensity of estrus in ewes to which the ram effect or other types of hormonal treatment schemes were applied varies from very good, normal, to weak, the variations being closely related to the type of hormone administered and its concentration [62, 63].

3. Conclusions

Induction and synchronization of estrus involves the application of different study methods that differ depending on numerous parameters: the breed of sheep, the welfare state of the animal, the type of exploitation, ensuring efficient feeding, the health of the female reproductive system, climatic conditions, respecting an optimal number of rams compared to the number of sheep in the case of natural mating. The use of hormonal preparations in the induction and synchronization of estrus in sheep represents the first step and the key element for stimulating reproductive function, the use of progesterone or its analogues, in association with other hormones can lead to remarkable effects of improving fertility, noted by increasing the number of calvings in the non-breeding season. Identifying efficient methods of induction and synchronization of estrus, followed by a quality mating can lead to increased fertility, fecundity, prolificacy and productivity from an economic point of view at the level of sheep farms.

References

1. Choudhary, K.K., Kavya, K.M., Jerome, A., and Sharma, R.K., "Advances in reproductive biotechnologies", Veterinary world, 2016, 9, 4, 388-395. DOI: https://doi.org/10.14202/vetworld.2016.388-395

2. Fonseca, J.F., Oliveira, M.E.F., Brandão, F.Z., Batista, R.I.T.P., Garcia, A.R., Bartlewski, P.M., and Souza-Fabjan, J.M.G., "Non-surgical embryo transfer in goats and sheep: the Brazilian experience", Reproduction, Fertility and Development, 2018, 31, 1, 17-26. DOI: https://doi.org/10.1071/RD18324

3. Đuričić, D., Benić, M., Žaja, I.Ž., Valpotić, H., and Samardžija, M., "Influence of season, rainfall and air temperature on the reproductive efficiency in Romanov sheep in Croatia", International journal of biometeorology, 2019. 63, 817-824. DOI: https://doi.org/10.1007/s00484-019-01696-z

4. Hasani, N., Ebrahimi, M., Ghasemi-Panahi, B., and HosseinKhani, A., Evaluating reproductive performance of three estrus synchronization protocols in Ghezel ewes, Theriogenology, 2018, 122, 9-13. doi: 10.1016/j.theriogenology.2018.07.005

5. Rosasco, S.L., Beard, J.K., Hallford, D.M., and Summers, A.F., Evaluation of estrous synchronization protocols on ewe reproductive efficiency and profitability, Anim Reprod Sci. 2019, 210, 106191. doi: 10.1016/j.anireprosci.2019.106191.
6. Yu, X., Bai, Y., Yang, J., Zhao, X., Zhang, L., and Wang, J., Comparison of Five Protocols of Estrous Synchronization on Reproductive Performance of Hu Sheep, Front. Vet. Sci., 2022, 9, 843514. doi: 10.3389/fvets.2022.843514
7. Caraba Ion Valeriu, Caraba Marioara Nicoleta, Dumintrescu Gabi, Dronca Dorel, Hutanu Delia, Preliminary tests on the induction of estrus during the non-breeding period in Turcana sheep International Conference on Environment and Natural Science, 2023 Turcia
8. Caraba Ion Valeriu, Caraba Marioara Nicoleta, Dumitrescu Gabi, Pet Elena, Popescu Roxana, Optimizing a protocol for inducing and synchronizing estrus in sheep during non-breeding season, SGEM Vienna GREEN International Scientific Coonfferrennce on Earth & Planetary Science. 2023.
9. Menchaca A, Rubianes E. New treatments associated with Timed Artificial Insemination in small ruminants. Reprod Fert Dev, 2004, 16, 403-414.
10. Menchaca A., dos Santos Neto P.C., Cuadro F., Estrous synchronization treatments in sheep: Brief update, Rev. Bras. Reprod. Anim., Belo Horizonte, 2017, 41, 1, 340-344.
11. Menchaca A., Miller V., Salveraglio V., Rubianes E., Endocrine, luteal and follicular responses after the use of the Short-Term Protocol to synchronise ovulation in goats. Anim Reprod Sci, 2007, 102, 76-87.
12. Vilariño M., Rubianes E., van Lier E., Menchaca A., Serum progesterone concentrations, follicular development and time of ovulation using a new progesterone releasing device (DICO) in sheep. Small Rum Res, 2010, 91, 219-224.
13 dos Santos Neto P.C., García Pintos C., Pinczak A, Menchaca A., Fertility obtained with different progestogen intravaginal devices using Short-term protocol for fixed-time artificial insemination (FTAI) in sheep. Livestock Sci, 2015, 182, 125-128.
14. Manes J, Hozbor F, Alberio R, Ungerfeld R. Intravaginal placebo sponges affect negatively the conception rate in sheep. Small Rumin. Res, 2014, 120, 108-111.
15. Martins L.T., dos Santos Neto P.C., Gaudêncio Neto S., Rauber L.P., Bertolini M, Vieira AD, Mezzalira A., Microbiological and functional evaluation of an alternative device (OB®) for estrous synchronization in ewes. Ciencia Rural, 2010, 40, 389-395.
16. Blaschi W., Lunardelli P.A., Marinho L.S.R., Max M.C., Santos G.M.G., Silva-Santos K.C, Melo-Sterza F.A., Baldassarre H., Rigo T.L., Seneda M.M., Effects of progestagen exposure duration on estrus synchronization and conception rates of crossbreed ewes undergoing fixed time artificial insemination. J Vet Sci, 2014, 15, 433-437.
17. Vilariño M., Rubianes E., Menchaca A., Ovarian responses and pregnancy rate with previously used intravaginal progesterone releasing devices for fixed-time artificial insemination in sheep. Theriogenology, 2013, 79, 206-210.
18. Taher J.K., Different oestrus induction methods in awassi ewes during the out of breeding season, Bas.J.Vet.Res. 2014, 1, 2.
19. King M., Mckelvey M.C., Dingwall W., Mattews W., Gebbi, K., Mylne F-E., Stewart E., Robinsons, J., Lambing rates and litter sizes following intrauterine or cervical insemination of frozen ⁄ thawed semen with or without oxytocin administration. Theriogenology, 2004, 62, 1236-1244.
20. HÙSEYİN, T and HAMIT, Y., Synchronization of oestrus in HAMDANI ewes: The use of Different PMSG doses. Bull Vet Pulawy, 2005, 49, 311-314.
21. Rosa, H. J. and M. J. Bryant, Seasonality of reproduction in sheep. Small Rumimant Research. 2003, 48: 155-171.
22. Boscos C.M., Samartzi F.C., Dellid S., Rogge A., Stefanakis A., Krambovitis E.., Use of progestagen gonadotrophin treatment in estrus synchronization of sheep. Theriogenology, 2002, 58, 1261-1272.
23. Timurkan H., Yildiz H., Synchronization of oestrus in Hamdani ewes. The use of different PMSG doses. Bull Vet Int pulawy, 2005, 49, 311-314.
24. Zimonetti L., Blanco M.R., Gardon J.C., Estrus synchronization in ewes treatment with sponges impregnated with different doses of medroxyprogesterone acetate. Small ruminant Research, 2000, 38, 247.
25. Vinoles C., Forsberg M., Banchero G., Rubianes E., Effect of Long – term and short – term progesterone treatment on follicular development and pregnancy rate in cyclic ewes. Theriology, 2001, 55, 993-1004.
26 Ungerfeld R., Rubianes E, Short term primings with different progestogen intravaginal devices (MAP, FGA, AND CIDR) for eCG- estrus induction in anestrus ewes. Small Ruminant Research, 2002, 46, 63-66.
27. Yu X., Bai Y., Yang J., Zhao X., Zhang L., Wang J., Comparison of Five Protocols of Estrous Synchronization on Reproductive Performance of Hu Sheep. Front. Vet. Sci. 2022, 9:843514. doi: 10.3389/fvets.2022.843514.
28. Rekwot P. I., Ogwu D., Oyedipe E. O., Sekoni V. O., The role of pheromones and biostimulation in animal reproduction, Anim Reprod Sci. 2001, 65(3-4):157-70. doi: 10.1016/s0378-4320(00)00223-2.
29. Wildeus, S., Current concepts in synchronization of estrus: Sheep and goats. Journal of Animal Science, 2000, 77: 1-14.
30. Kohno H, Okamoto C., Iida K., Takeda T., Kaneko E., Kawashima C., Miyamoto A., Fukui Y.,

Comparison of estrus induction and subsequent fertility with two different intravaginal devices in ewes during non-breeding season. Journal of reproduction and development, 2005, 51(6): 805-812.
31. Ustuner, B., Gunay, U., Nur, Z. and Ustuner, H., Effect of long and short-term progestagen treatments combined with PMSG on oestrus synchronization and fertility in Awassi ewes during the breeding season. Acta. Vet. Brno, 2007, 76: 391-397.
32. Jordan, K. M., Approaches to improve the ovulatory response and reproductive performance of ewes introduced to rams during seasonal anestrus. West Virginia: West Virginia University. 2005, 90.
33. Chanvallon, A., Blache, D., Chadwick, A., Esmaili, T., Hawken, P. A. R., Martin, G. B., Vinoles, C., Fabre-Nys. C. Sexual experience and temperament affect the response of Merino ewes to the ram effect during the anoestrous season. Animal Reproduction Science, 2008, Doi, 10:1016.
34. Skliarov, P., Pérez, C., Petrusha, V., Fedorenko, S., and Bilyi, D., "Induction and synchronization of oestrus in sheep and goats", Journal of Central European Agriculture, 2021, 22, 1, 39-53, DOI: /10.5513/JCEA01/22.1.2939S.
35. Ungerfeld, R., Suárez, G., Carbajal, B., Silva, L., Laca, M., Forsberg, M., Rubianes, E., Medroxyprogesterone primings and response to the ram effect inCorriedale ewes during the non-breeding season. Theriogenology, 2003, 60, 35-45.
36. Ungerfeld, R., Forsberg, M., Rubianes, E., 2004. Overview of the response ofanoestrous ewes to the ram effect. Reprod. Fertil. Dev. 16, 479-490.
37. Ungerfeld, R., Socio-sexual signalling and gonadal function: opportunities forreproductive management in domestic ruminants. In: Juengel, J.L., Murray, J.F.,Smith, M.F. (Eds.), Reproduction in Domestic Ruminants VI. NottinghamUniversity Press, Nottingham, UK, 2007, 207-221.
38. Delgadillo, J.A., Gelez, H., Ungerfeld, R., Hawken, P.A.R., Martin, G.B., Revisiting the dogmas surrounding the mechanisms involved in the male effect in sheep and goats. Behav. Brain Res., 2009, 200, 304-314.
39. Ungerfeld, R., Combination of the ram effect with PGF2 estrous synchronization treatments in ewes during the breeding season. Anim. Rep.Sci. 2011, 124, 65-68.
40. Ungerfeld, R., Sánchez-Dávila, F., Oestrus synchronization in postpartumautumn-lambing ewes: effect of postpartum time, parity, and early weaning.Span. J. Agric. Res. 2012, 10, 62-68.
41. Kenyon, P.R., Thompson, A.N., Morris, S.T., Breeding ewe lambs successfullyto improve lifetime performance. Small Rum. Res. 2014, 118, 2-15.
42. Gelez, H., Archer, E., Chesneau, D., Campan, R., Fabre-Nys, C., Importance oflearning in the response of ewes to male odor. Chem. Sens. 2004, 29, 555-563.
43. Corner, R.A., Mulvaney, F.J., Morris, S.T., West, D.M., Morel, P.C.H., WaCorner, P.R., A comparison of the reproductive performance of ewe lambs and matureewes. Small Rum. Res. 2013, 114, 126-133.
44. R. Ungerfeld, Reproductive response of mature and nulliparous yearling ewes to the ram effect during the non-breeding season, Small Ruminant Research 140 2016, 37-39.
45. Jaan Mohammad Wani, Utsav Sharma, Sajad A. Beig, Suhaib ul Haq Khan, Moien Javaid, Mudasir Bashir, A.K. Pandey, Umer Ali and Rouf Rashid Dar, Studies on Estrus Induction in Ewes during Non-Breeding Season. Int.J.Curr.Microbiol.App.Sci. 2017, 6(12): 1107-1115. doi: https://doi.org/10.20546/ijcmas.2017.612.125
46. Kashifalkitaa, H. F. A study of the effect of deep freezing with comparisons between AI and natural mating and hormone treatment on the conception rate of black Iraqi goats. Baghdad University- Coll. Vet. Med. Dept. Obst. Surg, 2003.
47. Kohno H., Okamoto C., Iida K., Takeda T., Kaneko E., Kawashima C., Miyamoto A., Fukui Y., Comparison of estrus induction and subsequent fertility with two different intravaginal devices in ewes during non breeding season. Journal of reproduction and development, 2005, 51(6): 805-812.
48. Juma, F.T., Effect of Prostaglandin and PMSG on prolificacy and some serum biochemical changes of Hamdani ewes synchronised with intravaginal progestagen. Al-Anbar Journal of Veterinary Science, 2010, 3(2): ISSN 1999-6527.
49. Alwan A. F., Thyroid hormones concentrations in relation to hormonal estrous induction, laparoscopical insemination and pregnancy out of breeding seasons. International Journal of Animal and Veterinary Advances. 2012, 4(5): 333-337.
50. Ataman, M. B., Akoez M., Akman O., Induction of synchronized estrous in Akkaraman cross-bred ewes during breeding and anestrous seasons, the use of short-term and long-term progesterone treatments. Rev. Med. Vet, 2006, 5: 257-260.
51. Dogan, I., Nur, Z., Different estrous induction methods during the non-breeding season in Kivircik ewes. Vet Med Czech, 2006, 51(4): 133-138.
52. Bogdan L., Groza, I., Pop, R., Petrean, Anamaria, Bogdan S., Induction and oestrus synchronization in sheep during breeding and non-breeding season. Bulletin UASVM, Veterinary Medicine, 2011 68(2).
53. Santos, G.M.G., Silva-Santos, K.C., Melo-Sterza, F.A., Mizubuti, I.Y., Moreira, F.B., Seneda, M.M. Reproductive performance of ewes treated with an estrus induction/synchronization protocol during the spring season. Animal Reproduction, 2011, 8(1/2): 3-8.
54. Sareminejad, P., Tabatabaei, S., Mamouei, M., Mirzadeh, K. And Boujarpour, M., The effects of short and long term Medroxy Progesterone Acetate (MAP) sponge treatments on reproductive performance during

the non-breeding season of Arabian ewes. Iranian Journal of Applied Animal Science, 2014, 4(4): 747-751.
55. Mellado, M., Olivas, R. and Ruiz, F., Effect of buck stimulus on mature and prepubertal norgestomet-treated goats. Small Ruminant Research, 2000, 36: 269-274.
56. Ungerfeld, R., Carbajal, B., Rubianes, E. and Forsberg, M., Endocrine and ovarian changes in response to the ram effect in MAP-primed Corriedale ewes during the breeding and non-breeding season. Acta Veterinaria Scandinavica, 2005, 46: 33-44.
57. Iida, K., Kobayashi, N., Kohno, H., Miyamoto, A., Fukui, Y., A comparative study of induction of estrus and ovulation by three different intravaginal devices in ewes during the nonbreeding season. J Reprod Dev, 2004, 50(1): 63-69.
58. Omontese, Bobwealth. Oakina., Rekwot, Peter. Ibrahim., Makun, Hussaina. Joan., Ate, Iyorhemba. Utim., Rwuaan, Joseph. Sankey, Induction of estrus in Sahel goats using Fluorogestone Acetate (FGA) sponges and equine chorionic gonadotrophin (ECG). Skoto j. vet sci, 2012, 10(2): 21-25.
59. Gardon, Juan. Carlos., Escribano, Begoña., Astiz, Susana., Ruiz, Salvador, Synchronization protocols in Spanish Merino sheep: reduction in time to estrus by the addition of eCG to a progesterone-based estrus synchronization protocol. Ann. Anim. Sci, 2015, 15(2): 409-418.
60. Marco-Jimenez, Francisco., Vicentel, Jose Salvador., Viudes-de-Castro, Maria Pilar., Effects of long-term fluorogestone acetate treatment combined with PMSG on oestrus synchronization and fertility in Guirra Ewes. Journal of Animal and Veterinary Science, 2014, 1(4): 25-29.
61. Ekiz, E. E. and Ozcan, M., Sexual behavior and hormone levels of Kıvırcık ewes after estrus synchronization during and out of the breeding season. Arch. Tierz., Dummerstorf, 2006, 49(6): 583-592.
62. Bhoraniya, B. L., Dhami, A. J. and Killedar, A., Influence of Estrus Synchronization Protocols on Fertility Plasma Progestrone and Biochemical Constituents in Kankrej Cows. Indian Journal of Animal Reproduction, 2012, 33: 2.
63. Amle, M. B., Nevkar, S. G., Birade, H. S., Gaikwad, S. M., Ulemale, A. H. and Bavaskar, M. S., Effect of CIDR + Ovsynch estrus synchronization protocol on estrus response and fertility rate in crossbred cows. National Symposium on Addressing Animal Reproduction Stresses through Biotechnological Tool and XXVIII Annual Convention of The Indian Society for Study of Animal Reproduction and National Symposium held at Assam Agricultural University, Khanapara Guwahati, 2012, 21-23, p. 114.