The effect of temperature on the HHA of spermatozoa of Buck (goot) semen extender with caprine blood serum

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Abstract
This experiment was conducted in two farms, the first one in Khartoum center for improvement goats breed (ministry of animal's wealth). The second farm in animal production research centre farm for small ruminant belonging to the Ministry of Animal Wealth. The result of this present study demonstrated the characteristics of 10% serum treated semen extended in egg yolk or skim milk and stored at 5° C under Sudan condition. Buck serum was collected from three breeds of goats, two to three years of age. Results of extender and blood serum illustrated significant (P≥0.05) effects of egg yolk on the motility, dead live, acrosome integrity and agglutination compared to skim milk. The effect of blood serum on the HHA phenomenon is well documented in this study. The results of this work demonstrated more HHA in the egg yolk extended semen compared to skim milk extended semen involving only and selectively sperms with entact acrosome.

Introduction

The number of goats in Sudan since 2011 is 30.649. 000 according to Ministry of Animal resource. In many countries, people consume more goats' milk than cow's milk, and goats are also important source of meat. There are five major kinds of domesticated goats: (1) the dairy goat is used largely for the production of milk and, to a lesser extent, for meat (2) the Angora goat is used mainly for the production of mohair and, to a lesser extent, for brush clearance and the production of meat: (3) the meat goat(male of Nilotic goats) is used for brush clearance, for the production of meat, and in some parts of the world, for skins and fine leather: (4) the Cashmere goat is noted for the soft cashmere fibers used in producing high-quality and (5) the pygmy goat is used as a laboratory ruminant animal and pet in the United States but is an important disease-resistant, meat and milk producer in West Africa (where it is known as the West African Dwarf) and other countries.(Makkawi1987).

Today A.I is one of the most valuable manager biotechnology available to beef and dairy cattle producers. Many bulls produce sufficient semen to provide enough sperm for 40,000 breeding units in one year (Bearden Fuquay, 1984).The use of A.I 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 used of (A .I), 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., water buffalo. Many extenders are currently being utilized in the preservation process. However, several problem still exist and, therefore, the ideal semen extender has as yet, not been described. (Robert et al 2001). The primary advantage of AI is

that it permits extensive use of outstanding sires to maximize genetic improvement. If the semen is frozen and stored for later use, hundreds of thousands of calves can be produced by a single sire (one calf per 1.5 units of semen) and many of these offspring can be produced long after the sire is dead. AI can be also used to control reproductive diseases, and sires can be used that have been injured or are dangerous when used naturally. The success of an AI program is dependent on superior heat detection, high levels of fertility, high quality of semen, and skilled semen handling and insemination technique. (Makkawi1987). In this view to determined the effect of temperature to HHA on the semen plus goat blood serum.

Semen Extenders

The storage time required for practical use of most semen in A.I programs varies from a few hours to weeks. With the discovery of egg yolk as a semen extenders (Philips, 1939), the metabolic activity of spermatozoa could be reduced and their livability could be prolonged be storage at 5°c Sodium citrate was found to be beneficial to sperm livability when added to the egg yolk (Salisbury et al, 1941). They reported that sodium citrate had an advantage over phosphate buffer in being a chelating agent and in dispersing the fat globules in egg yolk and skim milk thereby improving observation of the spermatozoa when examined under the microscope. The citrate yolk extender has been modified much time with varying concentration of sodium citrate and egg yolk, depending on the addition of other components, e.g. glycerol (Mukherjee Dott, 1960) and blood serum (Senger Saacke, 1976).Milk also in various forms has been used successfully as semen extender. Previous Many researchers have compared the effects of the different forms of milk as semen extenders (underbjerg e t al., 1942, Machajilov, 1950, Thacker Almquist, 1951, 1953). Flipse et al, (1954) reported the presence of toxic factor for spermatozoa in unheated skim milk .They indicated that the toxicity was associated with an albumin –containing factor (lactenin), and heating the milk at 92°c for 10 minutes eliminated the toxic effect. Determination of Acrosomal Changes during the Storage Period The technique developed by Watson (1975) of using Giemsa stain to detect changes in the acrosomes of buck spermatozo . Ten percent (v/v) buffered solution of Giemsa stain was used to stain the cells for two hours. The 10% Giemsa stain solution was prepared by filtering 10 ml of Giemsa stain in the graduated cylinder and to that, 90 ml double distilled water was added to make 100 ml 10% Giemsa stain solution. The Giemsa solution was stored at room temperature for one week. A drop of semen was smeared on a pre-warmed, labeled slide and dried in a current of warm air. The smears were then fixed by immersion in buffered formal saline for 15 minutes and washed in running tap water for 15 to 20 minutes. The smears were dried and then immersed in buffered Giemsa solution for at least two hours. They were rinsed briefly in distilled water and dried.

Acrosome reaction

The anterior half of the sperm head carries the acrosome ,which is moulded over the nucleus and is covered externally by the plasma membrane .Aspermatozoa with an intact acrosome cannot penetrate an egg-for this, the acrossome must react or break down ,releasing its contents, and then the reacted elements must be shed .Successive stages of the acrosome reaction are presented diagrammatically .First , the outer acrosome membrance and the plasma membrane of the sperm cell unite by fusion at a number of points over the front half of the sperm head. The spaces developing then provide exit ports for soluble acrosome contentents, probably at least a dozen different enzymes in all, and in a short time

the acrosome is emptied (Ed.G.M.J agiello and H.J.Vogel(1981) .The reacted shroud of fused vesiculating membranes is then shed in the process identified separately as acrosome loss ,and this seems to occur an appreciable time after the acrosome reaction .

The significance of the delay could be that the spaces (exit ports) between the vesicles enlarge quite slowly so that release of acrosomal enzymes is protracted, providing full opportunity for the lytic action of the enzymes to aid the passage of the spermatozoon through the egg investments. Loss of the acrosome and its visible content occur before the sperm head makes serious inroads into the substance of the zona pellucid.Of the dozen or so enzymes known to exist in the acrosome, the most important of sperm function in fertilization would appear to be hyaluronidase and a powerful trypsin –like enzyme called acrosin (Ed.G.M.J agiello and H.J.Vogel(1981). As Stan Meizel and his colleagues showed, acrosin is stored in the form of its zymogen pro-acrosin, which is converted into the active enzyme on its release in the acrosme reaction . Both enzymes could play important roles in sperm penetration of the egg coats,

Materials and Methods

Semen Collection

Semen sample were taken from foreign and local male goats which varied between (2-3) years. The interval period of collection is a week. The semen sample from foreign and local male taken by motivating male with female after that take the semen sample was transported to the artificial insemination lab in A.I center within the same vicinity and then identify the color, volume Consistency , Motility of spermatozoa, Concentration of spermatozoa and Wave motion characteristics .

Semen Extension

Each of the semen samples was extended with one of six extenders. The composition of those extenders was as following:

(1) Skim milk alone + (SSM)
(2) Egg yolk alone (SEY)
(3) Skim milk + 10% bucks blood serum (SSMbS)
(4) Skim milk + 10% doe blood serum (SSMdS)
(5) Egg yolk + 10% bucks blood serum (SEYbS)
(6) Egg yolk + 10% doe blood serum (SEYdS)

Preparing of Blood Serum:-

Blood from the jugular veins of Nubian identified male + female, Saneen and Shame all approximately two or three years of age. And then analysis with ELAZA test for Estrogen progesterone, and they were certificated to be free from tuberculosis and brucellosis. And then preparing the skim and Egg yolk .

Statistical Analysis Techniques Used :-

Data generated was subjected to statistical analysis system (SAS). One-factor Analysis of Variance (CRD) was performed. Means were tested and separated using Duncan' s Multiple Range Test (DMRT) referred to Steel et. Al.(1997).

Result and Discussion and conclusion

The negative effect of storage period on all semen characters is well illustrated in this study as there is the different tables.Since the results of this study showed significant (P≥0. 05) decrease in the percentage of motility ,acrosome integrity , and head to head agglutination

in the semen of the three of the buck's.The effect of storage period on the breed semen is also well quite clear.A significant (P≥0. 05) different between the three bucks.

Table (1) Effect of adding blood serum on agglutination (%) of male Nubian goats' semen

Blood serum level	Collections								
	1st			2nd			3rd		
	Days								
	1	3	7	1	3	7	1	3	7
$N.M_{0\%}$	85.00^{b} ±0.00	76.67^{c} ±2.89	50.00^{c} ±0.00	84.00^{b} ±0.00	75.67^{c} ±2.89	50.00^{d} ±0.00	82.00^{b} ±0.00	72.67^{b} ±2.89	49.00^{d} ±0.00
$N.M_{10\%}$	88.33^{ab} ±1.53	82.00^{b} ±0.00	52.00^{b} ±0.00	84.33^{b} ±1.53	82.00^{a} ±0.00	65.00^{b} ±0.00	86.33^{a} ±1.53	82.00^{ab} ±0.00	65.00^{b} ±0.00
$N.E_{0\%}$	89.33^{a} ±2.89	82.00^{b} ±0.00	56.67^{ab} ±2.89	88.33^{a} ±2.89	80.00^{b} ±0.00	57.67^{c} ±2.89	87.33^{a} ±2.89	72.00^{b} ±0.00	56.67^{c} ±2.89
$N.E_{10\%}$	90.00^{a} ±0.00	85.67^{a} ±0.58	68.67^{a} ±3.79	87.00^{a} ±0.00	84.67^{a} ±0.58	71.67^{a} ±2.89	85.00^{a} ±0.00	85.67^{a} ±0.58	70.67^{a} ±2.89
C.V%	1.99%	1.62%	12.79%	1.78%	1.59%	15.62%	2.06%	1.58%	9.68%
$Lsd_{0.05}$	1.075^{*}	1.066^{*}	2.3678^{*}	1.264^{*}	2.167^{*}	1.0695^{*}	1.362^{*}	2.1137^{*}	3.9645^{*}
SE	0.7529	0.3569	1.0974	0.6253	0.3224	0.9607	0.9068	0.2643	2.3346

Mean±S.D value(s) bearing different superscript letter(s) within columns are significantly

Table (2): Effect of adding female Nubian blood serum on agglutination (%) on the goats' semen

Blood serum level	Collections								
	1st			2nd			3rd		
	Days								
	1	3	7	1	3	7	1	3	7
$N.M_{0\%}$	27.00^{c} ±1.53	17.33^{c} ±1.53	15.00^{a} ±0.00	24.00^{c} ±1.53	11.33^{c} ±1.53	9.00^{a} ±0.00	22.00^{d} ±1.53	11.33^{c} ±1.58	10.00^{a} ±0.00
$N.M_{10\%}$	35.00^{b} ±1.53	17.00^{b} ±1.53	13.67^{a} ±1.53	36.00^{b} ±1.53	15.00^{b} ±1.53	11.67^{a} ±1.53	30.00^{b} ±1.53	14.00^{b} ±1.53	10.67^{a} ±1.53
$N.E_{0\%}$	28.33^{c} ±1.53	15.00^{b} ±1.53	11.67^{a} ±1.53	27.33^{c} ±1.53	16.00^{b} ±1.53	12.67^{a} ±2.89	26.33^{c} ±1.53	16.00^{b} ±1.53	10.67^{a} ±2.89
$N.E_{10\%}$	64.33^{a} ±1.53	28.00^{a} ±1.53	20.33^{a} ±2.89	65.33^{a} ±1.53	29.00^{a} ±1.53	18.33^{a} ±2.89	66.33^{a} ±1.53	27.00^{a} ±1.53	17.33^{a} ±2.89
C.V%	10.65%	1.65%	2.49%	10.81%	1.61%	2.53%	9.65%	3.96%	3.21%
$Lsd_{0.05}$	1.6684^{*}	2.8547^{*}	10.5863^{ns}	1.6634^{*}	2.8529^{*}	10.5865^{ns}	2.0964^{*}	0.8547^{*}	6.1485^{ns}
SE	0.8647	1.011	1.0627	0.8652	1.016	1.0630	1.1658	0.7721	2.1182

Table (3): Effect of adding blood serum on agglutination (%) of male Saaneen goats' semen

Blood serum level	Collections								
	1st			2nd			3rd		
	Days								
	1	3	7	1	3	7	1	3	7
$S.M_{0\%}$	26.67^{b} ±2.89	12.33^{c} ±2.08	13.33^{a} ±1.53	25.67^{c} ±0.00	12.33^{bc} ±2.08	9.33^{b} ±1.53	23.67^{a} ±2.89	10.33^{bc} ±2.08	7.33^{b} ±1.53
$S.M_{10\%}$	38.33^{ab} ±2.89	15.67^{b} ±5.15	10.00^{b} ±3.61	29.33^{b} ±0.00	13.67^{b} ±5.13	9.00^{b} ±3.61	28.33^{a} ±2.89	12.67^{b} ±5.13	9.00^{ab} ±13.61
$S.E_{0\%}$	24.33^{b} ±2.89	15.67^{b} ±1.53	10.00^{b} ±0.00	24.33^{c} ±2.89	13.67^{b} ±1.53	10.00^{a} ±0.00	24.33^{a} ±2.89	12.67^{b} ±1.53	8.00^{b} ±0.00

$S.E_{10\%}$	40.67^{a} ±1.00	22.67^{a} ±1.15	11.00^{ab} ±0.00	42.67^{a} ±2.91	23.67^{a} ±1.15	10.00^{a} ±0.00	44.67^{a} ±2.89	25.67^{a} ±1.15	11.00^{a} ±0.00
C.V%	9.86%	3.85%	3.48%	12.94%	2.22%	1.96%	26.54%	5.69%	7.85%
$Lsd_{0.05}$	6.9713*	3.2867*	1.5767*	3.6254*	4.5684*	0.9639*	23.4963ns	1.6879*	3.0635*
SE	1.6325	2.1398	1.6431	0.9876	1.9632	0.06325	6.5247	1.6276	1.5634

Table (4): Effect of adding female Saaneen blood serum on agglutination (%) on the goats' semen

Blood serum level	Collections								
	1st			2nd			3rd		
	Days								
	1	3	7	1	3	7	1	3	7
$S.M_{0\%}$	25.67^{c} ±2.89	12.33^{c} ±2.08	13.33^{b} ±1.53	26.67^{c} ±2.89	13.33^{c} ±2.08	10.33^{b} ±1.53	25.67^{c} ±2.89	11.33^{bc} ±2.08	8.33^{c} ±1.53
$S.M_{10\%}$	50.33^{b} ±2.89	15.67^{b} ±5.15	10.00^{c} ±3.61	$30..33^{b}$ ±2.89	14.67^{c} ±5.13	9.00^{b} ±3.61	29.33^{b} ±2.89	11.67^{bc} ±5.13	10.00^{b} ±13.61
$S.E_{0\%}$	24.33^{c} ±2.89	16.67^{b} ±1.53	10.00^{c} ±0.00	25.33^{c} ±2.89	16.67^{b} ±1.53	10.00^{b} ±0.00	25.33^{c} ±2.89	13.67^{b} ±1.53	10.00^{b} ±0.00
$S.E_{10\%}$	55.67^{a} ±1.00	23.67^{a} ±1.15	15.00^{ab} ±0.00	53.67^{a} ±2.91	24.67^{a} ±1.15	16.00^{a} ±0.00	52.67^{a} ±1.00	27.67^{a} ±1.15	14.00^{a} ±0.00
C.V%	9.86%	3.85%	3.48%	12.94%	2.22%	1.96%	26.54%	5.69%	7.85%
$Lsd_{0.05}$	6.9713*	3.2867*	1.5767*	3.6254*	4.5684*	2.9639*	9.4961*	1.6879*	3.0635*
SE	1.6325	2.1398	1.6431	0.9876	1.9632	0.06325	3.5242	1.6276	1.5634

Table (5): Effect of adding blood serum on agglutination (%) of male Shami goats' semen

Blood serum level	Collections								
	1st			2nd			3rd		
	Days								
	1	3	7	1	3	7	1	3	7
$Sh.M_{0\%}$	4.67^{c} ±2.31	4.00^{d} ±0.00	3.00^{c} ±0.00	5.67^{d} ±2.31	4.30^{d} ±0.00	5.00^{b} ±0.00	5.67^{d} ±2.31	4.00^{d} ±0.00	2.00^{c} ±0.00
$Sh.M_{10\%}$	16.00^{b} ±5.00	11.00^{b} ±0.00	7.00^{b} ±0.00	$20..00^{b}$ ±5.00	10.00^{b} ±0.00	6.00^{b} ±0.00	18.00^{b} ±5.00	10.00^{b} ±0.00	7.00^{b} ±0.00
$Sh.E_{0\%}$	15.67^{b} ±7.64	9.00^{c} ±0.00	6.67^{c} ±2.89	11.67^{c} ±7.64	8.00^{c} ±0.00	7.67^{b} ±2.89	10.67^{c} ±7.64	9.00^{c} ±0.00	8.67^{b} ±2.89
$Sh.E_{10\%}$	25.00^{a} ±5.00	15.33^{a} ±2.89	10.00^{ab} ±0.00	26.00^{a} ±5.00	14.63^{a} ±1.15	16.00^{a} ±0.00	25.00^{a} ±5.00	13.33^{a} ±2.89	10.00^{a} ±0.00
C.V%	9.86%	3.85%	3.48%	12.94%	2.22%	1.96%	26.54%	5.69%	7.85%
$Lsd_{0.05}$	6.9713*	3.2867*	1.7967*	3.6254*	4.5684*	3.9639*	9.4961*	1.6879*	3.0635*
SE	1.6325	2.1398	1.6431	0.9876	1.9632	0.06325	3.5242	1.6276	1.5634

Table (6): Effect of adding blood female Shami serum on agglutination (%) on the goats' semen

Blood serum level	Collections								
	1st			2nd			3rd		
	Days								
	1	3	7	1	3	7	1	3	7
$Sh.M_{0\%}$	50.67^{b} ±2.31	44.00^{a} ±0.00	36.00^{c} ±0.00	45.67^{c} ±2.31	34.00^{a} ±2.31	30.00^{d} ±0.00	31.67^{d} ±2.31	24.00^{d} ±0.00	1.00^{c} ±0.00
$Sh.M_{10\%}$	55.00^{a} ±5.00	35.00^{b} ±0.00	18.00^{b} ±0.00	$50..00^{d}$ ±5.00	35.00^{a} ±0.00	23.00^{a} ±0.00	55.00^{b} ±5.00	30.00^{b} ±0.00	12.00^{b} ±0.00

$Sh.E_{0\%}$	10.67^{c} ±7.64	8.00^{d} ±0.00	6.67^{c} ±2.89	10.67^{c} ±7.64	8.00^{c} ±0.00	8.67^{c} ±2.89	11.67^{c} ±7.64	9.00^{c} ±0.00	8.67^{b} ±2.89
$Sh.E_{10\%}$	50.00^{b} ±5.00	23.33^{c} ±2.89	12.00^{a} ±0.00	48.00^{b} ±5.00	23.33^{b} ±2.89	15.00^{b} ±0.00	42.00^{a} ±5.00	24.33^{a} ±2.89	16.00^{a} ±0.00
C.V%	9.876%	3.85%	3.48%	12.94%	2.22%	1.96%	17.58%	3.85%	9.71%
$Lsd_{0.05}$	6.9718^{*}	3.2867^{*}	1.7967^{*}	3.6254^{*}	4.5684^{*}	3.9639^{*}	4.6871^{*}	2.6582^{*}	2.0754^{*}
SE	1.3325	2.1398	1.6431	0.9876	1.9632	0.06325	1.0955	1.0965	2.6084

Mean±S.D value(s) bearing different superscript letter(s) within columns are significant different($P \leq 0.05$).

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 that breeds.Result of this study showed a significant ($P \leq 0.05$) effect of buck blood serum with improving semen motility. This concerted with result of Kreider et al.(1980).The result show that the Storage at 5°c maintained higher percent alive, motility , and head-to-head agglutination (HHA)for longer period of time , with no significant difference in the percent of acrosomal integrity for temperature and long period . The HHA declined with the passage of time, the temperature interaction demonstrated that at 5°C maximum HHA occurred after 12 hours of storage. The reduction in the percent HHA of this study as time elapsed confirming the findings of Senger and Saacke (1976) and Brown (1981). The significant decline in HHA that occurred during incubation supports the suggestion of Saacke et al (1976) that aging of spermatozoa dose occur during storage .The aging phenomenon occurred earlier with any temperature above 6°C.This event reflects a temperature influence on the sperm activities. Decline with temperature in percent alive and motility observed in this study confirm the conclusion of Salisbury (1978) that metabolic rates tend to be proportional to absolute temperature, and that storage at 5°C is the primary means of slowing the chemical reactions and prolonging the life of spermatozoa. Spermatozoa tend to be inactive at 5°C and hence less energy The result of this study agreed with the report of Salisbury et al (1978) that spermatozoa can tolerate temperature somewhat higher than body temperature for only a short period of time, due to the increased energy requirement and consequently increased accumulation of toxic products during the metabolic process which may affect the life of the spermatozoa this might support the result of this study. It also conclusion that the temperature of 5°C had a significant positive effect on motility and agglutination up to 7 days of storage .

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