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Research Article

DONOR AGE AND ITS RELATIONSHIP WITH SEMEN QUALITY IN GOATS

AMIT SHARMA* and PANKAJ SOOD
Department of Veterinary Gynaecology and Obstetrics,
CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur-176062 (H.P.), India

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ABSTRACT

Donor age and its correlation with seminal parameters were studied in 286 ejaculates from adult healthy post pubertal bucks (Gaddi n=106; Chegu n=180) belonging to either young (Y; <15 month old) or adult (A; >15 month old) with average age of (Y:1.22±0.03 and A:3.1±0.41) years for Gaddi and (Y:1.2±0.07 and A: 2.75±0.15) years for Chegu bucks, respectively. Semen was collected using artificial vagina techniqueand ejaculates were selected on the basis of standard quality parameters. The ejaculates were extended in Tris citrate egg yolk extender (TEY) containing 10% egg yolk (EY) and 6% glycerol to maintain a concentration of 150 X 106 spermatozoa/straw, processed as per standard protocol and were thawed at 37º C for 30 sec, 24 hrs post incubation to compare seminal attributes between fresh and post thaw semen along with percent change due to the processing. Data was analyzed using package R version 3.4.3. Adult than Y Gaddi bucks yielded significantly higher (P<0.01) semen volume (0.70±0.3 vs 0.50±0.03), lower concentration (2553.4±150.2 vs 4014.2±247.7) and mass motility (3.78±0.03 vs 4.15±0.04). Whereas, gross parameters were insignificantly higher in A than Y Chegu bucks. Volume was negatively correlated with concentration of ejaculate (r = -0.311, P<0.05) in Y bucks and positively correlated (r = 0.271; P<0.01) with mass motility in A Gaddi bucks. Whereas, in Chegu, volume was positively correlated (r = 0.334, P<0.05) with mass motility in Y bucks. The semen of A Gaddi bucks being more resilient than Y in terms of significantly higher post thaw progressive motility (37.21±0.64 versus 33.15±1.22; P<0.01), viability (50.75±1.13 versus 43.91±1.90; P<0.01) and HOST reactivity (54.98±1.32 versus 50.08±1.65; P<0.05). In conclusion, Adult Gaddi bucks produced higher semen volume along with lower concentration and mass motility but were more resilient for cryopreservation than Y bucks. Whereas, there was no effect of donor's age on cryo sensitivity of Chegu sperm.

Keywords: Age, Chegu goats, Cryopreservation, Gaddi goats, Semen

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Goats are important livestock species and contributes around 27.8 per cent of total livestock population (148.88 million; 20th Livestock census, 2019) of India. There is rich biodiversity among the indigenous goat in India, evident by more than 20 listed breeds of goat. A progressively declining cattle population in Himachal Pradesh (H.P.), India accentuates goat rearing under small household system. Several socio-economic reasons along with lack of developmental policies for breed conservation and improvement leading to loss of breed utility and marginalization of goat population (Dogra and Thakur, 2010), Chegu being worst affected and therefore have been put under "Endangered species" (20th livestock census, 2019). Unabated decline in males of superior genetic merit due to rampant slaughtering and inadvertent breeding (inbreeding) underscores the importance to conserve superior males and their use for artificial insemination programme. Researchers' believe that quality of bucks' semen is affected by various factors including breed (Qureshi *et al.*, 2013); season (Qureshi *et al.*, 2013; Gallego-Calvo *et al.*, 2015; Elsheikh and Elhammali, 2015; Kumar *et al.*, 2016; Sharma *et al.*, 2020); age of buck (Ferdinand *et al.*, 2012; Hashida *et al.*, 2013; Tabarez *et al.*, 2017); managemental practices (Hanmante *et al.*, 2009; Elhammali and Elsheikh 2014; Kumar *et al.*, 2016) and source of protein supplemented to ration (Elhammali and Elsheikh, 2014). There are differences in amount and quality of semen produced between animals of the same age and breed and hence, there is great variability in time of arrival of old age characterized by increased numbers of morphological abnormalities in bucks depending on health status, environment and their use (Leboeuf *et al.*, 2000). Hence, the present study was designed to understand the effect of donor age and its correlation with seminal quality parameters in Gaddi and Chegu goats.

*Corresponding author: vet50amy@gmail.com

MATERIALS AND METHODS

Present study was conducted on apparently healthy Gaddi (n=11) and Chegu (n=8) bucks at the University Livestock Farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur. All post pubertal animals were categorized to either young (Y:<15 month old) or adult (A:>15 month old) with average age of (Y: 1.22±0.03 and A: 3.1±0.41 years) for Gaddi and (Y: 1.2±0.07 and A:2.75±0.15 years), for Chegu bucks, respectively. Bucks were selected on the basis of breeding history, breeding soundness evaluation and testicular diameters. All the bucks were screened for diseases, Brucellosis, Chlamydiosis (AGPT, Chahota *et al.*, 2015) to eliminate the possible transmission of infection.

Experimental bucks were maintained under identical managerial conditions and fed as per the standards of Indian Council of Agricultural Research (ICAR 2013). All males had round the clock access to the clean drinking water.

A total of 286 ejaculates (Gaddi, n=106; Chegu, n=180) from healthy bucks were collected using an estrus doe. The semen collections were made twice weekly usingartificial vagina (AV) maintained at 42-43º C. The fresh ejaculates were evaluated for any gross abnormality including colour as well as volume and concentration (Caprine Photometer, IMV 1409®, France) followed by microscopic examination for mass motility. The suitability of ejaculates for further processing was done using the standard criterionof absence of any gross abnormality, milky to creamy semen colour, mass motility ≥ 3 and initial progressive motility ≥ 70 per cent (Rather *et al.*, 2016). The seminal plasma was removed as described previously (Nuti 2007; Sharma *et al.*, 2018). The semen pellet thus obtained was extended with two equal fractions of tris citrate egg yolk extender, TEY (TRIS 1.21 gms, Citric acid 0.685 gms, D-Fructose 0.5 gms, Benzyl Penicillin 1000 IU/ml, Streptomycin sulphate 1mg/ml), egg yolk (10%) and glycerol (6%). The TEY was added to semen at a gap of 2-3 minutes to yield a final concentration 150 ×106 spermatozoa/straw. The pH of the buffer was adjusted to 6.7 to 6.9. Extended semen was filled in 0.25 ml French mini straws (IMV Technologies, L'Aigle, Cedex, France), by aspiration using micropipette (Minitube, Germany) and subsequently sealed at free end with the help of polyvinyl alcohol (PVA) (IMV Technologies, L'Aigle, Cedex, France) powder. All the aforementioned steps of semen handling were undertaken at controlled air temperature of 30º C. The straws were laid on a stainless steel rack and placed in cooling cabinet 4º C (Macro Scientific works Pvt. Ltd. India) for 4 h and thereafter exposed to liquid nitrogen vapors for 7 minutes. Thereafter, the straws were plunged into liquid nitrogen for storage. The inventory of semen storage was also maintained. Thawing of semen straws was done at 37º C for 30 sec. (Sariozkan *et al.*, 2010; Sharma *et al.*, 2018) in a water bath.

The post-thaw cryopreserved semen samples were evaluated for progressive motility (Hafez and Hafez, 2000), viability, morphological abnormalities and HOST reactivity. Per-cent change in the aforesaid parameters between fresh and post-thaw semen was also calculated to determine the effect of cryopreservation.

The experiments were carried out after the approval of institutional ethical committee and the principles under Declaration of Helsinki were also taken into consideration.The data obtained were analyzed using package R version 3.4.3. Paired sample t-test was used to see the significant difference between fresh and post-thaw evaluation of quality seminal parameters in both Gaddi and Chegu breed. Independent sample t-test was applied between Y and A groups of both the breeds to observe the significant changes. Multivariate analysis was used to determine correlations between gross semen parameters and their significance was tested by using ANOVA. Results were presented as Mean ±SEM and atp <0.05 significance.

RESULTS AND DISCUSSION

Effect of donor age on gross seminal parameters of Gaddi and Chegu goats are summarized in Table 1. Study revealed significantly lower ($p<0.05$) average semen volume, higher sperm concentration ($p<0.001$) along with numerically high mass motility in the Y Group Gaddi bucks. Whereas, insignificant differences in gross seminal parameters were observed in Chegu bucks (Table 1).

Not many studies have evaluated the effect of age on the seminal qualities of bucks. Ferdinand *et al.* (2012) compared semen characteristics in West African Dwarf bucks (1-2 and 2-5 years old) and observed insignificantly higher volume (0.58±0.07 vs 0.55±0.09), concentration (4.73±1.33 vs 3.90±0.96) and mass motility (4.67±0.58 vs 4.50±0.58). Whereas, Tabarez *et al.* (2017) reported significantly higher volume (1.8±0.1 vs 1.0±0.1 ml) in older Blanca de Rasquera bucks. The findings pertaining to increase in semen volume with increased age corroborated to the bucks of present study, more so for Gaddi bucks manifesting a significant difference compared to Chegu bucks with a numerical increase in semen volume in A Group bucks. Semen production in any species is a function of animal breed, size, management and environment (Hafez and Hafez, 2000). Researchers' suggested variation in semen volume is mainly due to quantity of fluids secreted by the accessory glands and the epididymis, both of which are androgen dependent (Leboeuf *et al.*, 2000). Abd-Allah *et al.* (2007) stated that advancement of age increases significantly the body conditions score (body weight and height, crown rump length and girth) as well as scrotum circumference, testis-length and width which is thereby reflected on improvement of semen quality. They also suggested that low libido and semen quality in bucks of one year could possibly be due to the low level of circulating plasma testosterone which plays an indispensable role for regulating testis function and thereby semen quality. Correlations between various gross seminal parameters among different age groups were observed for Gaddi and Chegu bucks (Table 2). Study revealed significant negative correlation ($r = -0.311$, $P<0.05$) between volume and concentration of ejaculate among Y Group Gaddi bucks whereas, volume was positively correlated ($r = 0.271$; $P<0.01$) with mass motility in A Group Gaddi

Table 1. Effect of donor age on average (mean±SEM) gross seminal parameters of Gaddi and Chegu bucks

Breed	Age	Volume (ml)	Mass Motility (0-5)	Concentration ($\times 10^6$ sperms/ml)
Gaddi (n=106)	Young (n=49)	0.50±0.03	4.15±0.04	4014.2±247.7
	Adult (n=57)	0.70±0.03	3.78±0.03	2553.4±150.2
		p<0.001**	p<0.001**	p<0.001**
Chegu (n=180)	Young (n=75)	0.70±0.07	3.90±0.04	1896±205.4
	Adult (n=105)	0.82±0.06	3.94±0.05	2032±179.7
		p=0.19 (NS)	p=0.55 (NS)	p=0.52 (NS)

n= Number of ejaculates; **Highly Significant (p<0.01)

Table 2. Relationship of donor age with gross seminal parameters in Gaddi and Chegubucks

Breed	Gaddi (Young and Adult) (n=106)				Chegu (Young and Adult) (n=180)			
Parameter	Volume	Colour	Conc.	Mass motility	Volume	Colour	Conc.	Mass motility
Volume	1	0.045	0.112	0.271**	1	0.032	0.065	-0.097
Colour	0.132	1	0.146	0.023	0.179	1	0.492**	0.00
Concentration	-0.311*	-0.081	1	-0.115	0.203	0.408*	1	0.117
Mass motility	-0.122	-0.159	0.107	1	0.334*	0.00	0.052	1

Value in the upper matrix (above diagonal) corresponds to adult and values in lower matrix (below diagonal) corresponds to young buck of either breed;*Significant (p<0.05); **Highly Significant (p<0.01)

Table 3. Effect of Gaddi Buck donor age on average (Mean±SEM) seminal parameters of fresh and post-thaw semen

Parameters (%)	Fresh Diluted		Post Thaw		% change due to processing	
	Young (n=49)	Adult (n=57)	Young (n=49)	Adult (n=57)	Young	Adult
Progressive motility	74.41±0.79	72.86±0.49	33.15±1.22^a	37.21±0.64^b	55.4	48.92
Viability	76.47±0.85	74.93±0.58	43.91±1.90^a	50.75±1.13^b	42.6	32.2
Morphological abnormalities	7.0±0.31	7.06±0.22	8.29±0.32	7.58±0.27	18.4	8.2
HOST reactive	73.8±1.40	73.83±1.01	50.08±1.6^A	54.98±1.32^B	32.2	25.5

n= Number of ejaculates; A-B Values with different superscripts within same row differs (p<0.05)
a-b Values with different superscripts within same row differs (p<0.01)

Table 4. Effect of Chegu B buckdonor age on average (Mean±SEM) seminal parameters of fresh and post-thaw semen

Parameters (%)	Fresh Diluted		Post Thaw		% change due to processing	
	Young (n=75)	Adult (n=105)	Young (n=75)	Adult (n=105)	Young	Adult
Progressive motility	74.26±0.67	73.82±0.66	31.92±1.16	29.93±1.20	57.1	59.5
Viability	74.04±1.11	74.48±1.12	44.17±1.90	41.72±1.37	40.4	44.0
Morphological abnormalities	6.21±0.31	6.23±0.24	7.29±0.32	7.85±0.40	17.3	26.0
HOST reactive	63.17±2.02	60.04±2.25	37.34±1.55	37.76±1.80	40.8	37.1

n= Number of ejaculates; A-B Values with different superscripts within same row differs (p<0.05)

bucks. In case of Chegu bucks, volume of ejaculate was significantly correlated (r = 0.334, p<0.05) with mass motility among Y Group males, whereas, colour of ejaculate shows a significant positive correlation with concentration for Young Bucks (r = 0.408; p<0.05) and A (r = 0.492; p<0.01), respectively in same breed.

The seminal quality parameters for different age groups in fresh diluted and post-thaw semen amongst both the breeds (Table 3, Gaddi; Table 4, Chegu) were also compared in present study. There were insignificant differences amongst donor age groups on the seminal parameters in fresh diluted semen. However, the semen of Group A compared to Group Y Gaddi Goats exhibited better tolerance to cryopreservation protocol in terms of average progressive motility (37.21±0.64 vs 33.15±1.22; p<0.01), viability (50.75±1.13 vs 43.91±1.90; p<0.01) and HOST reactivity (54.98±1.32 vs 50.08±1.65; p<0.05). The semen of Adult bucks being more resilient than Young bucks was also justified by a reduced per cent change in progressive motility (48.92 vs 55.4), viability (32.2 vs 42.6), morphological abnormalities (8.2 vs 18.4) and HOST reaction (25.5 vs 32.2), respectively.

Alike Gaddi bucks; no significant effect of age was observed on the average seminal parameters in fresh diluted semen of Chegu bucks (Table 4). However, in contrast to Gaddi bucks, both Young and Adult Chegu bucks equally withstood the cryopreservation effect as indicated by similar corresponding values of progressive motility (31.92±1.16 vs 29.93±1.20), viability (44.17± 1.90 vs 41.72±1.37), morphological abnormalities (7.29± 0.32 vs 7.85±0.40) and HOST reactive sperms (37.34± 1.55 vs 37.76±1.80), respectively. Our observations of non-influence of age both in Young and Adult Chegu bucks was also supported by a lack of non-significant difference in value of per cent change in semen processing.

The increased semen quality with advancement in age of bucks has also been cited earlier in Murciano-Granadina bucks (Roca *et al*., 1991) and Damascus bucks (Al-Ghalban *et al.*, 2004), Jermasia bucks (Hashida *et al.*, 2013) and Rams (Garcia *et al.*, 2017). Researchers' (Abd-Allah *et al.*, 2007; Gallego-Calvo *et al.*, 2015; Tabarez *et al.*, 2017) observed increase in semen quality amongst older males, which could be attributed to increase in testicular weight and size, thereby increasing the efficiency of spermatogenesis in older males (Al-Ghalban *et al.*, 2004). Similarly, Hashida *et al.* (2013) observed significantly higher ($p<0.05$) sperm velocities in adult Jermasia bucks using CASA and hypothesized sperm motility changes as a consequence of altered testicular and epididymal age related changes which is associations of either structural, functional or both changes with increasing age. Whereas, contrasting results have also been observed in West African Dwarf goats (Ferdinand *et al.*, 2012), who observed better motility in 1-2 year than 2-5 year goats and attributes this to deterioration in prostate gland secretions with advancement in age. Adult male spermatozoa in Gaddi breed were more resilient to cryopreservation than Young ones in present study. Our results are in consonance to observation of Tabarez *et al.* (2017) and Hashida *et al.* (2013) and rams (Lymberopoulos *et al.*, 2010; Garcia *et al.*, 2017) who observed higher percentage of cells with plasma membrane integrity and functional mitochondria in mature animals.

In conclusions, Adult than Young Gaddi bucks yielded significantly higher volume, lower concentration and mass motility. The semen of adultbucks being more resilient than young in terms of significantly higher post thaw progressive motility, viability and HOST reactivity. There isno significant effect of age on gross as well as microscopic seminal quality parameters in Chegu bucks. Hence, it would be important to consider the potential effect of the donor age while creation of a sperm bank and be more prudent using animals older than one and half year.

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