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EFFECT OF BOVINE SERUM ALBUMIN AND OXYTOCIN ON QUALITY AND MEMBRANE INTEGRITY OF BULL LIQUID SEMEN

(With 4 Tables and 6 Figures)

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تأثير BSA والاوكسيتوسين على خاصية السائل المنوى المخفف وسلامة أغشية الحيامن فى العجول البقرى

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تمت دراسة تأثير كل من BSA والاوكسيتوسين على خاصية السائل المنوى المخفف للعجول البقرى وكذا على سلامة أغشية الحيامن أثناء الحفظ عند درجة ٤°م. تم تخفيف السائل المنوى باستخدام الستريت وصفار البيض للحصول على تركيز ١٠٠×١٠^٦ حيوان منوى لكل واحد ملليمتر. تم تقسيم العينة الى جزئين : أضيف BSA الى الجزء الأول بالتركيزات الآتية : ٥ ، ١٠ ، ٢٠ ملى جرام لكل ١٠٠×١٠^٦ حيوان منوى وأيضاً عينة بدون إضافة (العينة الضابطة). أضيف الاوكسيتوسين الى الجزء الثانى بالتركيزات الآتية : ٣ ، ٥ ، ١٠ وحدة دولية لكل ١٠٠×١٠^٦ حيوان منوى وأيضاً عينة بدون إضافة (العينة الضابطة). تم فحص العينات يومياً لمدة أربعة أيام بعد حفظها عند درجة حرارة ٤°م. أظهرت هذه الدراسة أن BSA وخاصة التركيزات العالية لها تأثير معنوى فى تحسين الحركة الذاتية للحيامن لمدة طويلة وأيضاً لها تأثير معنوى على كل من نسبة الحيامن الميتة ونسبة التغيرات الغير عادية فى الحيامن. وأيضا له تأثير ايجابي فى الأحتفاظ بحيوية الحيامن وكذالك مستوى الفركتوزفى بلازما المنوى. وأظهر الفحص

بالمجهر الالكترونى أن إضافة ٢٠ ملى جرام من BSA للسائل المنوى المخفف تحافظ على سلامة أغشية الحيامن فى كل من اليوم الثالث والرابع من الحفظ. كما وجد أن إضافة الاوكسيتوسين الى السائل المنوى له تأثير ضار على سلامة أغشية الحيامن.وقد أظهرت هذه النتائج إمكانية إضافة BSA إلى السائل المنوى المخفف للعجول البقرى لتحسين خاصيته وكذلك المحافظة على جودته خلال مدة حفظه.

SUMMARY

The effect of supplementation with bovine serum albumin (BSA) and oxytocin upon the quality of bull liquid semen as well as the degree of membrane integrity of spermatozoa were investigated during storage at 4°C. The semen was diluted by using egg yolk citrate to give $100x10^6$ sperm/ml and divided into two portions. The BSA was added to the first portion as 0.0 mg (control), 5, 10 and 20 mg/100 x 10^6 sperm/ml. The second portion was supplemented with oxytocin as 0.0 I.U (control), 3, 5 and 10 I.U/100 x 10^6 sperm/ml. All samples were stored at 4°C for 4 days as well as examined daily for sperm motility, livability and abnormality percentages. Fructose content, GOT activity and ORT were also determined. The obtained results revealed that, BSA especially the high concentrations, improved sperm motility ($P<0.05$) and alive sperm % ($P<0.05$) and decreased significantly ($P<0.05$) the sperm abnormalities %. BSA had a significant increasing effect on the fructose content, ORT and decreasing effect on the levels of GOT when compared with control. The membrane integrity investigation of the treated samples after 3 and 4 days storage revealed that, high concentration (20 mg) of BSA had a good protective effect for spermatozoa, however, the oxytocin with any concentrations had a very bad effect upon the spermatozoa membrane. These findings suggest the possibility of using the BSA with the diluent for improving the semen quality and prolonged sperm cell survival with good quality.

Key Words: *Bovine Serum Albumin, Oxytocin, Bull Semen*

INTRODUCTION

Artificial insemination (AI) has had a major impact on dairy cattle improvement. Besides the genetic progress, AI has resulted in the control of venereal diseases (Hopkins and Evans, 1989) and the reduction in frequency of undesirable recessive genes (Maxwell and Salamon, 1993). Preservation of semen has been a problem facing livestock breeders since AI was first considered. In vitro, semen can be extended considerably by storing the ejaculated semen under special conditions which ensure that the spermatozoa remain immotile without losing their potential to become fully motile again after storage (Mann and Lutwak-Mann, 1981). This could be achieved by methods that reduced or arrested the metabolism of spermatozoa and thereby prolonged their fertile life (Maxwell and Salamon, 1993).

Liquid storage of semen particularly the effect of decreased temperature on the physiology of spermatozoa has received much attention by early investigators (Nilovanov, et al., 1962; Mann, 1964). Moreover, liquid semen storage for several days has little effect on sperm quality than cryopreservation (Hammerstedt, 1993). Various additives have been incorporated into semen extenders, which might enhance sperm preservation, prolong survivability and fertility including vitamins, Tranquilizer and hormonal preparations (El-Gaafary et al., 1990; Alvareg and Storey, 1992; Vuurner et al., 1992 and Hammerstedt, 1993). Investigation using semen from various mammalian species and birds have indicated that bovine serum albumin (BSA) can promote sperm survival after dilution (Harrison et al., 1982; Klem et al., 1986; Bakst and Cecil, 1992). However, oxytocin injection in mammals appeared to have an immediate stimulation effect on the ejection of spermatozoa and seminal plasma during emission. The long term effect of oxytocin injection have adverse effect on spermatogenesis (Knight and Lindsay, 1970).

The aim of this study is to investigate the effect of addition of bovine serum albumin or oxytocin in liquid extender on sperm motility, survivability and abnormalities percentages. In addition, the changes in sperm plasma membrane were studied.

MATERIAL and METHODS

In the present study, semen samples were collected from three Baladi bulls, which maintained under identical nutritional and managemental conditions at the farm belonging to the Faculty of Vet. Med., Assiut University, Egypt. Bulls were sexually prepared and usually three ejaculates were collected at the early morning hours using artificial vagina and female in estrous as a teaser. Within 2 - 3 minutes after collections, the samples were transferred to the laboratory. The good quality samples were pooled before dilution and kept in water bath at 37°C for the subsequent examination.

The pooled semen was extended with egg yolk citrate to give a final concentration of 100 x 10^6 sperm/ml. Morphology and alive sperm percentages were assessed by using alkaline methyl violet and eosin-nigrosin stains respectively. Secondary abnormalities were recorded specially free lose head and bent tail. The extended semen was divided into two portions. Different BSA doses were added to the first portions as 0.0 mg (control), 5, 10 and 20 mg/100 x 10^6 sperm/ml. Different oxytocin doses were added to the second portions as 0.0 I.U (control), 3, 5 and 10 I.U/100 x 10^6 sperm/ ml. Three samples from diluted treated semen were prepared for each of the above mentioned concentrations in (BSA and oxytocin) and control. All samples (treated and control) were stored in refrigerator (4°C) and examined daily for 4 days for sperm motility, alive sperm % (using eosin nigrosin stain) and sperm abnormalities (especially lose head and bent tail, using alkaline methyl violet stain). The osmotic resistance test (ORT) was performed according to Revell and Mrode (1994). The standard method for testing diluted semen consisted of the incubation of 0.25 ml of extended semen in 1 ml of test solution [fructose 9.0 gm and trisodium citrate 4.9 gm in 1000 ml distilled water; osmolarity (100 mOsm kg^{-1})] for about 40 - 60 min. at 35°C. Following incubation, 10 µl drop was transferred to a warm, clean microscopic slide and cover with coverslip, then examined microscopically to determine (%) of cells showing residual activity.

After daily examination, the samples were centrifuged at 3000 rpm for 20 minutes. The supernatant fluid (seminal fluid and diluent) were collected, then kept at -20°C till used for determination of fructose content according to Dergmeyer (1974). Glutamic Oxaloacetic Transaminase enzyme (GOT) activity was determined spectrophotometrically by means of a test kit supplies by Sclavo, S.P.A. (Italia).

After centrifugation, the sediment was prepared, in the unit of Electronic Microscope, Assiut Univ., for examination by transmission electron microscope for any changes in the plasma membrane of spermatozoa after being stained by uranyl acetate and lead citrate.

RESULTS

The effect of bovine serum albumin (BSA) on bull liquid semen quality stored at 4°C are presented in Table 1 & 2 and Fig. 1 & 2. Sperm motility % increased significantly ($P<0.05$) for all days of storage when compared within each concentration of BSA (Table 1). Only high concentrations of BSA (10 mg and 20 mg) had a significant increasing effect ($P<0.05$) on sperm motility when compared with control samples at all days of storage (Fig. 1 A). However, high concentrations of BSA (10 mg and 20 mg) had a highest effect on alive sperm % especially at 2nd, 3rd and 4th day of storage when compared with control sample (Table 1 and Fig. 1 B). Sperm abnormalities (especially lose head and bent tail)% reduced by the addition of BSA to bull liquid semen in comparison to the control samples stored at 4°C among all days of storage. High concentrations of BSA (10 mg and 20 mg) had a highest reducible effect ($P<0.05$) upon sperm abnormalities (lose head and bent tail) during storage time (Table 1 and Fig. 1 C). The results presented in Table (1) and Fig. (2 A) show the effect of BSA on ORT of bull liquid semen. All concentrations of BSA had a significant increasing ($P<0.05$) effect upon ORT among storage time. The variation of fructose content of seminal plasma after addition of BSA was presented in Table (2) and Fig. (2 B). It was observed that, the overall means of fructose were increased significantly ($P<0.05$) when compared with the control samples in

each day of storage. The changes in the levels of GOT activity was presented in Table (2) and Fig. (2 C). The BSA had a significant decreasing effect on the levels of GOT in all treated samples when compared with control sample in all concentrations of BSA especially high concentrations among all days of storage.

The effect of oxytocin on bull liquid semen quality stored at 4°C are presented in Table 3&4 and Fig. 3&4. Sperm motility % was affected by the addition of oxytocin (Table 3 and Fig. 3 A). All concentrations of oxytocin had a significant increasing effect ($P<0.05$) on sperm motility % when compared with control samples at all days of storage. It was noticed that during 3rd and 4th day of storage, the sperm motility % decreased significantly ($P<0.05$) when compared with first day of storage at all concentrations of oxytocin, but increased significantly when compared with the control samples within the 3rd and 4th day of storage. The results presented in Table (3) and Fig. (3 B) showed the effect of oxytocin and days of storage on alive sperm %. A significantly decrease in alive sperm % ($P<0.05$) was noticed with all concentrations of oxytocin through all days of storage except the first day. However, alive sperm % increased significantly ($P<0.05$) during the 1st, 2nd and 3rd day of storage when compared with the control samples at the same day as well as it decreased significantly ($P<0.05$) at 4th day of storage when compared with the control samples. In the same manner, the sperm abnormalities (Table3 and Fig.3 C) % increased significantly with all concentrations of oxytocin during all days of storage. However, different concentrations of oxytocin had a significant reducible effect on sperm abnormalities % (free lose head and bent tail) among storage time in comparison with control samples. ORT of bull liquid semen (Table 3 and Fig. 4 A) stored at 4°C decreased during (2nd, 3rd and 4th day) storage time when compared with first day of storage. Moreover, the addition of oxytocin had a significant decreasing effect ($P<0.05$) upon ORT during different days of storage. Fructose content in seminal plasma are presented in table (4) and Fig. (4 B). The fructose content significantly decreased ($P<0.05$) after first day of storage. Moreover, the addition of oxytocin significantly decreased ($P<0.05$) fructose content especially at higher concentrations. The variation of GOT activity in seminal plasma of bull liquid semen stored at 4°C for 4

days with oxytocin are presented in Table (4) and Fig. (4C). However, the overall mean of GOT activity increased significantly ($P<0.05$) with days of storage. The GOT activity decreased significantly ($P<0.05$) when oxytocin concentration increased especially at 1st and 4th day of storage when compared with control sample at the same day of storage.

The ultrastructure investigation of the treated samples after 3 and 4 days storage revealed that, the high concentration (20 mg) of BSA had a good protective effect for sperm at 3 rd and 4 th days of storage (Fig. 5 A and Fig. 6 A) as compared with control (Fig. 5 C and Fig. 6 C) respectively. However, supplementation with oxytocin had a very bad action on the sperm (Fig. 5 B and Fig. 6 B). The changes were in the form of swelling and mild disintegration of the plasma membrane (Fig. 5 B) as well as severe disintegration of the sperm plasma membrane (Fig. 6 B).

DISCUSSION

Preservation techniques are becoming increasingly important for sperm cells (Wildt, 1989). The results in this study showed that, the addition of BSA significantly increased sperm motility %. This is in agreement with Bakst and Cecil (1992) who reported that the presence of BSA in fresh and stored turkey semen significantly increased several sperm motility characteristics. The percentages of sperm motility were significantly higher in all treated than in control samples. This finding coincide with that reported by Brillard and Bakst (1990).

Addition of BSA however, increased the motility of spermatozoa, it increased the fructose content in comparison to control samples. This could be attributed that after addition of coating agent (BSA), the motility of spermatozoa, is result of undulatory beating of a cylindrical axon, which required energy. This energy is generally provided by ATP which is generated by mitochondria in the basal or mid regions of sperm (Gibbons, 1983)

An alternative explanation for the obtained results is that, BSA may be coating the sperm (Wishart and Steele, 1990). Moreover, Harrison et al. (1982) and Klem et al. (1986) concluded

that the BSA stimulate sperm motility as well as is adsorbed to the sperm plasma membrane. Such a coating may render the sperm less subject to physical obstacles that could hinder motility (Bakst and Cecil, 1992).

The addition of BSA, especially the high concentration, improve the alive sperm %. This result is in agreement with Maxwell et al. (1997) who concluded that the percentages of alive and motile bull spermatozoa were higher when added BSA. The obtained observations are attributed to, the dilution of semen which contain BSA provides protection from the combined effects of dilution by sheath fluid and any physical damage to the sperm cells (Johnson, 1995). Moreover, the addition of BSA to the medium or extender prevented spermatozoa from binding to surface, this might explain their protective effect that helps to maintain the condition of sperm plasma membrane and prevent the membrane damaged cells (Ashworth, et al., 1994).

The obtained observation in the present study indicates that, the semen characteristics improved after adding BSA when compared with the control samples at 3 or 4 days of storage. This is supported by the findings of Weitze (1991) and Weitze and Petzoldt (1992) who reported that under normal practical conditions a decrease in the fertilizing ability can not be prevented after 3 days of storage, but after supplementation with BSA to the media, the reducible rate was somewhat inhibited.

In the present study, the leakage of GOT into the extracellular medium showed a significantly lower values, (especially at high concentration of BSA) when compared with control samples. This is supported by the present findings of ultrastructure investigation, which are in agreement with the previous findings of Chauhan et al. (1994). Such changes in the activity of GOT were attributed to sperm cell damage as well as to the increased membrane permeability, which lead to leakage of intracellular enzyme. This leakage of enzyme increased the sperm abnormalities and decreased motility (Dhami and Kodagali, 1988 and 1990). The high concentration of BSA had a significant increasing effect on motility and alive sperm % and a significant decreasing effect on sperm abnormalities and GOT activity. Our observations support the statement by Wishart and Steele (1990)

and Bakst and Cecil (1992) that BSA contained a free fatty acids which enhance the sperm metabolism and results in the increase of sperm motility. In addition, BSA coat the sperm which may render the sperm less subject to physical obstacle that could hinder motility (Bakst and Cecil, 1992).

Supplementation of diluted bull semen with oxytocin decreased significantly the alive sperm % and associated with the significant increasing GOT leakage into the extracellular media. Only one available literature agreed with the obtained result where the survivability of spermatozoa was significantly higher than control after when 20 I.U oxytocin was added (Ibrahim, 1988). The bad effect of oxytocin on sperm motility and condition of plasma membrane might be attributed to the blocked fructolysis in the tail which lead to decrease in the intracellular level of cyclic adnosine monophosphate (cAMP). This leads to decrease the mitochondrial protein and enzyme that are required for stimulation of sperm activity which associated with a decrease in RNA synthesis (Ahmed et al., 1984). In addition, blocked fructolysis stimulates phosphodiestrase enzyme activity which leads to a decrease of the cAMP with markedly depressed motility. On the other hand, cAMP acts directly on plasma membrane and regulates the exchange of inorganic phosphate and calcium. So, with a decreased CAMP, the plasma membrane will be damage (Barkay et al., 1984).

In conclusion, the results indicate that BSA added to diluted bull semen, especially 20 mg/100 x 10^6 sperm, improved significantly semen characteristics during storage for 4 days at 4°C. Furthermore, BSA had a good protective effect upon sperm cell membrane as well as increase lifespan of liquid bull spermatozoa with minimizing the membrane integrity.

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Table (1): Effect of different concentrations of bovine serum albumin (BSA) on bull liquid semen quality stored at 4°C.

Days of storage[ψ]	First day				Second day				Third day				Fourth day			
	C*	5 mg	10 mg	20 mg	C*	5 mg	10 mg	20 mg	C*	5 mg	10 mg	20 mg	C*	5 mg	10 mg	20 mg
Sperm motility (%)	65.00 ± 0.82^{a}	70.00 ± 2.45^{a}	74.00 ± 3.74^{a}	82.33 ± 2.05^{a}	62.00 ± 2.16^{a}	65.00 ± 1.63^{b}	72.67 ± 2.05^{b}	76.33 ± 1.24^{b}	52.00 ± 2.16^{b}	62.67 ± 2.05^{b}	65.00 ± 1.63^{c}	73.33 ± 3.40^{b}	32.30 ± 2.05^{c}	54.00 ± 2.94^{c}	62.00 ± 1.63^{c}	66.67 ± 0.93^{c}
Alive sperm (%)	75.50 ± 1.22^{a}	76.34 ± 0.35^{a}	77.94 ± 0.08^{a}	83.13 ± 1.29^{a}	71.41 ± 1.02^{b}	75.63 ± 0.55^{a}	76.10 ± 0.22^{ab}	78.45 ± 0.37^{b}	69.23 ± 0.25^{c}	75.26 ± 0.67^{a}	75.63 ± 0.46^{b}	76.96 ± 0.08^{c}	63.32 ± 2.40^{d}	66.17 ± 0.65^{b}	69.08 ± 0.46^{c}	72.71 ± 1.71^{d}
Abn.[$] sperm (%)	14.67 ± 1.31^{a}	13.10 ± 0.14^{a}	11.93 ± 0.07^{a}	10.50 ± 0.29^{a}	16.28 ± 0.29^{a}	15.92 ± 0.13^{b}	13.03 ± 0.17^{b}	13.09 ± 0.62^{b}	17.81 ± 0.24^{b}	17.66 ± 0.34^{c}	15.28 ± 0.63^{c}	13.32 ± 0.31^{b}	19.46 ± 0.76^{c}	17.05 ± 0.09^{d}	16.40 ± 0.35^{c}	15.47 ± 0.46^{c}
ORT[$$] (%)	40.67 ± 1.70^{a}	47.00 ± 0.82^{a}	48.67 ± 1.25^{a}	50.33 ± 1.25^{a}	34.33 ± 1.25^{b}	45.33 ± 1.25^{b}	45.67 ± 1.70^{b}	48.00 ± 1.63^{ab}	31.00 ± 1.63^{c}	42.00 ± 2.16^{a}	43.33 ± 2.05^{b}	45.00 ± 1.63^{bc}	25.33 ± 1.70^{d}	34.33 ± 1.70^{b}	36.67 ± 1.25^{c}	42.67 ± 2.05^{c}

Means with the same superscript letter in the same row are not significantly different.

[ψ] Just after dilution, sperm motility (85 %), alive sperm (86 %), abnormalities (13 %) and ORT (60 %)

* Control

[$] Secondary abnormalities

[$$] Osmatic Resistaure Test.

Table (2): Effect of different concentrations of bovine serum albumin (BSA) on fructose content (mg/100 ml) and GOT activity (U/L) of bull liquid semen stored at 4°C.

Days of storage[¶]	First day				Second day				Third day				Fourth day			
	C*	5 mg	10 mg	20 mg	C*	5 mg	10 mg	20 mg	C*	5 mg	10 mg	20 mg	C*	5 mg	10 mg	20 mg
fructose content	455.0 ± 1.63^{a}	459.10 ± 1.13^{a}	468.78 ± 1.26^{a}	477.86 ± 0.82^{a}	423.54 ± 0.97^{a}	452.69 ± 1.16^{b}	457.91 ± 0.22^{b}	427.45 ± 1.24^{b}	418.62 ± 1.08^{c}	447.43 ± 0.76^{c}	456.20 ± 0.79^{b}	462.94 ± 1.49^{c}	366.37 ± 3.18^{d}	397.37 ± 1.60^{d}	445.86 ± 1.04^{c}	425.91 ± 2.94^{d}
GOT	40.83 ± 0.66^{a}	39.19 ± 0.65^{a}	36.69 ± 0.58^{a}	33.62 ± 0.34^{a}	48.81 ± 0.52^{b}	39.01 ± 0.36^{ab}	37.54 ± 0.32^{a}	33.85 ± 0.20^{a}	52.03 ± 0.24^{c}	43.48 ± 0.56^{c}	41.82 ± 0.31^{b}	39.85 ± 0.15^{b}	49.61 ± 0.51^{d}	42.67 ± 0.41^{c}	38.77 ± 0.24^{c}	37.57 ± 0.33^{c}

Means with the same superscript letter in the same row are not significantly different.

[¶] Just after dilution, fructose content (493.6 mg/100 ml) and GOT (39.83 U/L).

* Control

Table (3): Effect of different concentrations of oxytocin on bull liquid serum quality stored at 4°C.

Days of storage[φ]	First day				Second day				Third day				Fourth day			
	C*	3 I.U	5 I.U	10 I.U	C*	3 I.U	5 I.U	10 I.U	C*	3 I.U	5 I.U	10 I.U	C*	3 I.U	5 I.U	10 I.U
Sperm motility (%)	65.00 ± 0.82[a]	76.33 ± 1.25[a]	76.67 ± 1.25[a]	76.66 ± 1.25[a]	62.00 ± 0.82[b]	70.67 ± 1.25[b]	76.00 ± 0.82[a]	75.67 ± 1.25[a]	51.66 ± 2.05[c]	61.33 ± 1.25[c]	62.00 ± 2.16[b]	66.33 ± 1.25[b]	30.35 ± 1.70[d]	29.67 ± 2.45[d]	42.67 ± 1.25[c]	41.33 ± 1.70[c]
Alive sperm (%)	75.50 ± 0.51[a]	80.70 ± 0.04[a]	82.42 ± 0.32[a]	86.69 ± 0.49[a]	70.39 ± 0.10[b]	73.60 ± 0.15[b]	71.47 ± 0.49[b]	69.86 ± 0.53[b]	69.92 ± 0.57[b]	73.24 ± 1.46[b]	70.57 ± 1.11[b]	69.00 ± 0.53[b]	62.75 ± 0.90[c]	50.22 ± 0.46[c]	43.90 ± 1.50[c]	40.23 ± 1.27[c]
Abn.[$] sperm (%)	14.67 ± 0.16[a]	13.51 ± 0.08[a]	12.11 ± 0.26[a]	11.21 ± 0.12[a]	16.48 ± 0.12[b]	15.54 ± 0.21[b]	14.24 ± 0.31[b]	13.05 ± 0.09[b]	17.06 ± 0.32[b]	17.56 ± 0.64[b]	16.70 ± 0.27[c]	14.84 ± 0.61[c]	18.80 ± 0.41[c]	18.15 ± 0.45[c]	16.78 ± 0.78[c]	16.56 ± 0.71[d]
ORT[$$] (%)	40.67 ± 0.54[a]	33.14 ± 0.75[a]	30.70 ± 0.26[a]	17.71 ± 0.35[a]	34.00 ± 0.09[b]	33.98 ± 0.95[a]	20.96 ± 0.05[b]	14.57 ± 0.48[b]	31.33 ± 1.09[b]	12.03 ± 0.19[b]	10.00 ± 0.09[b]	7.00 ± 0.16[c]	26.53 ± 0.45[c]	15.08 ± 0.76[c]	9.13 ± 0.95[d]	5.20 ± 0.67[d]

Means with the same superscript letter in the same row are not significantly different.

[φ] Just after dilution, sperm motility (85 %), alive sperm (86 %), abnormalities (13 %) and ORT (60 %).

* Control

[$] Secondary abnormalities

[$$] Osmatic Resistaure Test.

Table (4): Effect of different concentration of oxytocin on fructose content (mg/100 ml) and GOT activity (U/L) of bull liquid serum stored at 4°C.

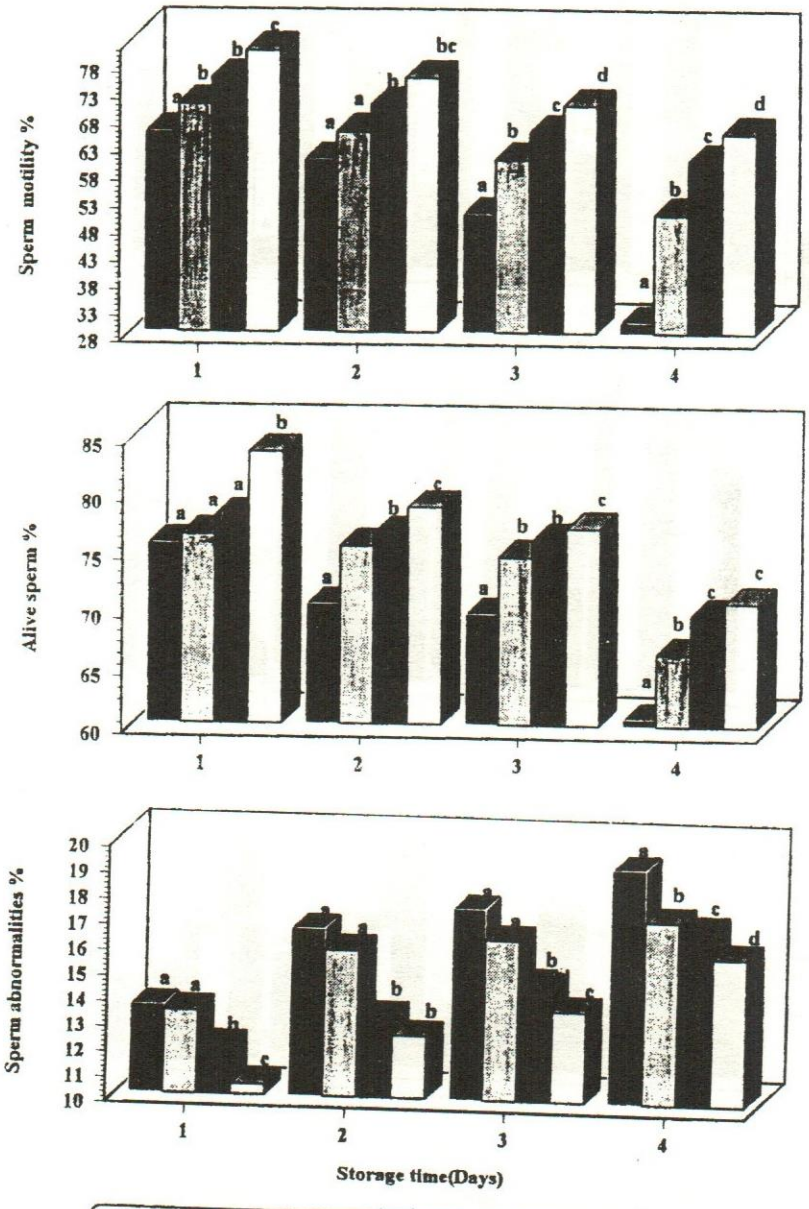
Days of storage*	First day				Second day				Third day				Fourth day			
	*C**	*3 I.U*	*5 I.U*	*10 I.U*	*C**	*3 I.U*	*5 I.U*	*10 I.U*	*C**	*3 I.U*	*5 I.U*	*10 I.U*	*C**	*3 I.U*	*5 I.U*	*10 I.U*
fructose content	455.39 ± 0.44^{a}	480.09 ± 0.13^{a}	435.85 ± 0.15^{a}	524.41 ± 0.89^{a}	421.76 ± 0.55^{b}	440.57 ± 0.49^{b}	428.94 ± 0.61^{b}	422.19 ± 0.86^{b}	418.62 ± 1.08^{c}	437.73 ± 0.52^{c}	427.54 ± 1.14^{c}	416.61 ± 1.19^{c}	363.18 ± 1.35^{d}	303.45 ± 1.30^{d}	274.33 ± 0.94^{d}	243.00 ± 2.95^{d}
GOT	40.89 ± 1.01^{a}	32.34 ± 0.36^{a}	28.11 ± 0.17^{a}	21.06 ± 0.16^{a}	46.02 ± 0.65^{b}	43.58 ± 0.42^{b}	43.95 ± 0.60^{abd}	46.13 ± 0.27^{b}	52.47 ± 0.37^{c}	46.04 ± 0.21^{c}	50.60 ± 0.87^{c}	52.15 ± 0.70^{c}	48.98 ± 0.70^{d}	40.37 ± 1.09^{d}	44.16 ± 1.05^{d}	45.12 ± 0.82^{bd}

Means with the same superscript letter in the same row are not significantly different.

* Just after dilution, fructose content (493.6 mg/100 ml) and GOT (39.83 U/L).

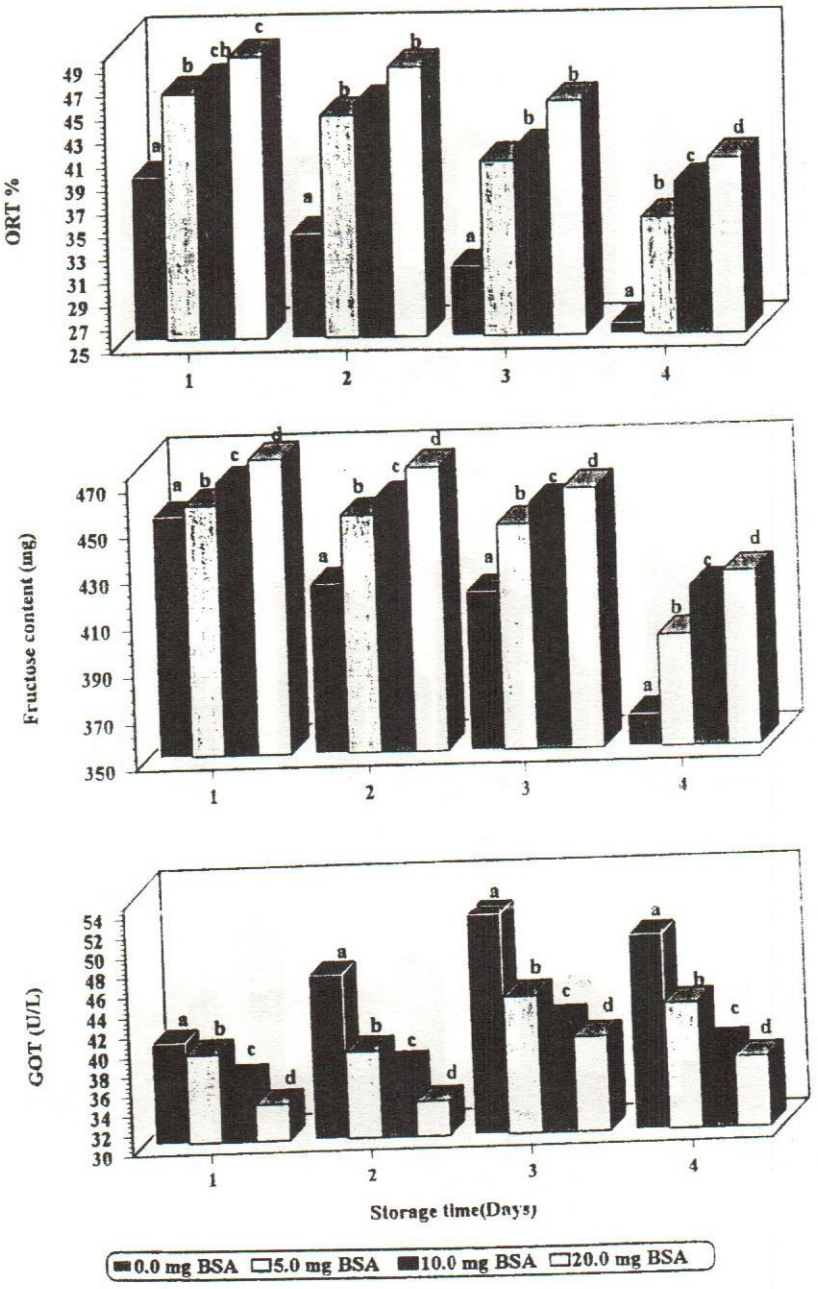
* Control

Figure (1) : Effect of different concentrations of Bovine Serum Albumin (BSA) upon :



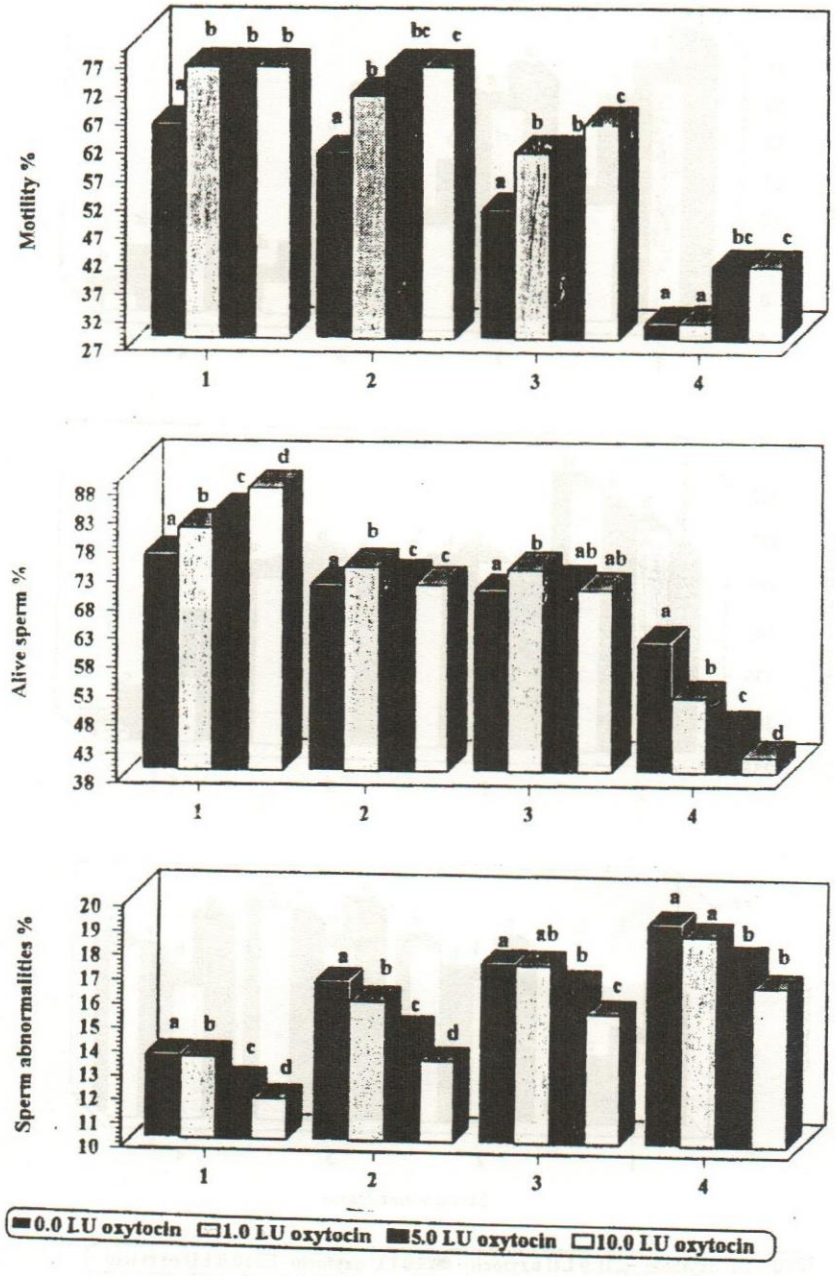
The same superscript letters on the column in the same day are not significant different.

Figure (2) : Effect of different concentrations of Bovine Serum Albumin (BSA) upon :



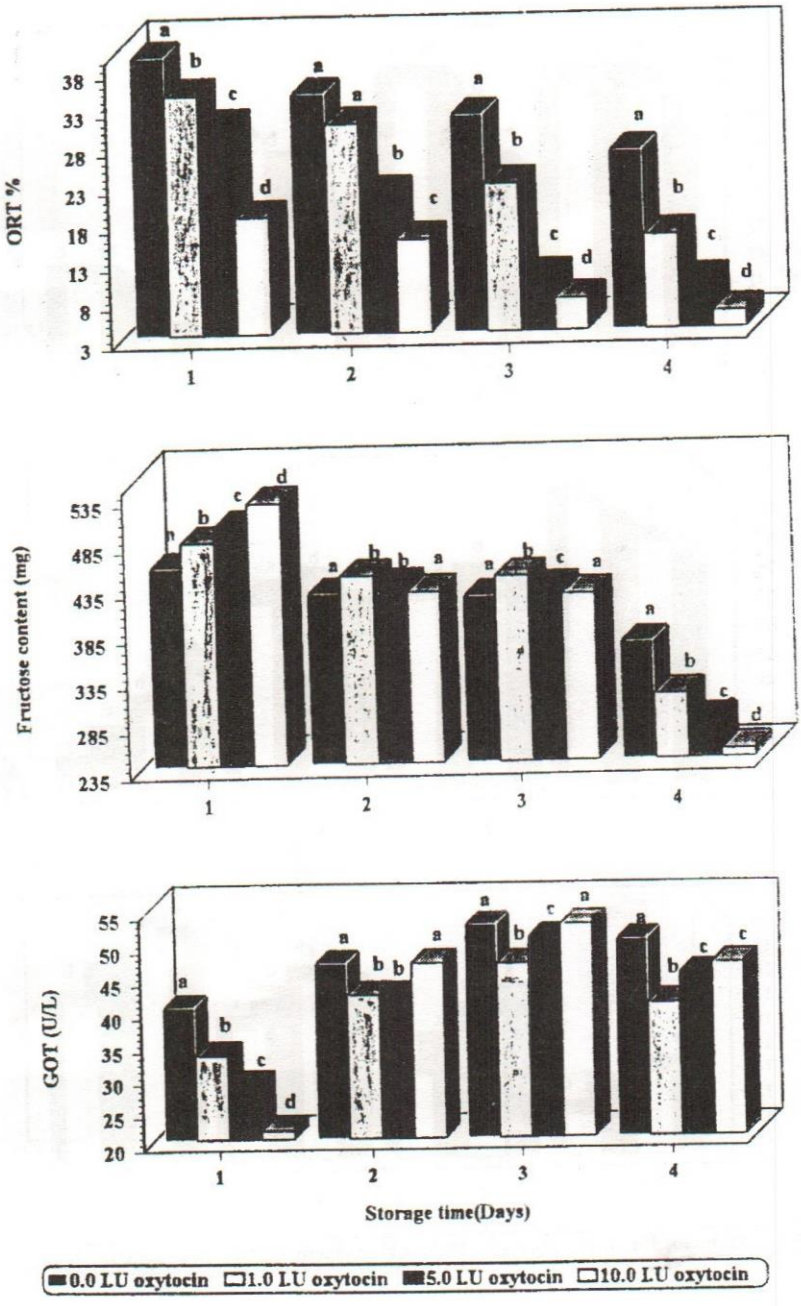
The same superscript letters on the column in the same day are not significant different.

Figure (3) : Effect of different concentrations of oxytocin upon :

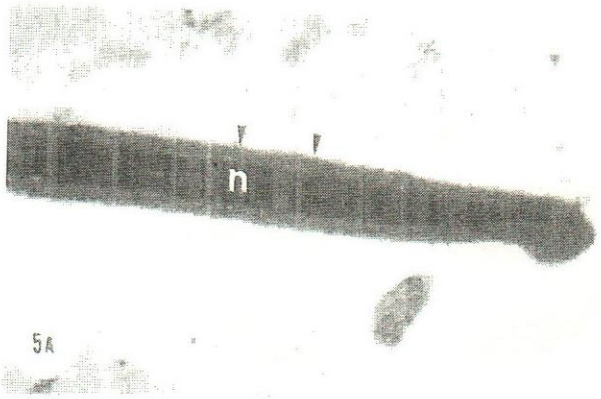


The same superscript letters on the column in the same day are not significant different.

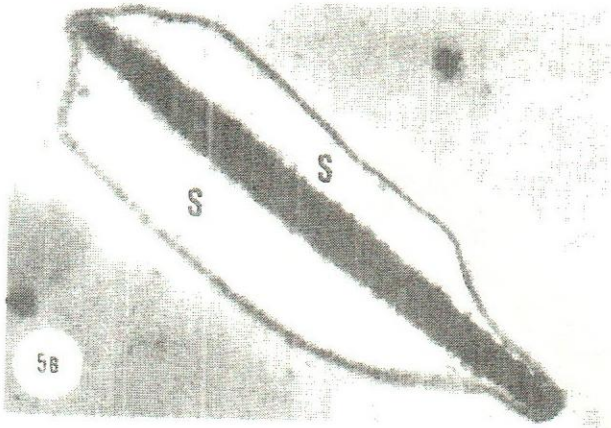
Figure (4) : Effect of different concentrations of oxytocin upon :



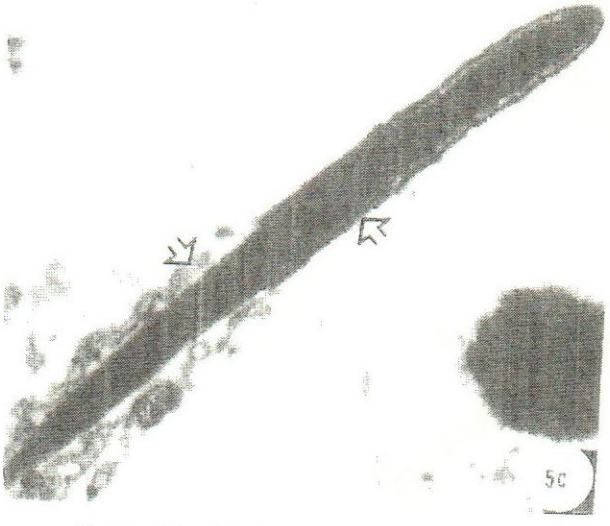
The same superscript letters on the column in the same day are not significant different.



Sagital section through the head of treated bull spermatozoa with 20 mg BSA. Note that a normal intact plasma membrane (arrow head)



Sagital section through the head of treated bull spermatozoa with 20 I.U oxytocin and shows a swelling with mild disintegration of the plasma membrane (arrow head)



Sagital section through the head of untreated bull spermatozoa. Note that a corrugate and rupture of the plasma membrane (arrow head)

Figure (5) : The changes of the ultrasturcture of treated and untreated bull spermatozoa after 3 days of storage.

Sagital section through the head of treated bull spermatozoa with 20 mg BSA. Note that a normal intact plasma membrane (arrow head)

Sagital section through the head of treated bull spermatozoa with 20 I.U oxytocin. Note severe swelling and broken plasma membrane (arrow head)

Sagital section through the head of untreated bull spermatozoa and note a complete loss of inner plasma membrane (arrow head) and severe disintegration.

Figure (6) : The changes of the ultrasturcture of treated and untreated bull spermatozoa after 4 days of storage.