The 6th International Seminar on Tropical Animal Production
Integrated Approach in Developing Sustainable Tropical Animal Production
October 20-22, 2015, Yogyakarta, Indonesia

Effect of Doe Blood Serum Supplementation to Buck Semen on the Head to Head Agglutination Test

Hassan Ishag Harren[1], Mohamed Abd Elmoneim Salih[1], Abdel Aziz Makkawi[2], and Hatim Idris[1]

[1]Faculty of Agriculture, Department of Animal Production, Omdurman Islamic University, Khartoum - Sudan P.O Box 382. .
[2]Faculty of Agriculture Studies- Department of Animal Production, Sudan University Of Science and Technology, Khartoum North, Shambat P.O Box: 407.
Corresponding Email: haren20101@hotmail.com

ABSTRACT: The aims of this study was to determine the effect of adding caprine blood serum on the extended semen quality of tow exotic breeds and one local Sudanese breed namely Nubian (local), Saaneen and Shami (exotic). This experiment was conducted in two farms, the first in Khartoum Center for Improvement Goat Breed (Ministry of Animals Wealth). The second farm in Animal Production Research Centre farm for small ruminants belonging to the Ministry of Animal Wealth. Semen and blood samples from 27 bucks and 27 doe were collected by use (AV). The goat blood serum was added to skimmed milk and egg yolk semen extenders, serum composed 10% of each extender. The effects of the goat blood serum, together with skim milk and egg yolk extenders for the stored semen were evaluated in vitro at temperatures of 5°C. The results demonstrated that the characteristics of 10% serum treated semen extended in egg yolk or skim milk and stored at 5°C was significant (P>0.05) effects on all studied traits compared with skim milk or yolk egg extended semen. The sex associated with skim milk and egg yolk in the three breeds on the motility, acrosome integrity and agglutination. Hormonal effect also observed in this study reflected sex influence on this phenomenon since demonstrating the significant effect of estrogen comparing to progesterone. The effect of blood serum and the conservation period on the stored semen are also quite clear in this study. A significant (P>0.05) difference showed the Nubian semen maintained better quality during 7 days of conservation compared to Saaneen and Shami semen.

Keywords: goat breeds, doe blood serum, buck semen, and (HHA) test.

INTRODUCTION

The population of goats in Sudan since 2011 is 30,649,000 according to Ministry of Animal resources (Robert and Thomas, 2001). AI in animal breeding programs provides an opportunity to accelerate genetic improvement though widespread use of desirable sires'. Concomitant with this advantage is the control and check of diseases and their spread. One of the major problems in the using of (AI) is the decreasing quality of semen during cryopreservation and thawing. Researchers have made considerable progress in correcting this problem, but have only been partially successful, especially in regard to certain animals, e.g, buffalo (Makkawi, 1987) (Robert Taylor and Thomas, 2001). The semen is usually mixed with an extender that dilutes to a greater the ejaculate volume which allows a single ejaculate to be processed into several units of semen. The extender is usually composed of nutrients such as milk, egg yolk, a citrate buffer, antibiotics, and glycerol (Robert Taylor and Thomas 2001). In the Sudan, most of the cattle and goats population is scattered in areas lacking facilities such as refrigeration plus the problem of improper

transportation must be considered, for an effective of artificial insemination program in those areas, fresh semen extension and transportation without loss of semen quality is essential for AI to be successful (Makkawi, 1987). Estrogens are the group of hormone derived from estradiol - 17 -b steroids, mainly secreted by ovaries, placenta, and even testes (Finlay, 1976). The main function of estrogens is the development, functioning and maintenance of the female genital organs, by stimulating protein synthesis and mitosis (Finlay,1976) while Hafez (1993) added that estrogens in the doe produced by theca enterna and granulose cells of the ovarian follicle under the positive control of LH and FSH. The secondary roles of estrogens include body confirmation and mammary gland development, Progesterone is produced by CL of non-pregnant doe and CL and placenta of the pregnant doe (Gordon, 1997 and Robinson, 1988). The effect of progesterone is obvious at the target tissue, after that tissue has been stimulated by estrogen which leads to synergistic response. When progesterone level in blood is constant, it will prohibit the pituitary gland from secretion of GNRH, which appears in the length of diestrus phase.

MATERIALS AND METHODS

The experiment was conducted in two farms are: Khartoum Center for Improvement of Goat Breed (Ministry of agriculture and Animals Wealth and Irrigation).The farm import two kinds of goats the first one is Saaneen (females) form Holland, and the second one is Shami breed, which comes from Cyperus and this breed seems to be big in shape and have long hair and comparatively low milk production .The second farm is Animal Production Research Centre Farm (small ruminants unite) belonging to the Ministry of Animal Resource and Fisheries for local breed (Nubian goats). Semen and blood samples from 27 bucks and 27 doe were collected by use (AV) using the technique developed by Salisbury and Willett (1940) The goat blood serum was added to skimmed milk and egg yolk semen extenders, serum composed 10% of each extender. The effects of the goat blood serum, together with skim milk and egg yolk extenders for the stored semen were evaluated in vitro at temperatures of 5°C. Blood from the jugular veins of three breeds centrifuged at 6000 rpm for 15 minutes to extract the serum. The serum was conserved in a water bath at 37°C for 30 minutes to avoid immunological reactions due to the blood compliments (Senger Saacke, 1976) and to destroy spermicidal factors (Chang, 1947, Aalseth *et al.*, 1978). The serum was identified by labeling according to the breed and stored in refrigerator until it was used. Then added Penicillin (act in both Gram +ve and Gram -ve bacteria and streptomycin were added), each 100 ml of skim milk extender contained 75 mg of penicillin and 50 mg of streptomycin. This blood serum was later used to constitute 10% volume of the skim milk or yolk egg. Each of the sera collected from does was used separately in an extended semen samples to compare the influence of sex and serum on the extended semen.

Semen Extension: each of the semen samples extended with one of six extenders.

The composition of those extenders as following:

(*1*) Skim milk alone + (SSM)
(*2*) Egg yolk alone (SEY)
(*3*) Skim milk + 10% doe blood serum (SSMdS)
(*4*) Egg yolk + 10% doe blood serum (SEYdS)

Preparing of blood serum and egg yolk

The skim milk was heated to 92-95°C for t 10 minutes and cooled in controlled room temperature (20 to 22°C).The heating was performed to destroy Lactenin, a spermicide (Flipse *et al.,*1954) recommended procedure of heating the milk by boiling water in a stainless steel pan, adding 200 ml of skim milk to a sterilized flask, placing it in the pan of hot water and controlling at 92 to 95°C for 10 minutes was used. The skim milk was then cooled to 5°C and antibiotics were added. The extender then ready for use (Skim milk 0.5% fat). The yolk of an egg was separated on a filter paper. Fresh eggs were always obtained, cracked gently to pour off the egg white and have separated the yolk. The egg yolk was then put on filter paper to be separated and collected. Statistical Analysis, Data was analyzed by (SAS) programming. One-factor Analysis of Variance (CRD) was performed. Means were tested and to separated treatment using Duncan's Multiple Range Test (DMRT) referred to (Steel *et al.*, 1997), correlation also was used.

RESULTS AND DISCUSSION

Table (1): Correlation (r) between mortality and agglutination of female goats' serum

Goat breed	Variable	Day of Replicate		
		1	3	7
Nubian	NM	0.65	0.62	0.60
	NE	0.83	0.81	0.80
Saaneen	SM	0.57	0.55	0.52
	SE	0.79	0.77	0.75
Shami	ShM	0.47	0.45	0.44
	ShE	0.70	0.67	0.65

Table (2):Correlation(r) between agglutination and acrosome integrity on serum of female goats'

Goat breed	Variable	Day of Replicate		
		1	3	7
Nubian	NM	0.59	0.56	0.54
	NE	0.81	0.82	0.83
Saaneen	SM	0.59	0.56	0.50
	SE	0.77	0.72	0.70
Shami	ShM	0.63	0.61	0.60
	ShE	0.65	0.63	0.61

NM = Nubian with skimmed milk, NE = Nubian with egg yolk, SM= Saaneen with skimmed milk, SE= Saaneen with egg yolk, ShM = Shami with skimmed milk, ShE= Shami with egg yolk.

Table (3): Correlation (r) between agglutination and estrogen on serum of female goats'

Goat breed	Variable	Day of Replicate		
		1	3	7
Nubian	NM	0.62	0.60	0.69
	NE	0.88	0.86	0.84
Saaneen	SM	0.60	0.59	0.57
	SE	0.84	0.82	0.80
Shami	ShM	0.58	0.55	0.52
	ShE	0.80	0.78	072

Table (4): Correlation (r) between agglutination and progesterone on serum of female goats

Goat breed	Variable	Day of Replicate		
		1	3	7
Nubian	NM	0.60	0.56	0.52
	NE	0.70	0.68	0.66
Saaneen	SM	0.48	0.46	0.42
	SE	0.60	0.58	0.55
Shami	ShM	0.36	0.33	0.30
	ShE	0.50	0.48	0.44

NM = Nubian with skimmed milk, NE = Nubian with egg yolk, SM= Saaneen with skimmed milk, SE= Saaneen with egg yolk, ShM = Shami with skimmed milk, ShE= Shami with egg yolk.

Table (5): Correlation (r) between acrosome integrity and estrogen on serum of female goats

Goat breed	Variable	Day of Replicate		
		1	3	7
Nubian	NM	0.60	0.56	0.52
	NE	0.70	0.68	0.66
Saaneen	SM	0.48	0.46	0.42
	SE	0.60	0.58	0.55
Shami	ShM	0.36	0.33	0.30
	ShE	0.50	0.48	0.44

NM = Nubian with skimmed milk, NE = Nubian with egg yolk, SM= Saaneen with skimmed milk, SE= Saaneen with egg yolk, ShM = Shami with skimmed milk, ShE= Shami with egg yolk.

Table (6): Correlation (r) between acrosome integrity and progesterone on serum of female goats.

Goat breed	Variable	Day of Replicate		
		1	3	7
Nubian	NM	0.60	0.56	0.52
	NE	0.70	0.68	0.66
Saaneen	SM	0.48	0.46	0.42
	SE	0.60	0.58	0.55
Shami	ShM	0.36	0.33	0.30
	ShE	0.50	0.48	0.44

NM = Nubian with skimmed milk, NE = Nubian with egg yolk, SM= Saaneen with skimmed milk, SE= Saaneen with egg yolk, ShM = Shami with skimmed milk, ShE= Shami with egg yolk.

Table (7): means of blood hormone (Estrogen and Progesterone) of female goats

Goat Breed	Estrogen	Progesterone
Nubian female	8 ±0.9	3 ±1.2
Saaneen female	7 ±0.9	2.7 ±1.2
Shami female	5.5 ±0.9	2.5 ±1.2

Mean ± SD value(s) bearing different superscript letter(s) within columns are significantly different ($P<0.05$).

Three breeds of goats namely Nubian (local) and Saaneen and Shami (exotic) were used in this study to determine the effect of adding caprine blood serum on the extended semen quality of those breeds. The results showed that sex associated with breed effect in all studied characters since there is a significant (P>0. 05) effect of doe's blood serum of Nubian goats, Saaneen and Shami respectively. Estrogen and progesterone in blood serum were studied to investigate the effects of semen extenders. Semen of Nubian buck containing egg yolk plus doe blood serum showed significant effect (P>0. 05) and high correlation between mortality and agglutination (Table 1), acrosome integrity and agglutination (Table 2), agglutination and estrogen (Table 3), agglutination and progesterone (Table 4), acrosome integrity and estrogen (Table 5) and acrosome integrity and progesterone (Table 6), this might be due to the effect of the hormones in the does blood serum and some enzymes in doe serum. This result agreed with Makkawi (1987) which reported the sex hormonal effect, and with Mariam (2000) and Austin (1981) repored that the female serum had some enzymes and hormones. The results obtained in this study also support the suggestion of Senger *et al.* (1981) that the marked difference in the degree and type of HHA caused by blood sera from the male and female due to the type of agglutinin present in the blood serum which might be under reproductive hormonal influence.

CONCLUSION

Does blood serum is recommended over buck serum for the extension of semen, which enhancing HHA test.

ACKNOWLEDGEMENT

This paper funded by authors.

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