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Influence of bovine serum albumin supplementation in semen extender on quality of stallion spermatozoa

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Abstract

The present work was designed to study the effect of different concentrations of bovine serum albumin (BSA) in INRA-82 extender on quality of equine cooled-stored and frozen spermatozoa. Semen was collected from four pure Arab stallions (10 to 12 years old) from Al-Zahraa horse stud on a regular basis (one ejaculate / week) duringAugust throughOctober, 2015. After collection, semen was evaluated using conventional methods. Centrifugation at 600xg for 15 mins, partial removal of seminal plasma leaving 5%, and dilution of pelleted spermatozoa using INRA-82 extender containing different concentrations of BSA (0, 10,15 and 20mg/ml) was carried out. Thenceafter, diluted semen was cooled at 5°C and was kept frozen at -196°C. Evaluation of chilled semen at 5°C revealed that inclusion of INRA-82 extender with BSA at 10, 15, 20 mg/ml diluent showed that, BSA 10mg/ml was significantly($p < 0.05$)higher (80.80%) than the other two concentrations (15, 20mg/ml) of BSA (77.98, 78.30%, respectively) and control (77.88%) in live sperm percent. Evaluation of frozen semen after thawing at 37°C for 30 sec, revealed that BSA 10mg/ml was significantly($p < 0.05$) higher in post-thaw motility, viability index and HOST than the other two concentrations and control. In view of the pesent results, it can be concluded that cold-storage (+5^0 C) and freeze-thaw processing of stallion semen in the presence of BSA antioxidant (10 mg/ml) achieved a significant amelioration in the maintenance of ejaculated sperm quality parameters.

Keywords: Stallion semen, bovine serum albumin, sperm viability, motility, HOST.,

Introduction

Modern equine reproduction, in most breeds, is based on the use of artificial insemination (AI). The widespread use of AI has accelerated genetic progress by making selected stallions available to breeders outside **et al., 2007).**

The storage of spermatozoa is associated with a reduction in cell viability and fertilizing capacity. In many species, including horses, peroxidation of plasma membrane lipids [lipid peroxidation (LPO)] and other cellular components is a major factor involved in sublethalcryodamage to the spermatozoa **(Aitken et al., 2007).** The particular susceptibility of the sperm plasma membrane to peroxidative damage is because of a high cellular content of polyunsaturated fatty acids. The fluidity of the plasma membrane that the spermatozoon needs to participate in the membrane fusion events associated with fertilization is attributed to its unsaturated fatty acids contents **(Lenzi et al., 1996; Flesch and Gadella, 2000).** However, these molecules of fatty acids are also vulnerable to attack by reactive oxygen species (ROS). Excess production of ROS during cryopreservation has been associated with reduce post-thaw motility, viability, membrane integrity, sperm function and fertility (**Uysal** and **Bucak, 2007**). **Aitken** the country or region where the stallion is located **(Paglet al., 2006).**Development in semen technology has become an area of renewed interest for the equine industry due to increase in use of AI using chilled or frozen–thawedsemen(**Samper**

and fisher (1994) and**Griveau and Lannou (1997)**reported that the impact of oxidative stress could be reduced by addition of molecules of antioxidant, and thus improve semen quality after thawing. **Ball et al. (2001)** mentioned that a wide range of enzyme scavengers and antioxidants have been used in attempts to block or prevent oxidative stress in a variety of cell systems. A number of antioxidant systems have been tested in attempts to prevent oxidative stress in semen from a variety of species **(Ball et al., 2001).**

Bovine serum albumin (BSA) has ability to protect sperm membrane integrity from heat shock during freezing- thawing of semen. In addition, it has been considered as a lipid peroxidation inhibitor **(Alvarez and Storey, 1993)** and is known to eliminate free radicals generations by oxidative stress **(Lewis et al., 1997; Uysal et al., 2007).** However, few reports have addressed application of BSA in preservation of equine semen. Therefore, the objective of the present work was to determine the ability of BSA -supplemented

in semen extender- to improve stallion sperm quality parameters during storage at 5°C and duringcryopreservation

Material and Methods

All chemicals used in the present work were purchased from Sigma (Madrid, Spain). The medium used for washing and centrifugation was INRA-82. It was prepared by mixing equal amounts of glucose-saline solution and ultra-heat skim milk **(Vidament et al., 2000)**. It contained antibiotics (Penicillin and Gentamycin).

Experimental Design

- **Stallions**

The experiment was carried out during August throughOctober, 2015 using four adult healthy pure Arabian stallions (10 and 12 years old) from Al-Zahraa horse stud. The animals were housed in individual boxes bedded with straw and were exercised daily. Each stallion fed 3-4 kg of a balanced grain ration plus 6-7 kg of barseem hay daily. Water was provided ad libitum.

- **Semen collection**

Semen was collected from stallions using a lubricated and pre-warmed (45-50^0 C) Missouri-model artificial vagina (All Vet. Supply Inc., USA) on a regular basis (one ejaculate / week). An in-line disposable nylon mesh gel filter was used to exclude the gel fraction of the ejaculate. Before beginning the experiment, the extra-gonadal sperm reserves were minimized and stabilized in each stallion by daily semen collections over a period of five consecutive days. Thereafter, ten ejaculates were collected from each stallion over a period of ten weeks. Semen collection was performed early in the morning and a mare in estrus was used as a mount animal.

- **Semen evaluation**

The semen samples were transported to the laboratory and placed in a water bath at 37^0 C for processing within 20 min after collection. The semen was examined for basic semen characteristics that included color, consistency, pH, volume (gel free fraction), sperm motility and concentration. Sperm morphology and viability were assessed using eosin–nigrosin stain **(Evans and Maxwell, 1987)**.

- **Semen processing**

Immediately after collection, the filter was removed. The filtered semen was then diluted at a ratio of 1:1 with a non-fat dry milk glucose- based semen extender (INRA-82). Only semen samples with more than 60% motile, viable, morphologically normal sperm and sperm density of > 250×10^6 sperm/ml were used for cryopreservation.

- **Evaluation of processed semen:**

The experiment was done to investigate the effect of bovine serum albumin (BSA) - supplemented in semen extender- on sperm quality parameters stored at refrigerator temperature (+ 5^0 C) and during cryopreservation (-196°c) .

For this purpose forty ejaculates were used. Each ejaculate was divided into four aliquots corresponding to different treatments: without any supplementation (control); supplemented with BSA in final concentrations of 10, 15 and 20 mg/mL. All aliquots were diluted 1:1 (v/v) with INRA 82 extender [INRA-82 consists of: 25 g / L glucose monohydrate, 1.5 g L−1 lactose monohydrate, 1.5 g / L raffinosepentahydrate, 0.4 g / L potassium citrate monohydrate, 0.3 g / L sodium citrate dihydrate, 4.76 g HEPES, pH 7.0, 500 mg / L penicillin, 500 mg /L gentamycin, and 0.15% skim milk **(Vidament et al., 2000)**. All diluted samples were centrifuged at 600 x g for 15 minutes using a microtube centrifuge (800 Electronic centrifuges, China). After centrifugation, nearly 95% of the supernatant was discarded immediately with minimal time delay. Post-centrifugation sperm pellets were re-suspended to a concentration of 100 x10^6 sperm cells / mL with the same volume of freezing medium (INRA 82 enriched with egg yolk 15% and glycerol 5%). Tubes with diluted samples were put in an incubator at 37^{0}C and then cooled slowly to +5^{0}C (for 90 min). Next, after 15 min equilibration at + 5^{0}C, the semen was filled into 0.5 ml plastic straws, sealed with a sealing powder and placed 4 cm above liquid nitrogen in the vapor phase in a foam box for 10 min before being plunged into the liquid phase **(Cristanelli et al., 1985)**. Straws were then transferred to liquid nitrogen (LN) container. The cryopreserved samples were stored in LN for a minimum of one week until examination.

For cooled (+5°C) semen: sperm motility, viability and functional membrane integrity were evaluated.

For post-thaw examination, two straws per treatment were thawed in a water bath at 37°C for 30 sec. Sperm motility, viability, acrosome integrity and hypo-osmotic swelling test (HOST) were assessed.

Individual sperm motility was recorded for cooled and just after thawing, 1, 2 and 3 hours post-cooling and post-thawing. The post-cooling and post-thawing viability indices were estimated according to **Milovanov (1962)**. Living sperm percent was evaluated using eosin – nigros in. Acrosome integrity was estimated using patent specific stain (Spermac; FertiPro N.V.,Beernem, Belgium) according to**Chan et al. (1999)**.

For evaluation of the functional integrity of the plasma membrane of the spermatozoa, hypo-osmotic swelling test (HOST) was performed **(Nie and Wenzel, 2001).**

Stallion No.	Volume (ml)	Motility (%)	Live sperm%	Concentration ($\times10^6$/ml)	Total sperm/ejaculate
1	52.30± 2.40 a	83.00± 0.82 a	87.70 ± 0.72 a	508.00 ± 12.25 a	26569.50 ± 1312.20 a
2	38.50± 1.83 b	76.00 ± 1.00 b	80.30 ± 0.97 b	431.20 ± 16.83 b	16550.00 ± 942.11 b
3	18.30± 1.36 c	72.00 ± 0.82 c	77.80 ± 0.83 c	423.10 ± 15.49 b	7677.40 ± 575.96 c
4	56.50± 2.12 d	68.00 ± 1.11 d	75.40 ± 0.83 c	518.60 ± 14.27 c	29237.00± 1163.45 a
Overall mean(N=40)	41.40 ± 2.56	74.75 ± 0.99	80.30 ± 0.841	470.21 ± 9.93	20.00×10^9± 1456.37

Semen samples were placed on a microscope slide and cover-slipped for examination using phase contrast microscopy (400 X) to evaluate 100 spermatozoa for evidence of swelling and curling changes.

Statistical analysis

The results are shown as means and standard errors. Data were normalized using arc-sine transformation. Statistical analyses were done according to **SnedecorandCochran (1989).**

Results

I-Fresh semen :

In general, the color of ejaculated semen of Arabian stallions was greyish whitish with PH around 6.7.

Table1: Fresh semen characteristics of Arabian stallions (Mean ±SEM)
(n=10 ejaculates/ stallion)

Means with different alphabetical superscripts (a, b, c…) within the same columns are significantly different at least at $P < 0.05$.

The values for raw semen characteristics are summarized in Table1. Stallion factor affected significantly ($p < 0.05$) all semen parameters examined.

II- Chilled semen

Table 2: Effect of BSA antioxidant on the studied parameters of chilled (5°C) stallion semen (Mean ± SEM).

Concentration of antioxidant	Progressivemotility (%)	live sperm (%)	Viability index
Control (n=40)	61.63^{a} ±0.68	77.88^{b} ±0.34	176.05^{ab} ±3.06
BSA1(10mg/ml (n=40)	62.62^{a} ±0.72	80.80^{a} ±0.46	183.30^{a} ±3.30
BSA2(15mg/ml) (n=40)	58^{b} ±0.86	77.98^{b} ±0.43	173.11^{b} ±4.03
BSA3(20mg/ml) (n=40)	56.25^{b} ±0.84	78.30^{b} ±0.48	168.56^{b} ±3.92

(n= Number of ejaculates) BSA = Bovine Serum Albumin

Means with different alphabetical superscripts (a, b, c…) within the same columns are significantly different at least at $P < 0.05$.

The results of supplementation of bovine serum albumin (BSA) in INRA- 82 equine semen extender at different concentrations (10, 15 and 20 mg/ml) on cooled-stored spermatozoa (+5^{0} C) are shown in Table 2. There was no significant difference between inclusion of BSA, 10 mg/ml, and control samples in terms of progressive motility and viability index. The other two concentrations (15 and 20 mg/ml) exhibited a significant ($p < 0.05$) decrease in progressive motility with no differences in live sperm percentage and viability index of spermatozoa compared to control. The results showed that INRA-82 diluent supplemented with BSA -10 mg/ml- was significantly superior to other concentrations (15 and 20 mg/ml) used for stallion semen cooled and stored at + 5^{0} C.

III- Frozen semen

progressive motility, structural membrane integrity (HOST) and viability compared to control.

As presented in table (3), the equine spermatozoa stored frozen in BSA (10 mg/ml) INRA-82 extender maintained significantly ($p < 0.05$) higher post-thaw

Table 3: Effect of BSA antioxidant on the studied parameters of frozen stallion semen (Mean ± SEM).

Concentration of antioxidant	Progressive motility (%)	live sperm (%)	Viability index	HOST (%)	Acrosome integrity (%)
Control (n=40)	47.63^{b} ±0.95	72.45^{ac} ±0.55	137.17^{b} ±2.94	73.73bd ±0.51	76.65^{ac} ±0.53
BSA1 (10mg/ml) (n=40)	51.38^{a} ±1.15	74.07^{a} ±0.66	148.49^{a} ±3.39	75.82^{a} ±0.64	77.85^{a} ±0.47
BSA2 (15mg/ml) (n=40)	45.50^{b} ±1.13	70.58^{b} ±0.74	127.81^{b} ±3.68	72.22^{b} ±0.52	78.18^{ab} ±0.47

BSA3 (20mg/ml) (n=40)	46.00^{b} ±1.16	72.02^{bc} ±0.62	129.66^{b} ±3.70	71.85^{bc} ±0.62	78.48^{ab} ±0.58

Means with different alphabetical superscripts (a, b, c...) within the same columns are significantly different at least at P< 0.05.

(n= number of ejaculates), BSA= Bovine Serum Albumin, HOST= Hypo-osmotic swelling tes

among the three levels ofBSA. The results demonstrated that spermatozoa exposed to BSA at 10 mg/ml in INRA-82 stallion semen extender and stored frozen were superior ($p < 0.05$) in progressive motility, live sperm percent, viability index and structural membrane integrity concentrations (15 and 20 mg/ml) used. Also, progressive motility, live sperm percent, HOST, and viability index of post-thaw spermatozoa improved by addition of BSA at 10 mg /ml concentration than those in any of the other two concentrations (table, 3). The acrosome integrity of post-thaw spermatozoa showed no significant difference

Discussion

The current work, which was performed on a total forty semen ejaculates obtained from four stallions, provides a detailed description of raw, cold-stored at + 5^{0} C and frozen semen characteristics in Arabian horse. Results of raw semen showed that the stallion significantly influenced all parameters evaluated in raw semen (table, 1). The large differences noted in semen quality correspond with finding in other horse breeds(**Dowsett andKnatt, 1996; Janett et al., 2003; Janett et al.,2012**).Age (**Dowsett and Knatt, 1996**), season of semen collection (**Magistrini et al., 1987**)and duration of sexual rest (**Magistrini et al., 1987;Siemeet al., 2002**) also may influence semen quality. The results of the present study showed that equine spermatozoa cold-stored at +5^{0} C and frozen in INRA-82 extender supplemented with 10 mg/ml BSA improved, to some extent, all parameters of sperm quality studied. This aspect is of high importance, since most studies dealing with stallion semen only evaluate post-thaw sperm motility. Moreover, the plasma membrane of spermatozoa acts as the principal physical barrier to outside environment and it is a main site for freeze-thaw damage (**Ricker et al., 2006**). Considering this evidence, the current work paid specific attention to the evaluation of the sperm membranes, both plasma and acrosome membranes. Also, live sperm (completely intact membrane) was considered. It known that INRA-82 extender is a common extender for equine semen preservation at +5^{0} C , and supplementation of this diluent with antioxidants was also beneficial for the preservation of stallion semen at +5^{0} C and freezing (**Klem at al., 1986**). Bovine serum albumin has been shown to possess antioxidant properties and has been incorporated into a number of semen extenders (**Kreider et al., 1985; Klem et al., 1986**). In the current study, addition of 10 mg/ml to INRA-82 extender at cold-stored and freezing of equine spermatozoa was significantly useful, and did enhance maintenance of progressive motility, viability, live sperm percent and plasma membrane integrity (HOST). These finding are in consistent with the observation of previous studies on equine (**Dixon and Kreider, 1981; Kreider, et al., 1985;Klem et al., 1986**) who also detected a positive effect of adding BSA to cooled, extended and frozen equine spermatozoa. Other studies on bulls (**Uysal et al. , 2007 andEl-kon, 2011**) rams(**Matsuoka, et al., 2006 ; Uysal and Bucak, 2007 ; Andrea, et al., 2009; Sariözkan, et al., 2009 ; Elsherbiny, et al.,2013 ; Akhter, et al., 2014**) and goats (**Bucak and Uysal ,2008 ; Andrea, et al., 2010**) recorded that inclusion of BSA to freezing extender at concentrations ranging from 5 to 20 mg/ml enhanced the post-thaw motility, live sperm percentage, functional membrane integrity, percentage of intact acrosome and viability index. The satisfactory results obtained with inclusion of bovine serum albumin to stallion semen freezing and chilling extender, in the present study, may be attributed to the direct evidence that bovine serum albumin adheres rapidly to the sperm membrane at time of dilution (**Blank, et al., 1976**), modifies the lipid composition of the sperm through lipid exchange or hydrolysis (**Davis, et al., 1979**) , promotes plasma membrane protein hydrolysis (**Davis and Gergely, 1979**), causes influx of calcium ions into the cytoplasm (**Blank et al., 1976**)and decreases the cholesterol and phospholipids ratio in the sperm plasma membrane (**Davis, et al., 1980; Singleton and Killian, 1983**).

Furthermore, it is well established that cholesterol efflux from the sperm membrane induces enhanced membrane fluidity **(Grunze, and Deuticke, 1974)**. Therefore, cholesterol efflux from sperm membranes by BSA would enhance the fluidity of sperm plasma membranes, before freezing and enhancing the cryo-survival, of the spermatozoa and regulates the susceptibility of sperm to cold shock **(Yamashiro et al., 2006)**. The sperm plasma membrane contains a large amount of unsaturated fatty acids and is, therefore, particularly susceptible to peroxidative damage, with subsequent less membrane integrity, impaired cell function and decreased motility **(Alvarez et al., 1987; Griveau et al., 1995)**. **Masuda et al. (2006)** suggested that BSA has the effect of maintaining some phospholipid fractions associated with the plasma membrane by reducing lipid peroxidation.Despite storage of equine spermatozoa at + 5^0 C or freezing in INRA-82 supplemented with BSA at 15 or 20 mg/ml, in the current work, improved sperm quality compared to control, some researchers in stallion did not observe improvement in sperm parameters of chilled-stored or spermatozoa frozen after addition of BSA to the extenders. The discrepancy in the results on the effect of bovine serum albumin on frozen and/or chilled semen might be attributed to species variation, diluents used, protocol of freezing, and the used concentrations of bovine serum albumin**(Baumber et al., 2000; Samper et al., 2007)**. The extent to which addition of BSA antioxidant (10 mg/ml) to INRA-82 extender is related to fertility of cooled-stored or cryopreserved stallion spermatozoa needs to be clarified in further studies. In view of results of the study, it can be concluded that cold-storage (+5^0 C) and freeze-thaw processing of stallion semen in the presence of BSA antioxidant (10 mg/ml) achieved a significant amelioration in the maintenance of ejaculated sperm quality parameters.

References

Aitken, J. and Fisher, H. (1994): Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. Bioessays, **16**: 259-267.

Aitken, R. J., Wingate, J.K., De Iulius, G.N. and Mclaughlin, E.A. (2007): Analysis of lipid peroxidation in human spermatozoa using BODIPY C11. Molecular human reproduction, **13**: 203-211.

Akhter, S., Rakha, B. A., Iqbal, R., and Ansari, M. S. (2014): Effect of bovine serum albumin on motility, plasmalemma, viability and chromatin integrity of buffalo bull spermatozoa. Pakistan J. Zool, **46**: 115-120.

Alvarez, J. G. and Storey, B. T. (1993): Evidence that membrane stress contributes more than lipid peroxidation to sublethalcryodamage in cryopreserved human sperm: glycerol and other polyols as sole cryoprotectant. Journal of andrology, **14**:199-209.

Andrea, A., Stela, Z., Coprean, D. and Sogorescu, E. (2009): The effects of cysteine, bovine serum albumin and Vitamine E on the qualitative parameters of frozen thawed ram semen. Annals of RSCB. **14**: 97-103.

Andrea, A., Stela, Z., Coprean, D., Dorina, N., Sogorescu, E. and Busuricu, F. (2010): The effects of antioxidants on the cytological parameters of cryopreserved buck semen. Romanian Biotech.,**53**: 294-298.

Ball, B. A., Medina, V., Gravance, C. G. and Baumber, J. (2001): Effect of antioxidants on preservation of motility, viability and acrosomal integrity of equine spermatozoa during storage at 5^0 C. Theriogenology, **56**: 577-589.

Baumber, J., Ball, B. A., Gravance, C. G., Medina, V. and Davies-Morel, M. C. (2000). The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. Journal of andrology, **21**: 895-902.

Blank, M., Soo, L. and Britten, J. S. (1976): Adsorption of albumin on rabbit sperm membranes. J. membr. Biol. **29**: 401-409.

Bucak, M. N. and Uysal, O. (2008): The role of antioxidants in freezing of Saanen goat semen. Indian veterinary journal, **85**: 148-150.

Chan, P. J., Corselli, J. U., Jacobson, J. D., Patton, W. C. and King, A. (1999): Spermac stain analysis of human sperm acrosomes. Fertilityandsterility, **72**, 124-128.

Cristanelli, M. J., Amann, R. P., Squires, E. L. and Pickett, B. W. (1985): Effects of

egg yolk and glycerol levels in lactose-EDTA-egg yolk extender and of freezing rate on the motility of frozen-thawed stallion spermatozoa. Theriogenology, 24:681-686.

Davis, B. K. and Gergely, A.F. (1979): Studies on the mechanism of capacitation: changes in plasma membrane proteins of rat spermatozoa during incubation in vitro. Biochem. Biophys. Res. Commun. **88:** 613-618.

Davis, B. K., Byrne, R. and Bedigian, K. (1980): Studies on the mechanism of capacitation: albumin- mediated changes in plasma membrane lipid during in vitro incubation of rat sperm cells. Nath. Acad. Sci. USA **77:** 1546–1550.

Davis, B. K., Byrne, R. and Hungund, B. (1979): Studies on spermatozo: the mechanism of capacitation. II. Evidence for lipid transfer between plasma membrane of rat sperm and serum albumin during capacitation in vitro. Biochem. Biophys. Acta. **558:** 257–266.

Dowsett KF, Knott LM. (1996): The influence of age and breed on stallion semen. Theriogenology; **46**:397-412.

El-Kon, I. (2011): Testing usability of Bovine Serum Albumin (BSA) for preservation of Egyptian buffalo semen. American-Eurasian J. Agric. & Environ. Sci. **11:** 495-502

Elsherbiny, H. E. R. (2013): Effect of Some Antioxidant additives on the Viability of Cryo-Preserved Ram Semen.M. V. Sc. thesis, Cairo University.

Evans, G. and Maxwell, W. C. (1987): Salamons' artificial insemination of sheep and goats (**No. Ed. 2**). Butterworths.

Flesch, F. M. and Gadella, B. M. (2000): Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochimica. etBiophysica. Acta. (BBA), **1469**: 197-235.

Griveau, J. F. and Lannou, D. (1997): Reactive oxygen species and human spermatozoa: physiology and pathology. Intern. J.Andro., **20**: 61-69.

Griveau, J. F., Dumont, E., Renard, P., Callegari, J. P. and Le Lannou, D. (1995): Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. J. Reprod. Fertil.,**103** : 17-26.

Grunze, M. and Deuticke, B. (1974): Changes of membrane permeability due to extensive cholesterol depletion in mammalian erythrocytes. Biochem. BiophysActa, **356:** 125-130.

Janett F, Thun R, Niederer K, Burger D, Hässig M. (2003): Seasonal changes of semen quality and freezability in the Warmblood stallion. Theriogenology; 60:453-61.

Janett, F., Sacher, K., Hassig, M. and Thun, R. (2012): Quality of raw and cold-stored in Icelandic stallions. Journal of Equine Veterinary Science, **72**: 1-6.

Klem, M. E., Kreider, J. L., Pruitt, J. B. and Potter, G. D. (1986): Motility and fertility of equine spermatozoa extended in bovine serum albumin and sucrose. Theriogenology, **26:** 569-576.

Kreider, J. L., Tindall, W. C. and Potter, G. D. (1985): Inclusion of bovine serum albumin in semen extenders to enhance maintenance of stallion sperm viability. Theriogenology, **23**: 399-408.

Lenzi, A., Picardo, M., Gandini, L. and Dondero, F. (1996): Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm functionto possible scavenger therapy. Human Reproduction Update, **2**: 246-256.

Lewis, S. E., Sterling, E. S. L., Young, I. S. and Thompson, W. (1997): Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertility and sterility, **67**: 142-147.

Magistrini M, Chanteloube PH, Palmer E. (1987): Influence of season and frequency of ejaculation on production of stallion semen for freezing. J ReprodFertil; **35**:127-33.

Matsuoka, T., Imai, H., Kohno, H. and Fukui, Y. (2006): Effects of bovine serum albumine and trehalose in semen diluents for improvement of frozen–thawed ram spermatozoa. J. Reprod. Dev.**52:** 675–83.

Milovanov, V. K. (1962): The biology of reproduction and the artificial insemination ofanimals.Biologijavosproizyedenijaiiskusstvennocoesemeneniezivotnyh.

Nie, G. and Wenzel, J. G. W. (2001): Adaptation of the hypoosmotic swelling test to assess functional integrity of stallion spermatozoal plasma membranes. Theriogenology, **55**: 1005-1018.

Pagl, R., Aurich, J. E., Müller-Schlösser, F., Kankofer, M. and Aurich, C. (2006): Comparison of an extender containing defined milk protein fractions with a skim milk-based extender for storage of equine

semen at 5°C. Theriogenology, 66: 1115-1122.

Ricker,J.V.,Linfor,J.L.,Delfino,W.J.,Kysar,P.,Scholtz,E.L.,Tabin,F., Crowe,J.H.,Ball,B.A., and Meyers,S.A (2006): Equine sperm plasma membrane phase behavior: the effect of lipid based cryoprotectants. Biol.Reprod. 74: 359-365

Samper, J.C., Estrada, A.J. and McKinnon, A.O. (2007): Insemination with frozen semen. In: Samper, J.C, Pycock, J.F and McKinnonAiO (eds), Current Therapy in Equine Reproduction. Saunders Elsevier USA, St. Louis MO, pp. 285–289.

Sariözkan, S., Tuncer, P. B., Bucak, M. N. and Ulutaş, P. A. (2009): Influence of various antioxidants on microscopic-oxidative stress indicators and fertilizing ability of frozen-thawed bull semen. Acta. Vet. Brno. 78: 463-469.

Sieme, H., Echte, A. and Klug E. (2002): Effect of frequency and interval of semen collection on seminal parameters and fertility of stallions. Theriogenology; 52:313-6.

Singleton, C.L. and Killian, G.J. (1983): A study of phospholipase in albumin and its role in inducing the acrosome reaction of guinea pig spermatozoa in vitro. J. Androl. 4: 150-160.

Snedecor, G.W. and Cochran, W.G. (1989): Statistical Methods. 8th Ed. Iowa State Univ. Press, Ames, IA, USA.

Uysal, O. and Bucak, M. N. (2007): Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. ActaVeterinaria Brno, 76: 383-390.

Uysal, O., Bucak, M. N., Yavas, I. and Varisli, O. (2007): Effect of various antioxidants on the quality of frozen-thawed bull semen. J. Anim. Vet. Adv., 6:1362-1366.

Vidament, M., Ecot, P., Noue, P., Bourgeois, C., Magistrini, M. and Palmer, E. (2000): Centrifugation and addition of glycerol at 22°C instead of 4°C improve post-thaw motility and fertility of stallion spermatozoa. Theriogenology, 54: 907-919.

Yamashiro, H., Wang, H., Yamashita, Y., Kumamoto, K. and Terada, T. (2006): Enhanced freezability of goat spermatozoa collected into tubes containing extender supplemented with Bovine serum albumin(BSA). J. Reprod. Dev. 52: 407-414.

الملخص العربي

أجري هذا البحث لدراسة تأثير التركيزات المختلفة لزلال سيرم الأبقار المضاف الي مخفف الانرا- 82 علي جودة حيامن الخيول العربية المبردة عند درجة 5 سيلزيوس والمجمدة عند درجة – 196 سيلزيوس . تم تجميع السائل المنوي من اربعة خيول عربية اصيلة تراوحت اعمارها من 10 الي 12 سنة وذلك في مزرعة الزهراء للخيول العربية . و قد تم التجميع بشكل منتظم بمعدل مرة اسبوعيا وذلك في الفترة من اغسطس الي اكتوبر2015. بعد التجميع مباشرة تم تقييم السائل المنوي با ستخدام الطرق التقليدية. بعد ذلك تم عمل طرد مركزي للسائل المنوي عند قوة 600 اكس جي لمدة 15 دقيقة لازالة البلازما المنوية وترك 5% منها فقط . تلى ذلك تخفيف الحيامن المركزة في قاع انبوبة الطرد المركزى باستخدام مخفف الانرا-82 مضاف اليه التركيزات المختلفة من زلال سيرم الابقار(0 , 10, 15, 20 مجم/ مل). بعد ذلك تم تبريد السائل المنوي المخفف عند درجة 5 سيليزية ثم تجميده باستخدام النيتروجين السائل عند درجة -196 سيليزيس. تم عمل تقييم للسائل المنوي المبرد و اوضحت النتائج ان اضافة زلال سيرم الابقار بتركيز 10مجم/مل أدت الي تحسن ملحوظ في معامل الحيوية المطلق للحيامن مقارنة بالتركيزات الاخري من زلال سيرم الابقار. اما بالنسبة للسائل المنوي المجمد بعد اسالته في الحمام المائي عند درجة 37 سيليزيوس لمدة 30 ثانية أظهرت النتائج التى تم الحصول عليها ان اضافة زلال سيرم الابقار بتركيز 10 مجم/مل أدت الي تحسن ملحوظ في كل من الحركة الأمامية ،معامل الحيوية المطلق وسلامة غشاء البلازما للحيامن مقارنة بالتركيزات الاخري. وفي ضوء هذه الدراسة نستطيع ان نجمل ان عملية تبريد و تجميد حيامن الخيول العربية في وجود زلال سيرم الابقار (10 مجم/مل) قد أدت الي تحسن ملحوظ في معاييرجودة الحيامن المقدوفة.