

ASSESSMENT OF MEMBRANE INTEGRITY AND ANTIOXIDATIVE ENZYME STATUS IN FRESH EJACULATED BARBARI BUCK SEMEN DURING BREEDING SEASON

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Abstract: Semen selection is very important step for artificial insemination. It has a positive correlation with conception rate. The post thaw semen outcome of chilled or frozen thawed semen largely depends upon quality of fresh ejaculated semen. But the data related to seminal attributes for indigenous breed of goat in country are lacking. So, the study was conducted to evaluate the physical seminal attributes and antioxidative enzyme status in Barbari bucks. The observed mean value (\pm SE) of mass motility was found to be 3.92 ± 0.15 while acrosomal integrity was 84.33 ± 0.76 % in freshly collected semen from Barbari bucks during their breeding season. Evaluation of capacitation like changes using CTC dye indicated that 69.83 ± 1.07 % spermatozoa exhibited Pattern F while 25.33 ± 1.11 % exhibited Pattern B and 4.83 ± 0.31 % exhibited Pattern AR indicating that majority of spermatozoa were uncapacitated and a small number exhibited acrosomal reaction. The concentration of catalase was 23.14 ± 0.42 mM H_2O_2 utilized/min/mg. protein, superoxide dismutase was 35.51 ± 0.43 U/mg protein, glutathione peroxidase was 9.29 ± 0.23 U/ml and glutathione reductase was 8.035 ± 0.21 U/ml in seminal plasma of freshly ejaculated Barbari buck semen.

Keywords: Antioxidative enzymes, Barbari buck, seminal attributes, semen.

Introduction

In current scenario of population explosion, food security is a major issue of concern especially in developing country like India. Goat being a potent food source has emerged as an alternate that has to be utilized to its maximum potential. There is an urgent need to formulate strategic approach to make this resource efficient. Propagation of superior genotype with enhanced productivity in terms of meat or milk and increased disease resistance can make goat domestication cost efficient (Hudson, 2010). The production of superior progeny will further intensify commercialized goat farming that will supplement food supply (Leboeuf et al, 2000).

The objective of genetic up gradation solely depends upon effective breeding policy required to develop and conserve superior genotype (Jabbar et al, 2010). This can be made

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possible through selection and mating of superior males and females (Bhatia and Arora, 2005). Since a male can only mate limited number of females makes natural breeding a limiting factor. Also the availability and identification of genetically superior animal in a geographical area is difficult tasks that can hinder/obstruct the success breeding programme. To overcome these problems, use of assisted reproductive technology *viz.* artificial insemination (AI) is utilized, where semen obtained from a desired male with superior genotype is preserved and later utilized to inseminate multiple numbers of females, enhancing germplasm propagation manifolds and also effectiveness of breeding programme with better progeny (Verma et al, 2012).

The selection of semen to be utilized for insemination is very important determining step to improve conception rate under field condition. Artificial insemination technique that utilizes diluted semen needs sufficient number of normal viable spermatozoa with forward progression for successful fertilization (Yotov et al, 2011). A presided assessment of fresh ejaculated semen for its admission to be used for insemination enhances the outcome of chilled or frozen thaw semen. The process becomes more important in goat due to persistence of lethal interaction between the seminal plasma and egg yolk in semen diluter (Anand et al, 2016). Further, the data related to seminal attributes for indigenous breed of goat in country are scarce. So, taking into account the importance about the knowledge of seminal attribute in fresh ejaculate semen and its relevance in determining chilled or post thaw semen quality, the experiment was conducted to evaluated the different physical seminal attributes and antioxidative enzyme status in breeding Barbari bucks.

Materials and Methods

The study was conducted to evaluate physical seminal attributes and antioxidative enzyme status in Barbari buck semen. Four healthy breeding bucks of similar age group (2-3 years) and body weight (25-35 kg) were selected as semen donor. Semen collection was made biweekly from each buck with the help of artificial vagina. A total of 24 ejaculates were collected from four bucks (six ejaculates from each buck) in the month of Feb.-Mar. 2015 with maximum temperature between 26 to 29 °C and minimum temperature between 11 to 16°C. Freshly collected semen after initial evaluation was pooled to eliminate individual variation. The pooled semen sample was divided into two parts. One part was utilized to evaluate the physical seminal attributes and the other part was subject to centrifugation at 1500 rpm for 15 minutes to separate the seminal plasma. Different seminal attributes studied in fresh ejaculated semen were mass motility, percent intact acrosome (Watson *et al.*, 1975)

and capacitation like changes using CTC dye. Three different patterns viz. pattern F, pattern B and pattern AR were recorded during the experiment (Collin et al, 2000). The concentration of different antioxidative enzyme viz. Catalase (CAT) as per Bergmayer (1983), Super oxide dismutase (SOD) as per Madesh and Balasubranian, 1998; Glutathione Peroxidase (GP_X) as per Paglia and Valantine (1967) with slight modifications and Glutathione Reductase (GSH-R) as per Goldberg and Spooner, 1983 were evaluated in seminal plasma. Statistical analyses were performed using Statistical Package for Social Science (SPSS[®] Version 20.0 for Windows[®], SPSS Inc., Chicago, USA). The data has been presented as mean \pm standard error (SE).

Results and Discussion

The observed mean (\pm S.E.) values of the seminal attributes and antioxidative enzyme evaluated for assessing the quality of freshly ejaculated semen from four Barbari bucks (total 24 ejaculates, 6 ejaculates per buck) has been presented in Table No.1 and 2. It was observed that the mean (\pm S.E.) value of mass motility was 3.92 ± 0.15 graded on 0-5 scale. Shakeel (1999) and Singh (2003) reported lower values while Tiwari (2000) reported higher values of mass motility in Barbaribuck semen. The probable reason for the difference may be testicular size, size of buck, age of buck, time of collection or seasonal and managemental variation (Sultana et al, 2013). The intactness of acrosome is determining factor for successful conception. Higher the spermatozoa with intact acrosome in prefreeze semen higher will be intact sperm after freezing. Therefore, ejaculates with higher intact acrosomes are better for deep-freezing. In our study the fresh ejaculates from Barbari bucks recorded 84.33 ± 0.76 % spermatozoa with intact acrosome. The recorded value during the experiment is in accordance with those reported in Majorera goat (Batista *et al.*, 2009) while a higher values were recorded in Damascus goat (El-Kon *et al.*, 2010) which probably point towards the breed variability.

The destabilization of sperm membranes were evaluated by tracking the distribution of Ca²⁺ in spermatozoa using chlortetracycline (CTC) which accumulate in organelles containing high concentrations of Ca²⁺. Neutral uncomplexed CTC easily crossed the membranes where it ionized to an anion and chelated Ca²⁺. The latter complex is bound preferentially to hydrophobic site, such as membrane and show increased fluorescence. The extent of binding to membrane depends on surface-to-volume ratio of vesicle and properties of lipid. Due to compartmentalization of plasma membrane of spermatozoa, several distinct staining patterns have been evaluated which are associated with functional status of

spermatozoon (Fraser *et al.*, 1995). A similar pattern of CTC staining has been observed across the other species. The three patterns are Pattern F, with uniform fluorescence on the head that indicating uncapacitated, acrosome intact spermatozoa; Pattern B, with a fluorescence-free band on post acrosomal region that indicates capacitated, acrosome intact spermatozoa; and pattern AR with uniformly fluorescence-free head and with a fluorescence band on equatorial region that indicate acrosome reaction. In our study $69.83 \pm 1.07\%$ spermatozoa exhibited Pattern F (Uncapacitated), while $25.33 \pm 1.11\%$ exhibited Pattern B (capacitated, acrosome intact) and $4.83 \pm 0.31\%$ exhibited Pattern AR (Acrosomal reacted) indicating that majority of spermatozoa were uncapacitated and a small number exhibited acrosomal reaction. Kadirvel *et al.* (2009) reported 14.32% spermatozoa to be capacitated in fresh buffalo semen. A higher percentage of capacitated spermatozoa exhibited by buck semen can be due to breed difference or antioxidative enzymatic status of the seminal plasma. A high level of ROS in human seminal plasma is related to poor sperm morphology, poor motility and a low sperm concentration (Aitkin, 1989). It has been also documented that enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione oxidase (GSH-Px) and glutathione reductase (GSH-R) are responsible for protection of spermatozoa against oxidizing agents like ROS, peroxide and -oxidases. Hence activity of these enzymes affects spermatozoa viability and membrane integrity. CAT is a heme-containing enzyme that catalyzes the conversion of H_2O_2 to H_2O and O_2 . SOD protects spermatozoa against spontaneous O_2 toxicity and lipid peroxidation (LPO) (Alvarez *et al.*, 1987). Superoxide dismutase (SOD) is an enzymatic antioxidant present in cytosol and mitochondria of cells, which converts superoxide radical to hydroperoxide by dismutation reaction (Halliwell and Gutteridge, 1999). CAT and SOD also remove (O_2^-) generated by NADPH-oxidase in neutrophils and may play an important role in decreasing LPO and protecting spermatozoa during genital-urinary inflammation (Aitken *et al.*, 1993). The mean CAT activity was 23.14 ± 0.42 mMH_2O_2 utilized/min/mg protein while mean SOD activity was 35.51 ± 0.43 U/mg protein.

Bilodeau *et al.* (2001) reported that glutathione is a large class of antioxidants and has -SH (thio) groups and antioxidant features. Therefore, it is able to react with many ROS directly and is also a co-factor for glutathione peroxidase (GPx) that catalyzes the reduction of toxic HO and hydroperoxides. On the other hand Glutathione reductase (GSH-R) is present in cytosol and mitochondria. In the anti-oxidative process, they reduce low molecular weight disulphide bond of glutathione reductase to form glutathione (GSH) in presence of NADPH

(Enkvelchakul, 1994). Selenium-Glutathione peroxidase and Glutathione reductase directly act as antioxidant enzymes involved in inhibition of sperm lipid peroxidation (Lenzi *et al.*, 1994). The mean Glutathione peroxidase (GP_X) activity was 9.29 ± 0.23 U/ml while mean Glutathione reductase (GSH-R) activity was 8.035 ± 0.21 U/ml.

Through this study, mass motility, percent acrosomal integrity, capacitation like changes, concentration of Catalase (CAT), Super oxide dismutase (SOD), Glutathione Peroxidase (GP_X) and Glutathione Reductase (GSH-R) were evaluated in Barbari buck semen during their breeding season and it was found that they are suitable candidates for deep freezing and extending artificial insemination through deep frozen semen. The baseline value developed will help many scientists to device suitable extenders keeping in view the sperm membrane integrity and anti-oxidative enzyme status.

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Table 1: Seminal attributes and capacitation like changes in freshly ejaculated Barbari buck semen (n=4, 24 ejaculates)

Parameters Replicate	Mass Motility (0-5)	Acrosomal integrity (%)	Capacitation like changes		
			Pattern F (%)	Pattern B (%)	Pattern AR (%)
1	4	84	73	21	6
2	3.5	86	71	25	4
3	3.5	87	66	29	5
4	4	82	72	24	4
5	4	84	69	26	5
6	4.5	83	68	27	5
Overall average values	3.92 ±0.15	84.33 ±0.76	69.83 ±1.07	25.33 ±1.11	4.83± 0.31

Table 2: Antioxidative enzyme status of freshly ejaculated Barbari buck seminal plasma (n=4, 24 ejaculates)

Parameters Replicate	Catalase (CAT) (mMH ₂ O ₂ utilized/min/mg. protein)	Superoxide dismutase (SOD) (U/mg protein)	Glutathione peroxidase (GP _x) (U/ml)	Glutathione reductase (GSH-R) (U/ml)
1	22.21	35.23	9.11	8.92
2	24.52	33.89	9.78	7.97
3	21.89	36.11	9.35	8.23
4	23.56	35.86	8.86	7.87
5	22.72	34.99	8.53	7.9
6	23.95	36.99	10.11	7.32
Overall average values	23.14 ±0.42	35.51 ±0.43	9.29 ±0.23	8.035 ±0.21