SEMEN QUALITY OF MATURE CARPATHIAN BUCKS DURING NON-BREADING SEASON

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Abstract

The recognition of the differences specific to each breed and those caused by the estrous season in terms of the breeding performance of the bucks, leads to the improvement of the management of individual semen samples used in the artificial insemination programs. The aim of the study was to evaluate the quality of the semen collected during non-breeding season, from 3 Carpathian bucks, 3 years of age. The semen was collected by artificial vagina between June and July 2019. Two methods of semen dilution, with and without seminal plasma were compared, in order to highlight the effect of seminal plasma in the refrigeration process. Tris-based extender was used, with and without egg yolk. The quality of the semen was evaluated immediately after collection by assessing the volume ejaculated, concentration, pH and motility and viability immediately after collection and also at 24 and 48 hours, after refrigeration at a temperature of 4^0 C. The results showed a decrease of the cytological parameters (motility, viability) by 15-20% every 24 hours of refrigeration in the semen samples from both methods. In conclusion, for insemination during non-breeding season with refrigerated semen, within 48 hours after collection, there is no need for centrifugation in order to remove the seminal plasma during semen's preparation process.

Key words: Semen quality; buck; non-breeding season

INTRODUCTION

The photoperiod controls the sexual activity and is the main environmental factor which established the seasonal feature of goats reproduction. Although the seasonality is less important at buck than goat, the bucks present a seasonal reduction of sexual behavior and spermatogenesis during the same period of the year when the females are in out of seasons [1].

The used extenders for buck semen will contain egg yolk or skimmed milk powder. However, the dilution of buck semen with extenders that contain egg yolk can be harmful for spermatozoa. This thing happens because the buck seminal plasma presents characteristics that differentiate it from other species, the most important one being the presence of photolipase A secreted by bulbourethral glands. This phospholipase is called also Eyce (Egg yolk coagulating enzyme) or BUSgp60 (bulb urethral gland secretion) and is responsible for spermatic cells viability decrease that have been diluted with extenders containing egg yolk or milk powder [2]. Because the seminal plasma is removed or diluted during processing and because this contains elements that prevent premature capacitation [3], it is considered to be a physiological fluid that could protect the spermatozoa from handling induced stress.

The objectives of recent research on animal reproduction wanted to establish the physiological functions of seminal plasma and to examine the possibility that this complex fluid can be used as a biotechnological tool in order to improve the function of semen during processing for the use in artificial reproductive technologies (ART). *In vitro* processing of spermatozoa for preservation by refrigeration, cryopreservation or sexing leads to important changes in the semen sample extracellular

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fluid medium. The most obvious result is the dilution of semen plasma proteins in the sperm environment due to the addition of optimized extender for reproductive technologies. *In vitro* manipulation attempts to copy the signals and protective aspects of the *in vivo* environment to maintain the shape and function of the semen. The processing of the semen also leads to fluctuations in temperature, pressure, osmolality and pH which can damage seminal plasma membranes and limit the fertilizing life of the processed spermatozoa. These *in vitro* modifications have many similarities with *in vivo* capacitation, including impaired lipid mobility, cholesterol efflux and tyrosine phosphorylation. Because these changes occur *in vitro* prior to deposition in the female tract, rather than at the site of *in vivo* fertilization (the oviduct), the fertilizing capacity of processed spermatozoa is considerably decreased [4].

The aim of this study was to evaluate the quality of the semen collected during non breeding season, after 48 hours of refrigeration at 4°C, with and without seminal plasma.

MATERIAL AND METHOD

The semen was collected from 3 Carpathian bucks of 3 years old, during June and July 2019. The semen was collected using female goats with estrus induced by hormonal treatments. From each male, two ejaculates were collected, at an interval of 15-20 minutes, which, after the first dilution, were processed together. If the motility differences between the ejaculates of the same buck were greater than 15%, the ejaculates were processed separately. The ejaculates were collected by artificial vagina.

The processing for refrigeration was done by two methods: the classical method, by centrifugation to remove the seminal plasma and dilution with Tris extender with 20% egg yolk and 1% glycerol and the method without centrifugation and dilution with Tris extender without egg yolk, with 1% glycerol.

In order to assess the quality of the semen, the physical and morphocytological parameters were evaluated at collection, and after 24 and 48 hours of cooling at 4 °C. The analysis of the morphological parameters was performed by optical microscopy techniques.

The volume of the ejaculate is established immediately after collection, by reading the divisions on the graduated test tube of the collecting glass. Only the volumes larger than 0.5 ml were used for processing. The concentration of spermatozoa in semen sample was estimated by haemocytometer method and is expressed in billions of spermatozoa/ ml semen.

In order to measure the motility, the semen is classified as non-motile, with progressive motility or with non-progressive motility. Also, the total percentage of spermatozoa with progressive motility is estimated. Motility was assessed by manual evaluation technique [5] in wet environment, under optical microscope (Novex, Holland) (x100 magnification) equipped with heating plate maintained at 37°C and camera.

Structural integrity of plasmatic membranes (viability) was assessed by the eosin-nigrosine staining method [6].

The morphological examination of the spermatozoa consists in establishing the number of spermatozoa with abnormal appearance.

The results were statistically processed using IBM SPSS Statistics 20 software.

RESULTS AND DISCUSSION

The morpho-cytological parameters of the semen collected during non-breeding season from the 3 Carpathian bucks are shown in table 1.

Table 1 showed that mean of semen volume per ejaculate ranged from 0.95±0.26 to 1.35±0.34 ml. Semen volume per ejaculate differed significantly ($p<0.05$) among the bucks. Highest semen volume was obtained in buck 3 followed by 2, and 1. The three bucks belonged to the same breed and of similar age, their management and nutritional status and general health condition were also similar. So, the difference in volume of semen might reflect their different genetic potentiality and genetically superior bucks could produce higher volume of semen. The result of the present study agrees with the studies of previous workers [7, 8]. In terms of sperm concentration, there were also significant differences ($p <0.05$) between all 3 males.

Table 1 Morpho-cytological parameters of Carpathian bucks' semen collected during non-breeding season

No.	Spermatozoa indices	Male 1	Male 2	Male 3
1.	Ejaculates (n)	32	30	35
2.	Volume (ml)	0.95^{a}± 0.26	1.1^{b}± 0.23	1.35^{ab}± 0.34
3.	*pH*	7.1± 0.06	7.1± 0.11	7.0± 0.21
4.	Concentration (billions/ ml)	4.99^{a}± 0.16	5.12^{a}± 0.19	3.23^{a}± 0.19
5.	Abnormalities (%)	7.66± 0.92	7.36± 0.12	7.21± 0.58

The results are presented as mean ± standard deviation. Means with different superscripts within the same row differed significantly ($p<0.05$)

Usually, the percentage of morphological abnormalities in the semen of a buck with normal fertility should be less than 5% during the breeding season. If collections are made during the summer, the percentage of abnormalities can be expected to be higher. The percentage of morphological abnormalities in the semen of below average and poorly fertile bucks may be 10-15%. Our study recorded values between 7.21 and 7.66%, which are normal for the season. There were no significant differences between the semen from the 3 males neither for anomalies nor for pH.

The results regarding the effect of refrigeration, in the presence and absence of seminal plasma, on the male 1 semen's quality are shown in table no. 2. According to these data, it can be observed that within the 2 days during which the semen was tested, for the batch with refrigerated semen in the presence of seminal plasma, without the addition of egg yolk, a decrease of viability by approximately 37% is observed, from 90.61% to 53.63%, while for the second alternative with the refrigerated semen without seminal plasma, there is a decrease of viability of 35%. There are no significant differences between the experimental options.

Motility is a very important sperm indicator on which the fertilizing capacity depends. The assessment of motility must take into account the speed, linearity and lateral movement of spermatozoa. During the 2 days of monitoring, there was a decrease of motility by 34% for the refrigerated batch without seminal plasma and by 35% in the refrigerated batch with seminal plasma (table 2).

Table 2 Motility (%) and viability (%) of the male no. 1 semen according to dilution method

Refrigeration period (h)	Motility (%)		Viability (%)	
	Without seminal plasma	With seminal plasma	Without seminal plasma	With seminal plasma
0	88.21±2.04	88.82±1.75	90.21±2.14	90.61±2.01
24	72.30± 2.11	72.42±3.02	74.40±2.27	73.92±1.96
48	54.31±2.66	53.33±2.41	55.40±3.62	53.63±3.06

The results are presented as mean ± standard deviation (n=32).

The results regarding the male 2 semen's quality are shown in table no. 3.

According to these data it can be observed that during the 2 days in which the sperm was tested, for the batch with refrigerated semen in the presence of seminal plasma, without the addition of egg yolk, a decrease of viability by approximately 37% was observed, while for the second alternative with refrigeration without seminal plasma, there is a 35% decrease. There are no significant differences between the experimental options. During the 2 days of monitoring, there was a decrease in motility by 35%, from 89.71% to 54.50%, in the refrigerated lot without seminal plasma and by 37% in the refrigerated lot with seminal plasma (table 3).

Table 3 Motility (%) and viability (%) of male no. 2 semen depending on dilution method

Refrigeration period (h)	Motility %		Viability %	
	Without seminal plasma	With seminal plasma	Without seminal plasma	With seminal plasma
0	89.71 ±1.88	90.50±1.35	90.71±2.00	91.30±1.25
24	72.73±2.16	73.52±2.75	74.00±2.66	74.02±2.35
48	54.50±2.79	53.13±2.02	55.90±2.33	54.11±1.53

Results are presented as mean ± standard deviation (n = 30)

The results regarding the male 3 semen's quality are shown in table no. 4. According to these data it can be observed that during the 2 days in which the sperm was tested, in the batch of semen refrigerated in the presence of seminal plasma, without the addition of egg yolk, a decrease of viability was observed by approximately 33,6% while for the refrigeration variant without seminal plasma, there is a decrease of 34%. There are no significant differences between the experimental options. During the 2 days of monitoring, there was a decrease of motility by 34,2%, from 84.61% to 50.45%, in the refrigerated lot without seminal plasma and by 35,5% in the refrigerated lot with seminal plasma (table 3).

Table 4 Motility (%) and viability (%) of the male 3 semen according to dilution method

Refrigeration period (h)	Motility (%)		Viability (%)	
	Without seminal plasma	With seminal plasma	Without seminal plasma	With seminal plasma
0	84.61 ±1.34	84.91±2.23	86.51±1.26	84.71±1.15
24	70.53±1.43	69.62±2.22	71.31±1.05	72.42±1.77
48	50.45±1.34	49.41±0.69	52.90±1.96	50.51±1.90

Results are presented as mean ± standard deviation (n = 35)

Refrigeration at low temperatures provides some suppression of semen metabolism compared to physiological temperatures, but the maintenance of energy metabolism following storage is still the most important criteria for successful preservation. Because glucose is an energy source for spermatozoa, higher efficiency energy metabolism would support sperm mobility during preservation. [9].

Researchers have long sought to identify the specific factors in seminal plasma that influence semen's function and fertility. As the information on seminal plasma function were accumulated, it has become clear that dilution of seminal plasma during semen's processing for artificial reproduction (eg, sexing and cryopreservation), to some extent, may explain the altered function and fertile status of the processed spermatozoa.

As a result, a considerable investigation of the effect of supplementation with seminal plasma on the survival and function of spermatozoa processed for controlled reproduction has been observed in recent decades Our study has shown that the decrease of semen's quality during refrigeration is the same in the presence and absence of seminal plasma. Other studies obtained higher percentages of motility and viability after 56 hours of refrigeration in the presence of seminal plasma [10]. Moreover, by avoiding centrifugation, a stress-generating stage is eliminated and the processing time is optimized. Our results recorded a 34-37% decrease in motility and viability without significant differences between the three males. Other studies have shown that semen's motility during refrigeration (24 h, milk at 15°C) is male dependent and may be correlated with the differences existing in the seminal plasma proteome. The same studies have shown that several seminal cell membrane proteins that interact with the cytoskeleton, glycolysis enzymes, and spermatozoa associated proteins involved in capacitation are positively correlated with good results after refrigeration and can be

considered as seminal biomarkers in semen conservation [11].

The proteomic evaluation of spermatozoa and the environment has made considerable progress toward these goals and allowed a better understanding of their physiological function [13]. Many plasma proteins have been identified as diagnostic predictors of semen's function and have been isolated and applied *in vitro* in order to prevent damage of semen due to the application of artificial reproduction technologies. A higher concentration of glycolytic enzymes in the seminal plasma of seminal samples that have high in vitro motility may be related to an increased abundance of glycolytic pathways in the spermatozoa or may represent an increased spermatozoa production [12]. Proteomic characterization of ram's seminal plasma has identified over 700 proteins [11], the most abundant seminal proteins being secreted by accessory sex glands. Comparative proteomic analysis has shown that spermatozoa-associated protein complexes are predominantly associated with higher semen's preservation capacity [14, 15]. Our research must continue to identify these proteins in the buck seminal plasma.

CONCLUSIONS

The proteomic evaluation of the spermatozoa and the environment has made considerable progress toward these goals and has allowed a better understanding of the physiological function of the spermatozoa. Our study has shown that the variation of semen's quality during 48 hours of refrigeration, in the presence and absence of seminal plasma, is the same, which demonstrates the importance of seminal plasma during preservation. Furthermore, by avoiding centrifugation, a stress-generating stage is eliminated and the processing time of the semen to be used in artificial insemination is optimized.

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