THE EFFECT OF BOVINE SERUM ALBUMIN ON SOME CHILLED SEMEN CHARACTERISTICS OF AWASSI RAMS

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ABSTRACT

This study was conducted on three Awassi rams to investigate effect of different levels of Bovine Serum Albumin (BSA) as the antioxidant on semen characteristics and the deterioration occurred by semen processing and preservation. Semen was collected by electro-ejaculator, pooled and subdivided to seven equal aliquots, then diluted with Sodium citrate and egg yolk diluent supplemented with 0.0, 5, 10, 15, 20, 25, and 30mg ml^{-1} of BSA as control(c) and T1 to T6 respectively. Semen quality was assessed by progressive motility, pH, dead, abnormal, and acrosomal defect percentages after dilution, cooling and storage in refrigerator (at 5c) for three consecutive days.

The results showed that any addition ranged between 5-30 mg ml^{-1} of BSA to the diluent could improve semen quality with greatest improvement occurred in the range of 10-20mg ml^{-1} of BSA. Except pH and dead sperm percentage, all other characteristics have been shown significant differences ($p<0.05$) during preservation stages. Semen preparation stages (dilution, cooling, preservation for 1, 2 and 3 days in 5c. resulted in a significant ($p<0.05$) decline in semen characteristics. The study concluded the importance of adding 10-20mg ml^{-1} of BSA to the extender and to be used as soon as possible after cooling.

INTRODUCTION

Awassi sheep is the most important local breed raised for dual purpose in Iraq and neighborhood countries for its favorite meat and milk, the information on reproductive physiology and artificial insemination capabilities are limited. Moreover, studies on ram semen preservation are rare.

Sperm, as other live cell, needs the oxygen to support metabolism, but this activity generate unfavorable factors usually called reactive oxygen species (ROS) which is increasingly accumulated in intracellular fluid and destroy the cell later. ROS were produced autonomously when semen prepared for storage (dilution, cooling, freezing, etc) and also along the preservation periods. Thus, it's necessary to alter this status by adding external antioxidants to the diluents to enable spermatozoa to tolerate the deterioration effects of ROS. While oxidative stress is very important cause for spermatozoa deterioration (1, 28), there are two other causes (cold shock and osmotic stress) (2, 34) have the same effects. First report on oxidative stress was appeared in 1943 (19) and revealed that human spermatozoa when incubated in high oxygenated aerobic condition, lose fastly motility it fastly lost their motility, a mechanism could be reversed by catalase addition.

Some antioxidants are existed naturally in the semen of mammals (21, 29), it and acts as trapping net to protect spermatozoa from destroying action of free radicals (ROS).

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Other mechanisms proposed by Anghel, et.al. (6) are prevention, interception or repair the spermatozoa membrane, all these mechanisms will be useless and inefficient when semen was prepared for preservation. Dilution, cooling, equilibration, freezing and thawing were found to be main causes responsible for viability, motility and membrane integrity decline as oxidation stress increased to maximum level (9, 23, 24).

Harmful effects of ROS in the oxidative stress could be lowered by high soluble protein Bovine Serum Albumin (BSA) which existed naturally in mammalian semen (12, 26), and were used as antioxidant in mammalian semen extender (3, 7, 33). BSA has a unique activities when trapping free radical through its multiple binding properties (26), Thus, researchers substituted BSA for egg yolk in the ram and buffalo semen diluents (20, 11) or as addition to the diluents of buffalo, bull, ram semen (25, 7, 33). Consequently, this study was proposed to investigate the ability of BSA to improve Awassi rams semen quality during chilled preservation at 5˚C and the deterioration occurred by semen processing and preservation, and was lined in series of studies on the effects of some antioxidants on Awassi semen quality after preservation.

MATERIALS AND METHODS

Semen from three Awassi rams aged 4.5 - 5.0 years and has body condition score of 2.50 - 3.50 were weekly collected by electro ejaculator during the period April 17 to May 26 /2016 in ruminant research department, AL-Zaafarania /20km south Baghdad.

Immediately after collection, semen was transferred to laboratory for primary evaluation. Ejaculate volume was measured by graduated tube, appearance by 1-5 scale (4), pH by litmus-paper, sperm concentration with special densimeter (591B). Mass motility was assessed with the 0-5 scale (8). Individual motility was estimated as percentage of progressive motile spermatozoa with x400 magnification (30).

Dead and abnormal spermatozoa percentages were determined with eosin – nigrosine stain (27), colorless spermatozoa was considered live, otherwise red or purple was dead. Spermatozoa acrosome defect was assessed with eosin-fast green fast stain (35), while the tail was light green color and the head red color, acrosome differentiated with opaque green. Ejaculates were ignored when having less than 3 degree mass motility, 70% progressive motility, 3 degree for appearance, 0.3 ml volume, and $1x10^9$/ml of concentration. Citrate-egg yolk extender stock was prepared using 2.37 gm trisodium citrate, 0.5 gm glucose dissolved in 100 ml distilled water and 20% egg yolk (v/v) then added. Pooled semen was divided into seven aliquots and each was diluted by the ratio of 1:4 (semen: diluents) by one of seven experimental diluents with addition of bovine serum albumin 0.0, 5, 10, 15, 20, 25 and 30mg ml^{-1} assimilating control (C) and T1 to T6 respectively. Diluted semen was cooled to 5˚C within 2 hours and kept in refrigerator for further evaluations during three consecutive days.

Data was expressed as, means ±standard error after statistical analysis using general linear model supplied by SPSS software (31), and differences were differentiated significant if it exceed 0.05 at Duncan multiple range test.

RESULTS AND DISCUSSION

Data in table (1) revealed raw semen characteristics of three Awassi rams used in the study, it seems that all these characteristics were within the natural limits for Awassi breed(4) and the ability of introducing antioxidants in the semen preparation techniques was presents, the goals of adding antioxidants to

the extenders were mainly to decrease oxidative and osmotic stress, and prevent cold and freezing shocks.

Table 1: Means of some fresh semen characteristics of Awassi rams.

Ram No.	Ejaculate Volume (ml)	App. [1] scale	Mass mot. scale	Progr. mot. (%)	pH	Conc. ($x10^9$/ml)	Dead sp. (%)	Abn. sp. (%)	Acro. defect (%)
185	1.20	4.6	4.07	87.83	7.2	2.38	12.75	9.66	3.74
193	2.04	4.0	3.98	85.14	7.2	2.15	16.18	7.45	6.83
195	0.84	4.1	3.66	80.86	7.0	1.55	17.98	8.93	7.94

[1]: appearance scale: 1, watery; 2, cloudy; 3, milky; 4, creamy; 5, thick creamy.

Some characteristics of Awassi ram semen (means independent of processing stages and preservation periods) have been shown in table (2). Statistical analysis revealed that there were no significant differences in pH and dead sperm percentage when BSA was added to citrate-egg yolk extender in different levels (from 0 to 30 mg ml^{-1}), while significant differences have been found in sperm motility, abnormal and acrosome defect percentages. Conformations were in line for the enhancement of post thawing ram semen characteristics when BSA was added to the extender (20, 33).

The non-significant differences of pH and dead sperm percentage clearly indicated that addition of BSA in the range of 5-30 mg ml^{-1} haven't any beneficial effect, and disagreed with the finding of other investigations (13, 32, 33), it seems that apoptosis which take place in early stages of dilution and cooling didn't blocked by adding BSA to the extender. In contrast, sperm motility percentage in all treatments was significantly increased in comparison with control. Moreover 15mg ml^{-1} of BSA showed the highest percentage of motility among all other treatments, it could be the balance occurred in this level between ROS production and scavenging system which is responsible for suitable sperm activity. This fact was previously referred earlier by Hamedanil, et.al. (14).

The improvement of sperm motility due to the addition of BSA to the extender was peak-like curve, the explanation for this phenomenon may be either the interaction of BSA with the extender components or the balance between ROS production and scavenging system. However, harmful effects of free radicals in oxidative stress could be protected by BSA adding (12, 26), but, it's very important to use suitable level to enhance sperm motility through preservation. Till 1986, (17) reported that the mechanism in which BSA stimulates sperm motility was unknown but recent studies (7, 26) revealed that BSA have a trapping properties, increment of antioxidant enzymes activity, total thiols and total antioxidant capacity. These theories may be true for the current results obtained in this study, futhermore to the fact that BSA as a macromolecular substance keeps sperm motility by protection from the detrimental effect of dilution (15).

One of the important traits for evaluating preserved semen quality is sperm abnormalities including head, midpiece, tail and acrosome defect percentages. All these anomalies were found in fresh semen and eventually increased due to dilution, cooling and storage. Sperm abnormalities for different treatments have shown significant differences ($p<0.05$). All BSA levels have improved abnormal sperm percentage in comparison to control, but there were no significant differences among them with superiority of 10, 20, 30 mg ml^{-1} of

BSA. These results were in agreement with (33) for post thawing ram sperm with 20mg ml^{-1} of BSA adding. Acrosome integrity was considered as prediction indicator for fertilizing capacity in ruminants. Differences in acrosome defect percentage were found to be significant ($p<0.05$). Control and T2 to T5 (10-25 mg ml^{-1} BSA) have the lowest percentages of acrosome defect indicating to suitable levels of BSA to keep good acrosome integrity. Lewis et al. (18) believed that BSA addition to the extender could reduce lipid peroxidation of sperm membrane which resulted in higher membrane integrity. Also, (13) observed highest acosome integrity after cryopreservation when 15mg ml^{-1} of BSA was included in the diluents.

Table 2: Effects of different supplementation of bovine serum albumin (BSA) on some semen characteristics of Awassi ram (means independent of processing stages and preservation periods).

Trait / BSA	pH	Progressive Motility (%)	Dead spermatozoa (%)	Abnormal Spermatozoa (%)	Acrosom Defect (%)
Control (C) (0.0mg ml^{-1})	6.49 a	39.17 c	37.40 a	17.13 a	9.07 b
T1 (5mg ml^{-1})	6.50 a	51.50 ab	32.47 a	14.47 ab	11.47 a
T2 (10mg ml^{-1})	6.55 a	53.00 ab	32.87 a	12.80 b	11.07 ab
T3 (15mg ml^{-1})	6.58 a	55.33 a	32.87 a	14.18 ab	9.93 ab
T4 (20mg ml^{-1})	6.56 a	53.67 ab	34.60 a	13.93 b	10.60 ab
T5 (25mg ml^{-1})	6.54 a	48.17 b	32.73 a	14.73 ab	11.00 ab
T6 (30mg ml^{-1})	6.52 a	52.00 ab	33.47 a	12.93 b	11.53 a

Different superscripts within column are significantly different ($P < 0.05$).

Data in table (3) revealed that all studied characteristics of Awassi ram semen were significantly ($p<0.05$) deteriorated (independent of BSA addition) due to dilution, cooling and storage for three consecutive days, but with different responses. Dilution process was significantly affected pH, motility and dead sperm percentages. Cooling process was significantly affected all semen characteristics except acrosome defect percentage. For first day of storage only acrosomal defect percentage were significantly increased. In the second day of storage motility and acrosomal defect percentage revealed significant difference. By the third day of storage all characteristics were significantly affected except dead sperm percentage. However, it seems that semen characteristics have different mode for response to semen preparation process and storage, this facts have previously been documented by several authors (4, 6, and 16). Therefore, best chilled preserved semen could be applied as short as possible of preservation period with high attention to preparation processes. ROS will be accumulated sooner or later as main products of lipids peroxidation (5, 10, 22), thus adding antioxidants to the diluents of raw semen will be of great benefit to keep good semen quality especially if BSA was used.

Up to our knowledge it is the first time to study the effect of adding BSA to Awassi ram semen, more studies are needs to establish promise techniques for Awassi rams semen preservation. In conclusion, Awassi rams semen could be

successfully improved by chilled technique at 5˚C using BSA, all BSA levels (5-30mg ml^{-1}) were found to be superior to control in semen characteristics and greatest preserved semen quality were applied by the range of 10-20 mg ml^{-1} of BSA. This study recommended using preserved semen as soon as possible after cooling.

Table 3: Effects of preservation stages on some chilled semen characteristics of Awassi rams (means independent of BSA treatment).

Trait / Preservation stages	pH	Progressive Motility (%)	Dead spermatozoa (%)	Abnormal Spermatozoa (%)	Acrosom Defect (%)
Fresh semen	7.14 a	84.61 a	15.58 d	8.68 a	6.17 a
After dilution	6.82 b	71.55 b	26.05 c	9.43 a	6.38 a
After cooling	6.62 c	55.71 c	33.05 b	12.38 b	7.86 b
Day 1 preservation	6.58 cd	51.90 c	33.48 b	14.43 b	10.24 c
Day 2 preservation	6.47 d	40.00 d	36.14 ab	16.62 c	13.24 d
Day 3 preservation	6.17 e	32.86 e	40.14 a	19.14 d	15.62 e

Different superscripts within column are significantly different ($p < 0.05$).

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تأثير البومين مصل دم الأبقار في بعض صفات السائل المنوي المبرد لكباش العواسي

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الملخص

أُجريت هذه الدراسة على ثلاثة كباش عواسي لدراسة تأثير إضافة الى مستويات مختلفة من البومين سيرم دم الأبقار(**BSA**) كمضاد أكسدة الى المخفف على بعض صفات السائل المنوي للكباش وكذلك للتعرف على المراحل التي يحصل فيها تدهور لنوعية السائل المنوي . تم جمع السائل المنوي بطريقة التحفيز الكهربائي وجمعت العينات للكباش مع بعضها ثم قسمت الى سبعة أجزاء متساوية . خففت بمخفف سترات الصوديوم وصفار البيض وبدون أية اضافة كمجموعة السيطرة (**c**) أو إضافة **5** ،**10**، **15**، **20**، **25**، **30** ملغم /مل من **BSA** للمعاملات من **T1** الى **T6**على التوالي. جرى تقويم نوعية السائل المنوي من خلال تقدير الحركة الأمامية، درجة الحموضة **pH**، النسب المئوية للنطف الميتة ، المشوهة ، وتشوهات الاكروسوم بعد التخفيف، التبريد، والحفظ بالثلاجة بدرجة **5** م لمدة ثلاثة أيام متتالية.

أظهرت النتائج ان أية إضافة تتراوح بين **5-30** ملغم /مل من **BSA** الى المخفف قد حسنت نوعية السائل المنوي وان افضل التراكيز هي عند إضافة من **10–20** ملغم/مل وفيما عدا **pH** ونسبة النطف الميتة فكل الصفات الاخرى تاثرت بشكل معنوي (**$p< 0.05$**) عند إضافة **BSA** بمستويات مختلفة. ان عمليات تحضير السائل المنوي من تخفيف وتبريد وحفظ لثلاثة ايام بالتبريد بدرجة **5**م كلها تسبب انخفاض نوعية السائل المنوي. خلصت الدراسة الى أهمية إضافة **BSA** من (**10–20** ملغم /مل) كمضاد أكسدة الى المخفف وان يتم استخدامه في أسرع وقت ممكن بعد التبريد.

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