Research Article/Artículo de investigación

EFFECT OF DIFFERENT CONCENTRATIONS OF GLYCEROL IN CRYOPRESERVATION OF GADDI GOAT SEMEN

EFECTO DE DIFERENTES CONCENTRACIONES DE GLICEROL EN LA CRIOPRESERVACIÓN DE SEMEN DE MACHOS CABRÍOS GADDI

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ABSTRACT

Goats have greater importance under small familiar systems in India. The unabated decline in Gaddi males of superior genetic merit due to extensive culling and unplanned breeding (inbreeding) accentuates the importance of conserving superior males and their use for artificial insemination. The available literature is unequivocal about the importance of glycerol during sperm cryopreservation. However, the optimal level seems to vary between species and breeds. The present study aimed to evaluate the effect of different concentrations of glycerol (6, 7, and 8%) on the quality of thawed semen from Gaddi bucks. A total of 180 ejaculates from six Gaddi buck were frozen in Tris citrate extender containing 10% of Egg Yolk with 6, 7, or 8% of glycerol. Sperm quality parameters in thawed semen (morphological abnormalities, sperm viability, progressive motility, and HOST response) were compared; in addition, the percentage of change in thawed semen with respect to fresh was determined. No effect of concentration of glycerol was observed on progressive motility and HOST response. Sperm viability was better in semen frozen at 6 and 8% (45.26±1.32% and 45.10±2.81% respectively) in comparison with 7% of glycerol (34.81±2.78%, P<0.05). Lower sperm morphological abnormalities were observed in semen frozen at 6% of glycerol (7.93±0.28, P<0.05) in comparison with 7% (9.18±0.69%) and 8% (9.90±0.55%). A fertility rate of 41.25% was achieved following AI with semen frozen containing 6% of glycerol. In conclusions, 6% of glycerol was a valid option to cryopreservation of semen from Gaddi buck, resulting in better viability and lower abnormalities. In addition, a good fertility response was observed.

Keywords: Gaddi goats, glycerol, extender, semen, cryopreservation, fertility.
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RESUMEN

Las cabras están teniendo mayor importancia en los pequeños sistemas familiares en India. Sin embargo, la disminución deliberada de machos Gaddi de alto merito genético debido a la eliminación excesiva y a los cruzamientos no planificados (consanguinidad) acentúan la importancia de conservar machos superiores y su uso para inseminación artificial. La literatura disponible ha confirmado la importancia del glicerol durante la criopreservación espermática. Sin embargo, el nivel óptimo parece variar entre especies y razas. El propósito de este estudio fue evaluar el efecto de diferentes concentraciones de glicerol (6, 7 y 8%) sobre la calidad del semen descongelado de machos de raza Gaddi. Un total de 180 eyaculados de seis machos fueron congelado en diluyente a base de Tris citrato conteniendo 10% de yema de huevo con 6, 7 u 8% de glicerol. Los parámetros de calidad espermática en el semen descongelado (motilidad progresiva, viabilidad, anomalías morfológicas y respuesta a HOST) fueron comparadas, además el porcentaje de cambio de cada parámetro con respecto al semen fresco fue determinado. No se observó un efecto del nivel de glicerol sobre la motilidad progresiva y la respuesta al HOST. La viabilidad espermática fue mejor en el semen congelado con 6 y8% de glicerol (45,26±1,32% y 45,10±2,81% respectivamente) en comparación con el semen congelado con 7% de glicerol (34,81±2,78%, P<0,05). Menos morfoanomalías fueron observadas en el semen congelado con 6% de glicerol (7,93±0,28, P<0,05) en comparación con el congelado con 7% (9,18±0,69%) y 8% (9,90±0,55%). Una tasa de 41,25% de fertilidad fue alcanzada luego de la inseminación con semen congelado con &% de glicerol. En conclusión, 6% de glicerol es una opción validad para la criopreservación de semen de machos cabríos Gaddi, resultando en una mayor viabilidad y menos morfoanomalías espermáticas. Además, una buena fertilidad fue observada.

Palabras clave: cabras Gaddi, glicerol, diluyente, semen, criopreservación, fertilidad.
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INTRODUCTION

Goats are assuming greater importance under the small household system in India. Accordingly, the Gaddi breed of goats is economically important for a large portion of nomadic and hilly tribes of Himachal Pradesh, India (Dogra & Thakur, 2010). A progressively declining cattle population accentuates goat rearing under a small household system. Sperm sensitivity to cryopreservation protocols varies among different breeds as well as the animals within the same breed and has been incriminated to variation in components of sperm plasma membranes (Bailey et al., 2000). In general, the sperms of small ruminants are more cryosensitive than other species (Purdy, 2006).

To determine ideal extender, different membrane-permeable cryoprotectants (Glycerol, Dimethyl Sulfoxide, Ethylene Glycol, and Propylene Glycol) and their combinations have been tested at different concentrations with buck semen (Leboeuf et al., 2000; Purdy, 2006; Gangwar et al., 2016; Rasad et al., 2017). Glycerol, however, remains the most frequently used penetrating cryoprotectant. Limited literature is available using variable glycerol concentration for semen cryopreservation in goats. Due to variability in reports regarding best glycerol concentration in extender, a varying number of ejaculates in the present study were utilized to establish the most promising glycerol concentration using 6, 7, or 8% concentration *per se* for Gaddi goat semen cryopreservation and its further usage for artificial insemination.

MATERIALS AND METHODS

Selection of animals and screening for diseases

The study was carried out on apparently healthy Gaddi bucks (n=11; aged 2.16±0.36 years, weighing 39.1±2.82 kg). These bucks were selected based on breeding history, breeding soundness evaluation, and testicular diameters. All the bucks were maintained under identical conditions and were screened for diseases, Brucellosis (RBPT, OIE guidelines, 2008), Chlamydiosis (AGPT, Chahota et al., 2015) to eliminate the possible transmission of infection.

Location of animals and period of investigation

The study was conducted at University Livestock Farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur (32.6°N, 76.3°E, altitude 1290.8m) from September-December 2016, January and March 2017 and September-December 2017, respectively. Average light:dark hours (h) and temperature (°C) during the period of the study comprised the average of the values considered on the day of semen collection and the preceding days. The average light:dark hours (h) and temperature (°C) ranged from 7.07±0.41:16.93±0.41 to 12.29±0.13:11.71±0.13 and 9.88±0.51°C to 21.94±0.18°C, respectively.

Animal housing and feeding

All bucks were subjected to grazing for five hours and remained under confinement in a shed where they were fed as per the standards of the Indian Council of Agricultural Research (ICAR, 2013). All males had rounded the clock access to the clean drinking water.

Semen collection and evaluation

A total of 180 ejaculates from eleven healthy adult bucks were collected using an estrus doe. The collections were made twice weekly with an artificial vagina (AV) maintained at 42-43°C. The fresh ejaculates were evaluated for any gross abnormality, including color, as well as volume and concentration (Caprine Photometer, IMV 1409®) followed by microscopic examination for mass motility. The suitability of ejaculates for further processing was determined according to Rather et al., 2016. Absence of any gross abnormality, milky to creamy semen color, mass motility of ≥3, and initial progressive motility of ≥70% were the criteria for selection of semen. The post-thaw cryopreserved semen samples were evaluated for progressive motility (Hafez and Hafez, 2000), viability (Hannock, 1951), morphological abnormalities (Bloom, 1977), and HOST reactivity (Pant et al., 2002). Percent change in the parameters described above between fresh and post-thaw semen was also calculated to determine the effect of cryopreservation.

Seminal plasma removal and extension

The seminal plasma was removed as described previously (Nuti, 2007; Sharma et al., 2018). The semen pellet thus obtained was extended with two equal fractions of tris citrate egg yolk extender, TEY (TRIS 1.21 gr, Citric acid 0.685 gr, D-Fructose 0.5 gr, Benzyl Penicillin 1000 IU/ml, Streptomycin Sulphate 1mg/ml), EY (10%) with variable glycerol concentrations (6, 7 or 8%). The TEY was added to semen at a gap of 2-3 minutes to yield a final concentration of $150x10^6$ spermatozoa/straw. The pH of the buffer was adjusted to 6.7 to 6.9.

Filling and sealing of straws

Extended semen was filled in 0.25 ml French mini straws (IMV Technologies, L'Aigle, Cedex, France), by aspiration using a micropipette (Minitube, Germany) and subsequently sealed at free end with the help of polyvinyl alcohol (PVA) (IMV Technologies, L'Aigle, Cedex, France) powder. All the steps of semen handling were undertaken at a controlled air temperature of 30°C.

Equilibration, vapor exposure, freezing, and thawing

The straws were laid on a stainless-steel rack and placed in cooling cabinet 4°C (Macro Scientific Works Pvt. Ltd. India) for four hours and after that exposed to liquid nitrogen vapors for 7 minutes. After that, the straws were plunged into liquid nitrogen for storage. The inventory of semen storage was also maintained. Thawing of semen straws was done at 37°C for 30 s (Sariozkan et al., 2010) in a water bath.

Artificial Insemination

It was decided to perform AI using semen found to be the best among the investigated glycerol concentrations. A total of 80 Gaddi goats were synchronized in two lots using Cloprostenol sodium @187.5µg (Pragma®; Intas Pharmaceuticals Ltd., India) IM. Sixty hours later, intra-cervical insemination was carried out using two 0.25 ml straws, each containing $150x10^6$ spermatozoa.

Ethical regulations

Proper ethical considerations related to animal handling and semen collections were observed and ensuring not to cause any injury during sampling.

Statistical analysis

The data obtained were analyzed using package R version 3.4.3. A paired sample t-test was used to determine significant differences in the ejaculates processed in varying glycerol concentrations. Pregnancy rates for different bucks were analyzed using the Chi-square test. A difference of $P<0.05$ or less was significant.

RESULTS

A perusal of the results (Table 1) revealed significantly lower morphological abnormalities (7.93±0.28%) along with minimal change (20.15) in the said parameter for the semen processed in 6% glycerol. Also, the sperm viability for 6% (45.26±1.32%) and 8% of glycerol (45.10±2.81%) were significantly higher than the samples processed in 7% of glycerol (34.81±2.78%). The numerical values for average progressive motility were also higher in the samples for 6% of glycerol (35.18±0.87%). There was no difference in HOST reactive sperms across different glycerol concentrations.

In the present study, 1.12 kids per doe (thirty-seven kids: males, 40.54%; females, 59.46%) were delivered. Buck no 3509 yielded best conception rate (53.84%), whereas buck no 3076 gave the lower (30.76%), with an overall conception rate of 41.25% (Table 2) following AI with frozen-thawed semen utilizing TEY extender containing 10% of egg yolk and 6% of glycerol.

DISCUSSION

Breed specificity to varying glycerol concentrations in goats, and accordingly, the quality of cryopreserved semen is on record (Purdy, 2006; Kulaksiz et al., 2013; Gangwar et al., 2016). Our findings using different glycerol concentrations, with 6% yielding the best results, corroborates to a recent observation (Rasad et al., 2017) in which 6% glycerol was better than 5, 7, 8 and 9% for goat (Etawah crossbred) semen cryopreservation. Similarly, 6% of glycerol was better than 4 and 8% of glycerol in Sirohi bucks (Sikarwar et al., 2015), as revealed by significantly higher motility and viability. Biswas et al. (2002) used 5, 7, and 10% of glycerol and found better motility and viability of frozen-thawed sperm in 7% than other glycerol concentrations. Similarly, Farshad et al. (2009) recorded better post-thaw semen quality with 5 and 7% glycerol than other glycerol concentrations (1 and 3 %) in Markhoz goat semen.

Table 1: Average (Mean±SEM) of post-thaw semen quality parameters extended with different concentration of Glycerol in Gaddi bucks.

Parameters (%)	Percentage of Glycerol		
	6% (n=106) (% change due to processing)	7% (n=37) (% change due to processing)	8% (n=37) (% change due to processing)
Progressive motility	35.18±0.87^{A} (52.03)	29.00±1.79^{A} (58.99)	34.70±3.03^{A} (51.63)
Viability	45.26±1.32^{A} (40.12)	34.81±2.78^{B} (53.30)	45.10±2.81^{A} (37.96)
Morphological abnormalities	7.93±0.28^{B} (20.15)	9.18±0.69^{A} (21.75)	9.90±0.55^{A} (30.26)
HOST reactive	52.48±1.43^{A} (26.26)	53.90±1.47^{A} (28.47)	53.40±1.50^{A} (30.01)

$^{A-B}$ Values with different superscripts within same row differs (P<0.05).
n=Number of ejaculates.

Table 2: Individual buck fertility.

Buck No	Does inseminated (Number)	Does pregnant (Number)	Fertility rate (%)
3067	14	5	35.71
3076	13	4	30.76
1201	15	7	46.60
3509	13	7	53.84
3002	12	5	41.60
3075	13	5	38.46
Overall	**80**	**33**	**41.25**

Chi-square value = 1.842; P value = 0.87

Kulaksiz et al. (2013) used variable concentrations of glycerol (5-10%) in different breeds of goats and concluded that 5% of glycerol is best for Angora, 5-9% for Kilis and 7% for Saanen breeds, respectively. Contrastingly, Deka & Rao (1986) showed that the different concentrations (i.e., 4, 6.4, and 9%) of glycerol did not differ in post-thaw sperm motility and morphological abnormalities in goat semen. Similar observations were recorded by Biswas et al. (2002) in Black Bengal goats.

Sperm membrane lipid composition might be a significant factor responsible for variability in susceptibility of spermatozoa to damage produced during the cryopreservation in different breeds of goats (Holt, 2000). Membrane destabilization occurs when the sperm plasma membrane undergoes a phase transition from the liquid crystalline phase to the gel phase due to a decrease in temperature, which could affect the semen during processing (Barrea-Compean et al., 2005). Glycerol remains to be the most effective cryoprotective compound for freezing goat semen, and no enhancement was shown by the addition of other compounds (Farshad et al., 2009). Ranjan et al. (2015) demonstrated the usage of a combination of egg yolk (10%) and glycerol (6%) to significantly (P<0.05) improve the percentage of intact acrosome in Jamunapari buck semen.

Rizal et al. (2013) reported the toxic effects of a higher concentration of glycerol. Similar observations about the harmful effects of higher concentrations of glycerol (9%; Etawah goats, Rasad et al., 2017) and (7%; Sonmez & Demira, 2004) in ram semen. Glycerol in higher concentrations can cause osmotic damage to spermatozoa because glycerol passes through the sperm membrane much slower than other cryoprotectants (Guthrie et al., 2002). The toxicity of glycerol in semen extender results in a reduction in sperm motility and alterations of the acrosome integrity by interfering with the permeability of the sperm membrane, which intern results in lower fertility rates when such semen is used for artificial insemination (Salmon & Maxwell, 2000).

Higher kids per doe have been observed by Gacitua and Arav, 2005 (Saanen goats; 1.78), Kharche et al., 2013 (Jamunapari goats; 1.64). Variable fertility rates (38 to 65 %) with the use of frozen-thawed semen, have been earlier observed in Indian and exotic breeds (Gacitua & Arav, 2005; Kharche et al., 2013). The reasons per se could be due to differences in freezability, a result of chilling injury (Drobnis et al., 1993) and fertilizing capacity of semen.

CONCLUSIONS

Extender containing 6% of glycerol was best concerning least morphological abnormalities and better progressive motility, viability for Gaddi goat semen cryopreservation. These results, together with the fertility of 41.25% achieved, indicate that glycerol at 6% represents an excellent alternative to preserve the quality of frozen-thawed semen from Gaddi bucks.

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