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NATURAL AND ARTIFICIAL INSEMINATION IN SHEEP - A REVIEW

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Abstract

In the temperate zone, sheep show an annual rhythm in their reproduction with alternating estrous and anestrous periods. In both seasons, biotechnical methods for intensification of the reproductive process include synchronization of estrous and ovulation through non-hormonal and hormonal methods and insemination. In sheep breeding practice, two methods of insemination are applied - natural and artificial insemination Factors influencing the efficiency of fertilization are: breed, age, season, feeding, physical condition of sheep and rams, method of synchronization, dose of gonadotropic preparation, method of insemination, time and frequency of insemination, use of fresh, chilled or frozen semen. In both methods of insemination, it is necessary to create good organization and control. The objective of the present review is to examine and summarize the factors influencing the result of the application of natural or artificial insemination of sheep.

Key words: *sheep, natural insemination, artificial insemination, laparoscopy.*

INTRODUCTION

Most sheep breeds in the world are seasonally polycyclic. It is customary for the anestrous season in the temperate zone to begin in mid March and end in late July or early August (Hristova, 2007). Seasonal anestrous is characterized by the absence of ovulation and sexual behavior and is a major factor hindering the year-round production of sheep's milk and lamb. There are sheep breeds that do not have a pronounced seasonal anestrous, such as Dorset Horn, Romanovska, Finnish Landrace, Ile de France, Northeast-Bulgarian fine-fleece, Merino etc. Establishing the presence of normal sexual cycling activity in all seasons of the year creates a basis for intensification of the reproductive process in these breeds (Goodman, 1994). Increasing the productivity of sheep breeding is essential for the economic efficiency of herds, which is associated with improved reproduction control (Nedjraoui, 2006). Assisted reproductive technologies are used to improve the reproductive characteristics of livestock and to accelerate genetic progress. These technologies include the application of various schemes for synchronization of estrous and ovulation, programmed insemination, freezing of sperm, transfer of embryos etc. Depending on the means used to synchronize estrous and ovulation, there are hormonal and non-hormonal methods. In sheep breeding practice, two methods of insemination are applied: natural and artificial.

Natural insemination can be performed with or without estrous synchronization. The advantages of the method are: cheaper, easier to perform, minimal stress for the animals, higher fertility (lambed ewes/inseminated ewes x 100) and no special equipment and trained specialists are needed (Baruselli et al., 2018). The disadvantages are the following: no rapid progress can be expected in the flock, faster depletion of the male breeder, because his intercourses cannot be regulated, a smaller number of sheep is covered by one ram (30-40 sheep), the period of use of the brood animal is shortened, covering one sheep repeatedly and the presence of unfertilized sheep, easier spread of sexually transmitted diseases in the flock, the date of insemination and the origin of the offspring cannot be registered. In sheep breeding practice, the so-called "hand release" is applied, in which the intercourses can be controlled, the ram is protected from exhaustion, fertility is increased, the date of insemination and the origin of the offspring are registered (Tyankov et al., 2000). The rams must be changed once every two years to avoid inbreeding.

Artificial insemination of sheep is a basic biotechnical method by which, in combination with the synchronization of estrous and ovulation, rapid genetic progress can be achieved through the use of a limited number of elite male sires (Alvares et al., 2015). In artificial insemination, fresh, chilled or frozen semen is used. According to the place of application of the sperm, are distinguished shallow cervical, deep cervical and intrauterine insemination (laparoscopic). To obtain good quality semen, it is recommended that rams perform 2-3 jumps per day for a period of 4-5 days, followed by 2 days of rest (Cueto and Gibbons, 2010). An important feature in the application of artificial insemination is the detection of ewes in estrous cycle. For this purpose, tester rams are used, which are raised separately from the sheep.

In France, more than 300,000 sheep are artificially inseminated annually, in Australia 500,000 sheep, 60,000 in Spain and 50,000 in Canada (Hernández Ballesteros et al., 2015). Artificial insemination of sheep is also well developed in Brazil, Argentina and Uruguay (Gibbons et al., 2019), while Bulgaria is one of the countries with poorly developed artificial insemination of sheep.

The advantages of the method are the following: maximum use of highly productive sires and fast transfer of their valuable hereditary qualities to a large number of offspring. 300-500 sheep can be inseminated by one ram. The date of insemination is registered, the paternity of the offspring is determined, individual selection and selection of the sheep, increase of the fecundity and twinning, protection of the animals from infectious, parasitic and venereal diseases are applied; reduction of infertility, the use of frozen semen avoids the import-export of valuable sires. Cryopreservation facilitates the long-term storage and transport of sperm. The frozen semen allows the conservation of endangered species or breeds, as well as in programs to eradicate various diseases (Tyankov et al., 2000; Amiridis and Cseh, 2012). The disadvantages of the method are: more expensive, more difficult to apply, more stressful for animals, tools, consumables and special equipment are needed, performed by qualified specialists, lower fertility, deep cervical insemination is difficult due to the anatomical complexity of cervix of sheep, reduced life expectancy of chilled sperm, the freezing procedure reduces the motility and viability of sperm, etc.

Allaouia et al. (2014) reported about fertility and fecundity (the number of born lambs (including all born lambs - live, dead and aborted)/lambed ewes x 100) in Algerian sheep breed Ouled Djellal of 86.70%, 116.54%, after natural insemination, 64% and 103% after artificial insemination.

The result of the application of natural and artificial insemination is influenced by a number of factors, such as: breed, age of sheep and rams, season, ambient temperature, farm, feeding before and after insemination, physical condition of sheep and rams, the method of synchronization, the dose of the gonadotropic preparation, the method of insemination, the period and frequency of insemination, the use of fresh, chilled or frozen semen, the place of application of semen in the female reproductive system, stress, the quality of semen, the experience of the insemination technician, etc.

RESULTS AND DISCUSSIONS

Some of the factors influencing the effectiveness of natural and artificial insemination in sheep will be discussed in more details in the present article.

The breed of sheep. The appropriate period for insemination is different for different breeds of sheep. This could be explained by the unequal period and rate of ovulation in different breeds (Fukui et al., 2010; Palacin et al., 2012), as well as differences in the physicochemical properties of cervical mucus, which can lead to difficulties in sperm movement (Kaabi et al., 2006; Richardson et al., 2011).

Karagiannidis et al. (2001) found that the optimal period for artificial insemination, after synchronization of estrous during the anestrous season in sheep of Chios, Vlachiki breeds and crossings (Chios x Vlachiki) is different. The most favourable moment for insemination of Vlachiki is the 48th and 60th hour, and for Chios and Chios x Vlachiki is the 48th and 72nd hour after removal of the vaginal sponge.

Artificial insemination administered at 60th hours after progestogen removal in Corridale sheep breed resulted in a higher fertility rate (Romano et al., 1997).

In Suffolk sheep, the optimal period for insemination was found to be 48th hours after removal of the vaginal sponge (Findlater et al., 1991). Whereas for Border Leicester × Scottish Blackface crossings, it is recommended to perform the artificial insemination at 54th -58th hours (Aitken et al., 1990). At Merino sheep it is recommended to be between 60th and 72th hours after the removal of the sponge, although the average ovulation time is the same in these two breeds (Eppleston and Roberts, 1986).

Age of animals. With age, fertility and fecundity in sheep decreases, which is associated with the presence of more reproductive disorders in older sheep, as well as the ovulation of fewer ova and lower quality than young sheep (Belkasmi et al., 2010).

In their study conducted on Churra, a Spanish milk breed, Anel et al. (2005) reported that as the age of the sheep increased, their fecundity decreased by 1.74% per year when using vaginal artificial insemination and by 2.07% when using the laparoscopic method.

Alabart et al. (2002), studied the influence of age on fertility in a total of 3819 sheep of the Spanish breed Aragonesa aged 1 to 12 years, artificially inseminated. In the present study, the authors reported a maximum fertility (56.7%) at 3 years of age, and in sheep aged 2 to 5 years the average fertility values were over 50%.

After vaginal artificial insemination with chilled semen in Chios and Lacaune sheep, Priskas et al. (2019) found that the animals of the 2nd (50.3%) and 3rd lactation (48.1%) had the highest fertility rate, which decreased with age.

According to Palacín et al. (2012) the fertility of sheep in cervical artificial insemination after the fifth birth is significantly reduced.

The age of ram affects the motility and concentration of sperm. Benia et al. (2018) reported that sperm motility was better in 3-4 year old males of Ouled Djellal breed.

A study on local rams in Bangladesh aged 1 to 4 years shows that with increasing age, sperm quality improves and stabilizes up to 3 years of age (Hassan et al., 2009). Ntemka et al. (2019) report that Chios rams maintain high sperm quality until the age of 13.

Breeding season. A characteristic feature of reproduction in sheep is seasonality. It is mainly determined by the length of daylight. Its reduction leads to the onset of the estrous period, during which the female animal is dispersed and fertilized. It is customary for the anestrous season in the temperate zone to begin in mid March and end in late July or early August. Seasonal anestrous is characterized by the absence of ovulation and sexual behavior (Hristova, 2007). Seasonal changes in temperature are of secondary importance. Fever can lead to ova death, embryonic death, and suppression of sexual reflexes (Hristova et al., 2011a).

Palacín et al. (2012) analyzed the results of 18,328 cervical artificial inseminations with chilled semen in sheep of the local Spanish breed Aragonesa. The authors reported the highest fertility in July, August, September and October (58.3%, 58.1%, 61.9% and 65.2%).

After artificial insemination in another Spanish breed (Churra), Anel et al. (2005) received lower fertility for the period July - August. The probable reason is that Churra breed, which is specialized in milk production, is more susceptible to heat stress than Aragonesa breed.

The season also affects the quality of semen in male animals, which can lead to reduced fertility and fecundity (Kukovics et al., 2011).

Higher values of sperm motility were found in the breeds Mountain Corriedale, East Friesian and Copper-red Shumen during the autumn-winter period and lower during the spring-summer period (Manolov and Georgieva, 2008).

In rams of the native South African breed Zulu, Ngcobo et al. (2020) received sperm with higher volume and motility during the estrous season (0.97 ml and 92.01%) compared to the anestrous (0.72 ml and 88.69%).

During the estrous season, the inseminating ability of rams is higher (Boykovski et al., 2017). Semen obtained only during the breeding season can be successfully frozen (Manolov and Georgieva, 2008). In intrauterine insemination of sheep with frozen semen, fertility is higher during the estrous season (60%) than in the anestrous season (45%) (Dovenski et al., 2012).

The body condition of the animals. In the sheep, which at the beginning of the random campaign had an BCS (body condition score) 2.5-3.5 points, a higher number and better quality ova were reported, as well as a higher fertility and fecundity rate (Fukui et al., 2010). While in weaker animals (BCS below 2.5) the number and quality of ovulated ova decreases, embryonic growth decreases and embryonic mortality increases (Abecia et al., 2014; Ridler et al., 2017).

Sheep weight is an important factor in determining the effect of estrous synchronization. Gizaw et al. (2016) reported that sheep with BCS of 2.5-3.5 points at the onset of estrous synchronization responded best to hormonal stimulation. These animals have shown the clearest signs of estrous and have the highest fertility. Identical results were obtained by Priskas et al. (2019) in Chios and Lacaune sheep breeds, after applied artificial insemination. The authors received 48.4% and 49.4% fertility in sheep with BCS 2.5 and 3.5.

Synchronization of estrous with progestogen and/or prostaglandin. Synchronization of estrous during the estrous and anestroul season has common principles, but there are also differences arising from the unequal hormonal status. The main difference is that after discontinuation of progesterone treatment in the breeding season, a large amount of LH and FSH is spontaneously released from the pituitary gland, causing complete estrous. During the anestrous season, FSH and LH peak do not appear on their own after treatment with progesterone or its synthetic analogues, additional administration of serum gonadotropins is required. Induced estrous at rest is single (Barrett et al., 2008).

Induction of estrous by prostaglandins can only be used during the breeding season (Fierro et al., 2017). Zonturlu et al. (2011) inseminated Awassi sheep naturally after synchronization estrous with vaginal sponges impregnated with progesterone and different doses PMSG (300, 400 and 500 IU). The reported fertility is similar in all three groups and is between 80.0-82.6% and fecundity is 100%.

Metodiev and Raicheva (2011) synchronization the estrous of Ile de France sheep breed in two schemes. One with progestogen for 6 days + prostaglandin + 250 IU PMSG, when removing vaginal sponges (1 group), and the other with progesterone and PMSG (2 group), after which the animals were artificially inseminated with fresh, undiluted semen, at a dose of 0.2 ml. The authors reported fertility and fecundity of 63.64% and 142% in the first group and 43.45% and 140% in sheep in the second group. In sheep of the same breed Metodiev (2019) induces estrous through different duration of short progestogen treatments (5, 6 and 7 days) + synthetic analogue of PGF2α + gonadotropin + single natural insemination. Fertility of 54.54-63.63% and fecundity of 171.43-200% were obtained.

Almeida et al. (2018) reported that fertility and fecundity in sheep were similar after two treatments with prostaglandins at different time intervals (7, 9, and 11 days).

After treatment with prostaglandins, fertility is higher in natural insemination (Metodiev, 2017). In the case of natural coverage (by hand), Sözbilir et al. (2006) reported fertility of 60.2% in the group with an interval of 10 days and 73.3% in the group with an interval of 14 days. While in artificial insemination fertility varies from 22% to 62% (Fierro et al., 2013).

The dose of gonadotropic preparation (PMSG) used. PMSG treatment causes rapid follicle growth, increases the number of ovulated follicles and synchronization estrous and ovulation (Eekass et al., 1989), and also allows to reduce follicular atresia (Hirsfield, 1989). The dose of the gonadotropic preparation needs to be adjusted according to the breed, the physical condition of the sheep and the season (Gibbons and Cueto, 2012). During the estrous season, PMSG dose should be higher in breeds with lower fecundity and lower in breeds with higher fecundity. Breeds with "deep anestrous" require higher doses of serum gonadotropin during the anestrous season (Bonev et al., 2002).

Aköz et al. (2006) found that treatment with 700 IU PMSG in the anestrous season in Akkaraman crossings resulted in increased fertility, fecundity and twinning (100%, 86.6%, 69.2%).

Hristova (2007) reported that in Tsigai sheep breed, during the anestrous season, doses of 400-500 IU PMSG are optimal.

Following the estrous synchronization with a progestogen and injection of different doses

(300, 400, 500 and 600 IU) of PMSG at the beginning of the estrous season, in combination with vaginal artificial insemination, the highest fertility, fecundity and twinning (67.9%, 126.2% and 58.9%) was reached in Kurdi, an Iranian sheep breed, at a dose of 600 IU PMSG (Nosrati et al., 2011).

Gibbons and Cueto (2012) report that in the breeding season, the recommended dose of the gonadotropic preparation for Merinos breed is 250-300 IU and 300 IU for Corriedale and Texel breeds.

The high dose of exogenously imported serum gonadotropins (1000 IU) leads to overstimulation of the ovaries and hence the other organs of the genital system, carries risks of cystic changes, which leads to disturbances in the processes of fertilization and implantation (Bonev, 2003; Ralchev et al., 2007).

Period and frequency of insemination after estrous synchronization – The optimal time for artificial insemination after gonadotropin injection 48th hours before removal of vaginal spongesis 36th - 48th hours, according to Hristova et al. (2011) and at 48th and 60th hours when applying the same at the time of removal of vaginal sponges.

A number of authors (Menchaca and Rubianes, 2004; Jha et al., 2020) recommend that artificial insemination in sheep should be performed twice, at the 54th and 60th hour after removal of the vaginal sponges.

According to Cseh et al. (2014) the most appropriate period for single or double artificial insemination is 55th and 50th-60th hours after sponge removal, respectively.

Cueto and Gibbons (2011) found that the most favourable period for cervical artificial insemination is between 53th and 56th hours after injection of the second dose of the prostaglandin analogue. While intrauterine insemination should be performed between 58th and 66th hours after hormonal treatment.

While Metodiev (2017) administered a single natural insemination at 49th hours after the second dose of prostaglandin.

The use of fresh, chilled and frozen semen. In sheep breeding practice, fresh semen is mainly used, with fertility ranging from 70% to 82% (Donovan et al., 2001; 2004; Ehling et al., 2003). The fresh semen should be used immediately after collection, as sperm viability decreases rapidly. It is used undiluted or diluted. It can be stored at 28-30°C in a thermostatic bath during insemination for a period of 30-60 min (Gibbons et al., 2019).

In Dorper sheep breed with synchronized estrous and artificially inseminated with freshly diluted semen at a dose of 0.1 ml, Zeleke et al. (2005) obtained 75% fertility and 94.6% fecundity.

After vaginal artificial insemination of sheep in Argentina with fresh semen, with a sperm concentration between 60 and 100 million per sheep, Naim et al. (2009) reported fertility of 60%, and Prieto et al. (2011) - 70%. Cueto and Gibbons (2010) obtained identical results for the same reproductive index. The authors conducted their study with sheep in Uruguay, which were inseminated with fresh, undiluted semen, at a dose of 0.2 ml and a concentration of 100-150 million sperm.

Insemination with chilled and frozen semen in sheep breeding is more limited (Salamon and Maxwell, 2000). In chilled semen, the speed of sperm transport in the sheep's genital tract is lower than that of fresh semen (Fernández et al., 2001).

A number of authors (Naim et al., 2009; Cueto and Gibbons, 2010) reported an average fertility of 65% obtained after vaginal artificial insemination with fresh undiluted semen and 40% with chilled. While Stefanov et al. (2006) reported higher values of the indicator after insemination with cooled semen - 64.2-73.33%. The use of artificial insemination with frozen semen is hampered by a number of factors, such as: the specific anatomy of the sheep's cervix, which is a physical barrier to sperm deposition in the uterus (Anel et al., 2005), the lower resistance of rams to semen freezing, sheep sperm are more sensitive to thermal stress than cold (Dinatolo, 2011), lack of proper cryopreservation and storage methods (D'alessandro and Martemucci, 2003; Santolaria et al., 2011; Gibbons et al., 2019).

Fertility in deep cervical insemination with frozen semen varies between 25-35% (Anel et al., 2005; Faigl et al., 2012), and in vaginal insemination it is even lower - 5-15% (Cseh et al., 2014).

Only through the laparoscopy method it is possible to deposit frozen-thawed semen in the uterine horns of sheep. Fertility in this method reaches approximately 60-75%.

When using cryopreserved semen, the ejaculate is pre-diluted in order to increase its volume and inseminate a larger number of sheep, as well as to provide a suitable nutrient medium necessary for its storage (Cueto et al., 2016).

In Australian merino sheep inseminated with fresh semen or frozen in granules or sequins, Hill et al. (1998) had fertility rates of 82.2%, 69.5% and 71.6%, respectively.

Place of sperm application in the female reproductive system - vaginal, deep cervical and laparoscopic (intrauterine).

Vaginal artificial insemination is most often used in sheep breeding because it is the easiest to apply and with the lowest equipment costs and a qualified team of specialists needed for laparoscopic insemination (Dovenski et al., 2012). In vaginal insemination, a dose of 0.1-0.2 cm^3 is injected, with 80-100 million live sperm with active translational movements.

Satisfactory results of deep cervical insemination have not yet been achieved, especially in the anestrous season and when using frozen semen. Probably the cause is the impaired transport of sperm through the cervix (Boland al., 1983). This problem can be overcome by intrauterine sperm deposition (Ishwar and Memon, 1996).

Using intrauterine insemination, the problem of the "cervical barrier" has been overcome, satisfactory fertility has been achieved by significantly reducing the number of sperm per insemination (Salamon and Maxwell, 2000). Insemination is performed based on monitoring of ovarian function, which leads to accuracy in fertilization (Yufeng, 2012). It is an alternative method of artificial insemination using frozen-thawed semen (Abdalbari et al., 2012).

Compared to vaginal at laparoscopic artificial insemination, higher fertility results were obtained (44.89% vs. 31.25%) in Churra sheep breed (Anel et al., 2005).

Taqueda et al. (2011) also reported higher fertility obtained after intrauterine, deep cervical and vaginal insemination with frozen semen - 45.8%, 25.7% and 15.4%. After deep cervical insemination with one or two inseminations, Kumar and Naqvi (2014) reported fertility of 20% and 26%, respectively. Bonev et al. (1991) achieved 75-83% fertility in laparoscopic insemination of sheep.

After estrous synchronization in Ghezel sheep, Najafi et al. (2014) applied two insemination methods - vaginal and laparoscopic. The authors received 60% fertility, 60% economic fecundity and 28.7% twinning in vaginal and 83.3%, 76.6% and 30.4% in laparoscopic insemination.

Dovenski et al. (1997) found a higher percentage of fertility and fecundity in goats inseminated intrauterine (80.95% and 182.35%) compared to vaginal inseminated (67.48% and 176.98%).

CONCLUSIONS

Establishing the links among all the mentioned factors in the different sheep breeds raised in different climatic areas is necessary for the proper regulation of the reproductive process, and hence for increasing the productivity of the flocks.

Estrous synchronization and ovulation are a key element of effective reproductive management in sheep. It allows planning and conducting the breeding and lambing campaign in a short time.

An important condition for increasing the efficiency of natural or artificial insemination is the use of rams with high sexual activity and sperm of good quality and high insemination capacity.

The highest fertility with frozen semen was achieved during intrauterine laparoscopic insemination.

In both methods of insemination, it is necessary to create good organization and control.

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