Vet.Med.J.,Giza. Vol.56, No1. (2008):109-122.

USE OF HYPOOSMOTIC SWELLING TEST FOR EVALUATING CAMEL SPERMATOZOAL MEMBRANE INTEGRITY IN RELATION TO DIFFERENT SOLUTIONS

ZEIDAN, A.E.B., M.M. EL-BAZ, E.A. AHMADI, N.A. ABD EL-AZIZ and ABD EL-SALAAM, A.M.

Animal Production Research Institute, Dokki, Giza, Egypt.

Received: 2. 3. 2008.

Accepted: 9. 3. 2008.

SUMMARY

Five male camels at 5 to 10 years of age and 400 – 600 kg body weight, were used in the present study.Semen was collected, evaluated, pooled and extended with seven different hypoosmotic solutions (glucose – yolk-citrate: GYC, fructose-yolk-citrate: FYC, lactose-yolk-citrate: LYC, sucrose-yolk-citrate: SYC, tris-yolk-fructose: TYF, skim-cow-milk: SCM and skim camel-milk: SLM) at a level of 100 mOsmol/L and then incubated at 37 °C for up to 60 minutes. After each incubation time (0, 5, 15, 30 and 60 minutes), the percentages of sperm motility, spermatozoa with intact acrosome, spermatozoa with coiled tails and swollen spermatozoa, were estimated.

The results showed that, the extended camel semen with FYC, SYC, TYF and SCM solutions at a level of 100 mOsmol/L, during an incubation of 37 °C for up to 60 minutes increased significantly ($P<0.01$) the percentage of sperm motility, spermatozoa with intact acrosome and swollen spermatozoa as compared to GYC, LYC and SLM solutions, however, the percentage of spermatozoa with coiled tails decreased significantly ($P<0.01$) in the extended camel semen with SYC and TYF solutions as compared to GYC, FYC, LYC, SCM and SLM solutions. The advancement of incubation time at 37 °C for up to 60 minutes with the all different solutions (GYC, FYC, SYC, LYC, TYF, SCM and SLM) at 100 mOsmol/L decreased significantly ($P<0.01$) the percentages of sperm motility and percentage of spermatozoa with intact acrosome, while was increased significantly ($P<0.01$) the percentage of swollen spermatozoa and spermatozoa with coiled tails. The maximum reactivity of the extended camel spermatozoa with the all different solutions to hypoosmotic swelling-test (HOS-test) was reached significantly ($P<0.01$) at 30 minutes of incubation at 37°C.

INTRODUCTION

Membrane integrity of spermatozoa is not only important for sperm metabolism, but a correct change in the properties of the sperm membrane is needed for sperm capacitating, acrosomal reaction and fertilization (Keel and Webster, 1990). Standard parameters used to assess male fertility (total number of spermatozoa, progressive sperm motility and morphology) have a limited capacity for predicting the potential of a semen ejaculate (Amann, 1989). Because the sperm membrane is of fundamental importance in the fertilization process, more attention has been dedicated to this area of study in recent years. Two tests have been available to evaluate membrane integrity supravital stain and the hypoosmotic swelling-test (HOS-test). When exposed to a hypoosmotic solution, the functional spermatozoa will undergo swelling to establish osmotic equilibrium, producing the typical swelling of the tail. Since fertilization will not occur if the sperm membrane is biochemically inactive, even if it remains structurally and intact. In addition, swelling test by media of different osmolarities is more reliable in assessing the outcome of *in vitro* fertilization (*IVF*) than other semen parameters and it has a higher individual specificity than other semen evaluation method (Zaneveld et al., 1990 and Zeidan, 2004).

The HOS-test has been tried in various species with apparent success (Kumi-Diaka, 1993, Correa and Zavos, 1994 and Zeidan et al., 2006). However, studies on the camel are still somewhat masked, especially with the different hypoosmotic solutions.

The present study aimed to evaluate the ability of camel spermatozoa to swell in the different hypo-osmotic solutions at a level of 100 mOsmol/L, during an incubation at 37 °C for up to 60 minutes.

MATERIALS AND METHODS

The present study was carried out in the Private Camel's Farm in Belbies City, Sharkiya Province, Egypt. Five male dromedary camel (*Camelus dromedarius*) 5 to 10 years of age and 400-600 kg body weight were used. The animals were healthy and clinically free of external and internal parasites. They were fed and watered *ad libitum*.

The experimental work was carried out to establish the optimum condition of osmotic pressure that caused the maximum number of identifiable swollen camel spermatozoa. The diluted solution was prepared by using GYC, FYC, LYC, SYC, TYF, SCM and SLM at a level of 100 mOsmol/L. The routinely osmolarity of media used for *in vitro* fertilization (*IVF*), during an incubation at 37 °C for 0, 5, 15, 30 and 60 minutes.

Semen was collected from five dromedary camels between 08 : 00 to 10 : 00 a.m. by using an artificial vagina (AV). A modified artificial vagina (30

cm long and 5 cm internal diameter, *IMV*, France) as described by Zeidan (2002) and Mosaferi et al. (2005). Ejaculate contact with the rubber liner of the AV was avoided, since Musa et al. (1992) reported that most rubber liners have a deleterious effect on camel spermatozoa. An additional disposable plastic inner liner is inserted to avoid contact with the rubber material. After passing the liner through the AV, 8 cm of cylindrical from (cut longitudinally) was placed between outer Jacket of the AV and liner at the end of the AV far from the water value according to Bravo et al. (2000). This was performed to imitate the internal cervix and provide more stimulation for the penis for proper erection and ejaculation. A shortened AV without collection funnel was used, allowing the semen to pass directly into a collection flask. The AV was filled with water at 55-60 °C. The temperature inside the inner liner was stabilized at 45-50 °C. Few drops of sterile Vaseline were smeared on the inner liner at the entrance to the AV top provide lubrication. A sexually receptive female couching with her front legs tied and teased by the male camel should be used. The olfactory contact should be allowed. The male is left to mount the female from behind on the right side. As soon as the male camel makes few thrusts, the operator who sits on the right side of the female grasps the males camel sheath and directs his penis into the AV. Ejaculation is completed after several thrusts, interspersed by periods of rest. The ejaculate usually comes in fractions. The collection flask containing the semen is protected by a towel or gauze, and immediately incubated in a water bath at 37 °C. Fresh camel semen that has a jelly-like consistency is left for liquifaction for about 30 – 60 minutes to let the spermatozoa attain their motility. Only semen samples with no less than 50% sperm motility, were used. After semen collection, it was placed inside an incubator set at 37 °C and evaluated immediately.

Semen was extended and aliquot protein (0.1 ml) of each sperm rich fraction was added to 0.9 ml of each of GYC, FYC, LYC, SYC, TYF, SCM and SLM solutions. The mixtures were incubated at 37 °C for up to 60 minutes. After each incubation period (0, 5, 15, 30 and 60 minutes), the percentages of sperm motility, spermatozoa with intact acrosome, swollen spermatozoa and spermatozoa with coiled tails were determined. The response of the camel spermatozoa to HOS-test was assessed using a solution prepared with fructose (1.25%) and Na-citrate (2.90%) in distilled water to give osmolarity 300 mOsmol/L using a freezing-point depression osmometer (Osmett A. Model 5002, Fisher Scientific, Pittsburg, PA, USA). The final osmolarity of the test solutions measured by freezing-point depression was modified to obtain 100 mOsmol/L via serial dilution hypoosmotic in the different solutions. Sperm swelling was assessed by placing 15 µl of well-mixed sample on a warm slide (37 °C). Slides were stained with eosin-nigrosin stain according to Hackett and Macpherson (1965). The smear was covered with

a cover glass before being examined under an immersion lens (100x). Two hundred spermatozoa per slide were counted. The percentages of swollen spermatozoa and coiled tails of spermatozoa were determined as follow : number of spermatozoa with swollen or coiled tails divided by the total number of spermatozoa counted multiplied by 100 (Vazquez et al., 1997).

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1982). The percentage values were transformed to Arc-Sin values before being statistically analyzed. Duncan's new multiple range test was used for the multiple comparisons (Duncan, 1955).

RESULTS AND DISCUSSION

Percentage of the camel sperm motility (%) :

Table 1 showed that, the effect of seven different solutions (GYC, FYC, LYC, SYC, TYF, SCM and SLM) at 100 mOsmol/L, during an incubation at 37 °C for up to 60 minutes on the percentage motile camel spermatozoa was highly significant ($P<0.01$). The percentage of sperm motility was significantly ($P<0.01$) higher with the extended camels semen with FYC, SYC, TYF and SCM solutions at 100 mOsmol/L than GYC, LYC and SML solutions. However, the different effects among FYC, SYC, TYF and SCM solutions or among GYC, LYC and SML on the percentage of sperm motility was nonsignificant. The highest ($P<0.01$) value of the percentage of sperm motility was recorded with the extended semen with TYF solution and the lowest ($P<0.01$) value was recorded with SML solution. Similar trend was reported by Neild et al. (1999) in equine spermatozoa. This phenomenon may be due to the differences in the different components of the solutions that may exist in the rate of active transport of the physical and biochemical compounds across the sperm membrane which is considered to have an important biochemical role for maintaining a high sperm viability and fertilizing capabilities (Keel and Webster, 1990). Moreover, an abrupt decrease in osmotic pressure results in loss of sperm motility (Zavos, 1983).

The advancement of incubation time at 37°C for up to 60 minutes decreased significantly ($P<0.01$) the percentage of sperm motility of the camel in all different hypoosmotic solutions at 100 mOsmol/L. The osmotic shock phenomenon caused by the exposure of the extended spermatozoa to hypotonic conditions is characterized by an increased coiling of the sperm tail which results in loss of the sperm motility (Zavos, 1983). Similar trend was reported by Al-Arifi (2005) in Friesian bull and Zeidan et al. (2006) in the dromedary camel spermatozoa.

Percentage of the camel spermatozoa with intact acrosome (%)

Data presented in Table 2 showed that, the effect

of seven different solutions (GYC, FYC, LYC, SYC, TYF, SCM and SLM) at 100 mOsmol/L, during an incubation at 37°C for up to 60 minutes on the percentage of camel spermatozoa with intact acrosome was highly significant (P<0.01). The percentage of the acrosomal intact was significantly (P<0.01) increased with the extended camel spermatozoa with FYC, SYC, TYF and SCM solutions at 100 mOsmol/L as compared to GYC, LYC and SLM solutions. However, the different effects among FYC, SYC, TYF and SCM solutions or among GYC, LYC and SLM solutions on the percentage of spermatozoa with intact acrosome was nonsignificant. The highest (P<0.01) value of the percentage of spermatozoa with intact acrosome was recorded with the extended semen with TYF solution and the lowest (P<0.01) value was recorded with GYC solution. Zeidan et al. (2006) found that the lowest (P<0.01) value of the percentage of spermatozoa with intact acrosome was recorded with LYC solution osmolarity of 50 and 100 mOsmol/L, during an incubation at 37°C for up to 60 minutes.

The advancement of incubation time at 37°C for up to 60 minutes decreased significantly (P<0.01) the percentage of camel spermatozoa with intact acrosome in the all different hypoosmotic solutions at 100 mOsmol/L. Similar findings were reported by Correa and Zavos (1994) in bovine and Zeidan et al. (2006) in camel spermatozoa. The response of spermatozoa to HOS-test is depending on cellular uptake (osmolarity level) per unit of time as reported by Zeidan (2004).

Percentage of the camel spermatozoa with coiled tails (%)

Table 3 showed that, the effect of seven different solutions (GYC, FYC, LYC, SYC, TYF, SCM and SML) at 100 mOsmol/L, during an incubation at 37°C for up to 60 minutes on the percentage of camel spermatozoa with coiled tails was highly significant (P<0.01). The different effects among GYC, FYC, LYC, SCM and SLM solutions or between SYC and TYF solutions on the percentage of camel spermatozoa with coiled tails at osmolarity of 100 mOsmol/L was nonsignificant. However, the percentage of spermatozoa with coiled tails was significantly (P<0.01) higher with the extended spermatozoa with GYC, FYC, LYC, SMC and SML solutions than SYC and TYF solutions. The lowest (P<0.01) value of percentage of spermatozoa with coiled tails was recorded with the extended semen with TYF solution and the highest (P<0.01) value was recorded with SLM solution. Similar trend was reported by Neild et al. (1999) in equine spermatozoa. A scoring system (HOS-test ranking), based on sperm swelling patterns is shown in Figure 1. Similarly, Zeidan et al. (2006) found that the lowest (P<0.01) value of the percentage of spermatozoa with coiled tails was recorded with solution osmolarity of 300 mOsmol/L and the highest (P<0.01) values were found with solution of 50 and 100 mOsmol/L when they extended dromedary camel semen with fructose –Na-citrate solution at the

different osmolarities (50, 100, 150 , 200 and 300 mOsmol/L), during an incubation at 37°C for up to 60 minutes. Jeyendran et al. (1984) found that the situations at which spermatozoa start coiling in response to the HOS-test, would imply normal membrane integrity of the HOS-test reacted spermatozoa. That is the ability between the fluid compartment of the spermatozoa and external environment. Similar results are in agreement with those of Vazquez et al. (1997), Moussa (1999) and Zeidan (2004).

The advancement of incubation time at 37°C for up to 60 minutes increased significantly (P<0.01) the percentage of coiled tails of camel spermatozoa in the all different hypoosmotic solutions at 100 mOsmol/L. Similar trend was reported by Al-Arifi (2005) in bovine and Zeidan et al. (2006) in the dromedary camel spermatozoa.

Percentage of swollen camel spermatozoa (%)

Data results obtained in Table 4 showed that, the effect of seven different solutions (GYC, FYC, LYC, SYC, TYF, SCM and SLM) at 100 mOsmol /L, during an incubation at 37°C for up to 60 minutes on the percentage of swollen camel spermatozoa was highly significant (P<0.01). The different effects among FYC, SYC and SMC solutions, among SYC, TYF and SCM solutions, between FYC and LYC solutions, between SYC and SCM solutions or between GYC and SML solutions on the percentage of swollen spermatozoa was nonsignificant. However, the percentage of swollen spermatozoa was significant (P<0.01) higher with the extended camel spermatozoa with FYC, SYC, TYF and SCM solutions than with other solutions. Similar trend was reported by Neild et al. (1999) who reported that the maximum numbers of swollen equine spermatozoa were observed with solutions of fructose, sucrose and lactose each at 100 mOsmol/L, while this was observed with sodium citrate solution at 25 mOsmol/L. The highest (P<0.01) value of the percentage of swollen spermatozoa was recorded with the extended camel semen with TYF solution and the lowest (P<0.01) value was recorded with SML solution at 100 mOsmol/L. These results may be attributed to that the sperm tail membrane bulges and swells in response to the hypoosmotic medium because of the influx of fluids into the spermatozoa as has been described by Schrader et al. (1986) in human spermatozoa. Differences observed in the percentages of spermatozoa swelling in sodium citrate solution and sugar solutions could have resulted from different influence on the availability of water for transport through the plasma membrane (Neild et al., 1999). Zeidan et al. (2006) found that, the highest (P<0.01) values of the percentage of swollen camel spermatozoa were recorded with solutions of 50 and 100 mOsmol/L and the lowest (P<0.01) value recorded with solution of 300 mOsmol/L when they extended semen with fructose Na-citrate solution at different osmolarities (50, 100, 200 and 300

mOsmol/L), an during incubation at 37°C for up to 60 minutes. Studies on water permeability of some mammalian sperm membranes in bull (Watson et al., 1992) and human (Curry et al., 1995) show that the osmotic water permeability coefficient is very high and that the associated facilitation energy is very low, which suggests a porous membrane and the presence of water channel proteins. In ram and human sperm membrane, glucose transporters may have a secondary water channel function (Curry et al., 1995).

The advancement of incubation time at 37°C for up to 60 minutes increased significantly ($P<0.01$) the percentage of swollen camel spermatozoa in the all different hypoosmotic solutions at 100 mOsmol/L. It is worth noting that the maximum reactivity of spermatozoa to HOS-test (spermatozoa with coiled tails and swollen spermatozoa) was reached at 30 minutes of incubation at 37°C. Similar trend was reported by Neild et al. (1999) in equine and Zeidan et al. (2006) in dromedary camels spermatozoa. The degree of spermatozoa swelling is dependent on cellular water uptake per unit of time. Under these conditions, the reliability of the assay was very high as reported by Jeyendran et al. (1984) in human spermatozoa. The same authors showed that the capability of human spermatozoa to swell in a hypoosmotic solution depended on the compounds in the solution.

In conclusion, the maximal number of spermatozoa with sperm motility, intact acrosome and the minimal number of spermatozoa with coiled tails and spermatozoa swelling were recorded in the extended dromedary camel spermatozoa with TYF solution at 100 mOsmol/L. The HOS-test is a simple, inexpensive, easily applicable technique and accessible evaluation method which, as in other species, could be a useful complement to routine semen analysis, identifying males infertility and in predicting the outcome of *in vitro* fertilization (*IVF*). It has the added advantages of being less susceptible to the immediate effects of cold shock and of evaluating individual sperm cells rather than the population as a whole, as does progressive motility.

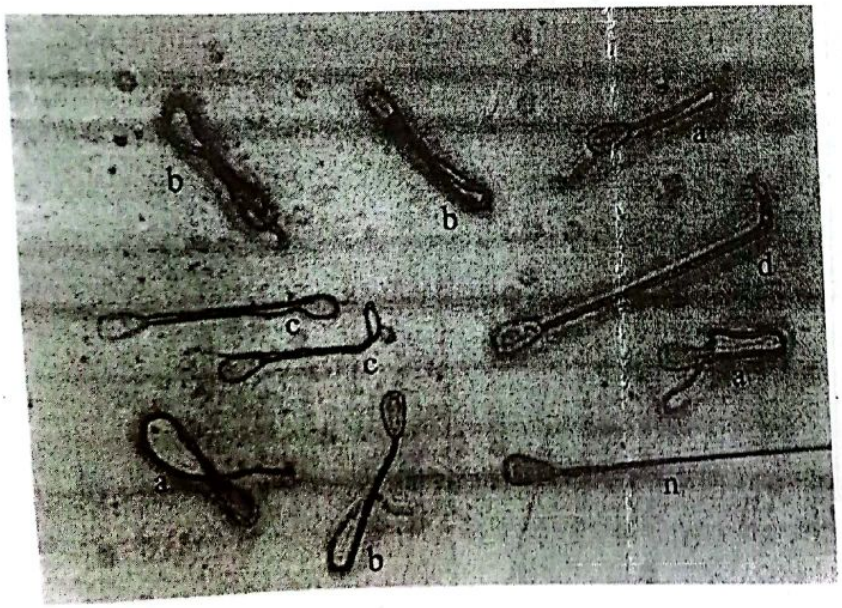

Figure1. Micrograph of spermatozoa swelling patterns as measured by the hypoosmotic swelling (HOS-test). Type a: represents maximal sperm swelling, Types b and c: represent intermediate sperm swelling stages, Type d: represents the initial sperm swelling response in the HOS-test and Type n: represents non-swollen spermatozoa are considered to have a functionally inactive or damaged sperm membrane (Cited after Zeidan, 2004).

Table 1. Percentage of motility of the dromedary camel spermatozoa with the different hypoosmotic solutions at 100 mOsmol/L, during an incubation at 37°C for up to 60 minutes.

Incubation time (minutes)	Hypoosmotic solutions							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	45.71±4.00	52.14±3.76	46.42±2.37	54.26±3.52	56.45±2.61	52.89±3.43	43.52±3.89	50.20±1.37^{A}
5	40.76±4.93	48.55±5.08	40.75±2.27	47.89±2.64	51.41±2.61	49.27±3.52	39.24±5.17	45.41±1.56^{B}
15	33.57±3.89	35.70±4.42	34.22±3.17	36.46±3.22	40.02±2.89	36.40±3.89	30.76±4.93	35.30±1.41^{C}
30	21.43±2.83	22.87±3.43	22.14±3.06	26.40±1.8	28.56±3.57	25.74±5.17	21.46±4.59	24.09±1.34^{D}
60	9.24±1.3	10.78±2.54	10.02±1.89	12.15±1.84	12.54±2.1	11.43±2.37	9.29±2.02	10.78±0.75^{E}
Means	30.14±2.73^{b}	34.01±3.14^{a}	30.71±2.52^{b}	35.43±2.82^{a}	37.80±2.91^{a}	35.15±3.06^{a}	28.86±2.78^{b}	33.16

a-b : Values with different superscripts with a row, are significantly different (P<0.01).

A-E: Values with different superscripts with a column, are significantly different (P<0.01).

GYC: Glucose-yolk-citrate.

FYC: Fructose-yolk-citrate.

LYC: Lactose- yolk-citrate.

SYC: Sucrose-yolk-citrate.

TYF: Tris-yolk-furctose.

SCM Skim-cow.milk

SLM Skim-camel.milk

Table 2. Percentage of intact acrosome of the dromedary camel spermatozoa with the different hypoosmotic solutions at 100 mOsmol/L, during an incubation at 37°C for up to 60 minutes.

Incubation time (minutes)	Hypoosmotic solutions							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	49.72±3.82	57.16±3.37	50.45±2.38	60.27±3.78	61.75±2.90	57.40±3.71	48.71±4.42	55.07±1.44^{A}
5	40.57±4.09	54.89±3.54	42.55±2.85	55.24±3.64	57.24±3.65	56.18±3.81	40.25±4.66	49.63±1.71^{B}
15	33.29±4.07	50.27±3.59	35.28±2.56	50.85±4.23	53.42±3.95	50.74±3.52	32.86±4.81	43.82±1.83^{C}
30	24.42±4.05	41.85±3.50	25.73±2.75	42.28±4.49	44.58±4.01	42.14±4.63	23.52±4.15	34.93±1.92^{D}
60	16.14±4.1	36.12±4.24	18.16±2.3	36.42±4.5	38.15±3.58	37.22±3.54	16.48±3.42	28.83±1.95^{E}
Means	32.83±2.64^{b}	48.06±2.06^{a}	34.43±2.25^{b}	49.11±2.29^{a}	51.03±2.12^{a}	48.74±2.11^{a}	32.36±2.68^{b}	42.37

a-b : Values with different superscripts with a row, are significantly different (P<0.01).

A-E: Values with different superscripts with a column, are significantly different (P<0.01).

GYC: Glucose-yolk-citrate.

FYC: Fructose-yolk-citrate.

LYC: Lactose- yolk-citrate.

SYC: Sucrose-yolk-citrate.

TYF: Tris-yolk-furctose.

SCM Skim-cow.milk

SLM Skim-camel.milk

Table 3. Percentage of coiled tails of the dromedary camel spermatozoa with the different hypoosmotic solutions at 100 mOsmol/L, during an incubation at 37°C for up to 60 minutes.

Incubation time (minutes)	Hypoosmotic solutions							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	34.14±2.63	33.43±2.00	33.56±1.49	22.27±1.78	20.89±1.18	32.26±2.2	34.28±3.31	30.12±1.10^{E}
5	41.71±3.91	39.16±2.30	39.12±2.30	28.59±2.27	27.24±0.97	39.82±1.78	42.54±3.42	36.38±1.24^{D}
15	49.29±4.02	48.25±1.84	48.24±1.7	33.16±2.20	30.23±1.11	47.18±1.73	50.16±3.99	43.86±1.45^{B}
30	57.18±3.79	56.17±1.47	56.42±2.45	42.86±1.7	41.59±0.90	55.43±1.9	58.45±3.76	52.59±1.30^{A}
60	46.54±4.25	42.28±1.36	44.18±3.08	30.41±1.69	28.78±1.91	41.59±2.64	49.11±4.56	40.41±1.50^{C}
Means	45.77±2.06^{a}	43.86±1.54^{a}	44.40±1.65^{a}	31.46±1.41^{b}	29.75±1.27^{b}	43.26±1.59^{a}	46.91±2.12^{a}	40.77

a-b : Values with different superscripts with a row, are significantly different (P<0.01).

A-E: Values with different superscripts with a column, are significantly different (P<0.01).

GYC: Glucose-yolk-citrate.

FYC: Fructose-yolk-citrate.

LYC: Lactose- yolk-citrate.

SYC: Sucrose-yolk-citrate.

TYF: Tris-yolk-furctose.

SCM Skim-cow.milk

SLM Skim-camel.milk

Table 4. Percentage of swollen of the dromedary camel spermatozoa with the different hypoosmotic solutions at 100 mOsmol/L, during an incubation at 37°C for up to 60 minutes.

Incubation time (minutes)	Hypoosmotic solutions							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	7.59+0.92	13.87+ 1.39	11.75+ 1.6	15.12+ 1.12	15.27+ 1.08	14.15+ 1.13	5.70+0.64	11.96+0.66 ^{B}
5	8.79+1.06	15.24+0.81	13.29+1.27	16.40+1.32	17.12+1.5	15.50+0.92	6.59+0.92	13.30+0.68 ^{D}
15	10.29+1.06	17.58+0.78	16.70+1.38	18.55+1.41	20.28+1.58	18.15+1.12	9.40+0.75	15.85+0.71 ^{C}
30	13.48+1.21	19.16+1.26	19.43+1.13	20.24+1.57	21.16+1.22	20.26+0.92	12.72+0.64	18.14+0.59 ^{B}
60	20.64+1.10	26.15+1.40	26.54+1.19	27.87+1.71	28.58+1.25	27.16+1.03	20.87+0.74	25.40+0.59 ^{A}
Means	12.16+1.02 ^{d}	18.40+0.88 ^{bc}	17.54+1.06 ^{c}	19.74+0.98 ^{ab}	20.48+0.96 ^{a}	19.14+0.88 ^{ab}	11.06+0.99 ^{D}	16.93

a-d: Values with different superscripts with a row, are significantly different (P<0.01).

A-E: Values with different superscripts with a column, are significantly different (P<0.01).

GYC: Glucose-yolk-citrate.

FYC: Fructose-yolk-citrate.

LYC: Lactose- yolk-citrate.

SYC: Sucrose-yolk-citrate.

TYF: Tris-yolk-furctose.

SCM Skim-cow.milk

SLM Skim-camel.milk

REFERENCES

Al-Arifi, Y.A. Al.M. (2005). Physical and biochemical studies on storage of Friesian bull semen. M.Sc. Thesis, Fac. of Agriculture., Cairo Univ., Egypt.

Anann,U.P.(1989). Can the fertility potential of a seminal sample be predicted accurately? J.Androl., 89-98.

Bravo, P.W.; Skidmore, J. A. and Zhao, X. X. (2000). Reproductive aspects and storage of semen in camelidae. Anim. Reprod. Sci., 62: 173-193.

Correa, J.R. and Zavos, P.M. (1994). The hypoosmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen – thawed bovine sperm membrane. Theriogenology , 42 : 351-360.

Curry, M.R.; Millar, J.D. and Watson, P.F. (1995). The presence of water channel proteins in ram and human sperm membranes. J. Reprod. Fertil., 104 : 297-303.

Duncan, D.B. (1955). Multiple range and multiple F-test. Biometrics, 11 : 1 – 42.

Hackett, A.J. and Macpherson, J.W. (1965). Some staining procedures for spermatozoa. Canadian Vet. J., 6: 55-62.

Jeyendran, R.S.; Van der Ven, H.H.; Perez- Pelaez, M. ; Crabo, B.G. and Zaneveld, L.J.D. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to the other semen characteristics. J. Reprod. Fertil., 70 : 219-225.

Keel, B.K. and Webster, B.W. (1990). Handbook of the Laboratory Diagnosis and Treatment of Infertility. CRC Press, Boston, USA, pp 80 – 96.

Kumi – Diaka, J. (1993). Subjecting canine semen to the hypo-osmotic test. Theriogenology, 39 : 1279-1289.

Mosaferi, S.; Niasari- Naslaji, A.; Abarghani, A.; Gharabaghi, A.A. and Gerami, A. (2005). Biophysical and biochemical characteristics of bactrian camel semen collected by artificial vagina. Theriogenology, 63 : 92-101.

Moussa, I.A. (1999). Evaluation of Nagdi rams spermatozoa using hypoosmotic test . Zagazig Vet. J., 27: 26-33.

Musa, B. ; Sieme, H. ; Merkt, H. and Hago, B.E.D. (1992). Artificial insemination in dromedary camels. Proc of 1st Intern. Camel Cong., Dubai, U.A.E., pp 179-182.

Neild, D.; Chaves, G.; Flores, M.; Mora, N.; Beconi, M. and Aguero, A. (1999). Hypoosmotic test in equine spermatozoa. Theriogenology, 51 : 721-727.

Schrader, S.M.; Platck, S.F.; Zaneveld, L.J.D. ; Perez- Pelaez, M. and Jeyendran, R.S. (1986). Sperm viability: comparison of analytical methods. Andrologia, 18: 530-538.

Snedecor, G.W. and Cochran, W.G. (1982). Statistic Methods. 7th Edition, Iowa State University Pres Ames., U.S.A., pp 593.

Vazquez, J.M.; Martinez, E.A.; Martinez, P.; Garcia-Artig C. and Roca, J. (1997). Hypoosmotic swelling of bo spermatozoa compared to other methods for analyzi the sperm membrane. Theriogenology, 47 : 913-922.

Watson, P.F.; Kunze, E.; Cramer, P. and Hammerstec R.H.J. (1992). A comparison of critical osmolarity a hydraulic conductivity and its activation energy in fo and bull spermatozoa. J. Andrology, 13 : 131-138.

Zaneveld, L.J.D.; Jeyendran, R.S.; Krajeski, P.; Coetze, K Kruger, T.F. and Lombard, L.J. (1990). Hypoosmot swelling test. In : Acosta A.A., Swanson, R.J. Ackerma S.B., Kruger T.F., Van Zyl J.A., Menkveld R. (eds), Hu man Spermatozoa in Assisted Reproduction Baltimor

استخدام اختبار انخفاض الأسموزية لتقييم سلامة الغشاء الخلوى فى الحيوانات المنوية للإبل تحت تأثير المحاليل المختلفة

علاء زيدان ، مصطفى الباز ، السيد أبو الفتوح أحمد ، ناصر عبد العزيز ، عاطف عبد السلام
معهد بحوث الإنتاج الحيوانى - الدقى - الجيزة - مصر

أجريت هذه الدراسة على عدد خمسة ذكور إبل عند عمر ٥ - ١٠ سنوات متوسط وزن الجسم ٤٠٠ - ٦٠٠ كجم. تم جمع وتقييم وتخفيف السائل المنوى باستخدام سبعة محاليل مختلفة (GYC, FYC, LYC, SYC, TYF. (SCM, SLM) عند مستوى ١,٠٠ مللى أوسمول/لتر. تم بعد ذلك تحضين السائل المنوى المخفف بهذه المحاليل على درجة حرارة ٣٧° م لمدة ٦٠ دقيقة. بعد كل فترة تحضين (صفر ، ٥ ، ١٥ ، ٣٠ ، ٦٠ دقيقة) تم تقدير النسبة المئوية لحيوية الحيوانات المنوية ، الحيوانات المنوية متماسكة الأكروسوم ، الحيوانات المنوية ملتفة الذيل والحيوانات المنوية المنتفخة.

أوضحت النتائج أن هناك زيادة فى النسبة المئوية لحيوية الحيوانات المنوية ، النسبة المئوية للأكروسوم المتماسك ، النسبة المئوية للحيوانات المنوية المنتفخة بدرجة معنوية (على مستوى ٠,٠١) وذلك عند تخفيف السائل المنوى للإبل بمحاليل TYF, SYC, FYC و SCM عند مستوى ١٠٠ مللى أوسمول/ لتر والتحضين على درجة حرارة ٣٧° م لمدة ٦٠ دقيقة بالمقارنة بمخففات LYC, GYC و SLM ، بينما أدى تخفيف السائل المنوى للإبل بمحلول TYF, SYC إلى انخفاض النسبة المئوية للحيوانات المنوية ملتفة الذيل معنوياً (على مستوى ٠,٠١) مقارنة بالتخفيف بمحاليل SCM, LYC, FYC, GYC و SLM. أدى تحضين السائل المنوى المخفف للإبل بجميع المحاليل (SLM, SYC, LYC, FYC, GYC, SCM, TYF عند مستوى ١٠٠ مللى أوسمول / لتر على درجة حرارة ٣٧°م لمدة ٦٠ دقيقة إلى انخفاض النسبة المئوية لحيوية الحيوانات المنوية ، والنسبة المئوية للأكروسوم المتماسك معنوياً (على مستوى ٠,٠١) مع زيادة النسبة المئوية للحيوانات المنوية المنتفخة والنسبة المئوية للحيوانات المنوية ملتفة الذيل معنوياً (على مستوى ٠,٠١). كان أقصى استجابة للحيوانات المنوية للإبل لاختبار انخفاض الأسموزية (HOS-test) بدرجة معنوية (على مستوى ٠,٠١) عند تحضين السائل المنوى المخفف بجميع المحاليل بعد ٣٠ دقيقة من التحضين على درجة حرارة ٣٧° م.