



Reproductive characteristics of Saanen and Alpine bucks

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Genetic variations among breeds within a species can impact not only productivity traits, such as milk yield and quality, but also animal health, including fertility. This study aimed to compare the reproductive characteristics of bucks from the Saanen and Alpine breeds. Sperm concentration and motility were assessed using light microscopy, viability was determined using eosin-nigrosin staining, and morphological parameters were evaluated using the *Spermac Stain* method. DNA fragmentation was measured using the *Halosperm* kit. Artificial insemination of goats was conducted with fresh semen during natural estrus. Statistical analysis was performed using the *Graph Pad Prism* software. The results revealed that Alpine bucks exhibited significantly higher semen volume, sperm concentration, viability, and motility ($P < 0.05$). No significant differences ($P \geq 0.05$) were observed between the breeds regarding the number of spermatozoa with normal morphology and the rate of DNA fragmentation. Cryobiological analysis of spermatozoa from Saanen bucks suggested a higher cryoresistance compared to the Alpine breed. Following artificial insemination of goats, the pregnancy rate for the Saanen breed was 61.8%, which was twice as high as that observed in Alpine goats — 28.8% ($P < 0.05$). These findings demonstrate significant differences in reproductive characteristics between Saanen and Alpine goats. Despite superior sperm characteristics, the pregnancy rate after artificial insemination was significantly lower in the Alpine breed compared to the Saanen breed. Consequently, it is crucial to consider these variations in essential reproductive characteristics when implementing breeding programs and employing reproductive biotechnology in animal husbandry to ensure their successful application and effectiveness.

Key words: sperm, reproductive characteristics, cryopreservation, pregnancy, goats, bucks, Saanen breed, Alpine breed



Introduction

Breeding in livestock makes it possible to develop breeds of domestic animals that have high productive characteristics and allows to improve their genetic value through breeding programs and reproductive biotechnology [5, 16]. Intraspecies genetic differences between

breeds can affect not only productivity characteristics, such as milk yield and its quality [8], but also animal health, particularly fertility [15]. Saanen and Alpine breeds of goats belong to the same species *Capra aegagrus hircus* [1]. The Saanen goat breed is a Swiss breed and is widely used in dairy farming due to its ability to produce large quantities of high-quality milk.

A common feature of this breed is its adaptability to different environmental conditions. The Alpine goat breed, also known as the French Alpine or Alpine Chamois, is originated from the French Alps. Alpine goats are known for their milk production, adaptability, and endurance [17].

Sperm cryopreservation is widely used in animal breeding as one of the methods of assisted reproduction, because it allows for more efficient animal reproduction [7, 18]. Cryotolerance of spermatozoa depends on their initial morphological characteristics, such as size and shape, and functional characteristics, such as motility, DNA integrity, and membrane lipid composition, which are seasonally dependent [3; 11].

Considering the importance of these characteristics when applying assisted reproductive technologies and choosing the most promising ones for further breeding, the study of reproductive peculiarities of different goat breeds is an essential task. Therefore, the aim of our study was to compare the sperm reproductive characteristics of Saanen and Alpine breeds of bucks.

Materials and methods

All manipulations with animals and their biological material were carried out according to the permission of the Bioethics Committee of the Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine (Protocol no. 1 from 28.01.2021) and in compliance with the main provisions of the Law of Ukraine “On Protection of Animals from Cruelty” (no. 3447-IV from 21.02.2006) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Ejaculates were obtained using an artificial vagina and a goat in estrus for attraction from four mature Saanen bucks (n=48) and three Alpine bucks (n=36) once a week during the breeding season (September-December) at the “Tetyana 2011” farm (Cherevky village, Kyiv region, Ukraine). All animals were kept under the same conditions and had the same nutrition [13].

The volume of semen was determined. The concentration and motility of spermatozoa were counted using a Makler chamber (*Sefi medical instrument*, Israel). Sperm viability was assessed in smears stained with eosin-nigrosin *VitalScreen (FertiPro)*, Belgium) smears under a light microscope ($\times 400$). The number of spermatozoa with normal morphological parameters was counted under a light microscope with a magnification $\times 1000$ in the smears, which were fixed and stained using the *Spermac Stain* kit (*FeriPro*, Belgium) according to the manufacturer's protocol. The level of DNA fragmentation was determined using the *Halosperm* kit (*Halotech*, Spain) according to the protocol provided by the manufacturer.

For the cryopreservation, sperm were isolated from the seminal plasma by centrifugation for 10 min at 200g, diluted with HEPES-based medium (*WASH, IVF Bioscience*, United Kingdom) supplemented with 10% glycerol

and 20% egg yolk to reach a final concentration of 200×10^6 sperm/ml. The sperm suspension was equilibrated for 15 min at room temperature (25°C), loaded into 0.25 ml straws (*Minitube*, Germany), and equilibrated again for 2 h at 5°C, placed horizontally 4 cm above the level of liquid nitrogen for 15 min, and then plunged into liquid nitrogen. Thawing was performed on a water bath at 37°C for 30 sec. The supernatant containing the cryoprotectant was removed after centrifugation at 200g for 5 min with 2 ml of washing medium (*WASH, IVF Bioscience*, United Kingdom). Sperm motility and viability, morphological characteristics, and DNA fragmentation level were evaluated after cryopreservation.

To perform artificial insemination of Saanen (n=131) and Alpine (n=78) goats aged 2–3 years in natural estrus, the obtained fresh sperm after analysis mentioned above was diluted with *Andromed* diluent (*Minitube*, Germany) to achieve a final concentration of 200×10^6 sperm/ml, loaded into 0.25 ml straws, and artificial insemination was performed using the cervical method. After 60 days, ultrasound diagnostics was performed to detect pregnancy.

Statistical analysis was performed using *Graph Pad Prism* software (*GraphPad Software*, USA). The data were presented as mean \pm standard deviation for all parameters except those related to pregnancy after artificial insemination. The data were tested for normality of distribution using the Shapiro-Wilk test. To compare two samples, a non-parametric test was used to compare samples with non-normal distribution — the Mann-Whitney U-test, the difference was considered significant at $P \leq 0.05$. Fisher's exact test was used to compare the parameters related to pregnancy, the difference was considered significant at $P \leq 0.05$.

Results and discussion

Comparing the parameters of the fresh semen of the studied breeds, we found a significant difference between the following reproductive characteristics: sperm volume, concentration, sperm motility and viability (table 1).

Thus, the Alpine bucks had a significantly higher semen volume, sperm concentration, sperm motility and viability compared to the Saanen bucks ($P < 0.05$). There was no significant difference between the breeds in the normal morphology rate of spermatozoa and the sperm DNA fragmentation rate.

It is considered that semen volume and sperm concentration are not affected by season and feeding [20]. Sperm motility also does not depend on the diet of the bucks, but varies in different breeding seasons. Our results may indicate that despite the similar physiological functioning of the reproductive system of different breeds, the genetic characteristics of the breed have an impact on some semen characteristics. This is confirmed by a study conducted with three different breeds, which demonstrated that Alpine bucks had higher quantitative sperm parameters (sperm volume, sperm concentration) but lower

qualitative characteristics (sperm motility) compared to the same parameters for Saanen and Damascus breeds [6]. Similar to the results of our study, there was found no difference in sperm DNA fragmentation rate between the Zaraibi and Baladi goat breeds [19].

Analyzing the morphofunctional parameters of spermatozoa after cryopreservation, no significant difference was found between the two breeds as for the motility and viability of spermatozoa, the amount of morphologically normal spermatozoa, and the number of spermatozoa with fragmented DNA. However, considering the parameters of sperm before cryopreservation, it can be concluded that the cryotolerance of spermatozoa of the Saanen goats is higher, although the difference is not significant (table 2).

In this study, conducted during the breeding season, fresh semen was used to inseminate goats. However, during the non-breeding season, it is planned to use cryopreserved biomaterial.

To ensure a high fertilization rate after cryopreservation, spermatozoa must have a high cryotolerance. Our previous studies have shown that to achieve higher fertilization rate using cryopreserved sperm of Saanen goats, it is recommended to collect semen during the breeding season [2]. In general, sperm resistance to cryodamage is associated with seminal plasma proteins that provide protection against oxidative stress during cryopreservation [18]. In addition, better sperm cryotolerance is demonstrated when the translation of aquaporin proteins (AQP3, AQP7, AQP11) is upregulated, as they presumably facilitate the penetration of cryoprotectants into the cell during equilibration [14].

After the artificial insemination of goats, the pregnancy rate was significantly higher ($P < 0.05$) in Saanen goats and twice as high as in Alpine goats. There was no significant difference in the occurrence of pseudopregnancy, singleton and multifetal pregnancy between the studied breeds (table 3).

The pregnancy rate in small ruminants depends on both the male factor, i.e. semen parameters, and the female factor, i.e. follicular development, egg maturation, and hormonal regulation of endometrial development. It has been shown that the expression of genes responsible for follicular development and atresia differs in different goat breeds, and thus is genetically determined [21]. However, the results of other groups of scientists also highlight the influence of environmental conditions, such as husbandry, climate, and feeding, on the pregnancy rate [10]. The incidence of pseudo-pregnancy in our study was much lower (approx. 7%) than in a study conducted on Saanen goats in northeastern Brazil (approx. 30%) [9]. This may indicate that environmental conditions have a considerable impact on this parameter. This is confirmed by a study conducted on three breeds of goats, which found no correlation between the pseudopregnancy rate and genetic or phenotypic traits of goats, but revealed a link with out-of-season reproduction, when estrus stimulation is performed by administering hormones [4]. Regarding the occurrence of singleton and multifetal pregnancies, the results of our study are consistent with the research of a group of scientists who

Table 1. Fresh semen parameters of Saanen and Alpine bucks

Semen parameters	Breed	
	Saanen	Alpine
Semen volume, μl	850 \pm 375	1575 \pm 275*
Sperm concentration, $\times 10^9/\text{ml}$	1.7 \pm 0.5	3.2 \pm 0.2*
Sperm motility, %	80.4 \pm 4.1	91.1 \pm 3.1*
Sperm viability, %	88.3 \pm 2.9	95.9 \pm 3.9*
Sperm with normal morphology, %	93.4 \pm 1.1	95.1 \pm 0.9
Sperm DNA fragmentation rate, %	2.8 \pm 0.16	3.1 \pm 0.2

Note. Here and further * — the difference is significant ($P < 0.05$) compared to Saanen goats.

Table 2. Sperm parameters of Saanen and Alpine bucks after cryopreservation

Sperm parameters after cryopreservation	Breed	
	Saanen	Alpine
Sperm motility, %	65.5 \pm 5.8	67.2 \pm 6.8
Sperm viability, %	85.2 \pm 5.1	83.2 \pm 4.8
Sperm with normal morphology, %	86.9 \pm 2.4	89.5 \pm 3.4
Sperm DNA fragmentation rate, %	6.2 \pm 1.9	7.1 \pm 2.4

Table 3. Parameters related to pregnancy after artificial insemination of goats in natural estrus with fresh semen of Saanen and Alpine bucks

Parameter	Breed	
	Saanen	Alpine
Pregnancy rate, % (n)	61.8 (81/131)	28.2 (22/78)*
Pseudopregnancy rate, % (n)	6.9 (9/131)	7.7 (6/78)
Singleton pregnancy rate, % (n)	46.9 (38/81)	40.9 (9/22)
Multifetal pregnancy rate, % (n)	53.1 (43/81)	59.1 (13/22)

showed the effect of hormonal stimulation on increasing the frequency of multifetal pregnancies, but did not find any difference for this parameter between the three breeds after artificial insemination in natural estrus [12].

Thus, the study of breed-specific reproductive characteristics allows us to identify specific genetic features of breeds that affect the effectiveness of reproductive biotechnology in livestock. This provides an opportunity to select males and females for further breeding, improve the genetic potential of the herd, and develop effective breeding programs.

The reproductive characteristics of spermatozoa of Saanen and Alpine goats differ significantly. Despite the better characteristics of the semen, the pregnancy rate for Alpine breed after artificial insemination was significantly lower than for Saanen. The spermatozoa of Saanen goats are characterized by a higher survival rate after cryopreservation compared to the same characteristic of the Alpine breed, which may have an impact on the effectiveness of artificial insemination with cryopreserved sperm. For the successful implementation of breeding programs and the effective use of reproductive biotechnology in animal husbandry, it is important to take into account the breed-specific differences in the main reproductive characteristics.

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Репродуктивні характеристики цапів зааненської та альпійської порід

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Внутрішньовидова генетична різниця між породами може впливати не тільки на характеристики продуктивності — наприклад, на кількість та якість надою, але й на здоров'я тварин, зокрема їхню фертильність. Мета дослідження — порівняти репродуктивні характеристики зааненської та альпійської порід. Концентрацію та рухливість сперматозоїдів визначали за допомогою світлової мікроскопії; життєздатність — у забарвлених еозин-нігрозином; морфологічні параметри — методом *Spermac Stain*, визначення рівня фрагментації ДНК проводили з використанням набору *Halosperm*. Штучне осіменіння кіз проводили нативним еякулятом в еструсі самиць. Статистичну обробку даних виконували у програмі *Graph Pad Prism*. Встановлено, що цапи альпійської породи мали статистично більший об'єм еякуляту, концентрацію сперматозоїдів, життєздатність та рухливість ($P < 0,05$). Не було виявлено статистично вірогідної різниці ($P \geq 0,05$) між породами у кількості сперматозоїдів з нормальними морфологічними характеристиками та рівнем фрагментації ДНК сперматозоїдів. Кріобіологічні характеристики сперматозоїдів цапів зааненської породи мали тенденцію до більшої кріорезистентності. Після штучного осіменіння кіз частота вагітностей у самок зааненської породи склала 61,8%, що вдвічі перевищувало частоту настання вагітності у кіз альпійської породи — 28,8% ($P < 0,05$). Репродуктивні характеристики сперматозоїдів цапів зааненської та альпійської порід статистично відрізняються. Незважаючи на кращі характеристики еякуляту, частота настання вагітності самиць альпійської породи після штучного осіменіння була вірогідно нижчою, ніж зааненської породи. Для успішного впровадження програм розмноження та ефективного використання біотехнологій у тваринництві важливим є комплексне врахування внутрішньовидової різниці основних репродуктивних характеристик.

Ключові слова: сперматозоїди, репродуктивні характеристики, кріоконсервування, вагітність, кози, цапи, зааненська порода, альпійська порода