https://doi.org/10.61900/SPJVS.2023.04.21

SYNCHRONIZATION OF OVULATION (FTET) IN TURCANA SHEEP AS EMBRYO RECIPIENTS

Ștefan-Gregore CIORNEI[1], Sebastian-Gabriel ȘCHIOPU [1], Ioana-Livia PRUTEANU [1], Dan DRUGOCIU[1], Florin NECHIFOR[1], Petru ROȘCA[1]

e-mail (first author): stefan_ciornei@yahoo.com

Abstract

Due to the fact that the farm wants to crossbreed sheep with meat breeds, it was opted for the fastest solution to bring genetic progress, namely embryo transfer, using sheep from the Turcan breed as embryo recipients and those from the Suffolk breed as embryo donors, so that finally the batch of receivers after parturition will produce a production of Suffolk lambs. Following the selection of recipients considering the standard criteria that include: general health, functional integrity of the genital tract and cyclic activity of the ovaries, two groups were formed for the induction and synchronization of estrus. The first batch consisted of 20 sheep and the second batch of 20. The first batch was subjected to a P4-PG-PMSG protocol and the second batch to the P4-PG-GnRH protocol. The two protocols consisted of the insertion of intravaginal sponges with flugestone acetate in a concentration of 20mg, to induce the progesterone phase, for 13 days, day 0 of the protocols being represented by the day when the progesterone sponges were applied. In continuation of the protocol, Prostaglandinf2α was used on day 12 in a double dose, in the morning and in the evening at a distance of 12 hours in a dose of 0.6 ml/administration/animal. On day 13, the progesterone sponges were removed and PMSG (Folligon) was administered in the case of the first batch at a dose of 600 IU/animal, and in the second batch GnRH (Receptal) was administered at a dose of 12 μg/animal (3ml/animal) followed by a dose of 600 IU HCG (Chorulon) 24 hours after GnRH administration. Ovulation was accurately assessed at the time of embryo transfer through laparotomy, due to the highlighting of the ovaries and their macroscopic analysis in the operative field, after identifying the CL on the ovary, their appearance and the number of CL on the ovary. However, the assessment of the rate of entry into heat was assessed 24 hours after the end of the protocol by biostimulating the receptors with the help of detector rams, thus the ewes that entered in estrus at 12 – 24 hours, 24 – 48 of hours or over 48 hours after completing the protocol. The results obtained in the case of the first batch having the highest rate of entering in estrus between 24 - 48 hours being 40%, followed by a rate of 30% between 12 - 24 hours, the lowest rate being 20% that entered in estrus after 48 hours, the second batch with GnRH had the highest rate of entry into estrus of 60% in the first 12-24 hours and the rate at 24-48 hours, respectively those that entered heat after 48 hours was 20%. Compared between the two groups, there were differences in the timing of ovulation assessment, as in the case of the first group with PMSG, a 60% ovulation rate was assessed with well-developed CL, 10% presented CL but these were unsuitable for embryo transfer, 10% presented ovarian cysts, and 10% did not ovulate, in the case of the second batch an ovulation rate of 60% was assessed and the rate of 40% represented the animals that did not ovulate, the difference between the two batches being the fact that it is observed in the case of the first batch rate of 10% with ovarian cysts, which indicates that the PMSG-based pharmaceutical is causing ovarian cysts. In conclusion, the therapeutic protocol used in the off-season for the induction of estrus and ovulation in Turcan sheep, in this study, resulted in the detection of estrus in 90% of the ewes subjected to the protocol, and the ovulation rate was 80% (60% with CL well developed and 20% were with poorly developed CL), regarding the first batch, and regarding the second batch 100% of ewes in oestrus were detected, but 60% of them ovulated with CL well developed. These results can be largely attributed to the reproduction seasonality of the sheep.

Key words: Țurcana, Embryotransfer, Receptors, Synchronization, Ovulation.

1. INTRODUCTION

The aim of the study was to induce and synchronize oestrus in Turcan sheep, in order to transfer embryos from the batch of donor sheep, from the Suffolk breed, to bring rapid genetic progress on the farm, in the off-season of sheep reproduction.

Regarding the success of assisted reproduction in the off-season, the farm also intervened with natural stimulation to imitate as much as possible the natural breeding season. In this sense, the light-dark ratio and the optimal temperature for reproduction in sheep were adapted.

[1] Reproduction, Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" University of Life Sciences, 6 Mihail Sadoveanu Alley, 700449 Iasi, Romania

The objectives of this study were the following:

• synchronizing the hormonal status of recipient sheep with the objective of survival, development and hatching of embryos from donor sheep.

• it is aimed that the recipient female is synchronized with the age of the embryo;

• calculation of ovulatory timing between donors and receivers, group synchronization of Țurcana receivers in the off-season.

• real estimation of ovulation through laparotomy and embryo inovulation (ET) 6 - 6.5 days after the onset of heat.

2. MATERIALS AND METHOD

2.1. Animals taken in the study

This study was carried out between June 25 and July 16, 2021, during the natural breeding off-season. The farm having a flock of 300 Turcana sheep, 2 batches of sheep were selected, one batch of 20 ewes and the second of 20, for the induction and synchronization of estrus according to the reproductive status (number of calvings, number of calvings with dystocia, degree of development of the female genital system, genital system conditions), as well as by maintenance status, body score, health status (comorbidities, possible infectious diseases, parasitic diseases, foot conditions). The selected ewes are lambs and primiparous, having BCS 3-4, with a weight of approximately 40-45 kg, and from a clinical point of view being healthy.

The sheep were kept in the stable, being fed in the specially arranged box, the fodder ration being made up of coarse, juicy and concentrated in a balanced ratio, in order to stimulate the reproduction and the needs of the animals, water being administered ad libitum. The light in the shelter was artificial in order to adjust the light-dark ratio, thus the selected animals were exposed to 12 hours of light and 12 hours of darkness (ratio 1:1), according to the natural winter season (autumn).

2.2. Off-Season Estrus Induction and Timing Protocol

In the natural off-season of breeding (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 breeding/insemination, it is necessary to stimulate sexual behavior and to initiate gestation as soon as possible (Pavlo Sklyarov et al., 2021).

Inducing oestrus during the summer or spring provides the additional chance to treat young ewes that have aborted or had non-viable lambs. Another goal is to shorten calving intervals to achieve three lambs in 2 years or 2 lambs per year (Pavlo Sklyarov et al., 2021).

To induce estrus in small ruminants during the off-breeding season, administration of progestin is the method of choice and causes prolongation of the luteal phase in these animals. As a result, they are synchronized and, after the cessation of hormone treatment, the animals enter estrus simultaneously. (Ajbazov et al., 2006; Aksenova et al., 2012).

The sheep selected for the study were grouped into two groups, the first group consisting of 20 sheep and the second group of 20 sheep. The two batches were subjected to two different estrus induction and timing protocols.

The first batch taken in the study were administered on day 0 intravaginal sponges with flugestone acetate (cronolon) in a dose of 20 mg, which have a progesterone effect, for 13 days. One day before the extraction of the sponges, they were treated with 2 doses of ProstaglandinF2α, administered in the morning and in the evening 12 hours apart, each 0.6 ml/dose/animal. On day 13 of the protocol, progesterone sponges were extracted and PMSG (Foligon) was administered at a dose of 600 IU/animal. 48 hours after the extraction of the sponges, the ewes were subjected to a biostimulation with the help of experimental rams, which were previously prepared not to intrude by applying an apron covering the furrow, identifying the ewes in estrus and dividing them according to how long has it been since the sponge extraction. The ewes taken in the study were not mounted or artificially inseminated in order to submit them to embryo transfer after 6 - 6.5 days after ovulation.

For the second batch, the intravaginal sponges with flugestone acetate (cronolon) in a dose of 20 mg were maintained as in the first batch for 13 days. On the 12th day of the protocol, ProstaglandinF2α was administered at 12-hour intervals in a dose of 0.6 ml/dose/animal. On day 13, the intravaginal sponges were removed and instead of PMSG as in the case of the first batch, the sheep received 12 μg/animal (3 ml/animal) of GnRH (Receptal), and 48 hours after the extraction of the sponges administered 600 IU of HCG (Chorulon), then the same procedure was followed as in the case of the first batch.

3. RESULTS AND DISCUSSIONS

3.1. Estrus expression and diagnosis in embryo-receiving Turkish sheep

Due to the fact that one of the main objectives of the study was the induction and synchronization of sheep from the Turcan breed, in the off-season, in order to use them in the embryo transfer protocol as recipients of embryos from Suffolk breed donors, ovulation was accurately assessed at the moment embryo transfer through laparotomy, due to the highlighting of the ovaries and their macroscopic analysis in the operating field, after identifying the CL on the ovary, their appearance and the number of CL on the ovary. But the assessment of the rate of entry into heat was assessed 24 hours after the completion of the protocol by biostimulation of the receptors with the

help of experimental rams, the ewes that entered estrus at 12 - 24 hours, 24 - 48 hours or more were assessed 48 hours after completing the protocol (Table 1).

Table 1

Appreciation of ewes in heat, from batch 1, in different periods of time

12-24h	24-48 h	> 48h
30% (6/20)	**40% (8/20)**	**20% (4/20)**

According to Table 1 a percentage of 90% (18/20) of the ewes in the first batch selected for the estrus induction and synchronization protocol went into heat, 10% (2/20) did not go into estrus. Their distribution according to the time period since the completion of the induction and synchronization protocol and the percentage in each time category can be found in Figure 1.

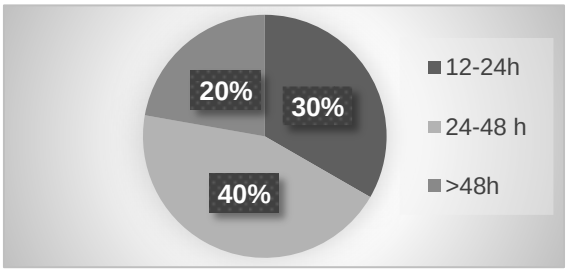

Figure 1 - **The percentage of ewes diagnosed in oestrus, from batch 1, in different time periods**

From the 20 ewes receiving embryos, from the Țurcana breed, selected in the first batch, following their identification according to the time period in which they entered estrus, a percentage of 30% were identified in the first 12-24 hours from finalization of the estrus induction and synchronization protocol, respectively 40% between 24-48 hours and 20% after 48 hours.

Table 2

Appreciation of ewes in heat, from batch 2, in different periods of time

12-24h	24-48 h	> 48h
60% (12/20)	**20% (4/20)**	**20% (4/20)**

The ratio of ewes in group 2, which were synchronized with GnRH, according to the time period can be seen in Figure 2. related to Table 2., so that 60% (12/20) entered heat between 12 – 24 hours, 20% (4/20) between 24 – 48 h, respectively 20% (4/20) after 48 hours.

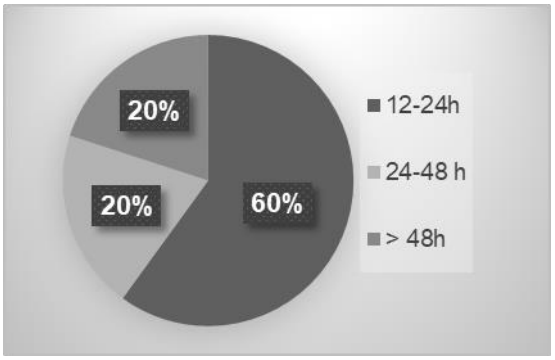

Figure 2 - **The percentage of ewes diagnosed in oestrus, from batch 2, in different time periods**

Comparing the two treatment regimens for estrus stimulation, we believe that both produced the expected results according to the specialized literature. Zamfirescu Stela at the Sheep and Goat Research Institute in Palas, Constanța obtained an estrus synchronization rate between receivers and donors between 80-95%, but in mestizo sheep between Tsiigai and Merino. The results obtained through this study show that these synchronization methods are validated and can be successfully used in other sheep breeds, the total mean of estrus induction 0% Turcana sheep was 95%, with limits between the two batches 80% at lot 1 and 100% for lot 2.

The difference between the groups is 20% in favor of group 2, it seems that the administration of GnRH on day 13 and hCG on day 15 led to the efficiency of estrus induction in Turcane.

Also in batch 2, the best grouping of estrus in the 12-24 hour interval was obtained, this being 60%. In ET sessions, a gap of maximum 12 hours is accepted between the estrus expressed between donors and recipients.

3.2. The actual ovulation rate of ewes of the Turcana breed

Following the embryo transfer protocol, the method used in small ruminants being the surgical one, through laparotomy, the presence and macroscopic appearance of CL on each ovary were highlighted, thus only the females that presented CL, on day 6 - 6.5 after ovulation, well developed have benefited from embryo transfer.

Following the surgery, it was found in the first batch of sheep used as embryo recipients (Figure 3) that 60% presented a well-developed CL, representing the fact that they had ovulated, but 20% ovulated but the CL was poorly developed following follicular dehiscence making these females unsuitable for embryo transfer, 10% of the possible recipients did not respond to the treatment

as CL was not identified on the ovaries, and in 10% of cases the presence of follicular cysts on the ovaries was identified (2/20).

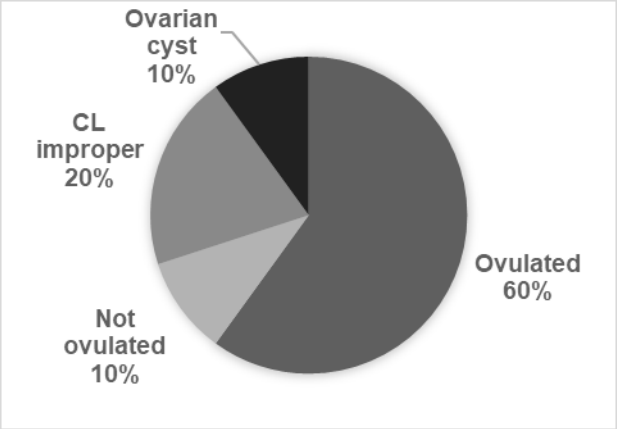

Figure 3 - **Ovulation rate of embryo recipients, from the first batch, following laparotomy**

In the second batch of embryo recipients, in which GnRH was used for estrus induction and synchronization, an ovulation percentage of 60% was found (Figure 4) with well-developed CLs on the ovaries, the respective females being used for embryo transfer and 40% of these did not show CL on the ovary which demonstrated that those females did not ovulate.

We conclude that the actual ovulation rate required for successful ET, assessed laparoscopically, was 60% and was identical in both groups. We note that in batch 2 no ovarian cysts were identified and no poorly developed CLs, the ovulations produced generated very qualitative CL. Due to the percentage of 40% of ewes that did not ovulate, although they were in oestrus (batch 2), it is necessary to increase the dose of hCG administered on the 15th day and change it with GnRH to force the ovulation of more follicles develop.

In the second batch of embryo recipients, in which GnRH was used for estrus induction and synchronization, an ovulation percentage of 60% was found (Figure 4) with well-developed CL on the ovaries, the respective females being used for embryo transfer and 40% of them did not present CL on the ovary, which demonstrated that those females did not ovulate.

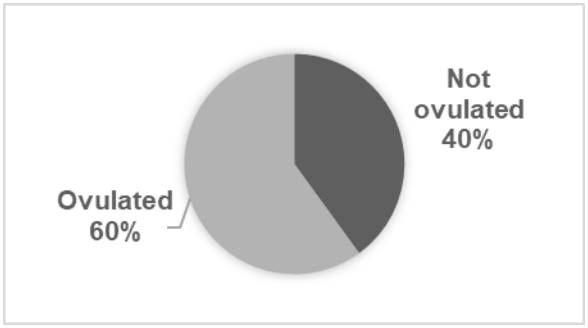

Figure 4 - **Ovulation rate of embryo recipients, from the second batch, following laparotomy.**

3.3. Discussions

Biehl et al. showed that estrous behavior in sheep appeared mainly within 48 h after withdrawal of the progestogen sponge (Xiaojie Yu et al., 2022), which is also found in this study. The estrus induction rate in the case of sheep from the Țurcana breed is the highest in the case of the first batch between 24 - 48 hours after the extraction of the sponges, followed by the interval of 12 - 24 hours. In the case of the second batch, most of the ewes entered estrus 12-24 hours after the extraction of the sponges.

Xiaojie Yu et al. showed that PMSG has direct action on the ovary, but it often caused ovarian cyst, even though it effectively facilitates follicular development in terms of induction and timing of ewes, thus affecting AI and embryo transfer (Xiaojie Yu et al. ., 2022). This is also found in this study in the ewes synchronized with PMSG from the first batch, having a share of 10% of the ewes with ovarian cysts, respectively 20% of the ewes synchronized with PMSG although they ovulated and developed CL on the ovary they have were inappropriate for the embryo transfer protocol. Xiaojie Yu et al., 2022 demonstrated that GnRH and its analogues through its action on the pituitary gland generate the secretion of FSH and LH without a difference in the rate of induction and synchronization compared to PMSG-synchronized ewes, but in in the case of ewes synchronized with GnRH, a shortening of the elapsed time from estrus to ovulation was observed, which was also observed in this study, in the second batch 60% of the ewes synchronized with GnRH ovulated and were used in the embryo transfer protocol , but estrus was mostly manifested 12-24 hours after progestogen sponge extraction in a percentage of 60%, no ewes with ovarian cysts were identified in this study following their synchronization with synthetic analogue of GnRH.

4. CONCLUSIONS

The protocol for inducing estrus and synchronizing ovulation in embryo recipient sheep aims at synchronizing their hormonal status with the objective of survival, development and hatching of embryos from donor sheep. The aim is for the recipient female to be synchronized with the age of the embryo, practically a perfect overlap of the time elapsed since ovulation and the degree of development of the transferred embryo is desired. At the end of this protocol, after 6-6.5 days from estrus, instead of AI, the embryo collected from the donor female will be directly inovulated (ET). Considering the fact that the sheep is a seasonal polyestrous animal (estrus occurs in autumn and spring), with the help of this protocol we make it possible to induce estrus and induced ovulation, and thus it is possible to carry out the transfer of embryos outside the breeding season. 10 Turcana sheep were selected as possible embryo recipients. Estrus induction in the off-season was carried out through a hormonal protocol: P4-PG-PMSG. Progesterone (P4) was administered for 13 days in the form of vaginal inserts (Chronogest), on the 12th day a dose of PG was administered, and on the 13th day of treatment PMSG (Folligon) was administered in dose of 300 IU. The ovarian response to follicular stimulation and the induction of the estrous phase was assessed starting 24 hours after the end of the therapeutic scheme, by using detection rams. Thus, 90% (9/10) of the receptors were diagnosed in estrus with their distribution as follows: 30% (3/10) between 12-24h, 40% between 24-48 hours and 20% (2/10) after 48h of to treatment. At the time of using the receptors in the ET protocol (6.5 days after estrus), ovulation was accurately assessed, according to the appearance and identification of the CL on the ovary. Of the total number of recipients in the ET protocol, 60% ovulated, 30% did not respond to treatment (without CL, did not ovulate) and in 10% of cases ovarian cysts were identified (1/10). The therapeutic protocol used to induce estrus and ovulation in Turcan sheep, in the off-breeding season, had an effect of 90% estrus and 60% ovulation rate.

The therapeutic protocol used in the off-season to induce estrus and ovulation in Turcana sheep, in this study, resulted in the detection of estrus in 90% of the ewes subjected to the protocol, and the ovulation rate was 80% (60% with well-developed CL , and 20% were with poorly developed CL), regarding the first batch, and regarding the second batch, 100% of ewes in estrus were detected, but 60% of them ovulated with well-developed CL. These results can largely be attributed to the seasonality of sheep reproduction.

The difference between the groups is 20% in favor of group 2, it seems that the administration of GnRH on day 13 and hCG on day 15 led to the efficiency of estrus induction in Turcane.

We conclude that the actual ovulation rate required for successful ET, assessed laparoscopically, was 60% and was identical in both groups. We note that in batch 2 no ovarian cysts were identified and no poorly developed CL, the ovulations produced generated very qualitative CLs. Due to the percentage of 40% of ewes that did not ovulate, although they were in oestrus (batch 2), it is necessary to increase the dose of hCG administered on day 15 and change it with GnRH to force the ovulation of more developed follicles.

REFERENCES

Ciornei S. 2021. Reproductive biotehnologies, practical guide, Editura "Ion Ionescu de la Brad", ISBN 978-973-147-435-9

Forcada F., M. Ait Amer-Meziane, J.A. Abecia, M.C. Maurel, J.A. Cebrián-Pérez, T. Muiño-Blanco, B. Asenjo, M.I. Vázquez, A. Casao, 2011, *Repeated superovulation using a simplified FSH/eCG treatment for in vivo embryo production in sheep*, Editura Theriogenology, Volume 75, pp. 769-776

Francisco A. Arrebola, Rafael Torres-Martell, Olga González-Casquet, Cesar A. Meza-Herrera and Carlos C. Pérez-Marín, 2022, Periovulatory Hormonal Profiles after Estrus Induction and Conception Rate by Fixed-Time AI in Payoya Goats during the Anestrous Season, https://doi.org/10.3390/ani12202853 (accesat la 22.05.2023).

Gavojdian Dinu, Szilvia Kusza, Ludovic Toma Cziszter, 2013, Comparative study regarding reproductive performance in Gyimesi racka and Turcana sheep breeds, https://www.researchgate.net/publication/260901920 (accesat 23.04.2023)

Hafez, E. S. E., 1952, Studies on the breeding season and reproduction of the ewe. *Journal of Agricultural Science*, nr. 42, pp. 189– 265.

Jose Alfonso Abecia, Matthieu Keller, Carlos Palacios Riocerezo, Francisco Canto, 2023, Reproductive management in small ruminants, https://www.researchgate.net/publication/369093900 (accesat 22.05.2023)

Mircu C., 2020, *Tratat de reproducție asistată*, Agroprint, Timișoara.

Robert S. Youngquist, Walter R. Threlfall, 2007, *Current Therapy in Large Animal Theriogenology*,

Naitana S., Ledda S., 2020, *Chapter 3 - Reproductive technologies in sheep*, Editura Academic Press, pp. 31-54.

Ciornei, Ș., Drugociu, D., Ciornei, L.M., Roșca, P., (2022). Ovarian response to P4-PGF-FSH treatment in Suffolk sheep and P4-PGF-PMSG synchronization in cross-bred ewes, for IVD and ET protocol. Veterinary Medicine and Science, Volume8, Issue2, ISSN:2053-

1095, Page726-734, DOI10.1002/vms3.705 https://doi.org/10.1002/vms3.705

Ciornei S.G., 2021. Embryo Transfer, in: Animal Reproduction. Edited by Prof. Yusuf Bozkurt (Ed.), Publisher: IntechOpen, DOI: 10.5772/intechopen.99683

Xiaojie Yu, Yuanyuan Bai, Jiangfeng Yang, Xiaokun Zhao, Lei Zhang and Jing Wang, 2022, Comparison of Five Protocols of Estrous Synchronization on Reproductive Performance of Hu Sheep, *Frontiers in Veterinary Science*, Volumul 9, nr. 843514, pp. 1-8.

Zamfirescu, S., 2010, The retrospective results of the research developments regarding reproduction biotechnologies in sheep and goat in Romania, *Romanian Biotechnological Letters*, **15** (3), pp. 3– 12.