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EFFECT OF SOYBEAN MILK AND TRIS EGG YOLK BASED EXTENDER ON SEMEN QUALITY PARAMETERS OF THARPARKAR BULL

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ABSTRACT

About 40 (n=10) ejaculates were taken from four Tharparkar Bulls (A, B, C, D) having age of 4-5 years, after the collection, ejaculates were examined for initial evaluation i.e. volume, color, pH, motility, wave motion, viability, morphology, membrane integrity and sperm concentration. Ejaculate having ≥ 70% motility, morphology, membrane integrity and viability were processed and extended into Tris based extender (Control) and Soyabean extender with 3, 6, 9, 12, 15ml concentration of soybean milk. frozen thawed samples were evaluated for motility, morphology, viability and membrane integrity. samples diluted in tris extender resulted in48.50±1.2 motility, 65.00±1.5 morphology, 67.25±3.4 viability and 63.75±2.9 membrane integrity. However, In Soyabean based extender at the quantity of9 ml frozen samples improved semen quality parameters 57.75±1.1 motility, 73.00±3.4 morphology, 72.25±0.3 viability and 70.25±1.3 membrane integrity. After lab-based evaluation, samples diluted with Tris and optimized concentration of 9 ml of Soyabean based extender were inseminated into 20 cows (n=10 each group). Pregnancy was followed after 60 days of AI (Artificial Insemination) Semen diluted with Soya bean-based extender resulted in improved conception rate up to 60%. It was concluded that the soyabean based extender improved the cooled and frozen thawed semen quality of Tharparkar bull semen.

1. INTRODUCTION

The Tharparkar cattle were derived from Thar Desert (Chand, 2011) in Sindh, Pakistan. It is a *Bosindicus*, lyre-horned breed found in India-Pakistan border area of Thar Desert and considered as the dual-purpose breed valued for its milk as well as draught utility (Gadara et al., 2015).

It is a stable, heat tolerant and somehow tick resistant indigenous breed. Tharparkar cattle are strongly built and medium size breeds. The Milk production is approximately 5-10 liters per day and 1135-2000 liters per lactation (Choudhary et al., 2018). The artificial Insemination (AI) technique is used for the deposition of semen in female reproductive tract artificially at proper time of heat in which semen is collected, and

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processed from well tested bulls (Kumar et al., 2017). AI becomes a toll for dissemination of genetic material of superior sires to huge number of females, it is one of main tool to prevent outbreak of venereal diseases i.e. brucellosis, but it depends on management practices of semen collection, storage and technique of insemination (Petruskaet al., 2014).AI assists in the effectiveness of herd production, profitability and genetics (Kaka et al., 2016).It has been utilized for the development of other Assisted reproductive technologies i.e. semen sorting, embryo transfer, and cloning (Kumar et al., 2017; Jelani et al., 2022).

Cryopreservation is a finely developed technology of Assisted Reproductive Technologies (ART) mostly applied for extending the viability of spermatozoa by reducing metabolism and toxin creation (Bailey et al., 2003). Ice crystals are formed during process of cryopreservation that alters the structure of plasma membrane and normal physiology of spermatozoa by ionic changes, disturbing protein-phospholipids bound and membrane permeability (Lessard et al., 2000), also leads to a decrease in viability, fertility (Wongtawan et al., 2006: Channo et al., 2022) and finally leads to death of spermatozoa (Bailey et al., 2003). Soya lecithin-based extender maintains the quality and fertility rate of caprine and bovine semen (Gil et al., 2003). Extenders help out in process of extension, preservation and pay a pivotal role in defense against the many shocks during the storage and transport of spermatozoa for Artificial Insemination. Moreover, extenders are used prolonging of viability and fertility after post thaw. Different kinds of extenders are easily available in the market for bovine, ovine, porcine, equine, caprine and human semen cryopreservation. They must be isotonic, maintain the pH, cold shock protective, energy possessing and antimicrobial during freezing and thawing (Raheja et al., 2018). This study was planned for comparative studies on the effect of soybean milk and tris egg yolk-based extender on semen quality of Tharparkar bull because there was deficit work done on the cryopreservation of Tharparkar bull semen extended in Tris and Soybeen Extender.

2. MATERIALS AND METHODS

This study was conducted on four Tharparkar bulls at Animal Reproduction Farm, Department of Animal Reproduction, Agriculture University Tandojam. Semen collection was performed two times a week for 08 weeks. After collection, samples were forwarded to the laboratory and then processed for initial semen evaluation. After initial evaluation semen was processed for Macroscopic and Microscopic evaluation.

Bull preparation and semen collection

Bulls were properly managed before collection and were cleaned; their preputial hairs were clipped and washed. An artificial Vagina (AV) was used for the collection of semen. Meanwhile, AV were maintained at 42-450C with the air pressure of 35mmhg.

Volume

The volume of semen was recorded directly from graduated collection tube per ejaculate.

Color

Visual examination was followed, color grading was classified as milky, creamy, creamy white and translucent.

pH

pH was assessed with the digital pH meter.

Wave motion

The wave motion was accessed on clean slide while putting a drop of undiluted semen with phase contrast microscope (Nikon, Germany) (10X). Recorded wave samples were classified as follows (Rehman et al., 2014)

Motility

The motility was determined by keeping a diluent of semen on pre-warmed slide with the rate of 1:100 (Semen: Normal Saline). Motility was assessed in percentage. Sample having >70% motility was processed for further procedure (Hafezet al., 2002). Moreover, diluent preparation and processing were followed as described by Kaka et al. (2012).

Concentration of sperm

The concentration of sperm was evaluated with hemocytometer and concentration solution was prepared as reported by Kaka et al. (2012).

Morphology and viability

Morphology and viability were determined for this a drop of fresh semen was mixed with 2-3 drops of staining solution on a pre warmed slide. Thin smear was made and incubated for 3 minutes at 37°C. Water was used to remove excess stain and slide was washed with ethanol to remove the water. Moreover, dried slide/film was examined by examining 4 microscopic fields and 100 spermatozoa were counted. The readings were categorized as normal and abnormal via their morphological characteristics.

The viability of sperms was accessed from same slide and sperms showing pink color were considered dead while white colored spermatozoa was considered to live and were categorized in percentage.

Membrane integrity

Hypo Osmotic Swelling Test (HOST) was used for the determination of Membrane integrity from fresh semen samples. One ml of HOST solution was poured into 100 µl of semen than incubation was followed for 1 hr. at 37°C, after that 15 µl of the solution was put on pre-warmed slide by following cover slip. Sperms were visualized under light microscope at magnification of 40X. Those spermatozoa that showed swelling in response to test solution were classified as normal spermatozoa. 100 spermatozoa from 4 microscopic fields were counted and determined in %.

Extension of semen

Samples having motility and normal morphology ≥ 70% and viability of ≥ 80% were extended in Tris (0) and Soyabeen milk base extender 3, 6, 9,12,15ml concentration of soyabeen milk.

Preparation of diluents

Tris based buffer system was prepared by following procedure of Kakaet al., (2012).

Table 1. Concentration of different integrant

Tris(hydroxymethyl-amino methane)	3.81gm
Citric acid	1.97gm
D (-) fructose	1.25g
Egg yolk	20ml
Glycerol	7ml
Penicillin	1000mg/ml
Streptomycin	1.00i.u/ml
Water	100ml

Table 2. Preparation of Soya milk extender

Soya bean milk	3, 6, 9, 12,15ml
D (-) fructose	1.25g
Glycerol	7ml
Penicillin	1000 i.u
Streptomycin	1.00gm
Water	100ml

Filling of semen straws

After preparation of extender, it was shifted to cold cabinet with temperature 5°C for 2 hrs, semen straws were filled with manual suction machine. The semen straws were sealed with polyvinyl hydrochloride powder (PVP). During filling equipment's, straws, sealing powder was stored in the cold cabinet to maintain a similar temperature and prevent shock.

Freezing

Freezing was made into a wide mouth container with 6 minutes holding time. The straws were plunged into a liquid nitrogen cylinder at -196°C and kept for 24 hours.

Thawing

Thawing process of frozen semen sample was carried out after 24 hours of freezability by placing semen straw at 37°C/15 sec in water bath.

Post thaw semen evaluation

The following parameter of the semen were evaluated after thawing process i.e., Motility; Morphology; Viability and Membrane Integrity.

Fertility Rate

Twenty cyclic Thari cows were selected from the surrounding of Tandojam for fertility trial. They were divided into two groups, Group A and Group B (n=10). All cows of both groups were synchronized by injecting prostaglandin (Serilin; Selmore) 2ml/animal. Fixed time artificial insemination technique was carried out after 72 hours of PG injection. In Group A cows were inseminated with post thawed Tri's egg yolk-based extender and Cow in Group B were inseminated with post thawed soya bean milk-based extender. Pregnancy rate was determined by rectal palpation after 60 days of insemination

Statistical analysis

Collected data were analysed to one-way analysis of variance (ANOVA) using Statistics (2006) and LSD used to determine difference means of different groups.

3. RESULTS AND DISCUSSION

Motility (+SEM) of post thawed semen is depicted in table 3. However, significant difference ($P< 0.05$) was observed in all treatments/groups level 0, 3, 6, 9, 12 and 15. Motility percentage increased linearly among treatment levels. However, the highest values were observed in level (9ml). The morphological mean (+ SEM) of post thawed semen is also presented in Table: 3. However, there was a significant difference ($P< 0.05$) in all treatment's levels 0, 3, 6, 9, 12 and 15. The morphological percentage increased linearly among treatment levels. However, highest values were observed in level (9ml). Moreover, the mean (±SEM) of membrane integrity obtained from each bull is represented in Table 3, there were also significant

differences (P< 0.05) in all treatment level 0, 3, 6, 9, 12 and 15. After that, highest values were observed in level (9ml). The mean (±SEM) of spermatozoas live dead ratio from each bull is depicted in table 3. There was a significant difference (P< 0.05) among all levels 0, 3, 6, 9, 12 and 15. Highest values were observed in level (9ml).

Fertility/ conception Rate

Table 3 shows the percentage conception rate after AI in both Tris and Soyabeen based extenders (9ml).

Thawing process usually causes a reduction in viability and liveability of spermatozoa up to 50 percent (Kaka et al., 2012). It's important to evaluate spermatozoa's post thawed quality parameters for the determination of spermatozoa fertility after chilling and thawing.

Soyabean extenders contain phospholipids, which may help in the maintenance of the structure and function of spermatozoa membrane (Trimecheet al., 1997). Phospholipids form a defensive film over sperm membranes to prevent them against lethal factors(Zhang et al., 2009). In this research frozen thawed motility, morphology with viability and membrane integrity were observed. Soyabean extender improves the post thawed characteristics of spermatozoa (Singh et al., 2012). Papaet al., (2011) recommended the extenders containing soyabean may be used over conventional extenders that are egg yolk based or skimmed milk based as an alternate. (Akhtar et al., 2011) find out motility and viability of buffalo spermatozoa at 5 ºC in soya lecithin-based extenders were improved as compared to egg yolk citrate, egg yolk tris-citric and milk-based extenders. (de Paz et al. 2010) also suggested soyabean lecithin extender has uniformity in the preservation of motility and viability of ram spermatozoa at 5 and 15ºC over egg yolk based extender.

There is a tight association between intact plasma membrane, fertilizing ability, post-thaw sperm motility, and acrosome spermatozoa. Soya Milk keeps the plasma membrane safe while restoring phospholipids that are impaired because of heat stress and protects the cryo viability (Campbell & Farrel 2007). Soyabean lecithin as a semen extender in freezing diluent might support cryoprotectants to distribute frequently and decrease its partial concentration, which leads to mitigating the toxicity of cryoprotectants on boar sperm through the thawing procedure. The sperm movement features in terms of acrosome integrity and plasma membrane integrity were used for detection of superiority of frozen-thawed boar sperm. In the current study, freezing diluent with 9mlsoyabean (milk) lecithin could give the best cryoprotective action for spermatozoa during cryopreservation, in comparison with egg yolk extenders.

This was the same to some studies on cryopreservation of sheep (Fukui et al., 2008) and bovine semen (Amiratet al., 2005; Gil et al., 2003).Many of scientists informed that the effect of the cryoprotective factors on soyabean lecithin on freezing bovine spermatozoa was the same to egg yolk extender (20%)Thun et al.,(2002);Aires et al., (2003).However, the effect of soyabean lecithin (9ml) on improving the sperm movement features, acrosome integrity and plasma membrane integrity was greater than tris egg yolk semen extender. Soya milk effects during cryopreservation firstly, egg yolk semen extender supplied deprived welfare for sperm through the process of cryopreservation contrasting for bovine spermatozoa (Bathgate et al., 2006), so the defensive factors of soyabean lecithin semen extender on frozen sperm were higher than those of egg yolk semen extender.

Debris particles in diluents and higher viscosity were hypothetical as appreciated aspects erode the spermatozoa fertilizing capability (Vishwanath and Shannon, 2000; Van Wagtendonk-de Leeuwet al., 2000). Therefore, soyabean lecithin semen diluent might play a supporting role for sperm throughout process of semen cryopreservation because of low viscosity and less debris. The Third reason was an ideal concentration of soyabean lecithin semen extender could play a defensive role for sperm membrane throughout the thawing procedure. Also, the spermatozoa should be capable of swim more simply in freezing diluents containing soyabean lecithin extender than in other diluents, which would lead to the best sperm movement.

4. CONCLUSION

Supplementation of Soyabean Milk enhanced chilled and frozen thawed semen quality parameters in Tharparkar cattle bull.

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 3. Effect of combination of Tris and Soyabeen milk baes extenders on post thawed semen characteristics (Mean % ± SEM).

Sperm Parameters %	0	3	6	9	12	15
Motility	48.50±1.2	47.50±0.8	53.50±0.8	57.00±0.5	49.75±1.1	45.50±1.2
Morphology	65.00±1.5	63.75±2.5	64.75±2.5	73.50±1.5	65.00±3.4	61.00±1.5
Membrane Integrity	63.25±3.4	60.25±3.4	65.25±3.4	70.00±0.1	65.25±0.3	62.25±3.4
Live dead ratio	67.75±2.9	64.25±0.4	69.25±0.4	72.00±0.8	67.25±1.3	57.75±2.9

Table 4. Conception Rate

Groups	Animals in each group	No. of pregnant animals	%
A	10	04	40%[a]
B	10	06	60%[a]