Vet.Med.J.,Giza. Vol.54, No.2. (2006):295-307.

ASSESSMENT OF THE EFFICACY OF SEPHADEX FILTRATION OF BUFFALO SPERMATOZOA FOR CRYOPRESERVATION

MAHA, S. ZIADA**, MONA, M.ALAA EL-DEEN*, A.A.H. ZAKY*,
KARIMAN, D.HASSANIN*
**Artificial Insemination and Embryo Transfer Dept.
*Biology of Reproduction dept. Animal Reproduction Research Institute.
Al-Haram, Giza, Egypt.

Received: 15. 2. 2006
Accepted: 28. 4. 2006

SUMMARY

In the present study, two experiments were conducted. The first experiment aimed to study the influence of buffalo bull semen filtration throughout different grades of sephadex columns (G-25, G-50, G-75, G-100 and G-200) on semen quality. Semen samples were collected from four buffalo bulls, diluted 1:20 with Tris buffer, loaded in different grades of sephadex columns and kept for 4-5 minutes at 37∞C. Filtered semen samples showed significantly ($p<0.0001$) higher individual motility (68.75 - 84.38 %) in different grades of sephadex when compared to control samples (63.13%). Percentages of live spermatozoa were found to be higher for filtered samples (71.38 - 88.63%) than that of control samples (65.94%). Percentages of major and minor abnormal spermatozoa were found to be decreased (2.37 - 4.5% and 1.56 - 5.12%, respectively) significantly ($p< 0.0001$) than that of control samples (5.56 and 6.31%, respectively). Percentages of spermatozoa with acrosomal defects were found to be significantly ($p<0.0001$) lowered (1.44 - 3.88%) significantly ($p<0.0001$) in comparison with control samples (4.63%). The loss in sperm number (0.67 to 0.99 vs 1.25 X 10^9 /ml) could be adequately compensated by obtaining higher values of all other important semen quality traits. The use of G-75 sephadex grade is recommended for wider use because it gives a more balanced picture of semen quality compared with other grades studied.

The second experiment aimed to study the efficacy of sephadex G-75 filtration of buffalo spermatozoa for cryopreservation. Semen samples were collected from the same bulls used in the first experiment. Split samples were diluted 1:20 with Tris buffer, filtered through sephadex G-75 columns and centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded and the sediments were re-diluted and frozen in Obtidyl diluent (Tris based diluent). Filtration was found to improve semen quality after dilution, freezing and thawing. Post-dilution, filtrated sperm motility

was significantly ($p<0.0001$) improved (86.25%) when compared with control samples (67.91%). Post-thaw sperm motility of filtered samples was significantly ($p<0.001$) higher (61.25%) than that of control samples (45.83%). Filtration was significantly enhanced sperm viability after thawing ($p<0.0001$), where viability indices were averaged 151.71 vs 90.83 for control samples. Percentages of spermatozoa with acrosomal defects were significantly ($p<0.0001$) reduced for filtered samples after dilution (1.92 vs 5.83%) and after freezing and thawing (7.75 vs 14.66%). Bull individualities were found prominent for some parameters; post-thaw sperm motility and viability indices ($p<0.05$).

From the present study, filtration of semen samples through sephadex G-75 column could be recommended to improve pre- and post-thaw sperm quality of buffalo bull semen samples.

INTRODUCTION

The negative influence of dead and abnormal spermatozoa on the remaining sperm population (Lindemann et al, 1982) as well as on fertility (Hancock, 1959) has long been known. Various centrifugation gradients (Lessley and Garner, 1983 and White et al., 1984), filtration columns (Graham and Graham, 1990 and Anzar and Graham, 1993) or methods based on active sperm movement, as swim up (Parrish and Foot, 1987) have been used to separate motile from immotile cells, and to enhance the quality of ejaculates. Graham et al. (1976) described a simple method for separating motile from immotile spermatozoa using sephadex G-15-120. The mechanism by which sephadex retains dead, damaged or capacitated spermatozoa (Samper and Crabo, 1993) is still not well understood. It is believed that sephadex particles provide a physical barrier, forcing immotile/dead spermatozoa to agglomerate (Graham et al., 1976). The separation of spermatozoa was probably on the basis of complex and interacting properties of sperm plasma membrane, the medium suspending the sperm and the sephadex particles (Landa et al, 1980). Samper et al (1995) speculated that there was a physico-chemical reaction between sperm plasma membrane bound proteins and sephadex particles. Yet, under the microscope, no binding was seen between sephadex particles and spermatozoa (Anzar and Graham, 1993). It reflects complex binding forces between sephadex and spermatozoal plasma membranes (Graham et al., 1976). This reaction probably reflects the presence of negative charge (acidic negative residues) on the plasma membrane (Cooper and Bedford, 1971 and Yanagiimachi et al., 1972). Graham and Graham (1990) were the first to report a significant improvement in fertility (as non-return rates) for low fertility bulls after removal of dead and abnormal spermatozoa. Therefore, the first experiment of the present study aimed at comparing various grades of sephadex for improving quality of buffalo semen through filtration technique.

Fresh ejaculates of buffalo bulls usually have greater than 65% motile spermatozoa with 85% having a normal morphology (Ziada, 1989 and 1994). With frozen-thawed semen, post-thaw motility (30 to 50 %) is lower with little percentage of spermatozoa with normal morphology. It was found that freezing and thawing did not destroy

the negative surface charge which did not depend on the cell being alive and was maintained after storage for one month at room temperature (Veres et al., 1976). However, El-Sheltawi (1989) found that there was significant correlation between killed cell counts of buffalo frozen semen with its fertility. Therefore, the second experiment was conducted to assist the efficacy of sephadex-filtered buffalo spermatozoa for cryopreservation.

MATERIALS AND METHODS

Experiment I: Comparative evaluation of various grades of sephadex for improving the quality of buffalo semen:

Semen samples were collected from four buffalo bulls, at the Animal Reproduction Research Institute farm, Al-Haram, Giza Province. Slurries of sephadex G-25 (12 % w/v), G-50 (6.0 % w/v), G-75 (4.2 % w/v), G-100 (3.3 % w/v) and G-200 (1.8 % w/v) were prepared by allowing them to swell in 3% sodium citrate buffer for 4 houres at 5∞C (Graham et al. 1976). Prior to use, the sephadex slurries were pipetted into Dispo-van syringes in 0.6 ml quantity. The syringes were placed in a test tube rack for allowing the free drainage of fluid into a collecting vessel (Faycmi et al, 1979). The Dispo-van syringes with external diameter of 1.5 cm containing 0.6 cm and the graduated glass-tubes were then placed in the test-tube rack and the rack was kept in an incubator at 37°C prior to filtration. The complete filtration process took about 4-5 min. in all columns. Semen samples (control and filtrated from different grades of sephadex) were evaluated using standard techniques for individual motility (%), sperm concentration (X109 /ml). Percentages of alive and abnormal spermatozoa (%) in stained smears using eosine-negrosine stain were recorded according to Blom (1983). Percentages of spermatozoa with abnormal acrosomes were counted in stained smears by fast green stain according to Wells and Awa (1970).

Experiment 2: Cryopreservation of sephadex filtred spermatozoa:

Semen samples were weekly collected from the same buffalo bulls used in the 1st experiment. Good quality semen samples (motility> 70% and conc.>0.8 X 10^9 sperm/ml) were divided into 2 aliquots, the 1st one was diluted with Tris buffer and filtered through sephadex 75 columns for 4-5 min at 37°C. The filtrate was then centrifuged at 1500 rpm for 5 min. The supernatant was discarded and the sediment was diluted with Obtidyl diluent (Tris based diluent with ionized egg yolk and antibiotics; pencilline, streptomycin, spectinomycin and lincomycin, made in EEC. Bio-Vet. Fleurance, France). All diluted samples (control and treated) were then cooled to 45°C through 45 min., left for equilibration at 5°C for 2 hours and packaged into French straws 0.25 ml. The straws were then frozen in liquid nitrogen vapour for 15 min in foam box, immersed and stored in liquid nitrogen containers according to Mohamed et al. (1998). After 24 hours, frozen samples were thawed at 37°C for 30 sec. Both diluted and samples were evaluated for individual motility and percentages of spermatozoa with acrosomal defects. Viability indices were estimated for thawed

samples which kept in water bath for 3 hours according to Milvanov (1962).

The obtained data were analyzed statistically, using Costate computer program, version 3.03, Copyright 1986 cottort software according to Snedecor and Cochran (1980).

RESULTS

The effects of filtration of buffalo bull semen through different grades of sephadex on semen quality [sperm motility (%), percentages of alive spermatozoa (%), spermatozoa with major, minor and acrosomal abnormalities (%) and sperm concentration (X 10^9 sperm/ml)] are presented in Tables 1, 2, 3, 4, 5 & 6, respectively.

On the other hand, the efficacy of filtered buffalo spermatozoa through sephadex G-75 for cryopreservation [post-dilution and post-thaw sperm motility (%), post-thaw viability indices and post-dilution and post-thawing acrosomal defected spermatozoa (%)] are presented in Tables 7, 8 & 9, respectively.

Table (1): Sperm individual motility (%) of buffalo semen samples after filtration in different grades of sephadex columns (means ± SE).

Bull no. → Sephadex ↓	Bull no. (1)	Bull no. (2)	Bull no. (3)	Bull no. (4)	Overal Means
Control	62.50±1.44	63.75±2.38	65.00±2.04	1.25±1.25	63.13±0.89^{d}
Sephade/25	71.25±1.25	66.25±2.39	70.00±3.53	67.50±1.44	68.75±1.16^{c}
Sephadex/50	72.50±1.45	71.25±2.38	72.50±1.44	72.50±1.45	72.18±0.78^{b}
Sephadex/75	80.00±2.04	86.25±1.25	86.25±2.39	85.00±2.04	84.38±1.11^{a}
Sephadex/100	78.75±2.39	82.50±1.44	85.00±2.04	83.75±2.39	82.50±1.12^{a}
Sephadex/200	68.75±1.25	75.00±2.04	70.00±2.04	73.75±2.39	71.88±1.10^{b}
Over-all means	72.29±1.38	74.17±1.84	74.79±1.86	73.96±1.87	73.80±0.87

a, b,....etc within columns are significantly different ($p < 0.0001$).

Table (2): Percentages of live spermatozoa (%) of buffalo semen samples after filtration in different grades of sephadex columns (m e a n s ± S E) .

Bull no. → Sephadex ↓	Bull no. (1)	Bull no. (2)	Bull no. (3)	Bull no. (4)	Overal Means
Control	62.75±1.10	69.25±2.05	68.25±2.32	63.50±1.55	65.94±0.95^{c}
Sephade/25	74.25±1.31	74.25±1.75	72.50±2.95	69.50±1.55	71.38±1.07^{d}
Sephadex/50	75.50±1.75	89.75±0.85	76.00±1.95	76.50±1.55	75.56±0.81^{c}
Sephadex/75	85.25±2.05	84.75±1.31	90.50±2.02	89.00±2.27	88.63±0.92^{a}
Sephadex/100	84.75±1.20	79.25±2.49	87.00±1.96	86.25±2.21	85.68±0.85^{b}
Sephadex/200	71.25±1.25	68.25±2.32	71.50±1.71	74.25±2.32	74.06±1.22^{cd}
Over-all means	76.12±1.56	77.25±1.84	77.63±1.89	76.50±1.98	76.87±0.90

a, b,....etc within columns are significantly different (p< 0.0001).

Table (3): Percentages of spermatozoa with major abnormalities of buffalo semen samples after filtration in different grades of sephadex columns (means ± SE).

Bull no. → Sephadex ↓	Bull no. (1)	Bull no. (2)	Bull no. (3)	Bull no. (4)	Overal Means
Control	5.50±0.95	5.50±0.64	5.51±0.64	5.75±0.63	5.56±0.33^{a}
Sephade/25	4.50±0.64	4.25±0.63	4.50±0.28	4.75±0.63	4.50±0.25^{b}
Sephadex/50	4.00±0.71	3.75±0.63	3.75±0.47	3.75±0.62	3.81±0.27^{bc}
Sephadex/75	3.50±0.29	3.00±0.41	3.50±0.64	3.25±0.47	3.31±0.21^{ac}
Sephadex/100	3.25±0.25	2.50±0.29	3.25±0.62	2.75±0.25	2.94±0.19^{cd}
Sephadex/200	2.75±0.25	2.25±0.25	2.50±0.29	2.00±0.41	2.37±0.15^{d}
Over-all means	3.92±0.28	3.54±0.29	3.83±0.27	3.71±0.32	3.75±0.14

a, b,....etc within columns are significantly different (p< 0.0001).

Table (4): Percentages of spermatozoa with minor abnormalities of buffalo semen samples after filtration in different grades of sephadex columns (means ± SE).

Bull no. → Sephadex ↓	Bull no. (1)	Bull no. (2)	Bull no. (3)	Bull no. (4)	Overal Means
Control	62.75±1.10	6.00±0.81	5.75±0.47	6.75±1.03	6.31±0.41[a]
Sephade/25	5.50±1.04	5.00±0.70	5.25±0.85	4.75±1.11	5.12±0.43[b]
Sephadex/50	4.00±0.70	4.00±0.41	3.75±0.48	4.00±0.41	3.94±0.23[c]
Sephadex/75	2.75±0.47	3.00±0.41	4.25±0.94	2.50±0.64	3.13±0.32[c]
Sephadex/100	2.00±0.57	2.25±0.47	1.75±0.48	2.00±0.41	2.00±0.22[d]
Sephadex/200	1.50±0.29	1.75±0.48	1.25±0.25	1.75±0.25	1.56±0.16[d]
Over-all means	3.75±0.47	3.66±0.37	3.67±0.41	3.63±0.44	3.67±0.21

a, b,.....etc within columns are significantly different ($p < 0.0001$).

Table (5): Percentages of spermatozoa with acrosomal abnormalities buffalo semen samples after filtration in different grades of sephadex columns (means ± SE).

Bull no. → Sephadex ↓	Bull no. (1)	Bull no. (2)	Bull no. (3)	Bull no. (4)	Overal Means
Control	5.50±0.95	4.75±0.85	4.50±0.96	4.75±0.48	4.63±0.30[a]
Sephade/25	4.25±0.85	3.75±0.63	3.25±0.48	4.25±0.85	3.88±0.34[ab]
Sephadex/50	3.75±0.75	3.50±0.65	3.50±0.65	3.50±0.65	3.56±0.30[b]
Sephadex/75	1.75±0.25	1.75±0.47	2.00±0.41	1.75±0.48	1.81±0.18[c]
Sephadex/100	1.50±0.29	1.50±0.28	1.75±0.25	1.75±0.25	1.63±0.12[c]
Sephadex/200	1.50±0.28	1.25±0.25	1.75±0.25	1.25±0.25	1.44±0.12[c]
Over-all means	2.87±0.34	2.87±0.33	2.79±0.27	2.88±0.34	2.85±0.16

a, b,....etc within columns are significantly different ($p < 0.0001$).

Table (6):Sperm concentration (x 10^9 /ml) of buffalo semen samples after filtration in different grades of sephadex columns (means ± SE).

Bull no. → Sephadex ↓	Bull no. (1)	Bull no. (2)	Bull no. (3)	Bull no. (4)	Overal Means
Control	1.25±0.09	1.27±0.59	1.25±0.06	1.23±0.05	1.25±0.03[a]
Sephade/25	1.01±0.04	1.03±0.04	0.97±0.02	0.96±0.02	0.99±0.01[b]
Sephadex/50	0.89±0.01	0.89±0.01	0.88±0.01	0.88±0.01	0.89±0.01[c]
Sephadex/75	0.81±0.02	0.81±0.02	0.79±0.02	0.81±0.03	0.81±0.01[d]
Sephadex/100	0.73±0.01	0.76±0.02	0.74±0.02	0.75±0.01	0.74±0.01[e]
Sephadex/200	0.67±0.01	0.67±0.01	0.64±0.01	0.68±0.01	0.67±0.01[f]
Over-all means	0.89±0.04	0.91±0.04	0.88±0.03	0.89±0.03	0.89±0.02

a, b,.....etc within columns are significantly different ($p< 0.0001$).

Table (7): Post-dilution and post-thaw sperm motility (%) of filtered samples in sephadex-75 and frozen in Obtidyl diluent (means ± SE).

Bull no.	Post-thaw sperm motility			Post-thaw sperm motility		
	control	sephadex	Over all means	control	sephadex	Overal Means
(1)	65.00±2.88	85.00±2.89	75.00±4.83	43.33±6.01	61.66±7.26	52.50±5.87[ab]
(2)	70.00±2.89	86.67±1.66	78.33±4.01	48.33±1.66	63.33±1.67	55.83±3.51[a]
(3)	68.33±1.66	88.33±1.67	78.33±4.59	55.00±4.99	70.00±2.88	62.50±4.23[a]
(4)	68.33±4.41	85.0±2.89	76.67±4.41	36.66±8.81	50.00±5.76	43.33±5.57[b]
Overall means	67.91±1.43[B]	86.25±1.09[A]	77.08±2.01	45.83±3.24[b]	61.25±3.02[a]	53.54±2.69

a, b,.....etc within columns and A, B and a,b within raws are significantly different for at least ($p<0.05$).

Table (8): Post-thaw Viability Idices of filtered samples in sephadex-75 and frozen in Obtidyl diluent (means ± SE).

Bull no.	control	sephadex	Over all means
(1)	93.33±±4.41	139.16±10.24	116.25±11.39^{ab}
(2)	90.83±8.21	156.00±10.39	123.41±15.72^{ab}
(3)	118.33±7.94	165.00±2.49	141.67±11.08^{a}
(4)	60.83±24.03	146.66±13.41	103.75±22.80^{b}
Overall means	90.83±8.41^{B}	151.71±5.17^{A}	121.27±7.97

a, b,...etc within columns and A,B within rows are significantly different for at least ($p<0.05$).

Table (9): Post-dilution and post-thaw sperm acrosomal defects (%) of filtered samples in sephadex-75 and frozen in Obtidyl diluent (means ± SE).

Bull no.	Post-thaw sperm motility			Post-thaw sperm motility		
	control	sephadex	Over all means	control	sephadex	Overal Means
(1)	6.00±1.15	2.00±0.57	4.00±1.06	13.66±1.45	7.33±0.88	10.50±1.61
(2)	6.00±0.99	1.66±0.33	3.83±1.07	15.00±1.73	7.33±0.66	11.16±1.91
(3)	5.33±0.88	2.33±0.66	3.83±0.88	14.66±1.85	8.00±0.57	11.33±1.72
(4)	6.00±0.57	1.66±0.66	3.83±1.04	15.33±1.76	8.33±1.20	11.83±1.83
Overall means	5.83±0.40^{a}	1.92±0.25^{b}	3.88±0.47	14.66±0.75^{A}	7.75±0.38^{B}	11.21±0.83

A, B and a,b within raws are significantly different ($p< 0.0001$).

DISCUSSION

The present study was conducted to assess the effect of different grades of sephadex filtration on sperm quality characteristics.

The results from the first experiment demonstrated that sephadex filtration improved sperm quality in terms of motility (Table 1). The significant ($p<0.0001$) improvement recorded in sperm motility of semen filtered through sephadex column supports the earlier findings of Chinnaiya and Sharma (1988) and Chauhan et al (1993). Further, live sperm counts were significantly ($p<0.0001$) higher (Table 3) in the filtrates of sephadex G-75, G-100 and G-200 than those of sephadex G-50 and G-25. This could be attributed to the better filtration efficiency of the higher and finer grades of sephadex column such as G-75, G-100 and G-200. This also showed that these higher grades had better power of separation of immotile and dead spermatozoa as compared to lower grades. It could probably be due to the firm nature of packed beads of smaller diameter of higher grades of sephadex as compared to lower grades viz. G-50 and G-25.The significant increase in live sperm count over the control in the semen-filtered through different columns support the observation of Heer and Tahir (1982), Kumar et al (1989) and Chauhan et al (1993).

Percentages of major and minor sperm abnormalities (Table 3 & 4, respectively) were found to be significantly ($p<0.0001$) lower (4.5 to 2.37 and 5.12 to 1.56, respectively) in semen filtrate of different grades of sephadex than those of control samples (5.56 and 6.31, respectively). Percentages of spermatozoa with acrosomal defects (Table 5) were found to be significantly ($p< 0.0001$) lower for semen samples filtrated through different grades of sephadex columns (1.44 to 3.88) than control samples (4.63). Effective removal of abnormal spermatozoa from cattle (Graham and Graham, 1990, Vyas et al, 1991 and Januskauskas et al, 2005) and buffalo (Kumar et al, 1989 and Chauhan et al, 1993) semen by passing it through sephadex columns has also been reported earlier.

On the other hand, filtration procedures significantly reduced the concentration of recovered spermatozoa (Table 6). The retention of spermatozoa in the columns of sephadex G-25 was significantly ($p<0.0001$) higher (0.99 x 109 /ml) than in the columns of sephadex G-50 and G-75 (0.89 and 0.81 x 109 /ml, respectively). Furthermore, the sperm retention in the sephadex G-100 and G-200 was significantly ($p<0.0001$) lower (0.74 and 0.67x 10^9 /ml, respectively) than the rest of the columns. The significant reduction in number of spermatozoa recovered after sperm sephadex separation was documented for both bull (Januskauskas, 2005) and for buffalo (Chauhan et al., 1993) semen. Although there was a significant decrease in sperm concentration values, this decrease was compensated by proportionate increase in the percentage of motile, live and morphologically active sperm which in turn could lead to the possibility of improving conception rate through artificial insemination. This encourages us to use sperm filtrates through sephadex columns for preparation of frozen buffalo semen.

The second experiment was conducted to determine the influence of sephadex gel filtration on sperm quality prior to and following cryopreservation. This could provide further proof of the value of the technique to recover post-thaw sperm of higher quality. The results of the present study demonstrated (Table,7) that sephadex filtration significantly ($p< 0.05$) improved sperm quality in terms of post-dilution motility (86.25 vs 67.91 %), post-thaw sperm motility (61.25 vs 45.83%). Filtration improvement in semen motility is dependent on semen quality before filtration. In accord with the present study, Anzar and Graham (1995) and Ahmad et al (2003) have reported significant improvement in sephadex filtered sperm motility after dilution of bovine and buffalo semen, respectively. Other studies (Januskauskas et al, 2005) did not observe any significant effect of filtration on motility values.

In the present study (Table 8), post-thaw viability indices were found to be significantly ($p< 0.0001$) improved for sephadex filtered semen samples (151.71) than that of control samples (90.83). Similar to our results, Januskauskas et al (2005) found significant enhanced viable sperm for filtered semen samples compared to non-filtered samples.

Irrespective to the control or filtration treatments, bulls were found to be significantly differed in their post-thaw sperm motility and viability ($p< 0.001$ and $p<0.05$, respectively). Moreover, it was observed that in frozen thawed semen samples, sperm motility and viability results were very close and significantly ($p<0.001$) correlated ($r=0.79$). The same correlation was proved by Ziada et al (1998) and Januskauskas et al (2005).

Freezing and thawing procedures are mostly harmful to sperm membranes (Ziada, 1994), since temperature and osmotically caused changes occur in the organization, fluidity, permeability and lipid composition of sperm membranes (Januskauskas et al, 2005). As intactness of the acrosome is significant to fertility (Saacke and White, 1972), studying the effect of filtration on acrosomal integrity was performed. Higher percentages of spermatozoa presenting acrosomal abnormalities were effectively removed by filtration. In the present study, acrosomal defected spermatozoa (Table, 9) were significantly ($p< 0.0001$) lower percentages after dilution of filtered samples (1.92 vs 5.83) and post-freezing and thawing (7.75 vs 14.66). Previous studies documented significant improvements in the percentage of morphologically normal acrosomes in filtered semen from cattle (Graham and Graham, 1990 and Anzar and Graham 1993 and 1995) and buffalo (Goyal et al., 1996; Ahmad et al., 2003 and Januskauskas et al., 2005).

In conclusion, filtration of buffalo semen samples through sephadex G-75 columns could be recommended to improve post-thaw sperm quality which may increase its fertilizing capacity.

REFERENCES

Ahmad, Z., Anzar, M., Shahab, M., Ahmad, N. and Andrabi, S.M. (2003). Sephadex and sephadex ion-exchange filtration improves the quality and freezability of low-

grade buffalo semen ejaculates. Theriogenol. 59:1189.

Anzar, M. and Graham, E.F. (1993). Filtration of bovine semen. I. Development of Sephadex ion-exchange filter. Anim. Reprod. Sci. 31: 187.

Anzar, M. and Graham, E.F. (1995). Effect of filtration on post-thaw quality of bull semen. Theriogenol. 43: 439.

Blom, E. (1983). The spermiogram of the bull. Nordisk Vet. Med. 35: 105.

Chauhan, S.S., Mohan, G., Kumar, S. and Sahni, K.L. (1993). Comparative evaluation of various grades of sephadex for improving the quality of buffalo semen. Indian J. Anim. Sci. 63: 346.

Chinnaiya, G.P. and Sharma, P.V. (1988). Comparative studies on different column sizes of glass wool and sephadex for improving the quality of semen by filtration technique. Abstract. National Symposium on Recent Trends in Fertility Management of Farm Animals and VII Annual Convention of Indian Society for the Study of Animal Reproduction Trichur,22-24

August, P. 44. Cooper, G.W. and Bedford, J.M. (1971). Acquisition of surface charge by the plasma membrane of mammalian spermatozoa during epididymal maturation. Anat. Rec. 169: 300.

El-Sheltawi, M. A. (1989). Evaluation of locally processed frozen semen. Ph. D., Vet. Thesis, Cairo Univ.

Fayemi, L.O., Crabo, B.G. and Graham, E.F. (1979). Assay of frozen boar semen with sephadex filtration. Theriogenol. 12:13.

Goyal, R.L., Tuli, R.K., Georgie, G.C. and Chand, D. (1996). Comparison of quality and freezability of water buffalo semen after washing of sephadex filtration. Theriogenol. 46: 679.

Graham, E.F. and Graham, J.K. (1990). The effect of whole ejaculate filtration on the morphology and the fertility of bovine semen. J. Dairy Sci. 73: 91.

Graham, E.F., Vasquez, I.A., Schmehl, M.K. and Evensen, B.K. (1976). An assay of semen quality by use of sephadex filtration. In: Proceedings of the Eighth Inter. Cong. Anim. Reprod. 4: 896.

Hancock, J. L. (1959). The morphologic characteristics of spermatozoa and fertility. Int J. Fert. 4:347.

Heer, C. and Tahir, N.M. (1982). Experiments on deep freeze preservation of water-buffalo semen using sephadex filtration as semen assay. Proceedings of buffalo Seminar on Reprod. Med. Production Tanuku. p. 55-63.

Januskauskas, A., Lukoseviciute, K., Nagy, s., Johannisson, A. and Martinez, H.R. (2005). Assessment of the efficacy of sephadex G-15 filtration of bovine spermatozoa for cryopreservation. Theriogenol. 63: 160.

Kumar, N., Pangankar, G.R. and Singh, J. (1989). Improvement in the semen quality of buffalo bulls with filtration technique. National Symposium on Applied Reproduction in Farm Animals and VIII National Convention of Indian Society for the study of Animal Reproduction. Gujarat Agricultural Univ., 10-12 November p. 21. (Abstract).

Landa, C.A., Almquist, J.O. and Amann, R.P. (1980). Factors influencing sephadex separation of bovine and ovine spermatozoa. J. Dairy Sci. 63:277.

Lessley, B.A. and Garner, D.L. (1983). Isolation of motile spermatozoa by density gradient centrifugation in Percoll. Gamete Res. 7: 49.

Lindemann, C.B., Fisher, M. and Lipton, M.A. (1982). A comparative study of the effects of freezing and frozen storage on intact and demembranated bull spermatozoa. Cryobiol. 19: 20.

Milvanov , V.K. (1962). Biology of reproduction and artificial insemination. (Trans. Title Mono-graph, Moscow.).

Mohamed, K.M.E., Ziada, M.S. and Darwish, G.M. (1998). Practical trails for freezing semen of buffalo and friesian bulls: Effect of various regimens of freezing, different milk extenders and types of straws packages on post-

thawing semen characters. Assiut Vet. Med. J. 77:70.

Parrish, J.J. and Foot, R.H. (1987). Quantification of bovine sperm separation by a swim-up method. Relationship to sperm motility, intergrity of acrosomes, sperm migration in polyacrylamide gel and fertility. J. Androl. 8:259.

Saacke, r.G. and White, J.M. (1972). Semen quality tests and their relationship to fertility. In: Proceeding of the Fourth Technical Conference on Arificial Insemintion and Reproduction, NAAB; p.22.

Samper, J.C. and Crabo, B.G. (1993). Assay of capacitated, freeze-damaged and extended stallion spermatozoa by filtration. Theriogenol. 39: 1209.

Samper, J.C., Hamilton, D.W., Pryor, J.L., Loseth, K.J., Trodsson, M.H. and Crabo, B.G. (1995). Mechanism of sephadex trapping of capacitated stallion spermatozoa. Biol. Reprod. Monogr. 1: 270.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods 7th ed. J. B. H. Publishing Comp. Oxford.Veres, I., Muller, E., Oscenyl, A., Turanyl, J., Szasz, F., Bernorider, G. and Kronreif, A.J. (1976).

تجميد السائل المنوى الجاموسى بعد تمريرها فى اعمدة السيفادكس

** مها سليمان زيادة *منى علاء الدين . * عادل أنيس حنا زكى
* كريمان دياب حسين

**قسم بحوث التلقيح الإصطناعى ونقل الإجنه

*قسم بحوث بيولوجيا التكاثر معهد بحوث التناسلبات الحيوانيه الأهرام جيزة

لقد تم إجراء تجربتين فى هذه الدراسة حيث إستهدفت التجربة الأولى إختبار تأثير تمرير السائل المنوى الجاموسى فى أعمدة السيفادكس المختلفه فى حجم بلورانها (جـ ٥٠، جـ - ٥٠ جـ - ٧٥، جـ - ١٠٠ ، جـ - ٢٠٠) على حيوية الحيامن بعد الترشيح وفى هذه التجربة تم تجميع السائل المنوى من أربع طلائق جاموسى وتم تخفيفها ١:٢٠ بإستخدام محلول الترس المتعادل ثم تمريرها فى أعمدة السفادكس المختلفه و تركت لمدة من ٤ إلى ٥ دقائق عند ٣٧° وقد وجد أن ناتج تمرير السائل المنوى فى أعمدة السيفادكس له من التأثير الإيجابى لتحسين نسبه حركة الحيامن من ٦٨.٧٥ إلى ٨٤.٣٨٪ بالمقارنه بالعينات الضابطه (٦٣.١٢٪) وقد وجد أيضاً أن نسبة الحيامن الحيه بعد التمرير فى أعمدة السيفادكس تتزايد (٧١.٣٨ - ٨٨.٦٣٪) عن مثيلاتها للعينات الضابطه (٦٥.٩٤٪) أما عن نسبة الحيامن ذات التشوهات الكبرى (٣٧. ٢-٤.٥) و الصغرى (١.٥٦ -٥.١٢) فكانت أقل من مثيلاتها فى العينات الضابطه (٥.٥٦، ٦.٣١٪ على التوالى) وقد وجدت نسبة الحيامن ذات القلنسوات المشوهه فى العينات الممررة فى أعمدة لاسيفادكس أقل (١.٤٤ - ٣.٨٨ ٪) من العينات الضابطه (٤.٦٣٪) . أما عن تركيز الحيامن بعد التمرير فى أعمدة السيفادكس فكان أقل (٠.٩٩٠٠.٦٧ X 10^9/ملى) من العينات (١.٢٥ X 109/ملى) . ومن مجمل نتائج التجربة الأولى وجد أن إستخدام أعمدة السفادكس جـ - ٧٥ من أفضل الأنواع المجربة من حيث حيوية الحيامن بعد التمرير

وقد إستهدفت التجربةالثانية دراسة قابلية تجميد السائل المنوى الجاموسى بعد تمريره فى أعمدة السيفادكس جـ - ٧٥ و فى هذه التجربة تم تجميع عينات السائل المنوى من نفس الطلائق المستخدمة فى التجربة الأولى وخففت ١: ٢٠ بمحلول الترس المتعادل ومررت فى أعمدة السيفادكس جـ - ٧٥ مثل التجربة الأولى بعد ذلك تم تدويرها فى جهاز الطرد المركزى عند ١٥٠٠ دورة فى الدقيقة لمدة دقائق ثم التخلص من الجزء العلوى وتخفيف الراسب بمخفف الأوبتيديل (مخفف أساس تركيبة الترس) وتجميد العينات بإستخدام سائل النيتروجين وجد من التجربة أن حركة الحيامن الممررة فى أعمدة السيفادكس أفضل بعد التخفيف (٨٦.٢٥٪) وبعد التجميد والإسالة (٦١.٢٥٪) عن مثيلاتها فى العينات الضابطة (٦٧.٩١ . ٤٥.٨٣٪ على التوالى). وجد أيضاً أن تمرير العينات فى أعمدة السيفادكس بحسن من حيويتها بعد الإسالة (١٥١.٧١) عنها فى العينات الضابطة (٩٠.٨٣) . أما بالنسبة للحيامن ذات القلنسوات المشوهة للعينات بعد التخفيف والإسالة فكانت نسبتها أقل (١.٩٢ . ٧.٧٥ على التوالى) للعينات الممررة فى أعمدة السيفادكس عنا فى العينات الضابطة (٥.٨٣ . ١٤.٦٦ على التوالى ووجد أيضاً بعض الفروق الفردية بين الطلائق متمثلة فى نسبة حركة الحيامن وحيويتها بعد التجميد والاسالة .

من الدراسة يمكن إستخلاص أن تمرير عينات السائل المنوى الجاموسى فى أعمدة السيفادكس جـ ٧٥ يمكن إستخدامه بصورة تطبيقية لما له من تأثير إيجابى لتحسين حيوية الحيامن بعد التجميد والإسالة