

PRELIMINARY RESULTS OF ARTIFICIAL INSEMINATION WITH FRESH DILUTED SEMEN DURING NATURAL ESTROUS AT EWES

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Abstract

Based on the context of the development and extension of the ewe's exploitation system and on the application of the European legislation en force regarding the conditions for reproduction and certification from genealogical registry, the main concern of the sheep breeders became the assisted breeding with rams of known origin and genetic value. Thus, the interest for artificial insemination has increased among sheep breeders who are facing a shortage of rams with known genetic value, pure breed, in the application of selection and breeding programs. By intensive use of 5 rams in the natural breeding of 195 Palas Merinos, the male exhaustion and a rate of return to estrous cycle of 53.84% were obtained. After a 5 days of stimulating feeding diet and sexual rest of the rams, the semen was collected and qualitatively analyzed. 105 ewes at the 2nd or 3rd estrous cycle were artificially inseminated. The insemination was performed during natural estrous, on the day of the detection of estrous, with diluted raw semen, with a motility of over 80%. The rate of non-returning to the estrous at 15-19 days from artificial insemination was of 84.76%. Diagnosis confirmation was made by transabdominal ultrasound at 45 - 50 days after artificial insemination and it indicated a gestation rate of 74.28%.

Key words: ewes, artificial insemination, semen

INTRODUCTION

The present study was carried out as a result of the need to perform artificial insemination with semen from purebred rams with high genetic value. By the European Regulation 1012/2016 en force, for keeping animals in the racial genealogical register, females are allowed to be bred only with purebred males. By applying selection and improvement methods, the hierarchy and selection of the Merinos de Palas rams was done, so that a number of sheep breeders were unable to perform assisted natural breeding with the available rams from their own herd. Rams' physical and/or reproductive exhaustion have been reached by their excessive and irrational use for

assisted natural breeding. In these conditions, it is necessary to verify the ram's semen quality in terms of mobility and viability [1], [2], [3] and to establish its suitability for artificial collection, dilution and insemination. Artificial insemination is an efficiently assisted breeding method that has expanded and become a common practice for sheep farmers in Europe. Thus, in France, in 2017, there were nine artificial insemination centers carrying out more than 800,000 insemination /year for ewes of dairy breeds [4], number which is maintaining almost constant for over 20 years. In France, where there is a network of insemination centers and semen collection and preservation centers, artificial insemination is performed with frozen seminal material, thus allowing a better diffusion in time and space of valuable genetic material. In our country there is only the Institute of Research - Development for

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the Breeding of Ewes and Goats from Palas, Constanta and six Research and Development Stations for the Breeding of Ewes and Goats, which can carry out the actions of collecting and preserving semen. Because, breeding of small ruminants on Romanian territory has developed by increasing the number of herds raised traditionally, without moving to intensive agriculture with the application of breeding biotechnologies, the requirement for artificial insemination is quite low. A greater number of artificial insemination was performed on goats, where the results were very good. So, after insemination with cooled semen achieved a rate of non-returning to estrous after two estrous cycles of 87% and a fecundity of 82% and after insemination with fresh diluted semen achieved a rate of non-returning to estrous after two estrous cycles of 92% and a fecundity of 88% [5].

MATERIAL AND METHODS

At the beginning of June, a sheep breeder from Constanta county, owner of a herd of 195 ewes and 5 rams Merinos de Palas, constituted 5 lots of females of 39 ewes, each lot being assigned a male. He carried out the assisted breeding by tracing the estrus every morning and evening, followed by the natural breeding of the ewes in estrus. The ewes found in estrus were not individually mounted, but on the day of the estrus they were kept together with the male, in a common box, for 5-6 hours, the ram carrying out the successive breeding of the same female. Through the male effect, the synchronization of the females in the second cycle was achieved, so that a ram could mount 7-10 ewes / day, without giving the rest pause and no additional stimulating feeding was applied before and during the breeding period. In this activity, the breeder did not take into account the number of ewes / male / day and did not grant the necessary sexual rest in case of intense use, respectively performing 4-5 mounts / day for 3-5 consecutive days. Thus, during July, some of the ewes already mounted showed estrus, which demonstrates that the mount was not fertile. In these conditions, the sheep breeder called on the specialists from ICDCOC Palas, Constanta to complete the 2019 breeding campaign.

The first action carried out by the specialists of the Laboratory of Reproduction and Biotechnologies of ICDCOC Palas, Constanta was to verify the semen of the rams. Thus, after a 10-day sexual rest and stimulating feeding with a good quality fan, 500 g barley and 100 g carrot/ ram/day, the semen was collected by artificial vagina and quantity (ejaculate volume) and quality (color, density, motility, viability) were evaluated. The estimation of the seminal material was achieved both at the beginning of the collection from all 5 males as well as of each ejaculation collected during the entire period of semen collection and artificial insemination. The quantitative assessment was made immediately after collection by reading the ejaculated volume directly on the gradations of the collecting glass, obtaining between 0.5 and 2.5 ml. The color was appreciated through the transparency of the collecting glass, ranging from slightly white-yellow (normal color) to white-brown (present in an ejaculate) [6],[7]. Once with appreciation of color, the degree of viscosity was established too, being between the normal consistency of the cream and liquid, when the ejaculates contained seminal liquid. The motility and density were assessed firstly by microscopic examination between blade and lamella of a drop of raw semen, and secondly after dilution with TRIS extender. Ejaculates with high and very high spermatozoa concentration, over 1.2×10^9 spermatozoa / ml (thick and very thick semen) are diluted with TRIS extender in proportion of 1:4 and those with average concentration are diluted in ration of 1:3, so that they can reach a concentration of 800×10^6 spermatozoa/ml, respectively 400×10^6 spermatozoa/ml for an insemination dose. For the density assessment of over 3.5 billion of spermatozoa/ml, correspondent for a thick and very thick semen, the distance between spermatozoa must be smaller or equal to a spermatozoa head length. In this situation the values of these parameters are similar to the results of the measurements made on the semen of 241 adult rams who recorded an average concentration of over 3×10^9 spz / ml with a motility of over 80% [8]. The motility expressed by percentage, estimates the quantity of the mobile spermatozoa that performs forward movements. Viability was assessed by the vital coloration of eosin-nigrosine, by

which the percentage of living spermatozoa was established. The qualitative parameters of the semen collected and diluted depends also on the type of extender used, respectively based on skimmed milk, citrate medium, TRIS medium) [9], [10] as well as on the period of time passed between collection-dilution and the time when artificial insemination is performed [11]. In the present study we used as dilution medium - TRIS solution and the insemination was performed at maximum 1.5 hours after collection, the diluted semen being maintained during this time on a water bath at 30-35°C [12]. Following the first evaluation of ejaculates from the 5 rams, one ram was removed from reproduction, having yellow-brown, very rare semen. This male was examined during the entire artificial insemination, period at an interval of 3-4 days, observing an improvement of the sperm parameters, with an average dense semen and a motility of 40-60%. In these conditions the ram can be used on natural breeding with a moderate rate of use but it is not recommended the collection of semen and its dilution for artificial insemination. The artificial insemination of the ewes was done during natural estrus, following the detection of the ewes in heat by rams in heat trying with luck. Insemination was performed with 0.5 ml of diluted raw semen (in a ratio of 1: 4), with an average concentration of 400×10^6 spermatozoa / dose. For artificial insemination the vaginal speculum was used with its own light, visualizing the place of semen deposition, respectively at the level of the involved flora. The penetration of the insemination dropper into the cervix may vary according to its opening, respectively by the moment of the estrous cycle. When the cervix was closed and the insemination dropper did not penetrate at least 0.5-1 cm, the semen was deposited at the vaginal level. These ewes showed estrus also the next day, so they were re-examined and artificially inseminated.

RESULTS AND DISCUSSION

During July and the first third of August, the ewes in heat were detected, establishing a percentage of 53.85% rate of returns (105 ewes returned out of 195 ewes naturally mounted). Of these ewes, 29 ewes (14.88% of the total number of mounted ewes and

27.62% of the returns respectively) were from the group assigned to the male who, when checking the semen, had the unfavorable results, having a very poor quality semen. The gestation rate of the naturally mounted ewes with this male was 25.64%. At the other 4 batches, between 17 and 20 females returned in estrus, and only 19-22 ewes / ram were considered pregnant, thus having a gestation rate of 48.72 - 51.28%. At the beginning of the action of estrus screening at ewes with trying rams, in all five groups of adult females from 2nd up to 4th parturition, a good synchronization of the estrous cycle is observed which demonstrates a good response to the male effect since the beginning of the breeding campaign. The manifestation of synchronized estrus in a large number of females and the lack of supervision of the breeding group explains the high rate of return to estrus [13]. At the beginning of the estrous screening followed by artificial insemination, the non-pregnant females were in the second or third estrous cycle. Table 1 presents the structure of the groups of females that have been artificially inseminated, and formed didactically to evaluate the results. Thus, 79 ewes (40.51%) were in the second estrous cycle and 26 ewes were in the third estrous cycle (13.33% already had two unfertilized natural mounts).

After insemination of these 105 ewes, a non-return rate of 15.24% was recorded in the next estrous cycle, respectively 12 ewes inseminated in the second estrous cycle (11.43%) and 4 ewes in the third estrous cycle (3.81%). Because it was practiced the assisted breeding in cluster (group) the rams often mounted the same ewes on the day when they were in heat having as consequence the physical and reproductive exhaustion of the males. The ewes that were in the second estrous cycle at the time of insemination had a delayed beginning of sexual activity and because the rams did not have the period of sexual rest for recovery they did not mount anymore or had improper ejaculation.

Table 1 Distribution of experimental batches of ewes

Total females	195	Return rate after NB* (%)	Return rate after AI*	
			Nr.	%
Total females artificially inseminated	105	53.84	16	15.24
Artificially inseminated ewes at the 2nd estrous cycle	79	40.51	12	11.43
Artificially inseminated ewes at the 3rd estrous cycle	26	13.33	4	3.81

NB* - natural breeding, AI* - artificial insemination

As a result of the artificial insemination with diluted raw semen, at the next detection of estrus, the first assessment of the gestation rate was performed between 16 and 20 days, which was 84.76% distributed in 63.81% of

artificially inseminated ewes on the second estrous cycle (67 ewes) and 20.95% ewes inseminated on the third estrous cycle (22 ewes). (Table 2).

Table 2 Gestation rate in artificially inseminated ewes

Specification	Ewes No. AI	No. of pregnant ewes	GR* %	No of pregnant ewes ECO*	GR-ECO* %
Artificially inseminated ewes at the 2nd estrous cycle	79	67	63.81	64	60.95
Artificially inseminated ewes at the 3rd estrous cycle	26	22	20.95	14	13.33
Total females artificially inseminated	105	89	84.76	78	74.28

AI*- artificial insemination, GR*-gestation rate, ECO*-ultrasound, GR-ECO*- GR after ECO

During September, at 45-50 days after the artificial insemination, ultrasounds were performed, establishing the number of safe pregnant ewes by visualizing the uterus and the caruncular uterus. Thus, confirmation of pregnancy was achieved for 78 ewes (74.28%), respectively 64 ewes (60.95%) in the second estrous cycle and 14 ewes (13.33%) in the third estrous cycle. For the ewes artificially inseminated in the 2nd estrous cycle, 95.52% the diagnosis was confirmed by ultrasound (64 pregnant ewes out of 67 ewes non-returned in the estrus), while for the ewes inseminated in the third cycle the percentage of ultrasound confirmation of gestation is of 63.63% (14 pregnant ewes out of 22 ewes non-returned in the estrus). This higher percentage and the difference between the two female categories indicates the female's infertility. These results are similar to data reported by other authors. Thus, fertility at Turcana ewes, estimated basis on the non-return syndrome is 94.8% and the pregnancy rate established

by ultrasound is 89.5% [14]. The rate of non-return to estrus for artificially inseminated goats, during non-breeding season, with hormonal induced estrous activity is of 94.3% and the gestation rate confirmed by ultrasound is of 84.3%.[15]. Other results regarding the variation of the reproduction indices according to the type of breeding and semen method of preservation indicate a gestation rate of 67% consecutive to natural breeding, 43% after artificial insemination with frozen semen and 78% after artificial insemination with freshly diluted semen when females had induced and synchronized estrus [16]. The gestation rate is influenced by maintaining the semen's viability during transportation, so that a longer time, of over 8 hours, from collection to artificial insemination causes smaller indicators. A gestation rate of 86.70% was obtained after natural breeding, compared to a gestation rate of 64% after artificial insemination about 8 hours after semen's collection [17].

CONCLUSIONS

Based on the applicability of the new European legislative provisions, several farmers, sheep owners registered in the Genealogical Register, are facing a deficit of purebred rams, tested by performance. In order to ensure the assisted reproduction, it is necessary to synchronize the estrous cycle on groups of females according to the number of available males or to screen the estrus with male testers and to collect the semen of the males so as to ensure the artificial insemination of a large number of females in the estrous phase of the sexual cycle. An essential condition for the success of the breeding campaign is the analysis of the ram's semen that will be used for breeding. The rams selected on genealogy and productivity criteria may have poor reproductive performance, a semen with low density and motility, with an increased percentage of spermatozoa's abnormalities or even azoospermia. Therefore, performing the spermogram before using it in the natural breeding is mandatory. Particular attention should be paid to breeding preparation not only of the females but also of the males that should be provided with proper feeding quality and quantities. Very important is the intensity of use in the natural breeding or in the semen collecting programs so that it does not reach the physical and sexual exhaustion of rams. It is compulsory to correlate the rhythm of use at the mount or the semen collection with the rest periods that will ensure the recovery of the semen reserve and the maintenance of an adequate livelihood, without excesses.

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