www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 4270-4277

www.thepharmajournal.com
Received: 19-04-2022
Accepted: 22-05-2022

Nancy Jasrotia
Ph.D Scholar, Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

MK Tandle
KVAFSU, Bidar, Karnataka, India

Anju Kujur
Ph.D Scholar, Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

RG Bijurkar
KVAFSU, Bidar, Karnataka, India

Nagaraj
Department of AH&VS, Veterinary Officer, Karnataka, India

Ambika
Department of AH&VS, Veterinary Officer, Karnataka, India

MD Suranagi
KVAFSU, Bidar, Karnataka, India

Shrikant Kulkarni
KVAFSU, Bidar, Karnataka, India

Corresponding Author
Nancy Jasrotia
Ph.D Scholar, Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

CASA assessment of sperm motility parameters of NARI-Suwarna rams semen after supplementation of semen extenders with almond and olive oil

Nancy Jasrotia, MK Tandle, Anju Kujur, RG Bijurkar, Nagaraj, Ambika, MD Suranagi and Shrikant Kulkarni

Abstract
The study was carried out to evaluate the effect of supplementation of almond oil (AO) and olive oil (OO) in Skim milk (SM) and TRIS egg yolk (TEY) extenders, on sperm quality parameters of the NARI Suwarna ram semen refrigerated for 72 h. Semen collected from five mature NARI-Suwarna rams was pooled and diluted to a final concentration of 0.8 x 10^9 spermatozoa/ml in SM and TEY extenders supplemented with AO and OO at 0.25% v/v (SM, SMAO, SMOO, TEY, TEYAO, TEYOO). Semen was then stored at 5 °C and several sperm motility parameters were assessed by Computer assisted semen analysis (CASA) at 0, 24, 48 and 72h of storage. Randomized Block Design (RBD) was used to test the significant difference between extenders. Extenders of TEY (TEY, TEYAO and TEYOO) were found significantly (P<0.05) better as compared to SM, SMAO and SMOO. Addition of olive oil (0.25%) in TEY extender had beneficial effect in preserving rapid progressive sperm motility percentage better than in other extenders. Assessment of sperm motility parameters by CASA revealed TEY as better extender as compared to SM for preservation of refrigerated NARI Suwarna ram semen for 72 h.

Keywords: Almond oil, CASA, motility parameters, olive oil, skim milk, TRIS egg yolk

Introduction
NARI-Suwarna was created by a government-funded agriculture science group called the Nimbkar Agricultural Research Institute (NARI), Maharashtra having about 90% Deccani breed proportion or 60% Deccani and 30% Madgyal (and only 10% Garole breed) proportion and is capable of producing and raising twin lambs due to the presence of *Fec* B gene from the Garole breed of Sunderban, West Bengal.

It is well known that "a sire is half of the herd." Semen dilution and storage are widely used in artificial insemination (AI) programs. Preservation of semen is done either in a liquid unfrozen state at reduced temperature to depress sperm metabolism or in a frozen state (sub-zero temperature). Liquid storage of semen prevents damage associated with freezing and thereby ensures greater sperm viability. The success of artificial insemination is mainly dependent on the development of satisfactory semen extenders which performs the same function as the seminal plasma and preserves the fertility of spermatozoa for an appreciable period of time. Cold shock results in irreversible damage when semen is stored at low temperature. The cold shock effect could be minimized by using additives such as egg yolk, milk, Tris, glycerol and antioxidants in the semen extenders during slow cooling (Salamon and Maxwell, 2000) [38].

Many kinds of extenders like Tris - egg yolk, sodium citrate – egg yolk, whole and skim milk, coconut milk, lactose in addition to many other traditional extenders were used successfully in preserving rams semen (Hegedusova *et al*., 2012; Fukui *et al*., 2008) [15, 11]. Efforts to improve the preservation of ram semen are focused on the modification of extenders (Marti *et al*., 2003) [25], as well as on the addition of various components to maintain motility, fertilizing capacity and preserve sperm membrane integrity (Riha *et al*., 2006; Sarlos *et al*., 2002) [35, 39].

Antioxidants are important for limiting damaging oxidative reactions in sperm cells. Olive oil contains a large amount of natural antioxidants, which provide oxidative stability during storage (Boskou *et al*., 2005) [4]. Among the antioxidants present in this oil can mention the tocopherols, sterols, carotenoids and phenolic compounds, being the o-dihydroxy-phenolic a potent antioxidant (Lopez-Miranda *et al*., 2008) [23]. Visioli (1998) [45] stated that the protective effects of olive oil could be ascribed not only to its high oleic acid content but also to the antioxidant properties of its polyphenols. Because of their lipid solubility, olive oil can

permeate the plasma membrane of spermatozoa and suppress free radical damage (Hazim *et al*., 2012) [12]. Oil composition of almonds are monounsaturated oleic acid and omega-9 fatty acid, linoleic acid a polyunsaturated omega six essential fatty acid and palmitic acid a saturated fatty acid (Berry *et al*., 1992) [3]. *Prunus amygdalus* mainly increases the sperm motility and sperm contents in the epididymis and vas deferns without producing any spermatotoxic effects (Qureshi, 1989) [29]. The antioxidant activities of almond oil may be related to the presence of flavonoids and other phenolic compounds (Mazinani *et al*., 2012) [26].

Computer assisted sperm analysis (CASA) has been introduced to veterinary andrology (Rijsselaere *et al*., 2003; Verstegen *et al*., 2002) [36, 44] which assures objective semen assessment and allow calculation of several motility and velocity parameters, which characterize movement of individual sperm cells.

The perusal of literature revealed scanty information on the antioxidant supplementation in preservation of NARI Suwarna ram's semen. Thus, the present study was carried out to evaluate the effect of addition of almond and olive oil (0.25%) as antioxidants in Tris-egg yolk-citrate and Skim milk extenders for preservation of semen at refrigeration temperature at 0, 24, 48 and 72 h.

Materials and Method

The study was carried out in five rams of NARI Suwarna strain maintained from February 2018 to May 2018 under Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bidar. All rams were maintained under uniform conditions and reared under semi intensive housing system. The rams were kept in a single flock and routine deworming and vaccination were performed as per schedule. The rams were allowed free grazing of 7-8 h daily, fed concentrate at 200 g per day per head and provided *ad libitum* drinking water throughout the day.

All the extenders were prepared one day earlier of semen dilution and stored at refrigeration temperature and thawed to room temperature (37 ^{0}C) in a water bath at the time of semen dilution. All the ejaculates were diluted with six different extenders (1:60) at room temperature.

Table 1: Different semen extenders with abbreviations

Group No.	Semen extenders	Abbreviations
I	Tris- Egg yolk	TEY
II	Tris-Egg yolk- Olive oil 0.25%	TEYOO
III	Tris-Egg yolk-Almond oil 0.25%	TEYAO
IV	Skim milk	SM
V	Skim milk- Olive oil 0.25%	SMOO
VI	Skim milk - Almond oil 0.25%	SMAO

Tris-Egg Yolk Extender (TEY)

A stock solution containing Tris (2.4g), Fructose (1g) and Citric acid (1.4g) was prepared after adding each ingredient to the distilled water in a beaker. 15% (v/v) egg yolk was added to it and mixed properly by using magnetic stirrer for 3-5 min. Double distilled water was added to make the final volume 100ml.

Tris-Egg Yolk-Olive oil 0.25% Extender (TEYOO)

This was prepared by supplementing Tris-Egg Yolk Extender with 0.25% (v/v) olive oil.

Tris-Egg Yolk-Almond oil 0.25% Extender (TEYAO)

This was prepared by supplementing Tris-Egg Yolk Extender with 0.25% (v/v) almond oil.

Skim milk Extender (SM)

This was prepared out of skim milk powder (10g) and distilled water (80mL), heating to 95 ^{o}C for 10 min, and the cooled to room temperature before the addition of fructose (0.9g).

Skim milk-Olive oil 0.25% Extender (SMOO)

This was prepared by supplementing Skim milk Extender with 0.25% (v/v) olive oil.

Skim milk-Almond oil 0.25% Extender (SMAO)

This was prepared by supplementing Skim milk Extender with 0.25% (v/v) almond oil.

Antibiotics

Streptomycin sulphate (100mg) and crystalline Penicillin G (1 lakh IU) were added to all the above extenders and mixed thoroughly.

Semen collection and evaluation

Fifty ejaculates (10 ejaculates from each ram) were collected (weekly once basis) from five mature NARI Suwarna rams using artificial vagina method as per standard procedure. All the ejaculates were evaluated for various tests viz. volume, colour, mass activity, sperm motility and presence of foreign bodies as described below.

Volume

The volume of ejaculate was measured immediately after collection using a graduated semen collecting tube (0.1 mL accuracy) in millilitres (mL).

Colour

The colour of semen was assessed in the collecting tube immediately after collection by naked eyes. The semen samples with abnormal colour were discarded.

Mass activity

A drop of neat semen on a clean glass slide without cover slip was examined under low power (100X) phase contrast microscope and mass activity was scored in 1-5 scale (Rahman, 2014) [30] as shown below (Table 2).

Table 2: Scoring of mass activity of the semen samples as per the wave pattern of spermatozoa

Score	Mass activity
1	No motion
2	Free spermatozoa moving without forming any waves
3	Small and slow moving waves
4	Vigorous movement with moderately rapid waves and eddies
5	Dense, very rapidly moving waves and distinct eddies

Presence of foreign bodies

The neat semen was immediately examined with naked eyes for presence of faecal particles, dust, urine, Vaseline, etc and if noticed then such semen samples were discarded.

Sperm motility

It was assessed using a phase contrast microscope (400X magnification), with a warm stage maintained at 37 ^{0}C. A wet semen mount (diluted semen) was made using a small drop of semen placed directly on a microscope slide and covered by a cover slip (Loskutoff and Crichton, 2001) [24]. The sperm motility was scored from the table as shown below (Table 3)

Table 3: Score chart for assessing semen motility of the semen samples

Score	Semen motility
0	No movement
1	No forward progression (only head movement)
2	Slow forward sperm progression (usually with laboured head movement)
3	Fast forward sperm progression
4	Faster forward sperm progression
5	Very fast forward movement

Semen dilution and analysis by CASA

After noticing the fresh semen characteristics as mentioned above, the collected semen samples of five rams were pooled together, divided into six equal aliquots and diluted with six different semen extenders mentioned in Table1. The diluted semen samples were preserved at refrigeration temperature and analysed by Computer Assisted Semen Analysis system, model Biovis-CASA 2000 **(**Expert Vision labs Pvt. Ltd. Mumbai, India**)** at 0, 24, 48 and 72 hours of storage.

A drop of extender-diluted semen was taken on clean grease free glass slide and covered by a cover slip and was focused under phase contrast microscope of 100X magnification. Biovis CASA software was turned on and after fine adjustment clicked on option capture which captured around 60 frames / minute and automatically analyzed for sperm concentration and various motility parameters.

The data obtained was statistically analyzed by RBD using SAS-Statistics Version, SAS Inc., Cary, NC; 1996 software and mean as well as standard error was calculated as per standard statistical procedure.

Results

The present work was conducted to evaluate the effect of addition of almond and olive oil (0.25%) as antioxidants in Tris-egg yolk citrate and skim milk extender for preservation of semen at refrigeration temperature at 0, 24, 48 and 72 h.

The semen volume ranged from 0.89±0.01 to 1.14±0.04 mL and mass activity score ranged from 4.0±0.15 to 4.8±0.13. The semen samples were creamy white to creamy in colour and sperm motility ranged from 3.9±0.18 to 4.7±0.15 in NARI Suwarna strain of sheep (Table No.4)

Table 4: Characteristics of NARI Suwarna ram semen

Ram No.	Volume (mL)	Colour	Mass activity (Score 1-5)	Sperm motility (Score 1-5)
1	0.89±0.01	Creamy	4.0±0.15	3.9±0.18
2	0.93±0.01	Creamy	4.3±0.15	4.3±0.15
3	0.95±0.02	Creamy white	4.7±0.15	4.6±0.16
4	1.14±0.04	Creamy white	4.8±0.13	4.7±0.15
5	0.94±0.03	Creamy	4.4±0.16	4.3±0.15

Motile sperm percentage in semen diluted with various extenders at 0, 24, 48 and 72 hours of storage at refrigeration temperature by CASA

The motile sperm percentage varied from dilutor to dilutor with significant difference at 0, 24, 48 and 72 hours of preservation. The motile sperm percentage was higher in TEY extender than SM, SMAO, SMOO, TEYAO and TEYOO extenders at 0, 24, 48 and 72 hours of preservation at refrigeration temperature (Table No.5 and Figure 1).

The motile sperm percentage declined with increase in holding time in all extenders (p<0.05).TEY extender was superior in retaining motility up to 72 hours as compared to SM, SMAO, SMOO, TEYAO and TEYOO extenders. There was no effect of addition of antioxidants, almond and olive oil (0.25%) in the semen extenders on motile sperm percentage.

Table 5: Motile sperm percentage (Mean ± S.E) in semen diluted with various extenders at 0, 24, 48 and 72 h of storage at refrigeration temperature

Extenders	0 hour	24 hours	48 hours	72 hours
SM	90.19 $^{a\,p}$ ±0.40	76.17 $^{b\,p}$ ±1.09	62.11 $^{c\,p}$ ±1.02	49.28 $^{d\,p}$ ±1.54
SMAO	90.94 $^{a\,p}$ ±0.61	79.3 $^{b\,prs}$ ±1.37	69.41 $^{c\,q}$ ±2.10	58.09 $^{d\,q}$ ±1.69
SMOO	91.03 $^{a\,p}$ ±0.29	79.78 $^{b\,prs}$ ±1.20	70.30 $^{c\,qru}$ ±2.07	60.67 $^{d\,qr}$ ±1.93
TEY	92.78 $^{a\,p}$ ±0.49	86.26 $^{b\,qrs}$ ±0.38	79.21 $^{c\,s}$ ±1.04	72.91 $^{cd\,s}$ ±1.20
TEYAO	90.06 $^{a\,p}$ ±1.47	83.73 $^{a\,rs}$ ±0.58	76.81 $^{b\,st}$ ±1.31	68.9 $^{c\,st}$ ±1.25
TEYOO	91.43 $^{a\,p}$ ±0.28	83.72 $^{b\,s}$ ±0.79	76.45 $^{c\,su}$ ±1.17	71.22 $^{cd\,su}$ ±1.56

Note: SM: Skim Milk, SMAO: Skim Milk Almond Oil, SMOO: Skim Milk Olive Oil, TEY: Tris Egg Yolk, TEYAO: Tris Egg Yolk Almond Oil, TEYOO: Tris Egg Yolk Olive Oil

Means with different superscripts differ significantly at p<0.05

^{abcd} superscripts indicate the difference between time interval (columns) within extenders (rows)

^{pqrstu} superscripts indicate the difference between extenders (rows) within time interval (columns)

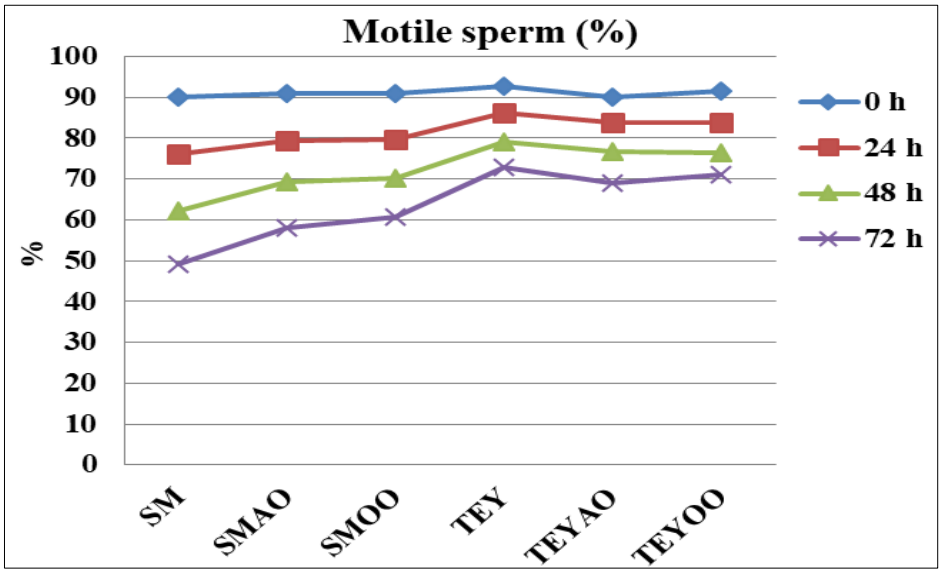

Fig 1: Motile sperm percentage in semen diluted with various extenders at 0, 24, 48 and 72 h of storage at refrigeration temperature

Rapid progressive sperm percentage in semen diluted with various extenders at 0, 24, 48 and 72 hours of storage at refrigeration temperature by CASA

The values for rapid progressive sperm percentage differed from dilutor to dilutor with significant difference at 0, 24, 48 and 72 hours of storage, however, differed non- significantly at 0 hour. The rapid progressive sperm percentage was significantly higher in TEYOO among all dilutors and lower in SM at 0 hour whereas higher in TEY and lower in SM at 72 hours of storage (Table No.6 and Figure 2).

The rapid progressive sperm percentage decreased with increase in storage time in all extenders. TEYOO was better in preserving rapid progressive sperm percentage at 0 and 48 hours, TEY at 24 and 72 hours of storage at refrigeration temperature. Addition of olive oil (0.25%) in TEY extender as an antioxidant had beneficial effect in preserving rapid progressive sperm percentage at 0 and 48 hours.

Table 6: Rapid progressive sperm percentage (Mean ± S.E) in semen diluted with various extenders at 0, 24, 48 and 72 h of storage at refrigeration temperature

Extenders	0 hour	24 hours	48 hours	72 hours
SM	63.01 $^{a\ p}$ ±2.11	46.28 $^{b\ p}$ ±2.53	28.88 $^{c\ p}$ ±3.35	20.02 $^{cd\ p}$ ±2.08
SMAO	63.90 $^{a\ p}$ ±3.20	45.60 $^{bcd\ pr}$ ±2.58	33.98 $^{cd\ pt}$ ±2.69	33.15 $^{d\ pt}$ ±2.24
SMOO	65.91 $^{a\ p}$ ±1.91	44.73 $^{bc\ pr}$ ±2.56	38.60 $^{c\ prst}$ ±3.31	31.39 $^{cd\ pu}$ ±1.92
TEY	66.33 $^{a\ p}$ ±2.71	62.33 $^{a\ q}$ ±2.94	47.87 $^{c\ qt}$ ±2.41	45.42 $^{bc\ qrst}$ ±3.00
TEYAO	64.67 $^{a\ p}$ ±3.18	61.48 $^{ab\ q}$ ±1.90	49.62 $^{bc\ qr}$ ±2.56	37.32 $^{c\ rstu}$ ±3.00
TEYOO	68.11 $^{a\ p}$ ±2.19	57.95 $^{ac\ pqr}$ ±2.88	51.48 $^{bc\ qs}$ ±3.20	44.45 $^{c\ stu}$ ±3.09

Means with different superscripts differ significantly at $p<0.05$

^{abcd} superscripts indicate the difference between time interval (columns) within extenders (rows)

^{pqrstu} superscripts indicate the difference between extenders (rows) within time interval (columns)

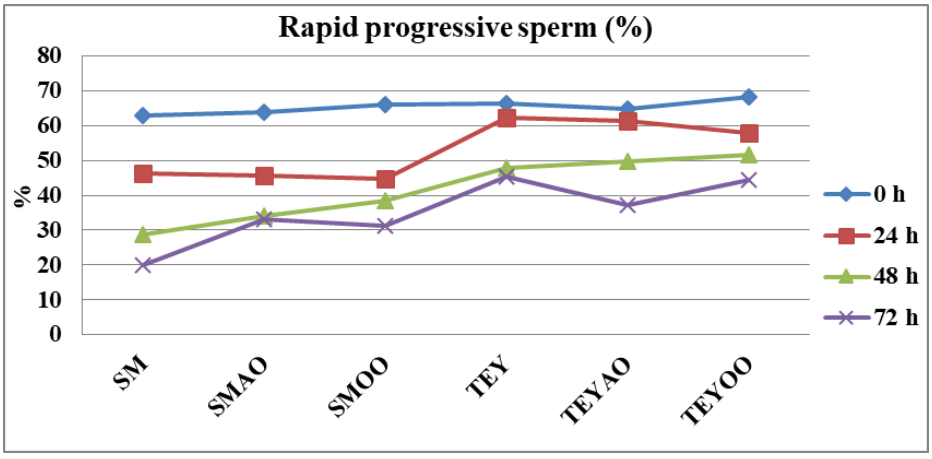

Fig2: Rapid progressive sperm percentage in semen diluted with various extenders at 0, 24, 48 and 72 h of storage at refrigeration temperature

Slow progressive sperm percentage in semen diluted with various extenders at 0, 24, 48 and 72 hours of storage at refrigeration temperature by CASA

The values for slow progressive sperm percentage differed from dilutor to dilutor at 0, 24, 48 and 72 hours of semen storage. The slow progressive sperm percentage was lower in SMOO at 0 hour, in TEYAO at 24 hours and in TEYOO at 48 and 72 hours of preservation (Table No.7 and Figure 3).

The slow progressive sperm percentage increased with increase in preservation time in all extenders. The decrease in slow progressive sperm percentage was more prominent in TEYOO at 0, 48 and 72 hours and in TEYAO at 24 hours of storage at refrigeration temperature. Addition of almond and olive oil (0.25%) in TEY extender had beneficial effect in reducing slow progressive sperm percentage.

Table 7: Slow progressive sperm percentage (Mean ± S.E) in semen diluted with various extenders at 0, 24, 48 and 72 h of storage at refrigeration temperature

Extenders	0 hour	24 hours	48 hours	72 hours
SM	16.81 $^{a\,p}$ ±1.58	21.52 $^{a\,p}$ ±2.95	26.64 $^{a\,p}$ ±2.84	24.33 $^{a\,p}$ ±1.84
SMAO	17.83 $^{a\,p}$ ±2.67	25.11 $^{a\,pq}$ ±2.03	27.51 $^{a\,p}$ ±1.66	19.70 $^{a\,p}$ ±1.61
SMOO	13.52 $^{a\,p}$ ±1.48	25.77 $^{b\,ps}$ ±1.76	23.44 $^{a\,p}$ ±2.08	23.37 $^{a\,p}$ ±1.87
TEY	14.63 $^{a\,p}$ ±2.61	14.20 $^{a\,pt}$ ±2.20	22.37 $^{a\,p}$ ±2.00	19.92 $^{a\,p}$ ±2.49
TEYAO	14.83 $^{a\,p}$ ±1.97	13.92 $^{a\,prt}$ ±1.46	18.67 $^{a\,p}$ ±1.88	24.42 $^{a\,p}$ ±2.49
TEYOO	12.83 $^{a\,p}$ ±1.68	15.74 $^{a\,p}$ ±1.81	17.18 $^{a\,p}$ ±2.42	18.79 $^{a\,p}$ ±1.88

Means with different superscripts differ significantly at $p<0.05$
^{abcd} superscripts indicate the difference between time interval (columns) within extenders (rows)
^{pqrstu} superscripts indicate the difference between extenders (rows) within time interval (columns)

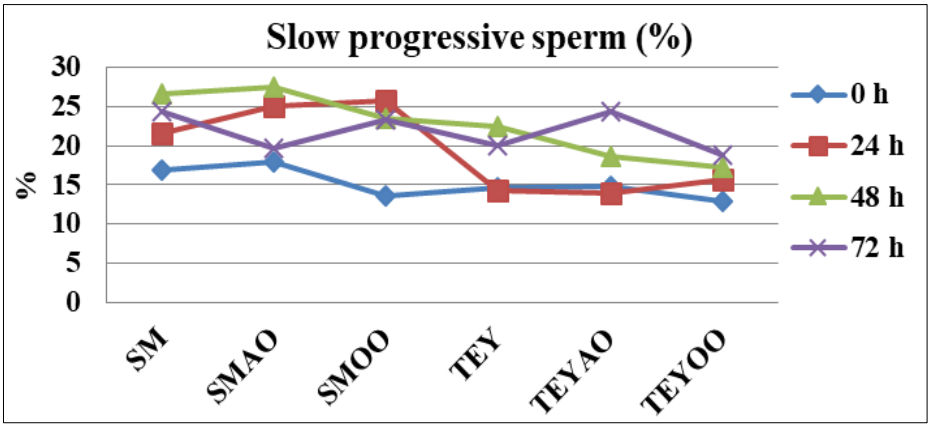

Fig 3: Slow progressive sperm percentage in semen diluted with various extenders at 0, 24, 48 and 72 h of storage at refrigeration temperature

Discussion

Evaluation of neat and diluted semen

The semen volume obtained in the present work is in accordance to that of the volume (0.80-1.4mL) reported by Chella *et al*., (2017) [6] in Zulu rams of South Africa, Kumar *et al*., (2007) [19] in Bharat Merino rams and Abbas *et al*., (2015) [1] in NARI Suwarna rams. However, Tejaswi *et al*., (2016) [42] reported lower semen volume ranging from 0.6 to 0.8ml in NARI Suvarna rams. The variation in ejaculate volume could be due to genotype differences, breed, seasonal variations (Olah, 2013) [27], frequency of semen collection (Jennings and McWeeny, 1976) [16], nutritional and health status of the animal.

The colour of semen varied from creamy to creamy white, which is in accordance with the earlier study of Tejaswi *et al*., (2016) [42]. The mass activity obtained is comparable to that of the mass activity (4.5-5) reported in Ghazal- Merinose rams by Soltanpour *et al*., (2014) [41] and in Malpura and Bharat Merino rams by Kumar *et al*., (2010) [20]. Lopez *et al*. (2000) [22] stated that the skim milk (M) diluent maintained a value of progressive motility (PM) ≥60% and motility score (MS) ≥ 3 up to 3 days of storage at 5 ^{0}C. Such variations may be attributed to nutritional and physical effects (Colas, 1981 and Toe *et al*., 1994) [7, 43], genetic and environmental changes (Gundogan *et. al.*, 2004) [13] and seasonal variation (Rege *et al*., 2000) [34] that can affect the semen quality.

Sperm motility parameter analysis by CASA

1. Motile sperm percentage

The motile sperm percentage varied from dilutor to dilutor with significant difference at 0, 24, 48 and 72 hours of preservation. The motile sperm percentage was higher in TEY extender than SM, SMAO, SMOO, TEYAO and TEYOO extenders at 0, 24, 48 and 72 hours of preservation at refrigeration temperature. The motile sperm percentage declined significantly with increase in holding time in all extenders ($p<0.05$).

Similar motility percentages were recorded by Rajashri *et al*. (2017) [32] when Deccani ram semen was stored in TCFEY extender at 5^{o}C for 48 h. In addition to this, Camara *et al*. (2011) [5] recorded that the mean percentage for total motility of 89.5 ± 1.4 in Santa Ines rams semen following dilution (0 h) in a Tris-egg yolk extender which is comparable to the present study.

The present findings were also in line with that of Gundogan (2009) [14] who reported that the spermatozoa motility could be maintained at greater rate than 50% for 4 days at 4 ^{o}C in the ram semen diluted with Tris-citrate-fructose-egg yolk extender. Salamon *et al*. (1979) [37] reported that the fertilizing ability is maintained for up to 10 days when ram semen is extended in Tris–fructose–egg yolk dilutor stored at refrigeration temperature. Lone *et al*. (2012) [21] concluded that Tris–citric acid–egg yolk–fructose (TCEYF) extender preserved sperm motility better than the other extenders, egg yolk–citrate (EYC) and egg yolk–citrate–fructose (EYCF) up to 72 h at 4 ^{o}C. The higher motility in TCFEY extender might be attributed to the better capability of the extender to support the spermatozoa passing through the various changes and physical stress during the time of dilution and preservation (Lone *et al*. 2012) [21].

However, El-Gaafary *et al*. (1987) [10] suggested that skim milk diluent was a good medium for preserving the fertilizing ability of ram spermatozoa during storage for three hours at 5 ^{0}C. Kaimal (2015) [18] also concluded that Skimmed Milk Powder Extender (SMPE) was better than Sodium Citrate Egg Yolk Extender (SCEYE) and Tris Egg Yolk Extender (TEYE) for preservation of semen of NARI Suwarna rams at refrigeration temperature for 72 hours.

Addition of almond and olive oil did not improve the motility parameters of TEYAO and TEYOO when compared to TEY extender whereas higher motility was recorded in SMAO and SMOO when compared to SM extender for 72 h of storage at refrigeration temperature. However, Hazim (2012) [12] stated that diluent supplementation with different levels of olive oil improved semen quality (motility, survival, and membrane and acrosome integrity) in aged roosters, when semen samples *in vitro* were stored at 5 °C for up to 72 h. Qureshi (1989) [29] stated that *Prunus amygdalus* mainly increases the sperm motility and sperm contents in the epididymis and vas deferns without producing any spermatotoxic effects.

2. Rapid progressive sperm percentage

The rapid progressive sperm percentage decreased with increase in storage time in all extenders. TEYOO was better in preserving rapid progressive sperm percentage at 0 and 48 hours, TEY at 24 and 72 hours of storage at refrigeration temperature. Addition of olive oil (0.25%) in TEY extender as an antioxidant had beneficial effect in preserving rapid progressive sperm percentage at 0 and 48 hours however had no beneficial effect at 24 and 72 hours of storage.

Similar results were reported by Deka and Rao, (1980) [8] who found that TRIS- egg yolk extender was superior in maintaining the progressive motility when the ram semen was stored at 4 °C. Joshi *et al*. (1999) [17] reported that the per cent rapidly motile spermatozoa for freshly diluted and for stored samples of Garole ram semen ejaculates were 83.2 % and 64.50%, respectively.

However, the present research findings were not in line with those of Ahangari *et al*. (2010) [2] who reported percentage of progressive motile spermatozoa as 75.5±1.65 for 15% of EY in Tris extender. El-Amiri *et al.* (2016) [9] also reported that ram semen diluted in skim milk extender showed higher progressive motility at 0 h, 8 h and 24 h (66.12%, 63.57% and 59.53%) compared with TRIS egg yolk extender at 0 h, 8 h and 24 h (54.06%, 35.69% and 34.06%). Lopez *et al*. (2000) [22] stated that the skim milk (M) diluent maintained a value of progressive motility (PM) ≥60% and motility score (MS) ≥ 3 up to 3 days of storage at 5^{0}C. Yaniz *et al*. (2011) [46] reported lower values of sperm progressive motility of 34.7, 28.5 and 27.8 per cent respectively for ram semen diluted in TRIS-based extender at 0 h, 24 and 48 h of storage at 15^{0}C using CASA.

3. Slow progressive sperm percentage

The values for slow progressive sperm percentage differed from dilutor to dilutor at 0, 24, 48 and 72 hours of semen storage. The slow progressive sperm percentage was lower in SMOO at 0 hour, in TEYAO at 24 hours and in TEYOO at 48 and 72 hours of preservation. The decrease in slow progressive sperm percentage was more prominent in TEYOO at 0, 48 and 72 hours and in TEYAO at 24 hours of storage at refrigeration temperature. Addition of almond and olive oil (0.25%) in TEY extender had beneficial effect in reducing slow progressive sperm percentage.

Silva *et al.* (2016) [40] also showed that olive oil at a concentration of 0.25% was found for the protection of boar semen against damage from freezing. These findings were in accordance with the findings of the present scenario.

Paulenz *et al*. (2002) [28], Gundogan (2009) [14], Rakha *et al*. (2013) [31] and Rather *et al*. (2017) [33] reported better spermatozoa motility and membrane integrity rates in a Tris-based extender than in the sodium citrate and skimmed milk extenders as was concluded in the present study.

Conclusion

The semen volume ranged from 0.89±0.01 to 1.14±0.04 mL and mass activity score ranged from 4.0±0.15 to 4.8±0.13. The semen samples were creamy white to creamy in colour and sperm motility ranged from 3.9±0.18 to 4.7±0.15 in NARI Suwarna strain of sheep. Extenders of TEY (TEY, TEYAO and TEYOO) were found significantly (P<0.05) better as compared to SM, SMAO and SMOO. There was no significant positive effect of addition of antioxidants; almond and olive oil (0.25%) in the semen extenders on motile sperm percentage and to reduce immotile sperm percentage however, addition of olive oil (0.25%) in TEY extender as an antioxidant had beneficial effect in preserving rapid progressive sperm percentage at 0 and 48 hours. Assessment of sperm motility parameters by CASA revealed TEY as better extender as compared to SM for preservation of refrigerated NARI Suwarna ram semen for 72 h.

References

1. Abbas Z, Sudha G, Krishnaswamy A, Honnappa TG, Ramesh PT. Effect of extenders on keeping quality of chilled ram semen. National symposium on current challenges and opportunities in animal reproduction, ISSAR Convention 2015, Bengaluru, 2015, 66p.
2. Ahangari YJ, Hamedani MA, Hassani SR. Effect of various concentrations of egg yolk on Dallagh Ram semen characteristics at 5 ºC. In Aust. Soc. Anim. Prod. 2010;28:88.
3. Berry EM, Eisenberg S, Friedlander Y, Harats D, Kaufmann NA, Norman Y, *et al*. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins the Jerusalem Nutrition Study. II Monounsaturated fatty acids vs carbohydrates. The American journal of clinical nutrition. 1992;56(2):394-403.
4. Boskou D, Blekas G, Tsimidou M. Phenolic compounds in olive oil and olives. Cur Topics Nut Res. 2005;3:125-136.
5. Câmara DR, Mello-Pinto MMC, Pinto LC, Brasil OO, Nunes JF, Guerra MMP. Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen. Small. Rumin. Res. 2011;100:44-49.
6. Chella L, Kunene N, Lehloenya K. A comparative study on the quality of semen from Zulu rams at various ages and during different seasons in KwaZulu-Natal, South Africa. Small. Rumin. Res. 2017;151:104-109.
7. Colas G. Variations saisonnierés de la qualité du sperme chez le belier Ile-de-France II. Fécondance: Relation avec les critéres qualitatifs observés *in vitro*. Reprod. Nutr. Develop. 1981;21:399-407.
8. Deka BC, Rao AR. Preservation of ram semen. Indian Veterinary Journal. 1980;57(2):130-134.
9. El-Amiri B, Ben Moula A, Badi A, El-Khalil K, Allai L, Gergatz E, *et al*. Could a extra long term boar semen

extender be successfully used during liquid storage of ram semen at 15 ºC. Anim. Reprod. 2016;13(3):568.
10. El-Gaafary MNSA, Chamberlain AG, Axford RFE. Survival rate and fertility of chilled ram semen supplemented with prostaglandin Fz, Anim. Breed. Abstr, 1987, 2196.
11. Fukui Y, Kohno H, Togari T, Hiwasa M, Okabe K. Fertility and artificial insemination using a soybean-based semen extender in sheep. Journal of Reproduction and Development. 2008;54:286-289.
12. Hazim-Al-Daraji HJ. Adding olive oil to rooster semen diluents for improving semen quality and storage ability during liquid storage. Baltic Journal of Comparative and Clinical System Biology. 2012;2:3-11.
13. Gundogan M, Yeni D, Ozenc E. Relationship between some reproductive parameters and biochemical properties of blood serum in Ram. Arch. Androl. 2004;50(6):387-390.
14. Gündoğan M. Short term preservation of ram semen with different extenders. Kafkas Univ. Vet. Fak. Derg. 2009;15:429-430.
15. Hegedűšova Z, Štolc L, Louda F, Čunat L, Vejnar J. Effect of different extenders on ram sperm traits during storage. Actauniv. agric. etsilvic. Mendel. Brun., LX6, 2012, 111-116.
16. Jennings JJ, Mcweeny. Effect of frequent ejaculation on semen characteristics in rams. Vet. Rec. 1976;98:230-233.
17. Joshi A, Bag S, Mittal JP. Assessment of semen characteristics of Garole rams. Int. J Anim. Sci. 1999;14:277-279.
18. Kaimal P. Studies on reproductive traits, semen preservation at refrigeration temperature and its evaluation in NARI Suwarna strain of sheep. MVSC Thesis. KVAFSU, Bidar, Karnataka, 2015.
19. Kumar D, Joshi A, Naqvi SMK, Kumar S, Mishra AK, Mauryaa VP, *et al*. Sperm motion characteristics of Garole × Malpura sheep evolved in a semi-arid tropical environment through introgression of Fec B gene. Anim. Reprod. Sci. 2007;100:51-60.
20. Kumar D, Joshi A, Naqvi SMK. Comparative semen evaluation of Malpura and Bharat Merino rams by computer-aided sperm analysis technique under semi-arid tropical environment. Int. J Anim. Veter. Adv. 2010;2(1):26-30.
21. Lone F, Islam R, Khan M, Sofi K. Effect of Different Egg Yolk-Based Extenders on the Quality of Ovine Cauda Epididymal Spermatozoa during Storage at 4 °C. Reproduction in Domestic Animals. 2012;47:257-262.
22. Lopez SA, Ortiz N, Gallego L, Garde JJ. Liquids storage (5 ºC) of ram semen in different diluents. Arch. Androl. 2000;44:155-164.
23. López MJ, Pérez-Jiménez F, Rose. Olive oil and health: Summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain). 2008;20(4):284-294.
24. Loskutoff NM, Crichton EG. Standard operating procedures for genome resource banking. The bill and Bernience-Grewcock Center for conservation and research omaha's zoo, 2001, 1-16.
25. Marti JI, Marti E, Cebrian-Perez JE, Muino-Blanco T. Survival rate of antioxidant enzyme activity of ram spermatozoa after dilution with different extenders or selection by a dextran swim-up procedure. Theriogenology. 2003;60:1013-1020.
26. Mazinani S, Hossain A, Rad E, Khaneghah AM. Determination and comparison the amount of tocopherols and phenolic compounds and fatty acids profile inedible nuts (pistachio, almond, and walnut) oil. Adv. Environ. Biol. 2012;6(5):1610-1619.
27. Olah J, Kusza S, Harangi S, Posta J, Kovacs A, Pecsi A, *et al*. Seasonal changes in scrotal circumference, the quantity and quality of ram semen in hungary. Archiv Tierzucht. 2013;56(10):102-108.
28. Paulenz H, Soderquist L, Perez-Pe R, Berg KA. Effect of different extenders and storage temperatures on sperm viability of liquid ram semen. Theriogenology. 2002;57:823-836.
29. Qureshi S, Shah AH, Tariq M, Ageel AM. Studies on herbal aphrodisiacs used in Arab system of medicine. Am. J Chin. Med. 1989;17(1-2):57-63.
30. Rahman Saidur. Preservation of indigenous ram semen with skim milk diluent. Bangladesh agricultural university, Thesis, 2014.
31. Rakha BA, Hussain I, Akhter S, Ullah N, Andrabi SM, Ansari MS. Evaluation of Tris–citric acid, skim milk and sodium citrate extenders for liquid storage of Punjab Urial (*Ovis vignei punjabiensis*) spermatozoa. Reproductive biology. 2013;13(3):238-242.
32. Rajashri M, Reddy KR, Kumari GA, Kumari NN, Srinivas G. Computer assisted semen analysis of deccani ram semen presevability at 5 ºC. Indian Journal of Animal Research, 2017.
33. Rather HA, Islam R, Malik AA, Lone FA, Banday MN. Effect of different extenders for preservation of ram semen at 4 °C. Indian J Anim. Res. 2017;51(6):982-987.
34. Rege JE, Toel FE, Mukasa-Mugerwa S, Tembley D, Amido RL Baker, Lahlou-Kassi A. Reproductive characteristics of Ethiopian highland sheep. II. Genetic parameters of semen characteristics and their relationships with testicular measurements in ram lambs. Small Rumin. Res. 2000;37(3):173-187.
35. Riha L, Apolen D, Pivko J, Grafenau P, Kubovicova E. Influence of implementors on sheep fertility out of season. Slovak J Anim. Sci. 2006;4:180-182.
36. Rijsselaere T, Van Soom A, Maes D, De Kruif A. Effect of technical settings on canine semen motility parameters measured by the Hamilton-Thorne analyzer. Theriogenology. 2003;60:1553-1568.
37. Salamon S, Maxwell WMC, Firth JH. Fertility of ram semen after storage at 5 °C. Anim Reprod Sci. 1979;2:373-385.
38. Salamon S, Maxwell WMC. Storage of ram semen. Anim. Reprod. Sci. 2000;62:77-111.
39. Sarlos P, Molnar A, Kokai A, Gabor G, Ratky J. Evaluation of the effect of antioxidants in the conservation of ram semen. Acta Vet. Hung. 2002;50(2):235-245.
40. Silva EF, Figueiredo-Cardoso T, Goulart-Dutra FL, Varela Junior AS, Leivas Leite FP, Dahl-Corcini C. Olive oil as an alternative to boar semen cryopreservation. CES Medicina Veterinary Zootecnia. 2016;11(1):8-14.
41. Soltanpour F, Moghaddam G, Asadpour R, Rafat SA. Effect of Antioxidant combinations on sperm quality of cross breed rams during liquid storage. International J Adv. Biol. Biomed. Res. 2014;2(3):732-740.
42. Tejaswi V, Swamy MN, Yathiraj S, Honnappa TG, Isloor S. Enzymatic Activities in Fresh Seminal Plasma and

Extended Refrigerated Semen in Nari Suwarna Rams. Theriogenology Insight. 2016;6(1):27.
43. Toe F, Lahlou-Kassi A, Mukasa-Mugerwa E. Semen characteristics of Ile-de-France rams of different age and physical condition. Theriogenology. 1994;42(2):321-326.
44. Verstegen J, Iguer-Ouada M, Onclin K. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology. 2002;57:149-179.
45. Visioli F, Galli C. The effect of minor constituents of olive oil on cardiovascular disease: new findings. Nutr Rev. 1998;56:142-147.
46. Yaniz JL, Mateos JA, Santolaria P. Zwitter ionic buffers preserve ram semen quality more efficiently than TRIS during storage at 15 ºC. Small Rum Res. 2011;95:54-60.