Asparagus racemosus aqueous extract improves seminal antioxidant status and sperm characteristics in buck semen at refrigeration temperature

chetna gangwar (chetnaom82@gmail.com)
ICAR-CIRG https://orcid.org/0000-0003-0826-0811
Ashok Kumar
Central Institute for Research on Goats
Anil Kumar Mishra
Central Institute for Research on Goats
S.D. Kharche
Central Institute for Research on Goats
Kamendra Swaroop
Central Institute for Research on Goats
R Pourouchttmane
Central Institute for Research on Goats
Rakesh Goel
Central Institute for Research on Goats
Shriprakash Singh
DUVASU: Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan

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Abstract

During semen preservation, with the passage of time, there is decrease in fertilization rate or conception rate. The decrease occurs due to the gradual deterioration of semen quality. Hence, the investigation was planned to evaluate the effect of *Asparagus racemosus* aqueous extract on buck semen quality during preservation. In the current study, 8 ejaculates from 8 Jakhrana bucks were collected (total 64 ejaculates). Good quality semen samples were pooled during each collection. Pooled semen samples were then divided into 4 equal parts, and diluted in TRIS buffer containing different concentration of Shatavari aqueous extract (Different groups, i.e., G I -500 mg, G II-250 mg, G III-125 mg, G IV-0 mg of Shatavari aqueous extract in 100 ml TRIS Buffer). All the diluted semen samples were kept at refrigerated temperature (5ºC) for seven days, and on each day diluted semen was evaluated for various sperm characteristics and antioxidant status. Shatavari aqueous extract exhibited significant ($P < 0.05$) enhancement in sperm viability, sperm motility, acrosomal integrity and plasma membrane integrity, whereas it reduced the sperm abnormality. G III (125mg Shatavari extract/100ml TRIS buffer) showed significantly ($P < 0.05$) better results in terms of sperm viability, sperm motility, acrosomal integrity and plasma membrane integrity. Along with this the longevity of sperm was also enhanced in Shatavari supplemented group.

1. Introduction

Artificial insemination (AI) is most widely used mean for fast dispersal of elite animal genetics. AI technique is a more successful technique than embryo transfer, and is economical as well as simpler too. Semen preservation is the indispensable step in AI, which prolongs the keeping duration and maintains sperm fertility, eases worldwide transfer of preserved semen, and ameliorate the reproducing potential of elite male animals (Wen et al., 2019). Either chilled or frozen semen, can be employed for AI however, liquid preservation leads to better pregnancy rates than frozen one (Maxwell and Salamon, 1993). Further, as duration of preservation increases, reduction in the conception rate occurs. The reduction occurs due to the regular downturn of semen quality parameters such as sperm motility, mitochondrial activity, acrosome and plasma membrane integrity (o'Hara et al., 2010). Oxidative stress is considered as the important reason of the deterioration in sperm quality on account of the generation of reactive oxygen species (ROS) at the time of semen preservation.

During semen preservation at refrigerated temperature, antioxidant in semen is exhausted and at the same time ROS is spontaneously formed by sperm aerobic metabolism (Gangwar et al., 2018) resulting in disparity between antioxidant and pro-oxidant activities, which eventually causes the lipid peroxidation of the polyunsaturated fatty acids of the sperm membrane. Lipid peroxidation causes irreversible damage to sperm, and makes the sperm sub fertile or infertile. This curtails the efficient use of semen cryopreservation or preservation technology. There is an obligatory requirement to optimize the buck semen extender to attain the better semen quality with higher fertility. Earlier in studies, various chemical antioxidants (Vit C (Gangwar et al 2015), Vit E, membrane chelating agent (Gangwar et al 2014), membrane stabilizer, enzymes like reduced glutathione (Gangwar et al 2018)) have been added in semen

diluter but such antioxidants sometimes disturbs the osmolarity of the diluter. The chemical antioxidants provide benefit in one way and may be harmful in another way (may stimulate inflammatory/immune reaction in females during artificial insemination. However, herbal antioxidants are not having such deleterious effects and this problem can be addressed by using the good quality antioxidant which is of natural (not chemical) origin.

Plants and their derivatives have constantly been subject of investigation for the research personnel, especially for the cure of numerous diseases, and in the invention of novel therapeutic medicines modalities. In the current scenario, it is capturing popularity as a novel way of therapy globally (Kuo et al., 2018; Mills et al., 2005). *Asparagus racemosus* (*A. racemosus*) is one such important medicinal plant having wide range of pharmacological and therapeutic effects, and it is called as a '*rasayana*' (plant drugs that enhances overall health by increasing cellular vitality and resistance) in the Ayurveda (Goyal et al., 2003). Shatavari is regarded as "100 spouses", signifying its potential to improve fertility and vitality, effective in treating reproductive impotency (Sharma et al., 2003; Thakur et al., 2009; Alok et al., 2013). *A. racemosus* is a recognized "Ayurvedic Rasayana", that retards senility, increases life expectancy, boost immunogenic protection, enhance mental health as well as vigor, and adds liveliness vitality to the body (Frawley, 1997; Gogte, 2000). Hence, after going through the available literature on the beneficial effects and potent antioxidant property of Shatavari (Kamat et al., 2000; Wiboonpun et al., 2004), the current research work was designed with the following objectives: 1. Effect of Shatawari aqueous extract on buck semen preservation and 2. Effect of Shatawari aqueous extract on antioxidant potential of seminal plasma. We first time examined the effect of Shatawari aqueous extract supplementation in buck semen diluter at refrigerated temperature.

2. Material And Methods

2.1. Method of aqueous extract preparation of Shatavari

A. racemosus is also called as Shatavari, Satawar or Shatuli, and comes under the family Asparagaceae. *A. racemosus* roots were purchased from the local market. *A. racemosus* aqueous extract was prepared from *A. racemosus* roots in the Veterinary Medicine Laboratory of the Institute (ICAR-CIRG, Makhdoom). Dried root powder was soaked in distilled water for 5 h at 80º C. The sediment was separated by filtration and the filtrate was concentrated by lyophilisation. The obtained aqueous extract of *A. racemosus* was processed with a small pulverizer, and kept at 25ºC for further use.

2.2. Semen Collection

The collection of semen was done as per the guidelines laid down by the Institute Animal Ethics Committee. In the present experiment, 8 Jakhrana bucks were used and the bucks were of 2 to 4.5 years of age, and weighing 35 ± 1.8Kg. From each buck, 8 ejaculates were collected. Semen was collected twice at weekly intervals with the help of artificial vagina in the morning hours.

2.3. Experimental design

The semen was diluted with tris–citric acid fructose diluents (tris, 3.604 g; citric acid, 1.65 g; fructose, 1 g; streptomycin, 100 mg; penicillin, 100,000 IU; triple distilled water, 100 ml; pH, 6.8–6.9) having 6% (v/v) glycerol as cryoprotective agent and 10% (v/v) egg yolk (Gangwar et al., 2014). The various concentration of Shatavari aqueous extract were added in the above extender (Different groups i.e. G-I -500mg, G-II-250mg, G-III-125mg, G-IV- 0 mg of Shatavari aqueous extract in 100 ml TRIS Buffer). The semen samples were diluted to maintain sperm concentration approximately 100–120 million/straw. All the diluted semen samples were kept at refrigerated temperature (5ºC) for seven days, and on each day, the semen samples were evaluated for various sperm characteristics and antioxidant status.

2.4. Semen Evaluation

The semen samples were placed in water bath at 37ºC till the primary evaluation. The semen samples were evaluated for volume, pH, colour, consistency, concentration and mass motility just after the collection. Quantity of each ejaculate was measured with the help of the graduated collection cups. Mass motility of the sperms in fresh semen was estimated at low power magnification (10X) using a compound microscope on microscopic thermo-stage maintained at 37ºC. The semen samples showing mass motility more than 3 were pooled, and diluted for further experimental study.

2.4.1. Effect of various concentration of Shatavari aqueous extract on sperm motility

A drop (10 µl) of extended semen was put on a clean pre-warmed slide (37°C) and covered with cover slip, and evaluated under 40× objective of microscope for observing the individual sperm motility. The mean of the values taken by the two experts was considered for calculating the progressive motility as reported by Gangwar et al. (2014).

2.4.2. Effect on sperm viability

Live sperm percentage was evaluated following the procedure mentioned by Bloom (1950). For sperm viability evaluation a drop (10 µl) of diluted semen was mixed in 2–3 drops of eosin-nigrosin stain, and tongue shaped smear was made on clean slide. Then slide was examined under the high power (100 X) lens of phase-contrast microscope with oil immersion. The unstained sperms were considered as live ones, whereas the sperms with pink or partial pink heads were classified as dead. Total 200 sperms were counted in various fields of the slide.

2.4.3. Effect on sperm acrosomal integrity

Giemsa stain was taken to evaluate the acrosomal integrity of buck spermatozoa as per the method described by Watson (1975). An amount of 10 µl of diluted semen was placed on clean slide, and thin smear was made. When the slide became dry, the smear was fixed in Hancock's fixative (Hancock, 1951) for 20 min inside BOD incubator and then, the fixed slide was kept for air dried after washing with slow flowing tap water. After that, the dried slides were placed in Giemsa working solution for 90 min. Extra stain was discarded with the slow flow of water. The slide was air dried, and examined under 100x oil

immersion objective of phase contrast microscope. Around 200 sperms were assessed in various fields of every slide.

2.4.4. Effect on sperm plasma membrane

The hypo-osmotic swelling test (HOST) was done to evaluate the integrity of the sperm plasma membrane. The sperm membrane integrity was examined as per the protocol used by Jayendran et al. (1984). For this 100 µl of the diluted semen sample from each treatment groups were incubated with 1 ml hypo-osmotic solution (100 mOsm) at 37°C for 1h. 10 µl of the incubated semen was examined with coverslip under phase contrast microscope for different types of curling of tails. Total 200 spermatozoa were enumerated in at least five different microscopic fields.

2.4.5. Effect on sperm abnormality

A fine dry smear of the sperm suspension was made on a clean, grease free glass slide at each day, respectively. Then slides were air-dried and fixed in 75% ethanol for 2 min before washing with gentle flowing tap water for 15–20 s. Then slides were stained in hematoxylin for 1 min as mentioned by Nayak et al. (2016). At least 200 spermatozoa per experimental group were examined under 100× oil-immersion objective of the light microscope.

2.5. Effect on Lipid peroxidation

Lipid peroxidation in sperm plasma membrane was indirectly measured by MDA (malondialdehyde) production assay as mentioned by Arangasamy et al. (2018) with certain changes. The stock solution containing equal volumes of trichloroacetic acid and 2-thiobarbituricacid was mixed with one volume of the semen sample in a capped glass tube, mixed properly, and heated for 20 min in a boiling water bath. After cooling, the supernatant was removed by centrifugation at 1000g for 10 min, and absorbance of supernatant was measured at 550 nm against blank using biophotometer. MDA levels were estimated at day 1 to day 7 of preservation, respectively, and denoted as nmol/dl.

2.6. Statistical Analysis

Data was given as mean ± S.E., and the statistical analysis was done by SPSS Software, IBM (SPSS Inc., Chicago, IL, USA). The differences in mean values were estimated assessed using Duncan’s multiple range tests and one-way analysis of variance (ANOVA). The semen ejaculates were considered as experimental units. The difference between means was significant at 95% level of significance ($P < 0.05$).

3. Results

3.1. Effect on sperm motility

After first day of preservation, all the experimental groups were having similar total sperm motility, whereas on the subsequent days, total sperm motility was significantly ($P < 0.05$) higher in Shatavari extract supplemented groups in comparison with the control group. However, significantly ($P < 0.05$)

enhanced sperm motility was observed in group III containing 125 mg of Shatavari aqueous extract per 100 ml of diluter. Thus, we can say the buck spermatozoa are more motile for longer duration in this group. Hence, it was concluded that Shatavari helped in maintaining the sperm motility for longer duration (Table 1).

Table 1

Total sperm motility percentage (Mean ± SEM) for different concentrations of Shatavari aqueous extract at different time intervals of buck semen preservation.

Days	Group I	Group II	Group III	Group IV
Day 1	89 ± 1.87	89 ± 1.87	90 ± 1.58	89 ± 1.87
Day 2	84 ± 1.01 ^{a}	88 ± 1.22 ^{a}	88 ± 1.22 ^{a}	79 ± 2.45 ^{b}
Day 3	72 ± 2.24 ^{b}	76 ± 2.24 ^{ab}	78.2 ± 1.22 ^{a}	64 ± 3.02 ^{c}
Day 4	60 ± 3.16 ^{ab}	67 ± 4.06 ^{a}	70 ± 3.16 ^{a}	52 ± 2.06 ^{b}
Day 5	38 ± 3.74 ^{bc}	47 ± 3.03 ^{ab}	51 ± 3.32 ^{a}	33 ± 3.05 ^{c}
Day 6	18 ± 2.06 ^{b}	28 ± 2.04 ^{a}	32 ± 2.07 ^{a}	12 ± 2.05 ^{c}
Day 7	7 ± 2.06 ^{bc}	13 ± 3.10 ^{ab}	18 ± 3.74 ^{a}	2.5 ± 2.10 ^{c}
Within the same preservation time, meanvalues with different superscripts differ significantly ($P < 0.05$) among treatment groups.				

3.2. Effect on sperm viability

Similarly, after first day of preservation, all the experimental groups showed similar sperm viability, whereas on the subsequent days, sperm viability percentage was significantly ($P < 0.05$) higher in Shatavari extract supplemented groups in comparison with the control group. However, significantly ($P < 0.05$) enhanced sperm viability was observed in group III during the whole -entire experimental period. Thus, we can say the buck spermatozoa are viable in more number for longer duration in this group. Hence, Shatavari helped in maintaining the sperm viability for longer duration (Table 2).

Table 2

Total sperm Viability percentage (Mean ± SEM) for different concentrations of Shatavari aqueous extract at different time intervals of buck semen preservation.

Days	Group I	Group II	Group III	Group IV
Day 1	92 ± 0.63	93.2 ± 0.73	91.8 ± 0.73	90.6 ± 0.81
Day 2	87 ± 0.32 ^{b}	90.8 ± 0.37 ^{a}	92.6 ± 0.40 ^{a}	83.6 ± 1.12 ^{c}
Day 3	76.4 ± 1.36 ^{b}	81.4 ± 1.29 ^{a}	84.6 ± 0.81^{a}	72 ± 2.50 ^{c}
Day 4	64.4 ± 2.77 ^{bc}	71 ± 3.21 ^{ab}	75.8 ± 2.63 ^{a}	57 ± 2.05 ^{c}
Day 5	42.8 ± 3.12 ^{bc}	50.8 ± 2.92 ^{ab}	56 ± 3.22 ^{a}	36.6 ± 3.61 ^{c}
Day 6	22.6 ± 2.23 ^{b}	32.8 ± 1.74 ^{a}	37.2 ± 1.66 a	15.4 ± 1.66 ^{c}
Day 7	11 ± 2.68 ^{bc}	16.8 ± 3.20 ^{ab}	22.2 ± 3.35 ^{a}	4.4 ± 2.23 ^{c}
Values with different superscripts (^{a,b,c}) differ significantly (P < 0.05) among treatment groups				

3.3. Effect on sperm acrosomal integrity

Likewise, after first day of preservation, all the experimental groups showed similar sperm acrosomal integrity, whereas on the subsequent days, sperm acrosomal integrity was significantly (P < 0.05) higher in Shatavari extract supplemented groups in comparison with the control group. However, significantly (P < 0.05) enhanced higher sperm acrosomal integrity was observed in group III during the entire experimental period and the buck spermatozoa with intact acrosome are more in number for longer duration in this group. Hence Shatavari helped in safeguarding the sperm acrosome, or minimize the early acrosome reaction (Table 3).

Table 3

Sperm acrosomal integrity percentage (Mean ± SEM) for different concentrations of Shatavari aqueous extract at different time intervals of buck semen preservation.

Days	Group I	Group II	Group III	Group IV
Day 1	87.4 ± 0.60	87.6 ± 1.02	88.2 ± 0.86	87.4 ± 1.08
Day 2	84.2 ± 0.86 ^{bc}	85.8 ± 0.86 ^{ab}	86.8 ± 0.49 ^{a}	83 ± 0.71 ^{c}
Day 3	79.2 ± 0.58 ^{c}	81.6 ± 0.75 ^{b}	84.6 ± 0.40 a	76.4 ± 0.50 ^{d}
Day 4	72.6 ± 1.66 ^{bc}	77 ± 1.34 ^{ab}	79.8 ± 1.32 ^{a}	69 ± 1.58 ^{c}
Day 5	65 ± 2.39 ^{bc}	71 ± 1.95 ^{ab}	75.4 ± 1.43 ^{a}	60.6 ± 2.38 ^{c}
Day 6	56.4 ± 3.78 ^{bc}	64.4 ± 2.42 ab	69.4 ± 1.63 ^{a}	51.8 ± 3.26 ^{c}
Day 7	49.4 ± 4.28 ^{bc}	55.4 ± 2.87 ^{ab}	61.4 ± 2.16 ^{a}	42.6 ± 4.83 ^{c}
Values with different superscripts differ significantly (P < 0.05) among treatment groups				

3.4. Effect on sperm plasma membrane integrity

On the day one of preservation, all the experimental groups showed similar sperm plasma membrane integrity, whereas on the subsequent days, sperm plasma membrane integrity was significantly (P < 0.05) enhanced in Shatavari extract supplemented groups in comparison with the control group. However, significantly (P < 0.05) enhanced sperm plasma membrane integrity was observed in group III during the entire experimental period. Thus, it was concluded that the buck spermatozoa were protected in more number for longer duration in this group. Hence Shatavari helped in protecting the sperm plasma membrane for longer duration (Table 4).

Table 4

HOST positive spermatozoa percentage (Mean ± SEM) for different concentrations of Shatavari aqueous extract at different time intervals of buck semen preservation.

Days	Group I	Group II	Group III	Group IV
Day 1	84.2 ± 2.08	83.8 ± 2.01	84.4 ± 1.6	83.8 ± 2.39
Day 2	78.4 ± 1.57 ^{bc}	82 ± 1.70 ^{ab}	84.6 ± 1.6 ^{a}	74.6 ± 1.72 ^{c}
Day 3	70.8 ± 1.36 ^{b}	74.4 ± 0.68 ^{a}	77.4 ± 0.68 ^{a}	65.6 ± 1.69 ^{c}
Day 4	55.8 ± 2.69 ^{bc}	61.4 ± 2.94 ^{ab}	65.2 ± 2.87 ^{a}	48.6 ± 2.54 ^{c}
Day 5	37.8 ± 3.56 ^{bc}	44 ± 2.66 ^{ab}	48.4 ± 2.09 ^{a}	30.8 ± 2.52 ^{c}
Day 6	18.8 ± 2.73 ^{b}	29.2 ± 2.01 ^{a}	34 ± 1.38 ^{a}	12 ± 1.92 ^{c}
Day 7	8.1 ± 2.70 ^{ab}	13.6 ± 3.66 ^{a}	17.6 ± 4.30 ^{a}	3.2 ± 1.96 ^{b}
Values with different superscripts differ significantly (P < 0.05) among treatment groups				

3.5. Effect on sperm abnormality

All the experimental groups showed similar sperm abnormalities for three days, whereas on the subsequent days, sperm abnormalities were significantly (P < 0.05) lower in Shatavari extract supplemented groups in comparison with the control group. However, significantly (P < 0.05) lower sperm abnormalities were observed in group II and group III during the entire experimental period. Thus, we can say the buck spermatozoa are protected in more number for longer duration in these groups (Table 5).

Table 5

Sperm abnormality percentage (Mean ± SEM) for different concentrations of Shatavari aqueous extract at different time intervals of buck semen preservation.

Days	Group I	Group II	Group III	Group IV
Day 1	6.4 ± 0.68	6.8 ± 0.95	6.4 ± 1.03	6.6 ± 0.51
Day 2	7.6 ± 0.75	7 ± 0.77	6.8 ± 0.8	8.2 ± 0.97
Day 3	8.8 ± 0.8	7.2 ± 0.86	7.2 ± 0.77	9.6 ± 0.93
Day 4	9.8 ± 0.86 ^{ab}	8.2 ± 0.84 ^{b}	7.6 ± 0.81^{b}	11 ± 0.95 ^{a}
Day 5	10.6 ± 0.98 ^{ab}	8.8 ± 0.86 ^{b}	8.8 ± 0.73 ^{b}	12.6 ± 1.36 ^{a}
Day 6	11.6 ± 0.98 ^{a}	9.8 ± 0.73 ^{b}	9.2 ± 0.77 ^{b}	13.6 ± 0.98 ^{a}
Day 7	12.4 ± 0.75 ^{a}	10.2 ± 0.84 ^{b}	9.8 ± 0.58 ^{b}	14.4 ± 0.6 ^{a}
Values with different superscripts differ significantly (P < 0.05) among treatment groups				

3.6. Effect on Lipid peroxidation (MDA estimation)

All the experimental groups showed similar MDA production for two days, whereas on the subsequent days, lipid peroxidation was significantly ($P < 0.05$) lower in Shatavari extract supplemented groups in comparison with the control group. However, significantly ($P < 0.05$) higher MDA production was observed in control group during the entire experimental period, while significantly ($P < 0.05$) low peroxidative damages occurred in Shatavari supplemented groups and buck spermatozoa were protected in more number for longer duration in these groups (Table 6).

Table 6

MDA concentration (Mean ± SEM) for different concentrations of Shatavari aqueous extract at different time intervals of buck semen preservation.

Days	Group I	Group II	Group III	Group IV
Day 1	2.64 ± 0.33	2.55 ± 0.35	2.61 ± 0.35	2.73 ± 0.35
Day 2	3 ± 0.32	2.98 ± 0.29	2.83 ± 0.28	3.56 ± 0.30
Day 3	3.20 ± 0.38^{b}	3.05 ± 0.37^{b}	3.01 ± 0.40^{b}	4.89 ± 0.32^{a}
Day 4	3.60 ± 0.32^{b}	3.47 ± 0.28^{b}	3.27 ± 0.32^{b}	5.24 ± 0.21^{a}
Day 5	3.92 ± 0.30^{b}	3.74 ± 0.32^{b}	3.67 ± 0.36^{b}	5.47 ± 0.33^{a}
Day 6	4.4 ± 0.18^{b}	4.13 ± 0.25^{b}	4.06 ± 0.28^{b}	6.24 ± 0.23^{a}
Day 7	4.67 ± 0.22^{b}	4.42 ± 0.18^{b}	4.25 ± 0.21^{b}	6.35 ± 0.21^{a}
Within the same incubation time, values with different superscripts differ significantly ($P < 0.05$) among treatment groups				

Table 7

Correlation among the different sperm parameters and MDA level in different treatment groups of buck semen.

	Sperm Motility	Sperm Viability	Acrosomal Integrity	HOST	Sperm Abnormality	MDA
Sperm Motility	1	0.996	0.938	0.99	-0.702	-0.762
Sperm Viability	0.996	1	0.945	0.992	-0.694	-0.75
Acrosomal Integrity	0.938	0.945	1	0.947	-0.744	-0.745
HOST	0.99	0.992	0.947	1	-0.706	-0.778
Sperm Abnormality	-0.702	-0.694	-0.744	-0.706	1	0.753
MDA	-0.762	-0.75	-0.745	-0.778	0.753	1

4. Discussion

Considerable success has been achieved in semen preservation of large animals over the past few decades; however, this is not yet much achieved in small ruminants. The decline, which occurs in semen quality during preservation, has not been solved out. Antecedent investigations have investigated that the inclusion of antioxidants can drastically enhance the semen quality by minimizing the ROS production (Maxwell and Salamon 1993). Earlier, various studies reported that addition of antioxidants in semen diluter improves the semen quality (Gangwar et al 2014, Gangwar et al 2015, Gangwar et al 2018). But now a days people are trying natural antioxidants in semen extenders (Wen et al 2019) and extract from Asparagus racemosus (Shatavari) roots have been used in many investigations as antioxidants (Kamat et al. 2000; Goyal et al., 2003). However, there is no evident research findings with respect to the protective effect of Shatavari aqueous extract on buck sperm as well as inhibition of lipid peroxidation. This is the first research work carried out to examine the semen quality and antioxidant activity in the bucks after supplementation of Shatavari aqueous extract in buck semen diluter.

In the present investigation study, herbal additives significantly improved the progressive motility, sperm viability, normal sperm morphology, acrosomal integrity and plasma membrane integrity. Moreover, we found reduced lipid peroxidation of sperm plasma membrane reflected by MDA level in Shatavari extract supplemented group. Though, this type of investigation has not been done or performed with herbal antioxidant supplementation in buck semen diluter in comparison with our results. Very little amount of data is present with regard to the specific use of herbs in laboratory animals, boar and human beings (Kopalli et al., 2015; Kumar et al. 2018; Ansari and Khan, 2017). Thakur et al. (2009) reported that *Asparagus racemosus*, *Chlorophytum borivilianum*, and rhizomes of *Curculigo orchioides* improved the mating behavior and aphrodisiac activity in male rats. During semen cryopreservation, there is imbalance between antioxidant and pro-oxidant activities, with the excess production of ROS and oxidation of polyunsaturated fatty acids in buck sperm membranes leading to formation of lipid peroxides, and ultimately formation of cytotoxic metabolic products, especially MDA (Zhao et al., 2009). In the current investigation, based on the negative correlation between sperm characteristics and MDA, however it is

positively correlated with sperm abnormalities (Table 7), hence it was concluded that Shatavari root extract adequately superoxide anions, and hindered lipid peroxidation.

Similar to our findings, Wen et al. (2019) reported that sperm motility, acrosomal integrity, mitochondrial membrane potential, plasma membrane integrity, total antioxidative activity in the 30 mg/L GSPE (Grape seed procyanidin extract) group was significantly enhanced, whereas MDA content was lower than as compared to the control group ($P < 0.05$). They also reported that AI results with GPSE supplemented semen showed higher litter size as compared to the control group ($P < 0.05$). Similarly, Zhao et al. (2009) reported the significant correlation between *Rhodiola sacra* aqueous extract and concentrations of GSH and MDA in frozen-thawed boar semen. As we know, that oxidative stress is a well-documented inducer of apoptosis and lipid peroxidation can initiate aging and a diminished longevity of the cryopreserved spermatozoa.

The results of the current study indicate that there is overall increase in sperm motility, sperm viability, acrosomal integrity, plasma membrane integrity in treatment groups as compared to the control groups whereas, there is significant increase in number of abnormal spermatozoa and MDA level in control group as compared to treatment groups which shows higher peroxidative damage in control group (Fig. 1). In our previous study, we found that dietary azolla supplementation significantly improves the semen quality and libido in bucks (Gangwar et al., 2019). Earlier study has shown that buck sperm motility was 68.59% after three days of storage at 5℃ (Wen et al., 2019); it reached 78.2% (a significant increase) after three days of liquid preservation with Shatavari supplementation. Hence, Shatavari extract could be able to protect the buck sperm from various types of cryo-damages.

Malo et al. (2011) found that by adding rosemary extract into the extender effectively increased the sperm penetration and oocyte cleavage rate, and also improved the effectiveness of the fertilization system. In addition to this, they also found that supplementation of exogenous rosemary to the freezing medium enhanced post-thawed semen quality in boar. Similarly, Zhao et al. (2009) reported that *Rhodiola sacra* aqueous extract (RSAE) exhibited strong scavenging activity against superoxide anion radical. They observed significant improvement in progressive motility, HOST response, fertility, and safeguarding the functional integrity of sperm plasma membrane. Antecedent findings indicated that the number of hypo-osmotic swelled spermatozoa is positively correlated with the oocyte penetration rate in human oocytes.

In Congruent with our results, El-Sheshtawy et al. (2016) demonstrated that addition of bull semen extender with 10% and 20% Pomegranate Juice provides adequate chilling, and boosted frozen-thawed semen quality. Similarly, Khan et al. (2017) reported that addition of green tea extract in semen diluter exhibited remarkable or noteworthy response on the post-thawed spermatozoa motility, viability and membrane integrity of Achai bull. Vahedi et al. (2018) found that supplementation of *Thymus vulgaris* extract at the rate of 4–8 ml/dl of diluter improves the attributes of ram sperm after freezing and thawing. Addition of 5% *Tribulus terrestris* extract in semen diluter improved the sperm motility in Afshari rams at 5°C (Pour et al., 2015). The findings of previous investigation reported that *T. terrestris* extract

had a noteworthy influence on the sperm motility and viability in human. However, in vitro studies on human reported that *T. terrestris* extract inclusion in extender had no effect on DNA fragmentation of (Kumar et al., 2018). Similarly, Mehdipour et al. (2016) reported that supplementation of *Camellia sinensis* extract at level of 10 mg/L can enhance post-thaw ram semen quality, when it is cryopreserved in a soybean lecithin extender.

In the present investigation, the enhancement of semen quality and antioxidant activity could be due to the richness of *A. racemosus* in advantageous polycarbohydrates, i.e., fructo-oligosaccharide with an average degree of polymerization. *A. racemosus* is also rich in sterols and steroidal saponins, Shatavarins I-IV (Alok et al., 2013). Shatvarin I is the main glycoside with 3-glucose and rhamnose moieties attached to sarsapogenin, effective in establishing the sufficient availability of hormone to gonads (Kukasawa et al., 1994). Diverse working of the herb can be a primary ground for a remarkable enhancement in the sexual attributes. After analyzing the results of the current investigation, it is concluded that *A. racemosus has* aphrodisiac property, and has potential to treat sexual dysfunction.

Conclusion

The results of the current study showed that Shatavari aqueous extract can be used as natural antioxidant in buck semen diluter and may be replaced with chemical antioxidants. Shatavari aqueous extract significantly enhanced the sperm motility, acrosomal integrity, plasma membrane integrity and increase the sperm viability for longer duration, thus improves buck semen quality during preservation at refrigerated temperature. However, the active ingredients responsible for the improvement in buck semen quality are needed to be investigated further.

Declarations

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Data availability: Data is available on request.

Author's contribution: Dr Chetna Gangwar conceptualized the study along with Dr Ashok Kumar, and did the semen preservation experiments, whereas Kamendra Swaroop performed the antioxidant studies. Dr Anil Kumar Mishra edited the manuscript and Dr Rakesh Goel did the statistical analysis. Dr Shriprakash

Singh microscopically evaluated the sperm abnormalities. Dr R Pourouchottamane reared, and managed the animals in uniform conditions during the trial and Dr S.D. Kharche monitored the experimental part of the study. All authors have gone through the manuscript, and accepted the content of the manuscript.

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Figures

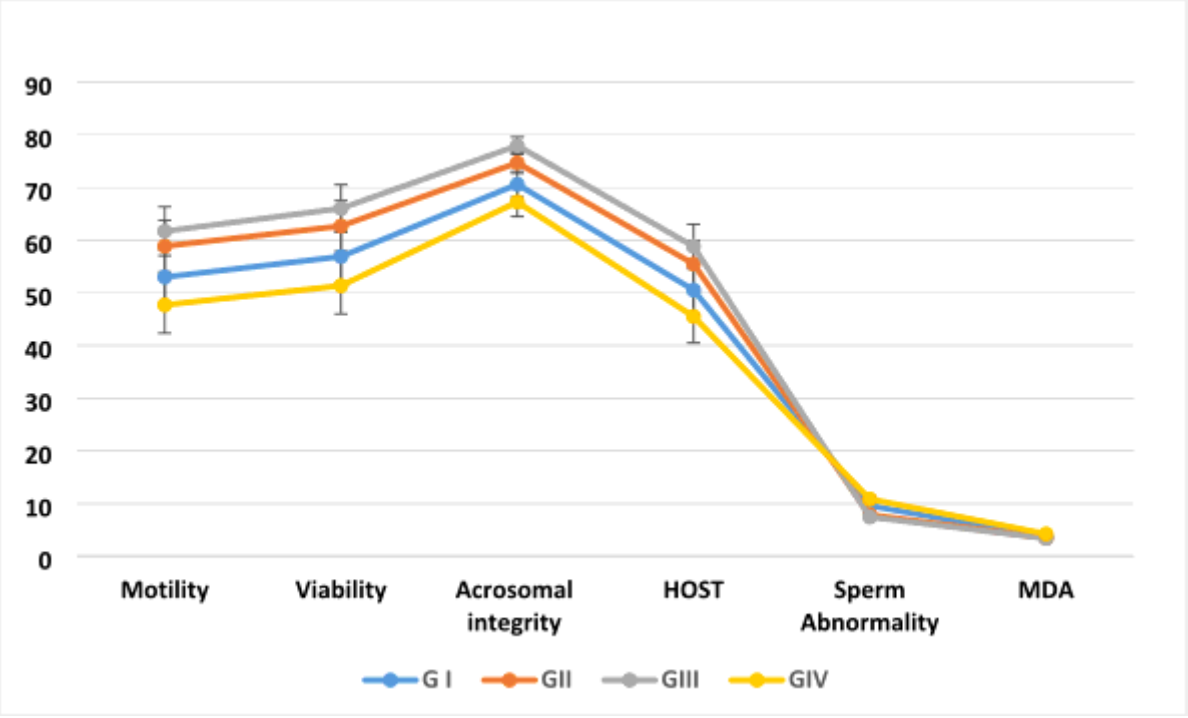

Figure 1

Overall sperm characteristics and MDA level during the entire period of experiment in buck semen supplemented with different concentration of Shatavari extract.