Comparison of laparoscopic and laparotomic methods in embryo transfer in Tuj ewes

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Abstract
The aim of this study was to transfer fresh embryos obtained through a superovulation protocol in Tuj ewes to recipient animals using either laparoscopic or laparotomic methods, and to evaluate the embryos' viability and survival rates. For this purpose, 10 Tuj ewes were used as donors and 20 Tuj ewes as recipients. Intravaginal progesterone-impregnated sponges were placed in the donor ewes during the breeding season for 5 days. FSH was applied in decreasing doses starting from the 8th day, and estrus ewes were mated with fertile rams. On day 7 following mating, embryos produced in vivo were collected surgically and evaluated under the stereo microscope. Intravaginal sponges were placed in the recipient sheep for 9 days for synchronization, and embryo transfer was performed on day 18 by laparoscopic and laparotomic methods. While the pregnancy rate was observed with the laparotomic method was 33.3%, the pregnancy rate with the laparoscopic method was determined to be 80% (P>0.05). In the evaluation of the obtained embryos, 15 morulae, 1 early blastocyst, 4 blastocysts, and 1 expanded blastocyst were determined. As a result, embryos obtained from Tuj ewes through superovulation protocols were transferred to recipient ewes via laparoscopic and laparotomic methods, and pregnancy was achieved. In the future, with more comprehensive studies using different protocols, the number of embryos obtained from Tuj ewes and transfer methods can be improved. It was also determined that this method could be used to protect local genetic resources of Tuj ewes.

Keywords: *Embryo transfer, FSH, laparoscopic, laparotomic, ewes, Tuj*

INTRODUCTION

Embryo transfer (ET) is an assisted reproduction method that involves producing multiple embryos from a female donor and then transferring them to multiple female recipients (Al Yacoub AN et al., 2011; Amiridis & Cseh, 2012; Köse et al., 2012). Under traditional sheep and goat breeding conditions, one or two offspring are obtained from a female per year. Therefore, six to eight offspring can be obtained from a female during her lifetime. ET can increase the reproductive potential of females by utilizing the large oocyte reserve in the ovaries. Hormonal stimulation of the ovaries induces multiple ovulations, which leads to superovulation. Thus, a significant number of offspring can be obtained in a short period (Mueller, 1993). Despite the low response rates from superovulation programs in sheep and goats, ET is considered an important biotechnological method in terms of achieving the desired genetic progress (Smith, 1986).

ET is one of the advanced reproductive biotechnologies applied to domestic animals. With this method, desired genetic traits can be rapidly transferred to herds, the risk of disease transmission can be reduced, and the protection of breeds or species can be ensured. In addition, genes that are at risk of being lost can be protected by storing genetic material (frozen embryos) in gene resource banks (Martemucci & D'Alessandro, 2013; Perera et al., 2008; Smith, 1986).

In sheep and goats, the cervix uteri has a narrow, complicated, and intertwined anatomical structure. Therefore, laparotomic (surgery) and laparoscopy are generally performed instead of the transcervical method for ET (Cordeiro et al., 2003; Kershaw et al., 2005). When all three methods are compared, they have advantages and disadvantages. Although the laparotomic method is preferred in terms of embryo recovery, this method causes adhesion of the ovary, oviduct, and uterus with the surrounding tissue. The embryo recovery rate decreases due to possible complications after repeated operations (Torres & Sevellec, 1987). The embryo recovery rate in this method is approximately 70-90% (McKelvey et al., 1986). Laparoscopy, which emerged as an alternative method to laparotomic, causes less trauma to the surrounding tissues and has a shorter operation time. Although it is preferred for transferring embryos to recipient animals, it includes some requirements, such as the need for expensive tools and equipment and the experienced personnel who will perform the procedure (Flores-Foxworth et al., 1992).

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One of the important assets of Türkiye is that it has local animal breeds. Due to the lack of importance given to breeding programs for small ruminants for many years, it has faced the risk of losing its existing values. The Tuj ewes we used in our study is a breed that belongs to the Caucasus region and is in danger of extinction (Akçapınar, 1994). It is found in the high settlements of the Northeastern Anatolia (Kars) Region in Türkiye. The Tuj breed, which has mixed and high-quality fleece, has a white fleece color and a fat thigh (Kopuzlu & Emsen, 2004; Ulusoy & Kaymaz, 2009). It is stated that the fertility of Tuj ewes are low, and multiple pregnancies are almost non-existent (5-10%) (Karaoğlu et al., 2001). Since the local people are mating with different breeds, their numbers are decreasing day by day (Akçapınar, 1994).

A review of the literature revealed that no studies have been conducted on embryo transfer in Tuj ewes. Therefore, there is a need for findings obtained from embryo studies specific to this breed. In this context, a study has been planned to evaluate the suitability of Tuj sheep for embryo transfer. The study aimed to transfer fresh embryos obtained from Tuj ewes during the breeding season to recipient ewes by both laparoscopic and laparotomic methods to determine the effect on the pregnancy rate obtained and to preserve these genetic materials.

MATERIALS AND METHODS

Ethical approval

This study was carried out after the approval received from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK / 2022-109).

Animals

In the study, 30 Tuj ewes that had given birth at least once, were clinically healthy, 3-4 years old, weighed 40-50 kg and had a body condition score (BCS) ranging from 2.5-3.5 (1: Extremely thin, 5: Obese) (Russel, 1984) were used (5 Tuj rams were included in the study to be used in matings during synchronization). 10 donor ewes and 20 recipient ewes were used. During the breeding season in which the study was carried out, the ewes were grazed in pastures and brought to the closed barn system in the evening. In addition to routine feeding, 0.5 kg ewes/day concentrate feed (16 HP, 2700 ME) was added to the ration. Water was given *ad libitum* during the study period.

Study design

Superovulation

On the day donor animals were taken into the treatment, a sponge containing medroxyprogesterone acetate (60 mg, Esponjavet®, Hipra, Spain) was placed in the vagina for superovulation, and PGF2α (160 mg, Enzaprost-T®, Ceva, Turkey) was injected. The sponges were removed on day 5, and pregnant mare serum gonadotropin (PMSG) (200 IU, Oviser®, Hipra, Spain) was injected. GnRH (8.4 mg, Ovarelin®, Ceva, France) was injected 36 hours after PMSG injection, and the first FSH (Stimufol®, Reprobiol, Belgium) injection was administered 24 hours later. 200 mg of FSH was administered per donor ewe. FSH was administered every 12 hours in six decreasing doses: 50, 50, 30, 30, 20, and 20 mg. PGF2α (80 mg) injection was performed on days 10 and 11, and GnRH (8.4 mg) injection was performed 12 hours after the last PGF2α injection. Ewes were mated with rams with determined fertility at 16-36 hours following the GnRH injection. Embryos produced in vivo on days 6-6.5 after mating were collected by laparotomic and evaluated under a stereo microscope by the criteria specified by the IETS (International Embryo Transfer Society) (Figure1).

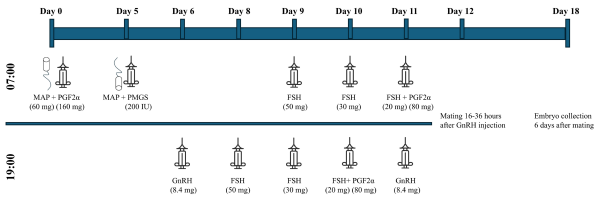

Figure 1: *Superovulation protocol of donor ewes*

Obtaining embryos by surgical method

Before the operation, donor animals were kept hungry and thirsty for 12 hours to prevent risks that may occur during the operation. The ewes were injected with subcutaneous atropine sulfate (Vetaş Atropine® 0.2%, Vetaş, Germany) at a dose of 0.1-1 mg/kg approximately 15 minutes before anesthesia for premedication. Then, 0.1 mg/kg xylazine (Rompun® 2%, Bayer, Turkey) and 2.2 mg/kg ketamine HCl (Ketasol®, Richter Pharma, Austria) were administered intramuscularly for anesthesia. The anesthetized ewes were laid on a laparoscopy table that could be tilted 30° for easier operation. Since the operations were performed with a median line incision, the abdominal region was shaved from the level of the umbilicus to the anterior part of the mammary lobes. Asepsis and antisepsis were applied before the operation. Then, a 5-8 cm midline incision was made starting from the cranial part of the mammary lobes to remove the uterus and ovaries. After the incision, the uterus and ovaries in the abdominal cavity were removed. First, the ovary was examined for follicles, cysts, corpus hemorrhagicum, or corpus luteum (CL). After the necessary information was recorded, uterine washing was started.

During the uterine washing procedure, a Foley catheter (Rüsch, no. 10, Teleflex Medical, Malaysia) and an intravenous (IV) cannula (IV cannula, 18 G, Bıçakçılar®, Turkey) were used. An incision was made at the level of bifurcation uteri, a Foley catheter was placed at the lower end of the uterine cornu, and an IV cannula was placed at the region where the ovary and uterine cornu meet, just below the oviduct. The medium used in the washing, evaluation, and transfer of the uterus in the study was obtained commercially. Each uterine cornu was washed with approximately 40 mL of DMPBS (Dulbecco's phosphate-buffered saline) (500 mL, Gibco®, USA) solution at 37°C. First, the medium was administered to the uterine cornu by IV cannula, removed from the Foley catheter into a sterile petri dish, and taken to the laboratory for evaluation. The same procedure was applied to the other uterine cornu. The uterus and ovary were

anatomically placed in the abdominal region. Finally, the incision site was closed according to the technique. PGF2α (80 mg) was injected intramuscularly to prevent possible pregnancies, and the operated animals were given antibiotics (Pan-Terramycin®, Zoetis, Türkiye) for 5 days postoperatively.

Embryo evaluation was evaluated under a stereo microscope according to morphological aspects. Embryos were moved using a micropipette or thin pipette to observe them from different angles. They were classified according to International Embryo Transfer Society (IETS) norms (excellent, good, poor, or degenerate). Embryos classified as excellent and good were transferred to freshly synchronized carrier animals by appropriate methods.

Synchronization protocol of recipient ewes

For synchronization of receptive ewes, sponges were inserted intravaginally on day 0. On day 9, PGF2α (150 mg) and PMSG (400 IU) were injected, and the sponges were removed from the vagina. Ewes were monitored for estrus for 2 days, and ewes in estrus were identified using Ram-seeking. Embryo transfer was performed on day 18 (Figure 2).

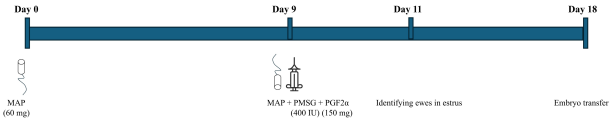

Figure 2. *Synchronization protocol of recipient ewes*

Transfer of embryos

Embryo transfer was performed on ewes with synchronized cyclic cycles showing signs of estrus and having at least one CL in one of their ovaries. Embryo transfer was performed on the 6th day after estrus was detected using laparotomic (n = 6) and laparoscopic methods (n = 5). Ewes selected for transfer were fasted for 48 hours and were not given water for 12-24 hours before the planned operation.

In the laparotomic method, the anesthesia of the animals, preparation for the operation, selection of the surgical site, and the exposure of the uterine horns are similar to the procedure described in "Surgical Collection of Embryos." Embryos were transferred to recipients based on the presence of a CL. During this procedure, a small incision was made in the uterine horn using a 16-gauge cannula, and fresh embryos contained in a straw and suspended in an appropriate transfer medium were transferred. Afterwards, the incision site was closed, and wound care was administered.

The embryo transfer was performed using a laparoscopic method under local anesthesia. The animal designated for embryo transfer was laid on its back at a 40-degree angle with its head facing downward. After disinfecting the surgical area, 2% lidocaine (Adokain®, Sanovel, Türkiye) was infused into the recommended puncture site for local anesthesia. A pneumoperitoneum was created by introducing carbon dioxide gas through a transabdominally placed Veress needle. Two incisions were made approximately 10 cm anterior to the mammary gland and 2–3 cm lateral to the midline. A laparoscope was inserted through one incision, and laparoscopic grasping forceps were inserted into the abdominal cavity through the other. The uterine horn containing the CL was held with the forceps, and the embryo transfer was carried out using a catheter inserted ventrally to the grasped horn. Following the transfer, the uterus was returned to the abdominal cavity, and the entry points were closed. Post-operative antibiotic (Pan-Terramycin®, Zoetis, Türkiye) injections were administered to the animals for 5 days.

Pregnancy

Pregnancy examination was performed 30 ± 3 days after embryo transfer using transrectal ultrasonography (5-7.5 MHz, iScan, Draminski®, Poland). Pregnancy examination was repeated on day 60 for confirmation.

Formulas used

Those with a total of four or more follicles and CL on the ovary were considered superstimulated, and those with four or more CL numbers among the superstimulated ones were considered superovulated. Superstimulation rate (SSR) = (number of animals responding to superstimulation*100)/total number of animals; superovulation rate (SOR) = (number of animals responding to superovulation*100)/number of animals responding to superstimulation; CL number (CLS) = total CL/number of animals responding to superovulation, total embryo retrieval rate (TEEO) = [number of embryos obtained + unfertilized oocytes (UFO)] *100/ Total CL number was determined by; minimum fertilization rate (MFO) = (number of embryos obtained *100)/total CL number (Köse et al., 2012).

Statistical analysis

The data obtained in the study are presented in mean ± standard error (SE) and frequency format. The distribution of the parameters by day was examined with the Shapiro-Wilk test, and it was determined that they did not show a normal distribution. The Chi-square test was used to compare pregnancy rates between the groups, and the Mann-Whitney U test was used to compare the parameters of the right and left ovaries. All statistical analyses were performed with SPSS software (Version 26.0, SPSS Inc./IBM Group, USA). In the analysis results, $P < 0.05$ was considered statistically significant.

RESULTS

Of the 10 Tuj breed ewes used as donors, 9 were superstimulated (90%), 9 were superovulated (90%), and the average CL count was 10.33. A total of 21 embryos and 21 UFOs (unfertilized oocytes) were obtained from superovulated animals. The total embryo retrieval rate was determined as 43.58%, and the minimum fertilization rate was defined as 22.58% (Table 1).

As a result of superovulation, numbers of CL 5.10±0.57 - 4.90±0.96, embryos 1.50±0.64 - 0.60±0.50 and UFO 1.20±0.44 - 0.90±0.38 obtained from left and right ovaries, respectively (P>0.05, Table 2).

In the evaluation of the total oocytes and embryos obtained, the number of morula was determined as 15 (10 excellent, 3 good, 2 poor), early blastocyst 1 (good),

blastocyst 4 (2 excellent, 2 good) and expanded blastocyst 1 (excellent) Also, number of morula 0.90±0.60 - 0.60±0.5; early blastocyst 0.10±0.10 - 0, blastocyst 0.40±0.30 - 0, expanded blastocyst 0.10±0.10 - 0 obtained from left and right ovaries, respectively (P>0.05, Table 3).

In embryo transfer, 4 pregnant ewes (80%) from 5 animals were obtained laparoscopically and 2 pregnant ewes (33.3%) from 6 animals were obtained laparotomically and there was no statistical difference between them (P > 0.05, Table 4). In addition, pregnant ewes were followed up and the birth process was completed healthily. A total of 20 ewes were included as recipients in the study, but since embryos could not be obtained from every donor and transfers were made on different days in terms of time, transfers were not made to every recipient.

DISCUSSION

Although embryo transfer offers many opportunities in sheep breeding, this assisted reproductive technique has not yet reached the desired level. The reasons for this are generally animal-dependent variations in superovulation response and embryo yield (Robinson et al., 1989; Shipley et al., 2007; Whyman & Moore, 1980). In sheep and goat breeding, there are still challenges in terms of productivity, and new developments and methods are needed to improve yield characteristics. Therefore, in order to increase profitability and performance in livestock production, new studies on embryo transfer should be conducted not only in cattle breeding but also in sheep and goat breeding (Alkan, 2021). According to the literature review, since there are no previous data on embryo transfer in Tuj sheep, the results obtained from this study

Table 1. *SSR, SOR, CLS, ES and UFO numbers and their effects on TEEO and MFO.*

N	SSR	SOR	CLS	ES	UFO	TEEO	MFO
10	%90	%90	10.33	21	21	%43.58	%22.58

N; number of animals, SSR; superstimulation rate, SOR; superovulation rate, CLS; corpus luteum number, ES; number of embryos obtained, UFO; unfertilized oocytes, TEEO; total embryo retrieval rate, MFO; minimum fertilization rate.

Table 2. *Numbers of CL, embryos, and UFO obtained from left and right ovaries.*

	Left Ovary	Right Ovary	Total
CL	5.10±0.57	4.90±0.96	5.00±0.54
Embryos	1.50±0.64	0.60±0.50	1.05±0.41
UFO	1.20±0.44	0.90±0.38	1.05±0.29

CL; Corpus Luteum, UFO; unfertilized oocyte.

Table 3. *Numbers of morula, early blastocyst, blastocyst, expanded blastocyst and UFO obtained from the right and left ovaries.*

	Left Ovary	Right Ovary	Total
Morula	0.90±0.60	0.60±0.5	0.70±0.40
Early blastocyst	0.10±0.10	0	0.10±0.10
Blastocyst	0.40±0.30	0	0.20±0.20
Expanded blastocys	0.10±0.10	0	0.10±0.10

UFO; unfertilized oocyte.

Table 4. *Pregnancy rates with laparoscopic and laparotom methods*

	Pregnant	Non-pregnant	Total
Laparoscopic	4 (%80)	1 (%20)	5
Laparotomıc	2 (33.3)	4 (%66.7)	6

are expected to reveal the reproductive characteristics of this breed in terms of embryo transfer.

Estrus synchronization and superovulation are the most critical steps affecting the success of embryo transfer (Rahman et al., 2008). This is because the response and number of embryos obtained following a superovulation protocol can vary from animal to animal or between different applications. Unpredictable variations in superovulatory responses negatively affect the success of embryo production programs in sheep and goats and may lead to increased costs. There are endogenous (antral follicle count, genetics, breed, age, follicular wave, breeding season) and exogenous (gonadotropins, method of administration, dosage, nutrition, etc.) factors that influence the superovulatory response (Amiridis & Cseh, 2012). Among these many factors, the breed of the animal is considered the most influential on the outcome of superovulation protocols (Köse et al., 2012). In superovulation studies conducted on local breeds such as Dağlı*ç*, Herik, Akkaraman, İvesi, and Norduz, the average number of corpora lutea obtained was determined to be 7.70, 10.3, 9.5, 8.9, and 9.30, respectively (Azawi & Al-Mola, 2010; Kayaalp, 2010; Köse et al., 2012). In the current study, the average number of corpora lutea in Tuj ewes was found to be 10.33. Although this outcome may be influenced by the hormones used and the specific superovulation protocol applied, it still demonstrates that the Tuj ewes shows good reproductive performance among local sheep breeds.

Animals with four or more corpora lutea (CL) on the ovaries are considered to be superovulated (Azawi & Al-Mola, 2010). Animals with four or more CLs and follicles were defined as superstimulated, and among these, those with four or more CLs were classified as superovulated. The rates of superstimulation and superovulation were reported as 80%, 77.7%, and 57.9%, and 76.6%, 66.6%, and 63% in the Norduz, Herik, and Dağlıç breeds, respectively (Köse et al., 2012). In the present study, the rates of superstimulation and superovulation were both found to be 90%. Today, the embryo retrieval rate is 6.6 in sheep (Cuadro et al., 2018; Mollo et al., 2017). Total embryo retrieval rates in uterine washing after superovulation vary in many studies. Embryo retrieval rates have been reported as 63.2% in Merino sheep, 62% in Saloia sheep, and 68.7% in Manchega sheep (Blanco et al., 2003; Chagas et al., 2003; Gonzalez-Bulnes et al., 2003). A similar rate of 62% has been reported in Norduz and Herik sheep (Köse et al., 2012). This rate was found to be 43.58% in Tuj sheep. The reason for the low embryo retrieval rate may be related to delayed ovulation, low viability of fertilized oocytes, or low fertilization rate. Additionally, individual animal variation may also play a role in this outcome. The timing of FSH administration in the superovulation protocol is critical. If FSH injections are initiated too early, some ewes may not have reached ovulation readiness at that point, which may negatively affect the superovulatory response due to the presence of a large dominant follicle.

The laparoscopic method has a number of advantages for the transfer of embryos in sheep compared to classical surgical methods associated with laparotomy. The use of laparoscopy shortens the duration of the operative procedure, reduces the rate of surgical trauma, and does not require the use of general anesthesia (Lukanina et al., 2024). Laparoscopic embryo transfer may have a higher success rate because it is a more noninvasive method compared to the laparotomy method (Alkan et al., 2017). The pregnancy rate after laparoscopic (46.1%) embryo transfer in goats is higher than that after laparotomy (26.8%) (Shin et al., 2008). In a study conducted in sheep, the pregnancy rates for laparotomy and laparoscopic methods were found to be 38.9% and 47.6%, respectively (Li et al., 2008). In the presented study, the pregnancy rate after laparoscopic embryo transfer was 80%, while the pregnancy rate after laparotomy embryo transfer was 33.3%. The lack of statistical difference between pregnancy rates may be due to the insufficient number of sheep in both application groups. It was stated that the laparoscopic method resulted in significantly less adhesion compared to open surgery (Tittel et al., 2001). In order for the embryos to continue their healthy lives after transfer, the sheep should return to their normal lives as soon as possible. For this, a less stressful postoperative process, fewer complications, and a faster recovery process are required. Since the laparoscopic method has many advantages such as these, pregnancy rates can be high.

In conclusion, embryos were successfully obtained from Tuj sheep through the application of superovulation protocols, and the collected embryos were transferred to recipient animals using both laparoscopic and laparotomic methods. It was determined that the pregnancy rates were similar between the two approaches. Significant individual variations (especially superovulation, embryo retrieval rate) were observed in embryo transfer studies conducted on Tuj sheep, indicating that further research focusing on identifying suitable animals for embryo transfer in this breed may be necessary. Additionally, it was determined that this method could be used in Tuj sheep to help preserve local genetic resources. In the future, embryo cryopreservation protocols can be developed, and studies can be conducted to protect this breed.

Conflict of Interest

None of the authors has declared any conflict of interest that may arise from being named as an author on the manuscript.

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Ethical Statement

Institution taken from: Kafkas University Local Ethics Committee for Animal Experiments. Date:25.05.2022 Number: KAFKAS-HADYEK / 2022-05

Author Contributions

MCD: writing – review and editing, validation, methodology, project administration, investigation, formal analysis, data curation. **CK:** writing – review and editing, investigation. **YÖ:** writing – review and editing, methodology. **SK:** methodology, investigation, formal analysis. **MK:** formal analysis, validation, editing, software. **UK:** methodology, resources. **HM:** methodology, resources. **SY:** data curation, investigation. **TG:**

resources, software. **MAK:** resources, data curation. **MSD:** investigation, writing – review & editing. **TG:** visualization, formal analysis.

REFERENCES

Akçapınar, H. (1994). *Koyun Yetiştiriciliği*. Medisan Yayınevi.

Al Yacoub AN, Gauly M, Sohnrey B, & Holtz W. **(2011)**. Fixed-time deep uterine insemination in PGF2α-synchronized goats. *Theriogenology*, *76*(9), 1730–1735. https://doi.org/10.1016/j.theriogenology.2011.07.005

Alkan, K. karakaş. **(2021)**. Koyun ve Keçilerde Embriyo Üretimi ve Transferi. *Turkiye Klinikleri Veterinary Sciences-Obstetrics and Gynecology-Special Topics* , *7*(3), 50–55.

Alkan, K., Özkan M, & Kaymaz M. **(2017)**. Küçük Ruminantlarda Yardımcı Üreme Teknolojilerinin Kullanım Seçenekleri. *Turkiye Klinikleri Veterinary Sciences-Obstetrics and Gynecology*, *3*(2), 113–122.

Amiridis, G. S., & Cseh, S. (2012). Assisted reproductive technologies in the reproductive management of small ruminants. *Animal Reproduction Science*, *130*(3–4), 152–161. https://doi.org/10.1016/j.anireprosci.2012.01.009

Azawi, O. I., & Al-Mola, M. K. M. A. (2010). A study on superovulation using FSH and eCG in Awassi ewes. *Tropical Animal Health and Production*, *42*(5), 799–801. https://doi.org/10.1007/S11250-009-9489-2

Blanco, M. R., Simonetti, L., & Rivera, O. E. (2003). Embryo production and progesterone profiles in ewes superovulated with different hormonal treatments. *Small Ruminant Research*, *47*(3), 183–191. https://doi.org/10.1016/S0921-4488(02)00245-6

Chagas e Silva, J., Lopes da Costa, L., Cidadão, R., & Robalo Silva, J. (2003). Plasma progesterone profiles, ovulation rate, donor embryo yield and recipient embryo survival in native Saloia sheep in the fall and spring breeding seasons. *Theriogenology*, *60*(3), 521–532. https://doi.org/10.1016/S0093-691X(03)00045-1

Cordeiro, M. F., Lima-Verde, J. B., Lopes-Júnior, E. S., Teixeira, D. I. A., Farias, L. N., Salles, H. O., Simplício, A. A., Rondina, D., & Freitas, V. J. F. (2003). Embryo recovery rate in Santa Inês ewes subjected to successive superovulatory treatments with pFSH. *Small Ruminant Research*, *49*(1), 19–23. https://doi.org/10.1016/S0921-4488(03)00059-2

Cuadro, F., dos Santos-Neto, P. C., Pinczak, A., Barrera, N., Crispo, M., & Menchaca, A. (2018). Serum progesterone concentrations during FSH superstimulation of the first follicular wave affect embryo production in sheep. *Animal Reproduction Science*, *196*, 205–210. https://doi.org/10.1016/j.anireprosci.2018.08.011

Flores-Foxworth, G., McBride, B. M., Kraemer, D. C., & Nuti, L. C. (1992). A comparison between laparoscopic and transcervical embryo collection and transfer in goats. *Theriogenology*, *37*(1), 213. https://doi.org/10.1016/0093-691X(92)90282-V

Gonzalez-Bulnes, A., Garcia-Garcia, R. M., Santiago-Moreno, J., Dominguez, V., Lopez-Sebastian, A., & Cocero, M. J. (2003). Reproductive season affects inhibitory effects from large follicles on the response to superovulatory FSH treatments in ewes. *Theriogenology*, *60*(2), 281–288. https://doi.org/10.1016/s0093-691x(02)01367-5

Karaoğlu, M., Macit, M., & Emsen, H. (2001). Tuj kuzularının büyüme ve gelişme özellikleri ile yaşama gücü üzerine bir araştırma. *Turkish Journal of Veterinary and Animal Sciences*, *25*(3), 261–266.

Kayaalp, E. (2010). *Akkaraman ırkı koyunlara FSH uygulaması sonucu elde edilen süperovulasyon cevabının değerlendirilmesi.* Selçuk Üniversitesi.

Kershaw, C. M., Khalid, M., McGowan, M. R., Ingram, K., Leethongdee, S., Wax, G., & Scaramuzzi, R. J. (2005). The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. *Theriogenology*, *64*(5), 1225–1235. https://doi.org/10.1016/j.theriogenology.2005.02.017

Kopuzlu, S., & Emsen, H. (2004). Tuj Koyunlarının Bazı Yapağı Özelliklerinin Değerlendirilmesi. *4. Ulusal Zootekni Kongresi*, *1*, 179–185.

Köse, A., Çizmeci, S.Ü., Tur, İ., & Güler, M. (2012). Evaluation of superovulation responses in some domestic ewe breeds. *Eurasian Journal of Veterinary Sciences*, *28*(4), 224–228.

Li, Q. Y., Guan, H., Hou, J., An, X. R., & Chen, Y. F. (2008). Technical note: Transfer of ovine embryos through a simplified mini-laparotomy technique1. *Journal of Animal Science*, *86*(11), 3224–3227. https://doi.org/10.2527/jas.2008-0846

Lukanina, V. A., Chinarov, R. Yu., Pozyabin, S. V., Shumakov, N. I., Cherkasova, O. V., & Singina, G. N. (2024). Comparative Study of the Effectiveness of Laparotomic and Laparoscopic Methods of Transplantation of Cloned Embryos in Sheep. *Russian Agricultural Sciences 2023 49:2*, *49*(2), S339–S344. https://doi.org/10.3103/S1068367423080128

Martemucci, G., & D'Alessandro, A. G. (2013). Efficiency of FSH/LH treatments for in vivo production of embryos and their cryopreservation by different methods in goats. *Small Ruminant Research*, *114*(2–3), 264–271. https://doi.org/10.1016/j.smallrumres.2013.07.006

McKelvey, W. A. C., Robinson, J. J., Aitken, R. P., & Robertson, I. S. (1986). Repeated recoveries of embryos from ewes by laparoscopy. *Theriogenology*, *25*(6), 855–865. https://doi.org/10.1016/0093-691X(86)90102-0

Mollo, M. R., Monteiro, P. L. J., Surjus, R. S., Martins, A. C., Ramos, A. F., Mourão, G. B., Carrijo, L. H. D., Lopes, G., Rumpf, R., Wiltbank, M. C., & Sartori, R. (2017). Embryo production in heifers with low or high dry matter intake submitted to superovulation. *Theriogenology*, *92*, 30–35. https://doi.org/10.1016/j.theriogenology.2017.01.015

Mueller J. **(1993)**. Utilización de la inseminación artificial y la superovulación con transferencia de embriones en el mejoramiento genético de ovinos. *Comunicación Técnica Del INTA CD Producción Animal,* 323, 1-8.

Perera, G. D. R. K., Pushpakumara, P. G. A., De Silva, L. N. A., & Alexander, P. A. B. D. (2008). Production of genetically superior goats through embryo transfer technology in Sri Lanka. *Tropical Agricultural Research*, *20*, 177–184.

Rahman, A., Ramli, A., & Embong, W. W. (**2008**). A review of reproductive biotechnologies and their application in goat. *Biotechnology*, *7*(2), 371–384.

Robinson, J. J., Wallace, J. M., & Aitken, R. P. (**1989**). Fertilization and ovum recovery rates in superovulated ewes following cervical insemination or laparoscopic intrauterine insemination at different times after progestagen withdrawal and in one or both uterine horns. *Reproduction*, *87*(2), 771–782. https://doi.org/10.1530/jrf.0.0870771

Russel, A. (**1984**). Body condition scoring of sheep. *In Practice*, *6*(3), 91–93. https://doi.org/10.1136/inpract.6.3.91

Shin, S. T., Jang, S. K., Yang, H. S., Lee, O. K., Shim, Y. H., Choi, W. Il, Lee, D. S., Lee, G. S., Cho, J. K., & Lee, Y. W. (**2008**). Laparoscopy vs. laparotomy for embryo transfer to produce transgenic goats (Capra hircus). *Journal of Veterinary Science*, *9*(1), 103. https://doi.org/10.4142/jvs.2008.9.1.103

Shipley, C. F. B. , Buckrell, B. C. , Mylne, M. J. A. , POLLARD, J. , & Hunton, J. R. (**2007**). Artificial insemination and embryo transfer in sheep. *Current Therapy in Large Animal Theriogenology*, 629–641. https://scholar.google.com.tr/scholar?hl=tr&as_sdt=0%2C5&q=Artificial+insemination+and+embryo+transfer+in+sheep&btnG=

Smith, C. (**1986**). Use of embryo transfer in genetic improvement of sheep. *Animal Science*, *42*(1), 81–88. https://doi.org/10.1017/S000335610001775X

Tittel, A., Treutner, K. H., Titkova, S., Öttinger, A., & Schumpelick, V. (**2001**). New adhesion formation after laparoscopic and conventional adhesiolysis. *Surgical Endoscopy*, *15*(1), 44–46. https://doi.org/10.1007/s004640000256

Torres, S., & Sevellec, C. (**1987**). Repeated superovulation and surgical recovery of embryos in the ewe. *Reproduction Nutrition Développement*, *27*(4), 859–563. https://doi.org/10.1051/rnd:19870612

Ulusoy, H., & Kaymaz, M. (**2009**). Koyunlarda gebelik muayenesi. *Vet. Hek. Derg.*, *80*(1), 31–36.

Whyman, D., & Moore, R. W. (**1980**). Effects of PMSG and the prostaglandin F-2α analogue, cloprostenol, on superovulation, fertilization and egg transport in the ewe. *Reproduction*, *60*(2), 267–272. https://doi.org/10.1530/JRF.0.0600267