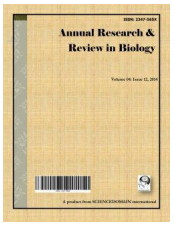

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An Objective Analysis of Factors Affecting Buck Semen Quality Attributes during Cryopreservation: A Mini Review

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Authors' contributions

This work was carried out in collaboration between both authors. Authors DJA and NK managed the literature searches and the analyses of the study. Author DJA wrote the first draft of the manuscript. Author NK critically revised the first manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

This paper aims to update recent findings related to the potential factors affecting the quality of buck semen during cryopreservation process. This paper describes cryopreservation of buck semen and it identifies different factors limiting the successful use of this technique in goats. Recently, several assisted reproductive technologies have been developed to control and to improve reproductive characteristics and productivity in goats. Semen cryopreservation is one of the most commonly used assisted reproductive technologies. This reproductive method allows an accelerated production of genetically valuable offspring. It is affected by various factors limiting its successful application. The handling practices prior to freezing, the composition of extenders, temperature and the length of storage have considerable effects on reproductive outcomes reducing the quantity and quality of spermatozoa. Furthermore, extensive investigations are needed to clearly assess the mechanisms between the temperature, and generation of reactive oxygen species during cryopreservation.

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1. INTRODUCTION

The important industrialization of milk, milk products, meat and meat products has led to a level of intensifying goat farming that involves the use of highly productive breeds subjected to new production strategies such as improved reproductive methods and selection programs. Cryopreservation is the method that preserves structurally intact living cells at very low temperature [1]. It is an important method used in biotechnology of reproduction to preserve male semen and store it in a bank for future use. It is a process during which all metabolic activities are minimized due to the freezing and the low temperature of storage [2]. The advantages of this technique include: indefinitely and widely use of frozen semen, the easier exportation and facilitation of international exchange of genetic material, reproduction of animals is made possible, even in non-reproductive seasons [3]. The potential advantages of semen cryopreservation have not been fully accomplished due to the limiting many factors such: semen concentration, storage temperature (optimum freezing and thawing rates), the composition of extension media and cryoprotectants used during the freezing process. Therefore this review paper discusses in detail these factors contributing to a successful semen cryopreservation.

2. CRYOPRESERVATION PROCESS OF BUCKS' SEMEN

2.1 Semen Samples Collection

Semen collection can be performed using electrical stimulation or artificial vagina to ensure the ejaculation. Semen collection with the artificial vagina is obviously depending on the libido of the buck and the skill and experience of the operator. Bucks are usually trained to mount a restrained estrous teaser doe to ejaculate into the artificial vagina. Training may take from two days to several weeks, but some bucks may prove impossible to train. During the breeding season, semen can be collected from trained buck once or twice per day [4]. The semen collection using sine-wave stimulator consists to stimulate buck using rectal probes with three longitudinal electrodes. The probe is lubricated, gently inserted into the rectum, and then orientated so that the electrodes are positioned ventrally. The penis is prolapsed beyond the prepuce, and semen can be collected using a sterile plastic container, which is kept warm by covering it with hand [5]. Bucks are able to ejaculate after reaching 3-5 volts of electrical stimulation that can be applied for intervals of 3-5 s and alternated with rest periods of similar duration [6,7].

2.2 Assessment of Semen Samples

The assessments of semen characteristics include evaluation of seminal attributes of the samples in order to determine the fertilizing potentialities of the spermatozoa. Generally, the major parameters include in semen characteristics assessment in term of quality are sperm morphology, sperm concentration and sperm motility [8]. This microscopic examination of sperm morphology, concentration and motility are subjective as the total integrity of spermatozoa is not analyzed [8,9]. Studies demonstrated that semen quality and characteristics are affected by many factors such as body weight, body condition score, age, scrotal circumference measurements and testicular parameters, the breed, management, climatic and seasonal conditions, nutrition, the method of semen collection, and degree of sexual stimulation [10,11,12,13,14,15,16,17].

2.3 Semen Extension and Packaging into Plastic Straws

Collected semen samples after evaluation are diluted using diluents containing a complex extender (egg-yolk, milk or milk-whey), cryprotectants (e.g., glycerol) [18]. Tris-glucose or skim milk extenders are most commonly used for cryopreserving goat sperm [19]. Semen is extended to a final concentration of approximately 250-500×10^6 spermatozoa/mL; if this involves an extension of less than 5:1, semen can be washed once before resuspending to the final concentration in the extender [20]. After extension, the semen is load into plastic straws and seal with polyvinyl acetate powder.

2.4 Cooling and Freezing of Semen

The straws containing the diluted semen are kept in a 500-mL beaker of water at ambient temperature and placed in a refrigerator for 1-2 h to allow slow cooling to 5ºC. The straws are introduced into the tank of liquid nitrogen where they are horizontally suspended 5 cm above the

surface of the liquid nitrogen for 7 min and then plunged rapidly into the liquid nitrogen. In the case of long-term storage, the straws are kept submerged in liquid nitrogen. Sperm samples may be stored indefinitely without any further loss of viability at -196ºC. However, degradation of the sample can occur as the result of a partial thawing and refreezing [20].

The nature of the extender, cooling and thawing rate, packaging, interactions between cryoprotectants, as well as the individual animal variation have been identified as potential factors influencing the success of sperm cryopreservation [21].

3. FACTORS AFFECTING SEMEN CRYOPRESERVATION

3.1 Effect of Medium Used for Semen Dilution

The success of semen cryopreservation largely depends upon the type, composition as well as the concentration of semen extender used during the process. In general, the medium used for semen dilution is constituted by a non-penetrating and a penetrating cryoprotectant, a buffer, and one or more sugars. Non-penetrating cryoprotectants are milk or egg yolk while penetrating cryoprotectants comprise glycerol (G), ethylene glycol (EG), or dimethyl sulfoxide (DMSO). The most commonly used buffer is Tris. Sugars comprise glucose, lactose, raffinose, saccharose, or trehalose [22]. Literature revealed that the commonly used extenders for cryopreserving goat sperm are made off Tris-glucose, egg yolk, skim milk, and glycerol. This has pH values ranging from 6.75 to 7, as mammalian semen has a pH ranging from 7.2 to 7.8 [19,21,23]. Skim milk based extender can maintain higher sperm quality as compare to semen stored in egg yolk based extender during liquid storage at 4ºC for 72 hours for fruitful AI and maximum for 96 hours [24].

On the other hand, studies showed that the levels of non-penetrating and penetrating cryoprotectants in extender have a critical role in freeze-thawed goat semen. For instance, 10% egg yolk has exerted better functional membrane integrity, acrosome intactness than the presence of 20% egg yolk after freezing and thawing of Jakhrana goat semen [25]. Moreover, glycerol concentration is an important factor affecting freezability of goat semen from different breeds [26]. Thus, the optimal values of glycerol concentration providing success of semen cryopreservation in Markhoz, Saanen, Angora and Kilis is 7%, 7%, 5 % and 5-9% respectively [22,26,27]. Additionally, at 37ºC the motility, progressive motility and viability of spermatozoa increase with an increasing of glycerol concentrations while the rate of morphological normal acrosome decreases significantly [27].

We can conclude that diluents used for freezing semen and fresh semen must have the similar biochemical composition and must contain cryoprotective agents in order to reduce intra and extra-cellular, biochemical and physiological changes and protect spermatozoa against cold shock leading to membrane damage during freezing.

3.2 Effect of Temperature and Freezing Rate

During cryopreservation spermatic membrane suffers a series of changes in fluidity due to changes in temperature. Evidence demonstrated the detrimental effects of cryopreservation on sperm motility, the integrity of membrane, DNA function, and mitochondrial function [28,29]. Buck sperms do not seem to be well-adapted to long-term cooling at low temperatures. A reduction of their post-thaw viability and consequently low fertility rate is observed. According to results obtained by Ahmad et al. [30] in Beetal buck, the cooling (from 37ºC to 4ºC in 90 min) of diluted sperm (37ºC) followed by a freezing and thawing causes considerable damage after freezing and thawing to motility (89.8±1.26% vs 42.3±7.5%), plasma membrane integrity (85.3±0.92% vs 50.1±8.7), acrosomal cap (87.7±1.3% vs 45.2±8.4%), live-dead ratio (92.6±0.68% vs 56.0±10.5) and morphology (96.8±0.36% vs 81.0±5.7) of the sperm. Additionally, Üstüner et al. [28] reported that in Saanen goats, the increase of freezing rate from 10ºC/min to 24ºC/min between +5ºC and -150ºC progressively reduced sperm motility and acrosome integrity. In terms of DNA fragmentation, they have found a non-significant difference between the freezing stages, except for freezing rate at 24ºC/min and 15ºC/min, where DNA fragmentation was 32.5% 19.1% respectively. They also reported that DNA damage observed for the lowest freezing rates (10ºC/min) and highest (24ºC/min) was higher than the other freezing rate groups (12ºC/min and 15ºC/min). They concluded that post-thawed DNA damage increases as the freezing rate from 10ºC/min to 24ºC/ min between +5ºC and -

150ºC. In conclusion, the survival of spermatozoa can be significantly influenced by cooling rate from temperature just above 0°C after freezing and thawing. For this, some have proposed to cool down the sperm beyond the traditional cooling temperature (4-5ºC) to allow sperm plasma membrane to accommodate those changes in fluidity without losing selective permeability [29].

3.3 Effects of Length of Semen Storage

Other leading factors that might alter post-thawed sperm characteristics are the duration of storage and concentration of sperm in a sample. In effect, it was well documented that sperm morphology, motility, viability and low fertility of frozen semen in buck are decreased progressively with increasing the period of storage. In a study on West African Dwarf buck raised in Sudano-Guinean area of Cameroon, Ngoula et al. [31] found that during storage at 4ºC, the sperm mobility declined with storage time that was 105 hours and 65 hours after semen preservation in Tris-based extender (from score 4 to score 0) and skimmed milk-based extender (from 3.57 at the time of dilution to a score 0) respectively. Similar results have been found by Wahjuningsih et al. [32] reporting a decreasing of sperm motility with increasing length of storage at 5ºC. Also, the same authors have stated that concentration of spermatozoa affects the motility of individual and they pinpointed the concentration of sperm $40x10^6$/ ml and length of storage 0 h in 5ºC showed the highest motility of spermatozoa.

3.4 Ameliorative Effects of Antioxidants Use

The negative effect of oxidative stress, due to over the generation of reactive oxygen species (ROS) can be reduced using various antioxidants (enzymatic and non-enzymatic antioxidants). The supplementation of antioxidants to control the level of ROS and improve seminal attributes (the motility, acrosomal integrity, viability and fertilization ability of the spermatozoa) has been successfully demonstrated. The study of Bucak et al. in Angora buck indicated that the addition of 2.5m/M glutamine and 500 µl/ml hyaluronan considerably reduced the superoxide dismutase (SOD) activity [33]. Furthermore, Bucak et al. demonstrated that the supplementation of methionine (2.5mM), carnitine (7.5 mM) and inositol (7.5mM) to extenders ensured the preservation of DNA integrity [34]. Investigating the effect of Vitamin C (acid ascorbic) and E supplementation on the post-thawed spermatozoa quality, Memon et al. and Sarangi et al. found that the spermatozoa motility, acrosomal and DNA integrity have been improved [35,36].

Recent studies showed the positive effect of pomegranate extracts including peel, juice, or seed on semen quality in male rats, rabbit bucks, roosters, cattle [37,38,39,40,41]. While in goat future studies need to investigate the effect of this improving effect of natural antioxidants such as antioxidants present in pomegranate. Despite several studies have been carried out on goat semen cryopreservation, the suitable protocols to enhance its greater and successful use in commercial farms is still less developed.

4. CONCLUSION

Cryopreservation of spermatozoa allows preserving fertility of high genetically valuable bucks. This allows the artificial insemination of goats and great reproductive performances. The semen cryopreservation consists of semen samples collection, the assessment of the collected sample for concentration, motility, and normal morphology, the dilution of the semen sample. The diluted semen is loaded into plastic straws and sealed with polyvinyl acetate powder and the straws are dipped into liquid nitrogen for cooling and freezing purposes. The interactions between cryoprotectants, type of extender, cooling rate, thawing rate and packaging, as well as the individual animal variation, have been identified as driver factors affecting the semen cryopreservation. More research should be done to investigate the effect of factors such as reactive oxygen species on the semen quality of freezing and thawing process.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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