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Kidding after Transfer of *in Vitro* Produced Saanen Goat Embryos into Local Ukrainian Breed Recipients in Different Seasons

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Abstract

In recent years, the demand for goat products has been growing due to the fact that goat milk has a number of advantages over cow milk, for example, it is low in lactose, and is considered less allergenic and easier to digest. To increase production during both breeding and non-breeding seasons and reduce the price of dairy products, it is necessary to effectively use reproductive management and assisted reproductive technologies. *In vitro* embryo production makes it possible to obtain a large number of eggs from goats, which for some reason are unable to conceive, but have genetic value. Afterward *in vitro* produced embryos can be transferred into recipient goats of other less genetically valuable breeds, such as the Ukrainian local breed. Therefore, the aim of the present study was to investigate the effectiveness of transfers of *in vitro* produced embryos of Saanen goats into surrogate sires of the Ukrainian local breed in different seasons. All manipulations with animals were carried out following ethical standards (Strasbourg, 1986). Six Saanen goats were selected as the oocyte donors. After the hormonal stimulation oocytes were retrieved by laparoscopic ovum pick-up. *In vitro* produced embryos were transferred laparotomically into 24 recipients of Ukrainian local breed. Fifty days after embryo transfers, pregnancies were determined by ultrasound diagnostics. Although the embryo development rate in the breeding season was 20% higher than in the non-breeding season, there was no difference in pregnancy and kidding rates between seasons. In conclusion, the transfer of *in vitro* produced Saanen goat embryos to recipients of the Ukrainian local breed gives the opportunity to achieve pregnancy and kidding regardless of

the breeding season, which will enable a faster and more efficient increase in the livestock of highly productive goats in Ukraine in the post-war period.

Keywords

Goats, *in Vitro* Produced Embryos, Saanen Breed, Goat Reproduction, Embryo Transfer, Kidding Rate, Breeding Season

1. Introduction

The conservation of biological diversity and sustainable development of genetic resources of farm animals has been recognized by the Food and Agriculture Organization of the United Nations (FAO) as crucial for balanced and harmonious development of livestock and agriculture [1]. In Ukraine, there has been an increasing demand for goat products [2], particularly goat milk, which offers several advantages over cow milk, including lower lactose content, making it suitable for people with hypolactasia, and being less allergenic and easier to digest [3] [4]. Dairy goat breeds have been developed through selective breeding programs to achieve high lactation performance [5] [6]. To meet the increasing demand for dairy products and reduce their prices, effective reproductive management is necessary [7] [8]. Assisted reproductive technologies (ART) have been widely used in animal breeding to increase livestock numbers [9]. In natural breeding for temperate latitudes, goat mating occurs in autumn, and kidding occurs in spring [8]. This necessitates the maintenance of a large number of males throughout the year, resulting in high costs. Multiple pregnancies in goats can also complicate pregnancy and kidding [10] [11]. The use of ART can bypass some of these limitations [12]. Recent advances in cryopreservation technology have significantly improved the effectiveness of ART in animal husbandry [13] [14]. Cryopreservation of goat sperm enables the use of sperm during any season by hormonally regulating the estrus of goats [15]. *In vitro* embryo production (IVEP) technology makes it possible to obtain a large number of oocytes from goats with a genetic value that are unable to conceive naturally [12]. Moreover, oocytes can be collected from slaughtered goats' ovaries and utilized for reproductive purposes. *In vitro* produced embryos can then be transferred into recipient goats of different breeds, such as the Ukrainian local breed. Therefore, the aim of this study was to investigate the effectiveness of transferring *in vitro* produced embryos of Saanen goats into recipient sires of the Ukrainian local breed during different seasons.

2. Materials and Methods

2.1. Ethical Approval

The animal experiments were conducted in accordance with the ethical standards outlined by the European Convention for the Protection of Vertebrate

Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the Law of Ukraine "On Protection of Animals from Cruelty" (Ukraine, 2006), and the guidelines set forth by the Committee on Bioethics of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Kharkiv, January 2021).

2.2. Animals

Six female Saanen goats, aged 4 - 5 years, weighing in average 65 kg and with an average milk production of 1150 L per year, were selected as oocyte donors for this study. The group of recipients was comprised of 24 female Ukrainian local breed goats, aged 2 - 3 years and weighing in average 50 kg, with 15 goats in the breeding season and 9 goats in the non-breeding season. All animals were kept under the same feeding and management conditions on a farm in Ukraine (50˚31'43.6"N 31˚39'44.4"E).

2.3. Estrus Synchronization and Laparoscopic Ovum Pick-Up

The experiment was conducted during the breeding season (September-October) and the non-breeding season (April-May). Estrus synchronization in both donors and recipients was achieved by administering intravaginal sponges containing 45 mg of flugeston (SYNCRO-PART®, Ceva, France) for a duration of 13 days. On the 7th day following the insertion of intravaginal sponges, hormonal stimulation for superovulation was performed in the egg donors. A total of six intramuscular injections of pregnant mare serum gonadotropin (Sergon 500, Bioveta, Czech Republic) were administered at 24-hour intervals, following this protocol: 500 IU, 500 IU, 500 IU, 500 IU, 250 IU, and 1000 IU. Laparoscopic ovum pick-up was conducted 36 hours after the final gonadotropin injection [16]. Recipient animals received an intramuscular injection of 300 IU of gonadotropin on the 12th day after the insertion of intravaginal sponges.

Laparoscopic ovum pick-up (**Figure 1**) was performed under general anesthesia using 0.5 ml of intravenous propofol (Fresenius Kabi, Germany) per 1 kg of animal weight, following prior sedation with 1 ml of 2% xylazine per animal (Alfasan International B.V, Netherlands). The animals were positioned in an inverted manner at a 30˚ angle on the operating table to prevent organ perforation during the insertion of trocars. Follicle aspiration was conducted using a 23G needle (Aspic, IMV Technologies, France) attached to a 20 mL syringe.

2.4. Oocytes and Sperm Retrieval

The follicular fluid was transferred to Petri dishes and examined under a stereomicroscope (AmScope, United States). The cumulus-oocyte complexes, isolated from the follicular fluid, underwent three washes in a culture medium (Wash, IVF Bioscience, United Kingdom) before being transferred to an oocyte maturation medium (BO-IVM, IVF Bioscience, United Kingdom) covered with mineral oil (Oil, IVF Bioscience, United Kingdom). *In vitro* maturation (IVM)

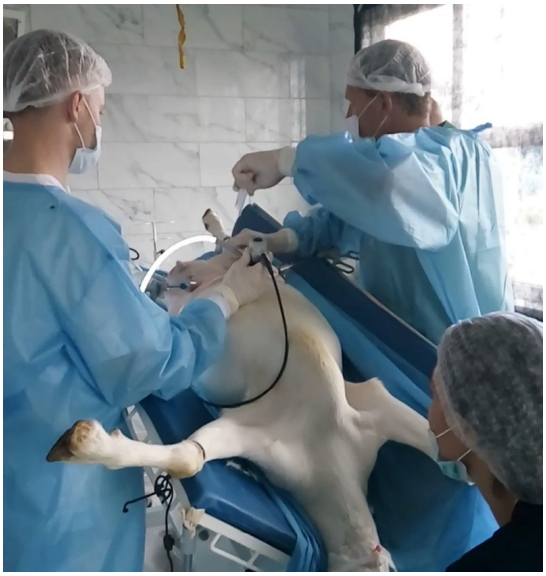

Figure 1. Laparoscopic ovum pick-up in donor goats under general anesthesia. The donor goat is fixed in an inverted position at an angle of 30˚. The doctor on the top right is performing an aspiration with a 23G needle connected to a 20 mL syringe. The doctor on the left is fixing an ovary and operating a camera to visualize inta-abdominal organs. The doctor on the bottom right is managing anesthesia.

was conducted in a CO_2 incubator at 38.8˚C, with a composition of 6.0% CO_2 and 21.0% O_2, for a duration of 22 - 24 hours.

Sperm was collected from a mature Saanen buck using an artificial vagina (Minitube, Germany) and a goat in estrus to attack the male during the breeding season (September-November). After assessing the concentration and motility of the spermatozoa, the sperm was diluted with a cryoprotective medium based on HEPES buffer, containing 10% glycerol and 20% egg yolk. The sperm suspension in the cryoprotective medium was then transferred to 250 µl straws (Minitube, Germany), with a final sperm concentration of 200 million per mL. Equilibration with the cryoprotectant was carried out at room temperature (+25˚C) for 15 minutes, followed by 2.5 hours at +5˚C, and a subsequent 15-minute exposure to nitrogen vapors at a distance of 4 cm from the liquid nitrogen, before being plunged into the liquid nitrogen. Thawing of the straws was performed in a water bath at +37˚C for 30 seconds. The cryoprotectant was removed by two cycles of centrifugation with the medium (BO-SemenPrep, IVF Bioscience, United Kingdom).

2.5. *In Vitro* Fertilization and *in Vitro* Embryo Culture

Fertilization of mature oocytes was accomplished by adding a sperm suspension to a fertilization medium (BO-IVF, IVF Bioscience, United Kingdom), covered with mineral oil (Oil, IVF Bioscience, United Kingdom), in a CO_2 incubator set at 38.8˚C, with a composition of 6.0% CO_2 and 21.0% O_2. Fresh sperm was used for *in vitro* fertilization during the breeding season, while cryopreserved sperm was utilized during the non-breeding season. After 16 - 18 hours, the fertilized

oocytes underwent stripping and three washes in a medium (Wash, IVF Bioscience, United Kingdom) and were subsequently transferred to an embryo culture medium (BO-IVC, IVF Bioscience, United Kingdom). The embryos were cultured for 7 days in a CO_2 incubator set at 38.8˚C, with a composition of 6.0% CO_2 and 6.0% O_2.

2.6. Embryo Transfers and Pregnancy Determination

The transfer of *in vitro* produced embryos (Figure 2) was carried out via laparotomy into the uterine horn of synchronized recipients following sedation with 1 ml of 2% xylazine per animal. To prevent multiple pregnancies, single embryo transfers were performed. The embryo, along with the medium (BO-IVC, IVF Bioscience, United Kingdom), was aspirated into a catheter (Tomcat, Minitube, Germany) connected to a 1 ml syringe (Vitromed, Germany).

Fifty days after embryo transfers, an abdominal ultrasound was performed to determine pregnancy. Subsequently, the kidding rate was calculated after a gestation period of 5 months.

2.7. Statistical Analysis

Statistical analysis was conducted using GraphPad Prism software (GraphPad Software, USA). The data were presented as mean ± standard deviation ($\overline{X} \pm \sigma$). The Shapiro-Wilk normality test was employed, followed by either the Mann-Whitney U-test (for all measurements except pregnancy and kidding rate) or Fisher's exact test (for pregnancy and kidding rate), to compare the data between the breeding and non-breeding seasons. A significance level of $P < 0.05$ was considered statistically significant.

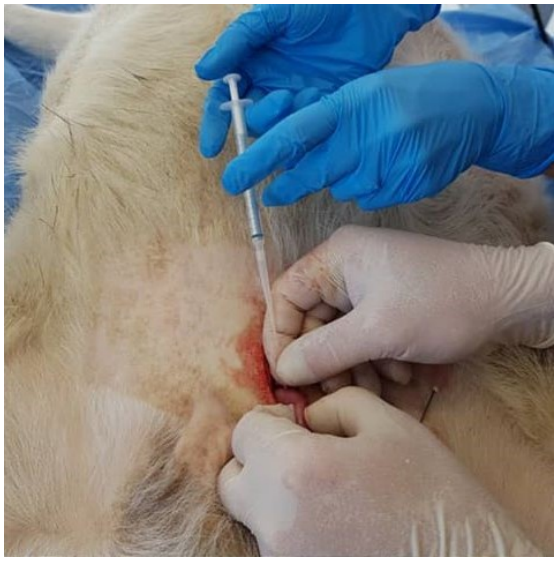

Figure 2. Laparotomic embryo transfer is performed to the uterine horn of the recipient goat. Initially, an incision is made in the uterine horn using an 18G needle. Subsequently, a tomcat catheter containing the embryo is carefully inserted into the incision, and the embryo is gently delivered into the uterine horn.

3. Results

3.1. Breeding Season

During the breeding season, a total of 41 cumulus-oocyte complexes (13.7 ± 1.5 per donor) were aspirated using laparoscopic ovum pick-up from 3 donor goats. Following *in vitro* maturation (IVM), 34 oocytes (83% ± 2.9%; 11.3 ± 1.2 per donor) were fertilized using fresh spermatozoa obtained from the ejaculate. The characteristics of the fresh sperm were as follows: ejaculate volume of 850 µl, sperm concentration of (3.08 ± 0.69) × 10^9 per mL, with 81.5% motility.

A total of 24 embryos, equivalent to 8.0 ± 1.0 per donor, (70.5 ± 4.2)% of all fertilized oocytes, continued to develop until day 7. Among them, 15 embryos were transferred to recipient goats via laparotomy, while the remaining embryos were cryopreserved for further studies.

After 50 days, a total of 5 pregnancies were confirmed, resulting in a pregnancy rate of 33.3%. Four of these pregnancies (26.7%) resulted in the birth of kids.

3.2. Non-Breeding Season

Performing laparoscopic ovum pick-up during the non-breeding season resulted in the retrieval of 39 oocytes (13 ± 4.6 per donor) (**Figure 3(a)**). Following IVM, 32 oocytes (82.4% ± 6.9%; 10.7 ± 3.8 per donor) exhibited an expanded cumulus complex (**Figure 3(b)**) and were subsequently fertilized *in vitro*.

The oocytes were fertilized with cryopreserved spermatozoa (**Figure 4(a)**), which were collected during the breeding season as our previous studies [17] [18] [19] demonstrated a decrease in sperm characteristics during the non-breeding season. After thawing, the sperm motility was observed to be (56.6 ± 10.1)%. The fertilized oocytes were easily stripped of cumulus cells, facilitating the visualization of polar bodies (**Figure 4(b)**).

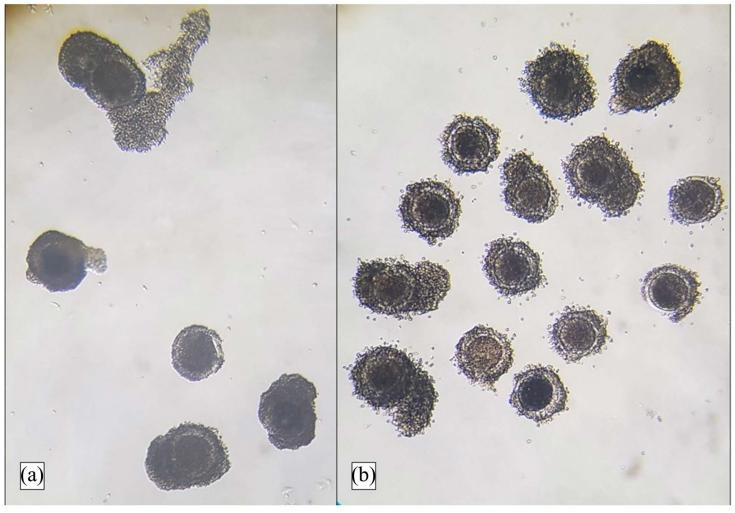

Figure 3. Goat oocytes before (a) and after (b) *in vitro* maturation. Compacted cumulus complex surrounding the oocytes developed into expanded cumulus complex following 22 - 24 hours of *in vitro* maturation.

Following 7 days of *in vitro* culture, a total of 16 blastocysts were achieved (5.3 ± 1.5 per donor) (Figure 5). The embryo development rate was determined to be (50.7 ± 4.5)% which is significantly ($P < 0.05$) lower than in the breeding season. Subsequently, nine of these embryos were transferred laparotomically into the uterine horn of recipient goats.

Among the recipient goats, three were confirmed as pregnant, resulting in a pregnancy rate of 33.3% during the non-breeding season. Out of these pregnancies, two (22.2%) successfully resulted in the birth of kids (Figure 6). It shows no significant difference in pregnancy and kidding rate between breeding and non-breeding seasons.

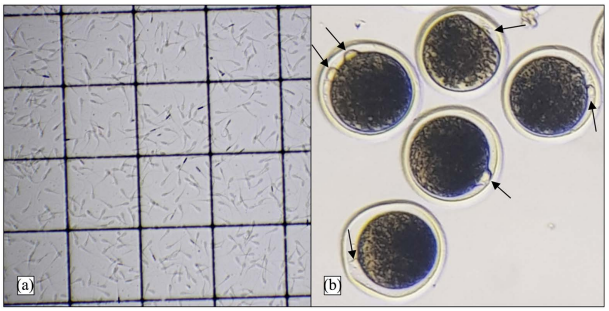

Figure 4. Goat spermatozoa after cryopreservation (a) and fertilized goat oocytes (b) with visualized polar bodies (black arrows).

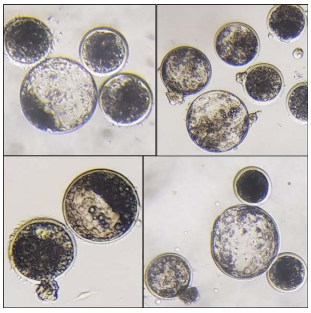

Figure 5. Goat embryos obtained on day 7 after *in vitro* fertilization of oocytes with cryopreserved spermatozoa.

Figure 6. A buck (a) and a goat (b) of Saanen breed alongside their surrogate sires of Ukrainian local breed.

4. Discussion

Among the 187 main dairy goat breeds in Europe [20], the Saanen breed is the most popular among Ukrainian goat farmers. The Ukrainian population of Saanen goats is derived from genotypes imported from European countries. However, due to the high cost of Saanen goats, there is interest in utilizing the Ukrainian local breed as recipient goats, considering their lower cost and higher availability.

The experiment was conducted during different seasons: breeding and non-breeding. Analysis of reproductive characteristics revealed that the number of obtained oocytes was comparable between the breeding and non-breeding seasons and had no statistical difference. The blastocyst development rate on the 7th day of culture in both seasons, after using fresh and cryopreserved sperm for fertilization, exceeded 50%, consistent with findings reported by other researchers [21].

The pregnancy rate observed in recipient goats in our study aligns with results from other research groups. For instance, Morais *et al.* [22] reported a pregnancy rate of 32.1% for Toggenburg breed goats in Brazil after non-surgical embryo transfer, with all pregnant goats successfully giving birth to kids. Although the season of the study was not specified, Morais *et al.* noted that embryo quality influences pregnancy rate. Another study [23] found a pregnancy rate of 26.67% in recipient goats of mixed breed (Boer × local breed) that had not undergone previous surgeries, such as laparoscopic ovum pick-up and surgical embryo transfer.

However, J. P. Greyling *et al.* [24] reported a pregnancy rate of 60% - 67% for two goat breeds in South Africa, which is nearly twice as high as our study. In experiments conducted by Chinese scientists [25] who studied the effect of transporting donors, recipients, and embryos between Chinese farms on the pregnancy rate of Boer goats, it was demonstrated that 42% - 80% of goats became pregnant after embryo transfer. Importantly, it should be noted that these studies involved *in vivo* produced embryos, obtained through flushing after superovulation induction and donor insemination. Such embryos have been shown to have higher potential for implantation compared to *in vitro* produced embryos [12]. Furthermore, it is important to note that in the aforementioned studies, embryos of one breed were transferred to recipient goats of the same breed. Our study involved embryos and recipients of different goat breeds. While all agricultural breeds of goats belong to the same species, Capra hircus, there are intraspecific differences in metabolic activity [26] [27] that may indirectly impact the expression of receptors on the surface of the endometrium [28] [29] and embryos, potentially leading to reduced implantation rates.

5. Conclusion

The transfer of *in vitro* produced Saanen goat embryos to recipients of the Ukrainian local breed offers the opportunity to achieve pregnancy and successful

kidding, irrespective of the breeding season. This method will enable a faster and more efficient increase in the livestock of highly productive goats in Ukraine in the post-war period. By utilizing cryopreserved sperm collected during the breeding season for fertilizing oocytes obtained in the non-breeding season, a satisfactory number of embryos can be obtained for subsequent transfers to recipient goat sires, resulting in the same pregnancy and kidding rate as in the breeding season.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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