# RESEARCH ON THE EFFICIENCY OF ARTIFICIAL INSEMINATION WITH FROZEN SEMEN OF MERINO PALAS SHEEP DURING THE BREEDING SEASON

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**Abstract**

*Artificial insemination is the most important reproductive biotechnology that helps to increase the genetic value of animals on a farm. It enables the rapid and massive diffusion of desired traits by using the semen of selected males with high productive potential. Correlating types of timing and oestrus induction in sheep with long-term semen preservation (cryopreservation) is the best way to increase productivity and ensure the dissemination of genetic progress. The aim of the present research was to identify the most effective treatment option for the application of artificial insemination in Merino Palas sheep, with frozen semen, in the normal breeding season. By using prostaglandins (Roflavol) for synchronizing females and identifying the optimal moment for performing artificial insemination, a calving rate of 32.14% was obtained with a prolificacy of 111.11%, and by applying the hormonal treatment Chronogest- Folligon to induce and synchronize oestrus with fixed point artificial insemination, the calving rate was 53.33% and the prolificacy was 131.25%. By artificial inseminating the ewes detected in natural oestrus with test rams' frozen semen, a calving rate of 31.67% and prolificacy of 110.53% was obtained.*

**Key words**: *ewes, oestrus synchronization, artificial insemination*

## INTRODUCTION

Sheep are seasonal polyestrous animals, presenting a succession of estrous cycles during the autumn-winter period (breeding season) and from April to July, the ewe does not exhibit estrous and is in a period of sexual rest called sexual counter-season or seasonal anestrus, photoperiodically controlled activity (Castonguay, 2018, Pascal, 2015, Chemineau et al., 2010).

The application of reproductive biotechnologies allows the monitoring of the breeding campaign according to the interests of the sheep breeders, by using the synchronization and induction of estrous in the normal season or in the counter-season of reproduction in association with artificial insemination.

In order to achieve artificial insemination, it is necessary to know the optimal moment of depositing the semen in the female genital tract. In this sense, the batch of females must be properly synchronized, respectively to be found at the end of the estrous phase (Costonguay, 2018). The most effective method of estrous synchronization proved to be the hormonal method (Chronogest-Folligan) with the advantage that it can be used both in the season and in the counter-season of reproduction and the exact moment of ovulation is known but with the disadvantage of relatively high costs for farmers, of the inconstancy on the market and especially the recommendation to use a maximum of two administrations in two consecutive years, due to the phenomenon of accumulation in the body and the need for intervention with anti-PMSG hormone from the third year (Bodin et al., 1997). An alternative to the use of gonadotropic hormones is the use of prostaglandins, products that are cheap and easy to obtain. The disadvantage of the method is given by its use only in the breeding season, as it achieves synchronization through the lysis of

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the corpus luteum and the resumption of estrous activity, synchronously, of the females in the programmed group. Prolificity, in the case of the use of prostaglandins, is specific to each breed, not being hormonally influenced as in the case of the use of gonadorelins (Folligan with the role of FSH and LH) which also causes a slight superovulation due to the exact lack of correlation between the dose of PMSG and the degree of lactation, the level of lactation, genetic determinism, etc.

Artificial insemination at a fixed point following the actions of synchronous induction of estrous is the most effective method of increasing the number of females inseminated in a single day with minimal labor consumption required for estrous detection procedures. The safest methods of spot-fixed heat induction are the use of progesterone-releasing devices and the administration of gonadorelins. Although the method of induction and synchronization is safe, studies are focused on establishing the optimal time to perform artificial inseminations and, as far as possible, to perform a single insemination. It is also aimed at decreasing the action time of intravaginal devices by creating short-term protocols (Menchaca et al., 2017).

Through the procedures of preserving semen from genetically valuable males by refrigeration, but especially by freezing, the diffusion of genetic progress can be ensured both over long distances and especially over time (Gibbons et al, 2019). Correlating types of timing and estrous induction in sheep with long-term semen preservation is the best way to increase productivity, save populations that are reduced in numbers, endangered or otherwise at risk.

Numerous studies have been conducted to develop semen cryopreservation methods, to create optimal freezing patterns, to find various cryoprotective media, to improve thawing techniques to improve the quality of cryopreserved semen. The technology of sperm cell cryopreservation has opened new opportunities in reproductive work due to the more intensive use of high-value genetic potential and the creation of frozen semen stores (Lebedeva et al., 2015).

## MATERIAL AND METHOD

The research carried out during the normal breeding season (July-August 2021) aimed to establish the efficiency of applying artificial insemination with frozen semen to 60 Merino Palas ewes synchronized by two hormonal methods and to establish the optimal moment of insemination.

The first batch of 30 ewes was synchronized by using synthetic progestagens with progressive release, namely Chronogest CR sponges (the company INTERVET INTERNATIONAL BV, Netherlands) impregnated with 20 mg of fluorogestone acetate (cronolone), which were maintained intravaginally for 14 days. The role of these progestagen-impregnated sponges is to block ovarian activity and upon their withdrawal, all females resume their activity from the same stage, thus achieving the synchronization of the estrous cycle. Once the sponges are withdrawn, gonadotropins or pituitary hormones are used: FSH or another synthetic product with the role of developing and maturing the ovarian follicles and LH to achieve follicular dehiscence. The product we used was Folligon (the company INTERVET INTERNATIONAL BV, Netherlands) which has equine serum gonadotropin as its active substance and fulfills the role of both FSH and LH. The administration of Folligan was carried out in a dose of 400 IU/head. Artificial insemination with frozen semen was performed 55 +/- 1 hour after the withdrawal of the Chronogest sponges (Fatet et al, 2008).

The second batch of 30 sheep were synchronized by the use of Prostaglandins, namely the intramuscular injection of 1 ml Roflavol (0.25 mg D-Cloprostenol sodium) (ROMVAC CO, Romania) at an interval of 8 days, the second prostaglandin injection taking place at 7 am. With a view to artificial insemination, 24 hours after the last administration of prostaglandin, estrous was detected with test rams provided with an apron, in the morning and in the evening, respectively at 7 o'clock before grazing and at 7 o'clock when they return from the pasture, an action which was repeated for three days. Ewes that were detected in heat were artificially inseminated twice, at 12-hour intervals.

Batch 3, considered a control batch, consisting of 65 Merino Palas sheep, were artificially inseminated with semen frozen on natural oestrus. Thus, every morning, at 7 o'clock, heat was detected with test rams provided with aprons. The sheep detected in the tubs were artificially inseminated at 12 o'clock and 24 hours after the heat detection (at 7 p.m. of the same day and at 7 a.m. the next day). The estrous detection work took place during 26 days until all the ewes in batch 3 were artificially inseminated.

In order not to interfere with the treatment options, i.e. the ewes in the control group to synchronize through the "male effect" or "female effect", the three groups were kept in the rearing shelter, in three separate boxes, at a distance from each other. The ewes from the 3 groups were selected based on their reproductive status, all of them being multiparous, at the 3rd gestation, they were kept in the same microclimate conditions and were given the same food ration. Artificial insemination was carried out with semen from a single male that was collected in February 2021 and the semen was processed with the same diluent and according to the same technology. At the time of artificial insemination, the frozen semen was thawed by placing the fine straw (0.25ml) in warm water at 38 C for 20 seconds, then it was wiped with water, the sealed end was cut off in the laboratory and loaded into the insemination torch, also heated to 37 C.

Regardless of the treatment option, the artificial insemination was carried out by the same team of researchers. For the deposition of the semen, the vaginal ostium of the cervix is visualized with the vaginoscope with its own light and by tunneling with the insemination pipette, the degree of opening of the cervix is assessed according to the stage of the estrous phase, respectively:

- open cervix - when the insemination pipette penetrates the cervix without encountering any difficulties and the semen is deposited deep intracervical,

- half-open cervix – when the insemination pipette penetrates the cervix 0.5-1.5 cm and encounters opposition in the total tunneling of the cervix, the semen being deposited at the entrance to the cervix,

- closed cervix - when the insemination pipette does not penetrate into the vaginal ostium of the cervix and the semen is deposited intravaginally, at the level of the involved flora.

The efficiency of the treatment option was assessed by establishing the main reproductive indices and establishing the efficiency of each method.

## RESULTS AND DISCUSSIONS

As the estrous synchronization works are carried out during the breeding season, from the 16th day after artificial insemination, estrous detection was carried out with test males to detect non-pregnant ewes, thus establishing the non-return rate (NR%). At an interval of 40-45 days after artificial insemination, pregnant females were diagnosed (DG%) by ultrasound examination (portable ultrasound WED 3000) equipped with a 3.5 MHz convex probe, and in December 2021- January 2022 was established the gestation (F%) and the prolificacy (P%) rate by tracking calvings. Table 1 shows the reproductive indices obtained in the 3 batches of females and the efficiency of artificial insemination for each experimental variant (ET%) is highlighted.

In the 1st batch of ewes synchronized by the Hormonal Chronogest-Folligan method, it can be observed that when detecting estrous, the percentage of pregnant ewes was 56.67% (no=17 ewes), while 53.33% (no=16 ewes) of pregnant ewes were identified during the pregnancy diagnosis. The difference indicates the existence of a female that has suffered embryonic loss, without clinical symptomatology. The pregnancy rate of 53.33% coincides with the efficiency of the application of artificial insemination on hormonally induced estrous, since all the females selected at the formation of the batch showed estrous before insemination at a fixed point and were inseminated. At the time of artificial insemination, the degree of opening of the cervix was observed, in all 30 females the frozen-thawed seminal material was deposited at the cervical level. This fact indicates a synchronous response of females to the hormonal treatment option. Taking into account that the artificial insemination was

performed transcervically with frozen-thawed semen, the results are good, comparable to the results reported in the international specialized literature. Thus, Azzarini et al, in 1988, in the case of intrauterine insemination, reported a fecundity ranging between 42 and 53%, Aybazov A.M. et al. published in 2019 a calving rate of 34% by transcervical insemination and 43.7-68.8% by laparoscopic intrauterine insemination of ewes with frozen semen. In 1997, Gergatz and Gyoker, report a fertility between 69.5 and 71.6% following laparoscopic insemination with frozen semen. Donovan et al published in 2004 the results of artificial insemination with frozen semen in ewes in natural estrous, obtaining a fecundity of 29% and 52% in hormonally synchronized ewes.

Table 1 Reproduction indices recorded in Merino Palas ewes artificially inseminated with frozen seminal materials, in the breeding season, depending on the method of estrous induction

| Batch | No of ewes | Treatment option | Ewes in estrous | NR | DG | F | P | ET |
|---|---|---|---|---|---|---|---|---|
| | | | | % | % | % | % | % |
| 1 | 30 | Chronogest | 30 | 56.67 | 53.33 | 53.33 | 131.25 | 53.33 |
| 2 | 30 | Roflavol | 28 | 32.14 | 32.14 | 32.14 | 111.11 | 30.00 |
| 3 | 65 | Natural estrous | 60 | 36.67 | 33.33 | 31.67 | 110.53 | 29.26 |

*NR% - non-return rate - the percentage expression of the number of females not returned in estrous 16-18 days after artificial insemination, compared to the number of inseminated females;*

*DG% - pregnant females diagnosed by ultrasound - the percentage expression of the number of pregnant females at ultrasound compared to the number of inseminated females;*

*F% - calving rate - the percentage expression of the number of females calved in relation to the number of inseminated females;*

*ET% - the efficiency of the treatment - the percentage expression of the number of females calved in relation to the number of females in the batch*

As the research was carried out during the normal breeding season, by administering the prostaglandin Roflavol, the lysis of the luteal bodies was achieved, obtaining the synchronization of the females and the resumption of the estrous cycle and the development and maturation of the ovarian follicles in a natural sequence, without intervening with medication to induce ovulation. Under these conditions, from the 30 ewes included in the batch, when detecting estrous with test rams, only 28 females showed estrous (93.33%) and were inseminated with frozen-thawed semen. Of these, 9 ewes did not show estrous when checking the returns to estrous, being considered pregnant, a fact also confirmed by the ultrasound examination. All 9 ewes carried the pregnancy to term, registering a fertility of 32.14%. Taking into account that not all females showed estrous through this method of synchronization, the efficiency of the treatment was 30%.

Table 2 shows the manifestation of estrous and the method of performing artificial inseminations in batch 2 of ewes treated with prostaglandins.

Table 2 Reproductive indices in Merino Palas ewes artificially inseminated with frozen semen in the breeding season following synchronization with prostaglandins

| Batch | Ewes in estrous | Time slot* | NR | | DG | | F | | P | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | nr. | % | nr. | % | nr. | % | nr. | % |
| 1 | 2 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 12 | 36 | 6 | 50.00 | 6 | 50.00 | 6 | 50.00 | 7 | 117.00 |
| 3 | 9 | 48 | 3 | 33.33 | 3 | 33.33 | 3 | 33.33 | 3 | 100.00 |
| 4 | 5 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 28 | | 9 | 32.14 | 9 | 32.14 | 9 | 32.14 | 10 | 111.11 |

*Time slot* - the number of hours since the last injection of Roflavol and the moment of estrous manifestation, respectively the male's acceptance,*

*NR – non-return rate*

*DG – ultrasound confirmed gestation rate*

*F - fertility, respectively percentage of females that calved compared to artificially inseminated females*

*P - prolificacy, respectively the percentage of lambs compared to the number of ewes that calved*

Manifestation of oestrus in the group of sheep synchronized with prostaglandins took place in the time interval 24-60 hours after the second administration of prostaglandin.

Since the artificial insemination was done 12 hours after the detection of estrous with repetition every 24 hours and correlating with the results obtained we can conclude that the optimal moment of insemination corresponding to the ovulatory peak is 36-48 hours after the second prostaglandin administration. Similar results regarding the effectiveness of synchronization treatments in the breeding season with prostaglandins were also obtained by other researchers who used a short action protocol, namely the administration of two injections of 75 mg D-Cloprostenol each, intramuscularly at an interval of 7 days in order to synchronize of ewes for fixed-point artificial insemination with refrigerated semen led to a treatment efficiency of 42.6%. (Olivera-Muzante J. et al., 2013).

It is very important to determine the optimal moment of administration, because the studies conducted by Olivera-Muzante J. et al. in 2013 demonstrated that the application of GnRH 24-36 hours after the second Prostaglandin injection led to a decrease in the efficiency of the treatment option, reaching 10.2% (GnRH 24 hours after the second PgF2α injection) and 33.7% (GnRH 36 hours after the second injection of PgF2α) based on ewes that calved following artificial insemination with refrigerated semen. Therefore, the results obtained in the present experiment, i.e. two administrations of Roflavol at an interval of 8 days without intervening with gonadorelin were in accordance with the data of the specialized literature.

In the case of the ewes from batch 3, the control batch, the artificial insemination was carried out 12 and 24 hours after the detection of the first estrous manifestations. Taking into account that the clinical manifestation of estrous in ewes is on average 24-36 hours and we did this detection once a day (once in 24 hours) and by correlating with the results obtained in batch 2 (table 1) we can conclude that the 22 ewes that did not return to estrous 16-18 days after insemination were detected approximately 12 hours after they went into heat. And in this lot, there were differences between the rate of non-returns in estrous and the ultrasound confirmation of gestation, which leads to the hypothesis of the existence of embryonic death in 2 females and one female aborted in the 4th month of gestation due to mechanical causes, obtaining a calving rate of 31.67% (no=19 ewes). Out of the 65 ewes selected for insemination on natural estrous, 5 had no visible signs of heat and did not accept the male. Therefore, the effectiveness of this variant of preparing the females for insemination was only 29.26%.

The prolificacy recorded in the 3 batches of sheep was between 111.11 and 131.25%, the highest value being recorded in the batch of hormonally synchronized ewes. By administering Folligon which has the role of FSH ensuring the growth and maturation of the oocytes but also the role of LH determining the ovulatory peak, a slight superovulation occurred in some females who were more receptive against the background of a lower milk production, therefore also low concentrations of LTH.

## CONCLUSIONS

The efficiency of the application of artificial insemination with frozen semen during the normal breeding season in Merino Palas ewes was different depending on the way the ewes were prepared. The best results were obtained through the protocol of inducing and synchronizing estrous through hormonal methods, the calving rate and the efficiency of applying this procedure being 53.33%, with a prolificacy of 131.25%. The method, although effective and applicable throughout the year, regardless of the breeding season, has the disadvantage that it can be repeated 2- maximum 3 consecutive years in the same animal, and the costs of hormonal treatment prevent it from being applied on a large scale to sheep owners with small herds and limited financial strength.

In the experimental version of estrous synchronization through the lysis of the luteal bodies, in the case of using prostaglandins (Roflavol), comparable results were obtained

with the artificial insemination of ewes in natural estrous (calving rate 31.14% vs 31.67% and prolificacy 111.11% vs 110.53%) with the advantage that the breeding campaign and therefore also the calving campaign were combined, and by reducing the execution time of directed breedings, a better management of the labor force in farms is achieved.

## REFERENCES


1. Aybazov A.M., Malmakov N.I., Selionova M.I., Mamontova V. (2019). Fertility of ewe following intrauterine laparoscopic insemination with frozen-thawed semen, Conference on Innovations in Agricultural and Rural development, Series: Earth and Environmental Science vol. 341, doi:10.1088/1755-1315/341/1/012163.
2. Azzarini M., Valledor F. (1988). Inseminacion intrauterina o cervical con semen congelado o fresco en ovejas en celo natural, Prod Ovina no. 1, p 1–8.
3. Bodin L., Drion P.V., Remy B., Brice G., Cognié Y., Beckers J.F. (1997). Anti-PMSG antibody levels in sheep subjected annually to oestrus synchronization, Reprod. Nutr. Dev., 37, p 651-660.
4. Castonguay F. (2018). La reproduction chez les ovins, pp. 26-33.
5. Chemineau, P., Bodin, L., Migaud, M., Thiéry, J.C., Malpaux, B. (2010). Neuroendocrine and genetic control of seasonal reproduction in sheep and goats. Reproduction in Domestic Animals. 45 (Supplement 3), p 42-49.
6. Donovan A., Hanrahan J.P., Kummen E., Duffy P., Boland M.P. (2004). Fertility of the ewe following cervical insemination with fresh and frozen-thawed semen at natural and synchronized oestrus, Animal Reproduction Science, 84, 359-368.
7. Fatet A., Leboeuf B., Freret S., Druart X., Bodin L., Caillat H., David I., Palhière I., Boué P. Lagriffoul G. (2008). L'insémination dans les filières ovines et caprines, Renc. Rech. Ruminants, No. 15, p. 355-358.
8. Gergatz E., Gyoker E. (1997). Cervicouterinal insemination method with cooled and deep frozen ram semen, Book of Abstract, Annual meeting of EAAP , Vienna, 48, p 39-325.
9. Gibbons A.E., Fernandez J., Bruno-Galarraga M.M., Spinelli M.V., Cueto M.I. (2019). Technical recommendations for artificial insemination in sheep, Animal Reproduction, vol 16, p 803-809.
10. Lebedeva L.F., Atroshchenko M.M., Burmistrova S.A. (2015). The main factors affecting the resultant insemination of mares with sperm, cryopreserved by Russian and foreign technologies Agricultural Biology, vol. 50:4, p. 476–85.
11. Menchaca A., Pedro C. dos Santos Neto, Cuadro F. (2017). Estrous synchronization treatments in sheep: Brief update, Rev. Bras. Reprod. Anim., Belo Horizonte, vol 41:1, p 340-344.
12. Olivera-Muzante J., Gil J., Vinoles C., Fierroa S. (2013). Reproductive outcome with GnRH inclusion at 24 or 36 h following a prostaglandin F2α-based protocol for timed AI in ewes, ELSEVIER, Animal Reproduction Science, 138 (2013), pp 175-179.
13. Pascal C., 2015. Sheep and goat breeding book, Edit. "Ion Ionescu de la Brad", p, 466-503.