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Article

The Effects of Bovine Serum Albumin (BSA) Supplementation on Post-thaw Quality of Cryo-preserved Bull Semen

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Abstract

The present work aimed to study the effect of cryopreservation on individual motility, mitochondrial activity, plasma membrane, acrosome, and DNA integrities of bull spermatozoa in response to the addition of different concentrations of bovine serum albumin (BSA) to frozen semen extender. Four adult fertile healthy bulls were enrolled in this study, semen samples were collected weekly from each bull for six successive weeks using an artificial vagina (of 42°C temperature). After semen evaluation, ejaculates from each bull were diluted in a Tris-based buffer egg yolk extender and cooled to 5°C. The Semen extender was supplemented by five different concentrations of BSA;0 (as a control group), 0.5, 1, 1.5, and 2 g/100 ml. Samples were processed for cryopreservation. Each cooled ejaculate was split into five equal aliquots and diluted with BSA supplemented extender; then semen was packaged in 0.25 ml straws and placed 4 cm above liquid nitrogen before being plunged into a liquid nitrogen tank and stored. Frozen straws were thawed in a water bath at 37°C for 30 sec and then evaluated for assessment of individual motility, mitochondrial activity, and plasma membrane and acrosome integrities. The results of the present study showed that post-thaw sperm motility and integrity of plasma membrane were significantly better (P<0.05) in semen samples containing 1.0% BSA (60.00±2.88 and 59.67±1.45 respectively) than in control sample (52.33±1.45 and 53.67±1.85, respectively). Moreover, BSA 1%-supplemented group had better motility and plasma membrane integrity than the other three groups; supplemented with BSA 0.5%-, BSA

1.5%-, and BSA 2%. Also, acrosome integrity of spermatozoa was significantly better (P<0.05) in the semen sample containing 1.0% BSA (62.33±2.60) than in the control one (57.00±1.53) and the other three groups; BSA 0.5%-, BSA 1.5%-, and BSA 2%-supplemented groups. Increasing the concentration of BSA above 1% appeared to have a deleterious effect on sperm DNA expressed as an increase in the percentage of fragmented DNA, DNA content in the tail of the comet, tail length, and Olive tail moment. These results suggested a protective role of BSA 1% supplementation in bull frozen semen extender that is, in turn, reflected in the improvement of individual motility, mitochondrial activity, and plasma membrane, acrosome, and DNA integrities after the freezing-thawing cycle.

Keywords: Keywords: BSA, post-thaw semen, cryopreserved bull semen, DNA.

1. Introduction

In developing countries, the use of assisted reproductive technologies to increase reproductive performance and livestock productivity needs to be more generalized (Bailey *et al.*, 2003). Use of cryopreserved semen in artificial insemination (AI) technology had become one of the most effective methods used for the improvement of bovine productivity and genetic quality (Watson, 2000). Many challenges are limiting the maximization of using frozen semen from highly superior bulls in AI; one important issue that occurs during cryopreservation is the reduction in the quality and fertility of bull semen due to the multiple physiological and mechanical stresses (Bailey *et al.*, 2003). Even with the application of controlled semen freezing protocols, sperm cells are still facing a lot of changes during cryopreservation such as pH and osmotic changes, cold shock, and cryo-damages (Bailey *et al.*, 2003) which are associated with oxidative stress that in turn is harmful to mitochondria (Pena *et al.*, 2009), plasma and acrosome membranes of spermatozoa (Meyers, 2005) resulting in significant deleterious effects on the structural integrity, functional activity (Andrabi, 2009) and compromising the fertilizing capacity of bull sperm (Gillan and Maxwell, 1999).

Production of reactive oxygen species (ROS) molecules in high levels is stimulated greatly during the cryopreservation process (Baumber *et al.*, 2005) due to lipid peroxidation of the plasma membrane of non-viable spermatozoa (Kankofer *et al.*, 2005); respiration of live spermatozoa during the freeze-thaw process and molecular oxygen that present in semen extenders (Bamba and Cran, 1992). Other stressors such as thermal shock or removal of seminal plasma also led to a high level of ROS (Bailey *et al.*, 2003). Excessive levels of ROS molecules can deteriorate different traits of bull semen such as (motility, plasma membrane, and acrosomal integrity) (Nair *et al.*, 2006).

Supplements added to the semen extender to enhance the semen-keeping quality are called semen additives; these additives can be used for the protection of male gametes from stressful conditions during the cryopreservation process (Andreea and Stela, 2010). The indigenous antioxidant system of the sperm cell isn't enough (Baumber *et al.*, 2005; Nichi *et al.*, 2006) to protect the sperm from in vitro oxidative stress-induced lipid peroxidation (Nair *et al.*, 2006). Furthermore, freezing-thawing cycles also reduce the indigenous antioxidant levels in bovine semen (Stradaioli *et al.*, 2007). Supplementation of antioxidants in the freezing extender may decrease the oxidative stress of ROS production and can improve different sperm parameters after thawing (Donoghue and Donoghue, 1997). Previously, Cocuzza *et al.*, (2007) noted that small levels of ROS are physiologically important for the regulation of normal sperm functions. Therefore, the addition of inappropriate high concentrations of antioxidants could alter

the production of the required level of ROS needed for the completion of sperm physiological processes (Hu *et al.*, 2010). After the above-mentioned stressful conditions occurring during bull semen cryopreservation, the question is to what extent supplementation of semen extender with extracellular cryoprotectant would be useful in overcoming the damaging effect of freezing-thawing process and protecting semen traits during cryopreservation?

BSA is a highly soluble protein that naturally occurs in mammalian semen and can protect the sperm cell from the harmful effects of free radicals in oxidative stress (Fukuzawa *et al.*, 2005). BSA has become a substance of scientific importance due to its antioxidant properties and protective effects on different cells (Roche *et al.*, 2008). Therefore, this current work was conducted to study the effects of different concentrations of BSA supplementation in freezing extender on sperm individual motility, mitochondrial activity, and plasma membrane and acrosome integrities in post-thawed bull semen.

2. Materials and Methods

2.1. Chemicals and Semen Collection

BSA and other chemicals used in this study were obtained from Sigma-Aldrich, USA. This trial was performed on 4 adult healthy bulls that were maintained on the experimental farm of the Animal Reproduction Research Institute (ARRI). Their age ranged between 3 and 4 years and their body weight were 450-700 Kg. Throughout the experimental period, the animals were kept under similar conditions of nutrition and management, each animal received a daily ration composed of about 15 kg green berseem, 4-5 kg concentrates mixture in pellet form, and 4-5 kg rice hay. Water was allowed ad libitum.

In the present study, ejaculates were collected early in the morning, using the pre-warmed artificial vagina (42˚c), and semen samples were collected weekly from each bull for six successive weeks (September – October 2022). Two successive ejaculates with about 10 min intervals were collected from each animal on one occasion. Immediately after collection, semen samples were evaluated for ejaculate volume, and individual motility (%) using a pre-warmed stage of phase-contrast system microscope.

2.2. Processing of Semen for Cryopreservation

After microscopic evaluation, the two ejaculates from each bull were pooled together. Each pooled semen sample was diluted with a Tris-based egg yolk extender. According to (Reddy *et al.*, 2010), Tris-based buffer comprised of Tris 33.2 g/l, citric acid 18.3 g/l, dextrose 7.8 g/l, 7% (V/V) glycerol, 20% (V/V) egg yolk and antibiotics (gentamycin sulphate 500µg/mL, tylosin tartrate 100µg/mL, lincomycin HCl 300µg/mL, and spectinomycin HCl 600µg/mL (Akhter *et al.*, 2011) and double distilled water to make a volume of 200 mL at 37 ˚C in an incubator in an appropriate dilution rate to obtain a concentration of 40×106 sperm/ml for all the ejaculates. Samples were cooled slowly to 5˚C over 1.5 hours.

BSA was added in the above-prepared extender to obtain five different final concentrations: 0, 0.5, 1, 1.5, and 2 g/100 mL (control, T1, T2, T3, and T4, respectively). Each pooled ejaculate was split into five equal aliquots and diluted with BSA supplemented extender; then packaged in 0.25 mL straws (IMV, France). The straws were sealed by using an automatic filling and sealing machine and placed 4 cm above liquid nitrogen in the vapor phase in a foam box for 15 minutes before being plunged into the liquid phase, Khalifa (2001). Straws were transferred to a liquid nitrogen tank and stored. Frozen straws were thawed in a water bath at 37°C for 30 sec. immediately after thawing; straw contents were evaluated.

2.3. Motility of Frozen-Thawed Semen

Immediately after thawing, semen samples were evaluated for individual motility (%) using a pre-warmed stage of phase-contrast system microscope.

2.4. Acrosome Integrity and Abnormalities

Acrosome integrity and abnormalities were evaluated by a dual staining procedure. According to (Didion *et al.*, 1989) after thawing, spermatozoa were incubated with an equal volume of 0.2% trypan blue for 10 min and washed twice (centrifugation at 700g for 6 min) with PBS (phosphate buffer saline). Smears were made on glass slides and dried quickly on a warm stage. Slides were stained with 10% Giemsa stain for 40 min. They were rinsed under a stream of distilled water, air-dried, and covered with coverslips. At least 200 sperm cells were counted. Spermatozoa were classified as acrosome intact [light purple - dark pink acrosome] and damaged/lost acrosome [unstained or blue acrosome]. The abnormal spermatozoa were classified as head, mid-piece, and tail abnormalities (Cevik *et al.*, 2007).

2.5. Plasma Membrane Integrity

The hypo-osmotic swelling test (HOST) was used to evaluate the functional integrity of sperm membrane; the procedure described by (Jeyendran *et al.*, 1984) was used to determine the percentage of HOST-positive cells in each sample based on curled and swollen tails. This was performed by incubating 30 mL of semen with 300 mL of hypo-osmotic solution which is prepared as follows (0.735g of sodium citrate dihydrate and 1.351g of fructose in 100 mL of sterile, de-ionized water). The sample was incubated at 37°C for 30-45min in a 2 ml micro-centrifuge tube. After incubation, 0.2 mL of the mixture was spread with a cover slip on a warm slide for examination by using phase-contrast microscopy. Then 200 sperm were examined with 400x magnification. Sperm with swollen or coiled tails were recorded (Buckett *et al.*, 1997).

2.6. Sperm Mitochondrial Activity

MTT (3-(4, 5-dimethyl-thiazolyl-2)-2, 5-diphenyltetrazolium bromide) test used to determine spermatozoal mitochondrial activity, The MTT reduction assay depends on the ability of metabolically active cells to reduce tetrazolium salt (3[4, 5-dimethylthiazol-2-y1]-2, 5-diphenyltetrazolium bromide) to formazan. MTT assay was performed according to the method of Mosmann (1983)**.** For each frozen-thawed semen sample, six wells of the 96-well microplate were used. The 100 µl of semen sample plus 10 µl of MTT stock solution (5 mg MTT/ml of PBS) was placed in each well. The rates of MTT reduction were determined using an ELISA reader (Thermo Reader) at a wavelength of 550 nm. The optical density of semen samples was measured three times (immediately after thawing, after half an hour, and after one hour of incubation at 37 ◦C). MTT reduction rates (optical density) for each semen sample were calculated by concurring the difference between the first and each of the second (MTT1) and third (MTT2) readings of the ELISA reader.

2.7. Sperm cell DNA integrity

The alkaline comet assay (single-cell electrophoresis) for spermatozoa was carried out according to (Hughes *et al.*, 1996). Full frosted glass slides were covered with 100 µl of 0.5% normal melting point agarose (Sigma), a coverslip was added, and the agarose was allowed to solidify. The coverslips were removed and 1x105 sperm cells in 50 µl PBS (7.2 pH) were mixed with 50 µl of 1.2% low melting point agarose and used to form the second layer. The slides with coverslips removed were then placed in lysis buffer for 1 h (2.5 M NaCl, 100 mM Na EDTA, 10 mM Tris, 1% Triton X at a pH of 10). The slides were then incubated at 37°C in 100 µl/ml of proteinase K in lysis buffer overnight. After draining the proteinase K solution from the slides,

they were placed in a horizontal electrophoresis unit filled with freshly prepared alkaline electrophoresis solution containing 300 mM NaOH and 1 mM EDTA for 20 min to allow the DNA to denature Electrophoresis was performed at room temperature, at 25 V (0.714 V/cm) and 300 mA, obtained by adjusting the buffer level, for 10 min. The slides were then washed with a neutralizing solution of 0.4 M Tris at pH 7 to remove alkalis and detergents. After neutralization, the slides were each stained for 5 min with 50 µl of 20 µg/ml ethidium bromide and mounted with a coverslip. A total of 200 sperm cells were examined under a fluorescent microscope (Leica, Germany, 400X, at 254 nm wavelength). The percentage of non-fragmented (compact sperm head) and fragmented (elongated tail of a comet) sperm nuclear DNA was calculated. The intensity of the fluorescent stain in the comet tail region is presumed to be related to the DNA content, and DNA damage is estimated from measurements of the percent DNA in the tail, tail length, and tail moment, using an image analysis system (Comet-Score program). Spermatozoa with fragmented DNA showed increased migration of the DNA from the sperm nucleus towards the tail forming a "comet" as shown in Figure 1, while spermatozoa with intact DNA do not form a “comet” as shown in Figure 2.

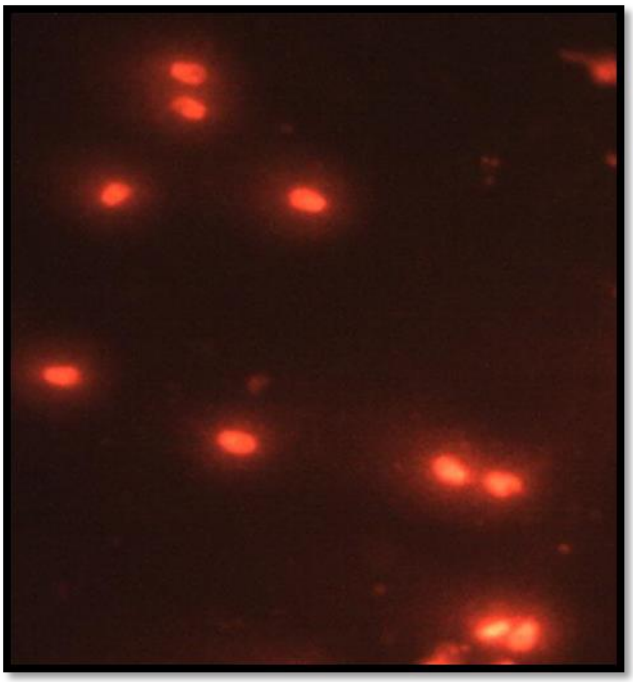

Figure 1. Sperm cells with comet (With DNA fragmentation).

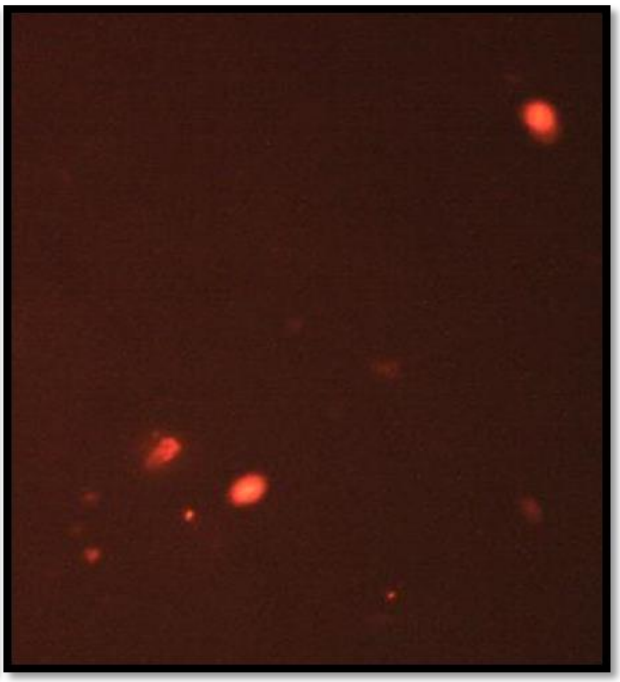

Figure 2. Sperm cells without comet (Without DNA fragmentation).

2.8. Statistical analysis

Data were presented as mean ± SEM (standard error of the mean). Independent-sample T-test and Duncan's multiple range tests were done for the obtained data using the SPSS program version 16.0 and $P \leq 0.05$ was considered statistically significant.

3. Results

The effects of the addition of different concentrations of bovine serum albumin (BSA) in the Tris citric acid (TCA) extender on Individual motility, viability index, mitochondrial activity, total abnormalities, the integrity of plasma membrane and acrosome of bull sperm at post-thawing are shown in Table 1. Most of the tested parameters were significantly better in the semen sample containing 1.0% BSA than in others containing (0.5% - 1.5% - 2% BSA) and control samples ($P<0.05$). Post-thaw sperm motility and integrity of plasma membrane were significantly better ($P<0.05$) in semen samples containing 1.0% BSA than that in the samples

containing (0.5% - 1.5% - 2% BSA) and also better than the control sample. Acrosome integrity of spermatozoa was significantly ($P<0.05$) better in semen extender containing BSA 1.0 than in other BSA concentrations (0.5% - 1.5% - 2% BSA) and control samples under study. Results also revealed that some of the tested parameters did not differ significantly between the control group and other BSA treatments (0.5 – 1.5 – 2) %, also BSA supplementation was not equally beneficial at all different concentrations.

Effects for the addition of different concentrations of bovine serum albumin (BSA) in the Tris citric acid (TCA) extender on DNA integrity (different comet parameters) of bull spermatozoa at post-thawing. are presented in Table 2. The addition of 1% BSA in the Tris citric acid (TCA) extender resulted in the highest ($P \leq 0.001$) DNA integrity (94.85±0.32 %), While the addition of 0.5% BSA to the semen extender was found to exert no significant difference from the control group on DNA in the tail of the comet, percentage of non-fragmented DNA, tail length and olive tail moment. On the other hand, the addition of 1.5% and 2% BSA resulted in the lowest DNA integrity (91.33±0.41% and 90.3±0.39% respectively).

Table 1. Effect of different concentrations of bovine serum albumin (BSA) in the Tris citric acid (TCA) extender on Individual motility, viability index, mitochondrial activity, total abnormalities, the integrity of plasma membrane and acrosome of bull sperm at post-thawing.

Semen trait	Bovine serum albumin (BSA) concentrations					
	Control (0%)	T1 (BSA 0.5%)	T2 (BSA 1%)	T3 (BSA 1.5%)	T4 (BSA 2%)	P-value
Individual motility (%)	52.33±1.45^{b}	48.67±2.33^{c}	60.00±2.88^{a}	48.00±2.08^{c}	46.33±1.85^{d}	0.009
Viability index	149.83±6.65^{b}	146.00±7.76^{b}	175.00±6.29^{a}	134.00±8.12^{d}	135.83±4.64^{c}	0.011
Plasma membrane integrity (%)	53.67±1.85^{b}	50.00±1.15^{ab}	59.67±1.45^{a}	49.66±1.45^{ab}	47.65±1.45^{c}	0.002
Acrosome Integrity (%)	57.00±1.53^{b}	52.33±1.45^{bc}	62.33±2.60^{a}	54.67±1.45^{c}	49.00±0.58^{d}	0.002
MTT reduction rate	0.50±0.01^{b}	0.43±0.02^{d}	0.56±0.01^{a}	0.44±0.02^{bc}	0.46±0.01^{c}	0.001
Total Abnormality (%)	9.00±0.58^{c}	10.67±0.88^{ab}	7.00±0.57^{d}	11.66±0.67^{a}	12.33±0.33^{a}	0.001

Values are mean ±SE. a,b,c,d Values with different superscripts within rows differ significantly, $p < 0.05$ (one-way ANOVA).

Table 2. Effects of different concentrations of bovine serum albumin (BSA) in the Tris citric acid (TCA) extender on DNA integrity (different comet parameters) of bull spermatozoa at post-thawing.

Semen trait	Different concentrations of bovine serum albumin (BSA)					
	Control (0%)	T1 (BSA 0.5%)	T2 (BSA 1%)	T3 (BSA 1.5%)	T4 (BSA 2%)	P-value
Sperm with intact DNA (%)	93.87±0.26^{ab}	93.13±0.46^{b}	94.85±0.32^{a}	91.33±0.41^{c}	90.3±0.39^{c}	0.0001
DNA in the head of Comet (%)	94.49±0.30^{ab}	93.63±0.49^{bc}	94.85±0.15^{a}	93.21±0.39^{cd}	92.41±0.53^{d}	0.001
DNA in the tail of Comet (%)	5.51±0.30^{cd}	6.37±0.49^{bc}	5.15±0.15^{d}	6.79±0.39^{ab}	7.59±0.53^{a}	0.001
Comet tail length (pixels)	5.63±0.51^{c}	5.26±0.29^{c}	4.70±0.31^{c}	8.61±0.48^{b}	11.69±0.74^{a}	0.0001
Olive tail moment	0.59±0.04^{b}	0.58±0.03^{b}	0.44±0.03^{c}	0.66±0.04^{b}	0.86±0.06^{a}	0.0001

Values are mean ±SE. a,b,c,d Values with different superscripts within rows differ significantly, $p < 0.05$ (one-way ANOVA).

4. Discussion

In the current study, BSA supplementation (1 g/100 mL) in semen extender has increased sperm motility characteristics, the integrity of plasma membrane, and acrosome. These results are consistent with the results of (Lornage *et al.*, 1983, and Uto and Yamahama., 1996) who found higher percentages of motile and viable spermatozoa in groups with BSA supplementation.

Post-thaw sperm motility and integrity of plasma membrane were better in semen samples containing 1.0% BSA than that in other samples containing (0.5% - 1.5% - 2% BSA) and also better than the control sample. Our findings are in line with the results reported by (Ashrafi *et al.*, 2013). BSA had beneficial effects on motility, viability, normal morphology, and plasma membrane integrity in bull spermatozoa after a freeze-thaw process. Also agree with what had been assessed by previous studies on bovine semen in which higher sperm plasma membrane integrity was reported after the addition of BSA in the extender (Uysal *et al.*, 2007). It is believed that BSA addition reduces lipid peroxidation of the membrane system that resulted in higher membrane integrity (Lewis *et al.*, 1997). Deterioration of semen variables for tested treatments containing BSA concentrations higher or lower than 1g/100 mL in the semen extender (0.5% - 1.5% - 2% BSA) may be rendered to what was previously mentioned by (Cocuzza *et al.*, 2007) small levels of ROS are physiologically important for regulation of normal sperm functions. Therefore, the addition of inappropriate higher or lower concentrations of antioxidants could alter the production of the required level of ROS needed for the completion of sperm physiological processes (Hu *et al.*, 2010).

Acrosome integrity of spermatozoa was better in semen extender containing BSA 1.0 than in other BSA concentrations (0.5% - 1.5% - 2% BSA) and control samples under study. The obtained results are in a similarity to findings noted by (Ashrafi *et al.*, 2013) who observed that out of four concentrations of BSA tested 1.0% was most beneficial for bull semen in citrate-egg yolk extender that resulted in higher post-thaw motility, the integrity of plasma membrane and acrosome compared with BSA at a concentration of 0.5 %. In contrast to obtained results, (Rahman *et al.*, 2015) reported that Post thaw motility, the integrity of plasma membrane, and acrosome of spermatozoa in 0.5% BSA were better than that in 1.0% BSA ($P<0.05$). As well as, the acrosome integrity of spermatozoa frozen in 0.5% BSA was also better than that in control semen ($P<0.05$). Results of the present work also revealed that some of the tested parameters did not differ significantly between the control group and other BSA treatments (0.5 – 1.5 – 2) % and this is similar to the results recorded by (Rahman *et al.*, 2015) who stated that neither of post-thaw sperm progressive motility or integrity of plasma lemma differed significantly between control and semen containing 0.5% BSA.

The addition of 1% BSA in the Tris citric acid (TCA) extender resulted in the highest DNA integrity, While the addition of 0.5% BSA to the semen extender was found to exert no significant difference from the control group on DNA in the tail of the comet, percentage of non-fragmented DNA, tail length and olive tail moment. On the other hand, the addition of 1.5% and 2% BSA resulted in the lowest DNA integrity. Different methods have been developed for detecting DNA strand damage (Charles, 2005). Comet assay has been established to be a very sensitive method for detecting DNA strand breaks in human sperm (Duty *et al.*, 2002).

The comet assay parameters, tail length, and tail moment provide additional evidence about the level of DNA damage during cryopreservation and therefore increase the sensitivity of the comet assay in detecting low levels of DNA damage (Fraser and Strzezek, 2007).

5. Conclusions

In conclusion, supplementation of (1 g/100 mL) BSA in the freezing medium has beneficial effects on the individual motility, normal morphology, mitochondrial activity, and plasma membrane & acrosome integrities in bull spermatozoa after freeze-thaw process and needs further investigation.

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مقال

الملخص العربي

تأثير إضافة مصل ألبومين الأبقار على كفاءة تجميد السائل المنوي لطلائق الأبقار

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يهدف العمل الحالي إلى دراسة تأثير الحفظ بالتبريد على الحركة الفردية، ونشاط الميتوكوندريا، وغشاء البلازما، والأكروسوم، وتكامل الحمض النووي لحيوانات الثور المنوية استجابةً لإضافة تراكيز مختلفة من مصل الألبومين البقري إلى مخفف السائل المنوي المجمد. تم استخدام أربعة ثيران بالغة سليمة في هذه الدراسة، وتم تجميع عينات السائل المنوي أسبوعياً من كل ثور على مدى ستة أسابيع متتالية باستخدام مهبل اصطناعي (بدرجة حرارة 42 درجة مئوية). بعد تقييم السائل المنوي، تم تخفيف السائل المنوي من كل ثور في مخفف صفار البيض وتم تبريده إلى 5 درجات مئوية. تم عمل خمس تركيزات مختلفة؛ 0 (كمجموعة تحكم)، 0.5، 1، 1.5 و2 جم / 100 مل. تمت معالجة العينات للحفظ بالتبريد. تم تقسيم كل قذفة مبردة إلى خمسة أجزاء متساوية وتم تخفيفها باستخدام المخفف الذي يحتوي على مصل الالبومين البقري؛ ثم تمت تعبئة السائل المنوي في قصيبات 0.25 مل، ووضعها 4 سم فوق النيتروجين السائل قبل تخزينها في النيتروجين السائل. تم تسييح القصيبات المجمدة في حمام مائي عند 37 درجة مئوية لمدة 30 ثانية ثم تقييمها لتقييم الحركة الفردية ونشاط الميتوكوندريا وغشاء البلازما وتكامل الأكر وسوم. أظهرت نتائج الدراسة الحالية أن حركة الحيوانات المنوية بعد الذوبان وسلامة غشاء البلازما كانت أفضل بكثير (P <0.05) في عينات السائل المنوي المحتوية على BSA 1.0٪ (2.88 ± 60.00 و59.67 ± 1.45 على التوالي) من عينة التحكم (52.33 ± 1.45 و53.67 ± 1.85 على التوالي). علاوة على ذلك، كان لدى المجموعة المدعومة بـ 1 BSA٪ قدرة حركية أفضل وسلامة غشاء بلازما أفضل من المجموعات الثلاث الأخرى؛ مكمل بـ 0.5 BSA٪ - و1.5 BSA٪ - و2 BSA٪. يبدو أن زيادة تركيز BSA فوق 1٪ له تأثير ضار على الحمض النووي للحيوانات المنوية معبرًا عن زيادة في النسبة المئوية للحمض النووي المجزأ ومحتوى الحمض النووي في ذيل المذنب وطول الذيل وعزم ذيل الزيتون. اقترحت هذه النتائج دورًا وقائيًا لمكملات 1 BSA ٪ في مخفف السائل المنوي المجمد للثور والذي ينعكس بدوره على تحسين الحركة الفردية ونشاط الميتوكوندريا وغشاء البلازما وتكامل الحمض النووي والأكر وسوم بعد التجميد.

الكلمات الأساسية: BSA، السائل المنوي بعد الذوبان، السائل المنوي للثور، الحمض.