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Research Article

Comparative Effects of Cryopreservation on Semen Quality of Tapri Goat Buck, Extended in Tris Extender with Duck Versus Fayoumi Chicken Egg Yolk

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Abstract | The egg yolks play a significant role in preventing temperature related damages to the sperm's acrosome and plasma membrane during cryopreservation. The present study aims to evaluate various egg yolk extenders in relation to quality indicators of post-thawed semen from Tapri goat bucks. Thus, total 60 ejaculates (n= 15/ animal) were collected from four healthy Tapri goat bucks entitled with A, B, C, and D. Semen samples were evaluated macro and microscopically and pooled in two groups viz; A and B. While preparing the Tris-based egg yolk extender; the egg yolk from duck was used in group "A" and Fayoumi chicken in group "B". The diluted semen was packed in two different color straws (French straws) of 0.25 ml size, equilibrated for 2 hours at 5ºC then frozen deep in the liquid nitrogen (at -196°C). The thawing of straws was attained at 37ºC for 20 sec. The percentages of post-thawed total sperm motility, viability, normal morphology, and acrosomal membrane integrity were found significantly ($p<0.05$) better in group A (46.7±67, 49.33±1.397, 48.87±1.028, 50.60±1.302), then group B (42.67±0.6738, 43.73±0.9394, 44.73±1.112, 47±00), respectively. In conclusion, the extender containing duck egg yolk demonstrated significantly better ($P<0.05$) post-thaw quality parameters for Tapri goat bucks semen compared to that of fayoumi chicken.

Keywords: Cryopreservation, Tapri goat, Semen extenders, Fayoumi, Chicken, Duck

Introduction

Tapri goats are small in size, and their major population is found in Sanghar, Mirpurkhas, Khairpur, and Hyderabad districts. The average body weight of Tapri males and females is 22 to 18 kg. The body color of reddish brown and camel colored but mostly seen in white. Heads and ears are small, long spiraled horns in males and twin births are common in females (Ghaffar & Ashfaq, 2017).

Bucks are being used for natural breeding services or their ejaculates in artificial insemination (AI). Semen extenders used in cryopreservation frequently include a variety of vital elements such as egg yolk, buffer, sugars, glycerol, and antibiotics. Each of these elements is essential to efficiently keeping and protecting the spermatozoa (Purdy, 2006).

Egg yolk is a common and generally accepted component in semen extenders because it protects sperm. The addition of egg yolk to semen extenders improves sperm preservation, particularly in South American camelids. This protective effect is attributed to its ability to interact with the plasma membrane lipid bilayer, preventing phase transition events of membrane lipids (Bertuzzi et al., 2020).

The successful semen cryopreservation depends on some common steps to follow the protocols, such as collection and extension, the addition of cryoprotectants, equilibrium, storage, and thawing procedure (Curry, 2007). Nevertheless, sperm motility may be influenced at various stages, with occasionally distinct differences observed even among individuals. Consequently, it becomes crucial to examine modifications in sperm motility at each step (Jiménez-Rabadán et al., 2012). The process of cryopreservation allows sperm cells to be stored for an extended duration before their utilization. Additionally, cryopreservation of buck semen prolongs a

buck's reproductive capacity beyond its own lifespan (Rahman et al., 2008).

In general, frozen semen tends to have lower fertility compared to fresh semen (Lemma, 2011; Apu et al., 2012) due to irreversible changes in sperm structure, leading to reduced membrane integrity, motility, and fertilizing ability (Talaei et al., 2010). To ensure successful implementation of artificial insemination (AI) in goat breeding, it is crucial to adopt an appropriate cryopreservation protocol and ensure the quality of the cryopreserved semen. This will help maintain reproductive efficiency and genetic progress in goat breeding programs. The spermatozoa are significantly impacted by the changes in osmotic pressure that occur during the preparation of semen for cryopreservation. This aspect is thought to be the main barrier to sperm survival throughout the process of cryopreservation (Watson, 2000).

The quality of a good diluent for cryopreservation is to provide energy sources to the sperm cells, protecting from biochemical and physical establish a favorable environment for survival during cryopreservation. The extenders for the cryopreservation of goat sperm typically consist of a non-penetrating cryoprotectant (milk or egg yolk), a penetrative cryoprotectant (propylene glycol, glycerol, or dimethyl sulfoxide), a (Tris) buffer, sugar (fructose, glucose, lactose, or trehalose), salt (sodium citrate), organic acid (citric acid), and antibiotics (penicillin, streptomycin). Egg yolks are one of the several cryoprotectants frequently used to preserve domestic animal sperm. When egg yolks are combined with other ingredients during the preservation process, a beneficial effect is observed (Amirat et al., 2004).

Therefore, the current study set out to examine the different egg yolk extenders in terms of quality indicators of post-thawed semen from Tapri goat bucks.

Materials and Methods

In this study, four healthy and trained male Tapri goats, aged between 2 to 3 years, were selected and kept at the farm of the Department of Animal Reproduction, under a semi-intensive farming system. The bucks were utilized as a semen source, and they were kept in conditions of natural light with a consistent and uniform nutritional regimen. Each buck received a daily diet consisting of concentrated fodder, green grass, and water without any restriction. Semen collection occurred twice a week from each buck in the morning for two consecutive months. Total of 60 (n=15/animal) ejaculates were collected.

Semen Collection: Thorough cleaning procedures were followed for the bucks. Before each semen collection from the donor and teaser animals, the fore skin hairs were trimmed, and the area was thoroughly cleansed to prevent contamination. Additionally, the teaser animal hindquarters and back were also properly washed in the same manner. The artificial vagina underwent rigorous washing and sterilization procedures. To maintain the appropriate temperature, warm water at 42-45°C and air at a pressure of 35 mmHg were used to fill the artificial vagina, while the inner liner's surface was lubricated with petroleum jelly (Deen et al., 2003).

Semen collection took place twice a week for a duration of two months, early in the morning. Immediately, after being collected, the samples of semen were delivered to the lab and put in a water bath with temperature maintained at 37 °C. Initial analyses of the semen's volume, color, pH, wave motion, motility, shape, and membrane integrity were performed in the lab.

Fresh Semen Evaluation: The parameters listed below were recorded. The volume was calculated by measuring the amount of ejaculate in a graduated tube. Color evaluation was conducted through visual inspection, the semen was classified based on its appearance as white, milky, creamy, yellowish, or watery, following the method described by Hafez (2000). A digital pH meter was used to determine the pH of the semen. The evaluation of wave motion was conducted using a clean, warm, and dry slide. On the slide, a drop of pure semen was inserted and examined under low power magnification (10X) with a phase contrast microscope from Nikon, Germany. The classification of the observed wave patterns in the semen was done following the procedures described by (Avdi et al., 2004; Memon et al., 2012). Accordingly, 0 = Absence of mass activity, 2+ = fewer than 20% of sperm are moving, 3+ = About 40 to 60 percent of the waves move slowly, 4+ = Around 60 to 80% showing movement with more intense waves, and 5+ = 80 to 100% of them are showing movement with eddies that create waves. To determine the total sperm motility percentage, the ratio of normal saline to the semen was 1:100. A cover slip was placed over a drop of diluted semen that had been applied to a preheated slide. Using low magnification (20X), at least 100 spermatozoa moving in a straight direction were randomly chosen for assessment. Spermatozoa moving backward or in circles were not included in the evaluation, and the motility percentage results were expressed. Samples with motility exceeding 70% were chosen for further examination, following the methods described by (Sundaraman & Adwin, 2008; David et al., 2015). Normal Sperm morphology and viability were determined in a smear of Eosin nigrosine staining techniques per standard staining procedure of sperm morphology was determined as described by (Memon et al., 2012). The concentration of sperms was assessed by the hemocytometer method, as outlined by (Kaka et al., 2012). The method used to assess acrosomal membrane integrity in a fresh semen sample was the hypo-osmotic swelling test (HOST) described by (Ahmad et al., 2014).

Extension of semen: The samples of fresh semen were pooled and examined. To achieve a final concentration of 100 million sperm per milliliter, they were then diluted using two different types of extenders: group A with duck egg yolk and group B with Fayoumi chicken egg yolk. After cooling the diluted semen from both groups to 5 ºC in a cold cabinet for two hours, there was a two-hour

equilibration phase. Then, the semen was stuffed into 0.25 ml straws, with a different color of semen being added to each straw to help differentiate them. These straws were placed in a container with liquid nitrogen at a height of 4 to 6 cm, and they were then subjected to nitrogen vapors for 10 minutes. The straws were then preserved by being submerged in liquid nitrogen and kept (Ahmad et al., 2014) for storage. The composition of tris-based chicken and duck egg yolk extenders is given in Table 1.

Table 1. Composition of tris-based chicken and duck egg yolk extenders

Component of extender	Composition in chicken egg yolk extender	Composition in Duck egg yolk extender
Tris	3.81gm	3.81gm
Citric acid	1.97gm	1.97gm
D (-) Fructose	1.25gm	1.25gm
Egg yolk	15ml (Sharma et al, 2020)	15ml (Sharma et al, 2020)
Glycerol	7ml	7ml
Penicillin	1000 I.U/ml	1000 I.U/ml
Streptomycin	1000 ug /ml	1000 ug /ml
Distilled Water	78ml	78ml

Equilibration: After cooling the duration of equilibration period was 5ºC for 2 hours.

Filling and sealing of straws: In each group, 0.25ml French straws of red and blue colors were utilized. The straws were filled using a filling machine, and the open ends were manually sealed with polyvinyl chloride powder (PVC).

Post Thaw Evaluation: The assessment of a thawed semen sample after thawing was performed to ascertain the condition of sperm morphology, viability, membrane integrity, and motility. This was accomplished by introducing a small amount of the thawed semen into a solution for analysis (Revell & Mrode, 1994).

Statistical analysis: The statistical computation, ANVOA-1 on the semen characteristics was done by graph pad prism V.5 software.

Results

The present study was conducted on four Tapri goat bucks, which were trained at the department of Animal Reproduction SAU Tandojam. The semen samples were processed and extended in two different extenders; i.e. Fayoumi chicken Tris-based egg yolk and Duck Tris-based egg yolk.

Assessment of fresh Tapri buck semen: The mean volume (ml) of each buck was A (0.76 ± 0.029), B (0.68 ± 0.022), C (0.5867 ± 0.021) and D (0.4867 ± 0.019) shown in Table 2. Significant difference (P<0.05) in the semen volume was observed among all the Tapri goat bucks (A, B, C and D). The color of the semen collected from all four Tapri goat bucks was observed creamy white (Table 2). The mean pH of semen was evaluated from selected bucks (Table 2). Our results showed no significant (p>0.05) difference among all the four bucks.

Table 2. Macroscopic values of fresh Tapri goat buck semen (mean%±SEM)

Parameter	Buck A	Buck B	Buck C	Buck D
Color	Creamy white	Creamy white	Creamy white	Creamy white
Volume	0.76 ± 0.03a	0.68 ± 0.02b	0.59 ± 0.02c	0.49 ± 0.02d
pH	6.74 ± 0.01a	6.71 ± 0.02b	6.69 ± 0.03c	6.68 ± 0.02d

Note: Means followed by similar letters in each row are non-significant at p>0.05

Microscopic characteristics of fresh semen of Tapri goat bucks (mean%±SEM): The swirling movement of the normal fresh Tapri buck semen under microscopic fields is called wave motion. The wave motion was recorded as ++++ which is interpreted as showing rapid dark waves and eddies in all semen samples of Tapri goat bucks and presented in Table 3. The mean total motile sperm percentages were analyzed for all semen samples and presented in Table 3. The total motile sperm percentage of buck B (87.6 ± 0.55) was found highest in comparison to buck A (86.13 ± 1.06), C (86.93 ± 0.55), and D (85.33 ± 0.88). Statistically, no significant difference (P>0.05) was recorded among all the bucks, and the lowest value was seen Buck D (85.33 ± 0.88). The mean normal sperm morphology of Tapri goat bucks was recorded in A (88.8 ± 0.56), B (87.7 ± 0.61), C (88.73 ± 0.64) and D (88. 7 ± 0.51) presented in Table 3. There was no significant difference (P>0.05) seen between all the semen samples. The mean sperm viability percentage recorded in all bucks showed no significant difference (P>0.05) between all Tapri goat semen samples (A, B, C and D) shown in Table 3, however, highest viability was observed in C (86.93 ± 0.64) and lowest in buck B (85 ± 0.54). The mean value of sperm acrosomal membrane integrity was recorded in A (83.2 ± 0.78), B (81.73 ± 0.62), C (82.93 ± 0.36) and D (82.53 ± 0.50) shown in Table 3. No Significant difference (P>0.05) was observed among all the bucks. However, the highest viability was observed in buck A (83.2 ± 0.79) and the lowest in buck B (81.73 ± 0.62). The mean spermatozoa concentration (millions/ml) of fresh Tapri goat buck semen was recorded as in A (3756 ± 29.46), B (3654 ± 30.18), C (3705 ± 32.18) and D (3686 ± 25.46) shown in Table 3. No significant difference (P>0.05) was observed between all bucks. Although, the highest sperm concentration was observed in buck A (3756 ± 29.46) and lowest in C (3654 ± 30.18).

Table 3. Microscopic characteristics of fresh semen of Tapri goat bucks (mean ± SEM)

Parameter	Buck A	Buck B	Buck C	Buck D
Wave motion %	++++ [a]	++++ [a]	++++ [a]	++++ [a]
TMS %	86.13 ± 1.06[a]	87.6 ± 0.55[ab]	86.93 ± 0.55[c]	85.33 ± 0.88[cd]
NM %	88.8 ± 0.56[a]	87.67 ± 0.61[cd]	88.73 ± 0.64[ab]	88.67 ± 0.51[c]
Viability %	85.33 ± 0.41[cd]	85 ± 0.54[c]	86.93 ± 0.64[a]	86.47 ± 0.27[ab]
AMI %	83.2 ± 0.79[a]	81.73 ± 0.636[bc]	82.93 ± 0.36[b]	82.53 ± 0.50[cd]
Sperm concentration % (Millions/ml)	3756 ± 29.46[a]	3654 ± 30.18[c]	3705 ± 32[b]	3686 ± 25.46[cd]

Note: Means followed by similar letters in each row are non-significant at alpha 0.05
++++ = Wave with more intense showing movement 60 to 80%

Assessment of post-thaw semen parameters: The post thaw total motile sperm percentage of Tapri goat buck semen extended in two different avian species egg yolk extender, Tris based duck egg yolk and Tris-based fayoumi chicken egg yolk extender shown in Figure 1. Significant difference (P<0.05) higher total motile sperm percentage was observed in group A (46.7 ± 1.406) tris based duck egg yolk than group B (42.67 ± 0.67) Tris based fayoumi chicken egg yolk. The post-thaw viability percentage of Tapri goat buck semen extended in two different avian species egg yolk extender, Tris based duck egg yolk and Tris based fayoumi chicken egg yolk extender shown in Figure 1. Significant differnce (P<0.05) higher viability percentage was observed in group A (48.87 ± 1.028) tris-based duck egg yolk than group B (44.73 ± 1.11) Tris based fayoumi chicken egg. The significant difference (P<0.05) was observed between group A and B. The post thaw normal sperm morphology percentage of Tapri goat buck semen extended in two different avian species egg yolk extenders, Tris based duck egg yolk and Tris based fayoumi chicken egg yolk extender shown in Figure 1. Significant difference (P<0.05) higher normal sperm morphology percentage was observed in group A (49.33 ± 1.39) tris based duck egg yolk than group B (43.73 ± 0.93) Tris based fayoumi chicken egg. The significant difference (P<0.05) were observed between group A and B. The post thaw acrosomal membrane integrity percentage of Tapri goat buck semen extended in two different avian species egg yolk extender, Tris based duck egg yolk and Tris based fayoumi chicken egg yolk extender shown in Figure 1. Significant difference (P<0.05) higher acrosomal membrane integrity percentage was observed in group A (50.60 ± 1.302) tris based duck egg yolk than group B (47.00 ± 0.0) Tris based fayoumi chicken egg. The significant difference (P<0.05) was observed between group A and B.

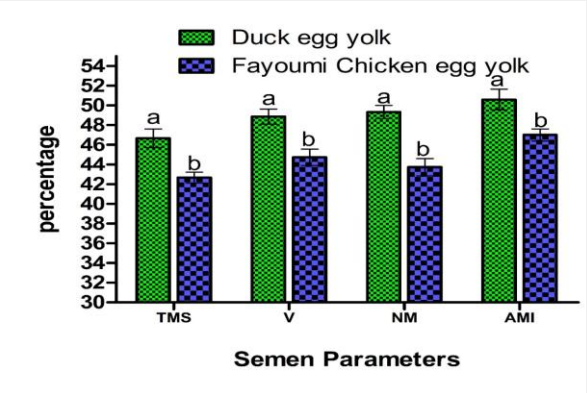

Figure 1. Post thaw sperm parameter percentages of Tapri goat buck semen extended in tris-based egg yolk mixed with duck and Fayoumi chicken eggs *(TMS: Total motile sperms, V: Viability, NM: Normal morphology and AMI: Acrosomal membrane integrity).*

Discussion

Semen cryopreservation enables the broad distribution of valuable genetic material, making it accessible even to small flocks through artificial insemination (AI), resulting in enhanced genetic improvement. Nonetheless, the usage of frozen semen for the cervical AI in goats is hindered by low fertility rates. The process of freeze-thawing spermatozoa leads to decreased cell motility, viability, and fertilizing capacity (Atessahin et al., 2008).

Egg yolk is a widely used component in extenders for preserving mammalian semen through cryopreservation, because it provides protection to sperm against thermal shock. This protective effect is attributed to the presence of low-density lipoproteins (LDL) found in egg yolk, which protect the sperm's cellular membrane during the cryopreservation process, helping to preserve the integrity of the sperm membrane (Bispo et al., 2011).

According to Kulaksiz and Daşkin (2010), egg yolks from various avian species exhibit varying amounts of fatty acids, phospholipids, and cholesterol. Consequently, the diluent with a higher proportion of margaric and linolenic fatty acids and a lower proportion of palmitoleic and myristic fatty acids might be accountable for the enhanced post-thaw semen quality.

The normal color of the buck of different breeds is creamy white to milky white, but it is species wise variations, depending on the sperm concentrations and presence of carotenoids and pigments in the semen plasma (Deori et al., 2018). Similarly, the semen color of Mahabadi bucks was also observed milky white, creamy-

grainy (Salmani et al., 2013). The pH of fresh semen was evaluated, and consistent values were reported by others, which ranges from 6.5 to 7.4 (Nuti, 2016; Lukusa & Lehloenya, 2017).

The mean semen volume of bucks of our study falls in the range of previously published reports by sultana et al. (2013); Nuti (2016), they observed mean volume of semen 0.58 ± 0.17 to 1.04 ±0.11 in black Bengal bucks. The semen volume may vary with the management practices, environment and buck age.

The wave motion was recorded as ++++, which is interpreted as showing rapid dark waves and eddies in all semen samples of Tapri goat bucks. The semen mass activity of the Tapri buck was very fast. Almost similar ranges of fresh semen wave motion were recorded by (Khalil et al., 2014).

The sperm motility in our study are in agreement with previously reported outcomes by Anjum, (2017) in Tapri goats buck semen, who observed it as 91.7±0.21 to 85±3.53. In parallel, Gojen Singh et al., (2016), studied sperm motility and found 84.14 ± 0.30 % of it in the Black Bengal buck semen. However, the motility of the semen depends on the age, health condition, breed, season and feeding practices of the animal (Khalili et al., 2009).

The mean sperm normal morphology of Tapri goat bucks found in our study is in line with Ramachandran and Singh (2017) in jamunapari bucks semen, and Ferdinand et al. (2012) in West African dwarf buck semen. However, sperm viability percentages are consistent with the Gojen Singh et al. (2016) in Black Bengal buck semen, and Anjum (2017). Whereas the corresponding values for AMI were observed by Kalyani et al., (2015); Anjum (2017). The variations in the AMI might be due to breed, season and age of the animals. The average mean spermatozoa concentration (millions/ml) of Tapri goat buck recorded are in agreement with Ferdinand et al. (2012); Anjum (2017).

Post thaw assessment of Tapri goat bucks semen: Egg yolk has been commonly employed as a universal extracellular cryoprotectant in the semen extender for animals (Sharafi et al., 2009). The post thaw total motile sperm of Tapri goat bucks semen observed in our study are in agreement with the reported observations of Salmani et al. (2013); Khalifa (2015); Gojen Singh et al. (2016); Malak (2022), they observed mean post thaw total motile sperm as 58 ± 2.94, 54 ± 0.89, 52.35 ± 0.59, 52.78 in buck semen respectively. Some variations in the reported data may be due to the presence of large amounts of unsaturated fatty acids in the spermatozoa membrane of the different species and may be due to usage of different diluents during semen processing techniques (Malak, 2022). However, the post thaw normal sperm morphology found in Tapri buck semen was similar to previous reported by Salmani et al. (2013); Anjum (2017); Malak (2022), they noted percent morphology as 55.7 ± 4.3, 65.00±0.894 and 74.45 respectively in buck semen. Our results for post thaw sperm viability are consistent with Salmani et al. (2013); Sharma et al. (2020); Malak (2022), they reported post thaw sperm viability as 55.00 ± 0.894, 45.26 ± 1.32, 66.89 respectively in buck semen. Whereas similar observations for post thaw acrosomal membrane integrity were observed by Salmani et al. (2013); Anjum (2017); Malak (2022), they noted percent acrosomal membrane integrity as 54.7 ± 4.1, 64.27, 54.00 ± 0.894 respectively in buck semen.

Conclusion

It was concluded from our study that, the semen extended in Tris-based duck egg yolk extender showed significantly ($p<0.05$) higher post-thaw quality parameters in terms of total motile sperms, normal morphology, sperm viability and acrosomal membrane integrity than the Tris-based fayoumi chicken egg yolk. Our study supports the use of duck egg yolk in the semen extenders in comparison to fayoumi chicken egg yolk.

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Author Contribution

SAM: Conduct of experiment and data collection, NAK: Conceptualization, data analysis, writing and editing of manuscript; AK, IHL, SAW, HDM, MUK: Overall help in experiment, laboratory analysis, and writeup of various sections of manuscript. All authors approved and assumed the responsibility of the content of MS.

Conflict of Interest

No competing interests are disclosed by the authors.

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