J. Agric. Sci. Mansoura Univ., 34 (8): 8657 - 8666, 2009

SOME PHYSICAL CHARACTERISTICS AND RESPONSE TO HYPO-OSMOLAITY LEVELS OF EPIDIDYMAL CAMEL SPERMATOZOA STORED AT 25 OR 5°C.

Abdel-Khalek, A. E.*; M. A. El-Harairy*; Sh. M. Shamiah; M. A. Abu El-Hamd** and W. A. Khalil*.**
*** Anim. Prod. Dept., Fac. Agric., Mansoura Univ., Egypt.**
**** Anim. Prod. Res,. Institute, Agric. Res. Center, Egypt.**
abdelkhalk2004@yahoo.com

ABSTRACT

This study was carried out to evaluate the effect of different storage periods at 25 or 5°C of camel testes (*camelus dromedais*) after slaughter on motility, livability, abnormality and the response to hypo-osmotic swollen test (HOS-test) of epididymal spermatozoa. Twelve testes of camel bulls (6-10 years) were collected from the abattoir after slaughtering and placed immediately into plastic bag in thermos at 25°C or into icebox at 5°C, then transported to the laboratory. The time between the removal of the testes and arrival at laboratory was approximately 6 h. Camel spermatozoa were recovered from the tail of epididymis by aspiration of the testes stored at 25 for or 5°C for 6, 12 or 24 h after slaughtering. Results show that the percent of motility (61.6 *vs.* 43.3 %) and livability (54.3 *vs.* 51.6%) were higher, while the percent of sperm abnormality (19.0 vs. 20.0%) was lower for spermatozoa recovered from testes stored at 25°C for 6 than 12 hours. The differences were significant ($P<0.05$) only for sperm motility. In testes stored at 5°C, percents of motility and livability were decreased by increasing storage period from 6 up to 24 hours, while sperm abnormality increased from 14.0% after 6 h. The differences were not significant to 25% after 24 h. Spermatozoa recovered from testes stored at 25°C for 6 or 12 hours showed similar response to HOS-test, being 46.3 and 46.9%, respectively. Whoever, testes stored at 5°C, percent of curled spermatozoa gradually decreased ($P\geq0.05$) from 51.9 up to 49.9% by increasing storage period from 6 up to 24 h. Percent of curled spermatozoa recovered from testes stored at 25°C increased ($P<0.05$) from 20.1% at 300 mOsmol/l to 69.78% at 50 mOsmol/l. The highest response was observed by decreasing osmolarity level from 200 to 150 mOsmol/l, while the lowest response was found between 100 and 50 mOsmol/l. At 5°C, percents of curling were 19.2% at 300 mOsmol and 76.9% at 50 mOsmol. Percent of curling increased ($P<0.05$) by increasing testing time from 10 up to 30 min for both epididymal spermatozoa recovered from testes stored at 25 or 5°C. All interactions on percentage of curled spermatozoa were not significant at 25 or 5°C. The current study indicated the possibility of recovering epididymal spermatozoa with acceptaple percentages of motility, livability and membrane integrity from testes stored at 5°C for 24 hours collected from slaughtered camels.

Keywords: Camel, epididymal sperm, physical characteristics, osmolarity.

INTRODUCTION

Application of assisted reproduction technologies in camels, such as artificial insemination and embryo transfer, has been slow in comparison to that for other livestock species. In Egypt, there are few attempts to establish IVF-techniques in dromedary camel. (Abdoon, 2001; Ali and Abdel-Razek, 2001; Torner *et al.*, 2003 and Mahmoud *et al*, 2003).

The ultimate goal in reproduction is to produce pregnancies and the method that will produce the best results is always going to be natural mating or at least the use of ejaculated semen. However, in many cases natural mating is not an option and ejaculated semen is unavailable, due to difficulty of handling the animal, death prior to collection or obstructive azoospermia preventing ejaculation (Drouineaud *et al.*, 2003). In these cases, the best alternative source of viable, reproductively capable sperm are those stored in the cauda epididymidis. Research as shown that cauda epididymal sperm, when used with AI can produce offspring in a multitude of species, for example, goat (Blash *et al.*, 2000), dog (Hori *et al.*, 2005). When used with intracytoplasm sperm injection (ICSI) epididymal sperm has produced offspring in a few species including cattle (Goto *et al.*, 1990) and rats (Hirabayashi *et al.*, 2002).

Kolbe and Holtz (1999) reported that using ICSI with epididymal sperm on in vivo or in vitro matured pig oocytes can result in cleavage, however, using fresh ejaculated sperm produced significantly higher cleavage rates than did epididymal sperm. More recently, Probst and Rath (2003) reported the birth of piglets using ICSI and epididymal sperm.

The hypoosmotic swelling test (HOS-T) was used to evaluate functional integrity of the sperm membrane of most mammalian species because spermatozoa with a biochemically active membrane will swell when hypoosmotically stressed, due to the influx of water. The HOS-T is a simple, inexpensive and easily applicable technique (Jeyendren *et al.*, 1984). However rare data are available on the response of camel spermatozoa (ejaculated or epididymal) to HOS-T.

Therefore, the aim of the present work was to study the effect of, storage period of camel testes stored at 25 or 5°C after slaughter, on characteristics and response to hyo-osmotic swelling test (HOS-test) of epididymal spermatozoa.

MATERIALS AND METHODS

The present study was carried out at the Laboratory of Biotechnology, Animal Production Department, Faculty of Agriculture, Mansoura University during the period from February to April 2008.

Total of 12 testis of camel bulls aged from 6 to 10 years were collected at the abattoir after animal slaughtering, then placed immediately into plastic bag in thermos at 25°C or into icebox at 5°C, and transported to the laboratory. Approximately six hours was the time between the removal of the testes and arrival to laboratory.

In the laboratory, each testicle was dissected away from its tunica vaginalis and other extraneous tissues, washed 3 times by tab water and once by alcohol 70%. Various incisions in the tail of epididymis were performed with a scalpel and then, by pressing that region manually, the spermatozoa were released and collected by aspiration with sterile disposable (5 ml) syringe containing 2 ml semen extender. The recovered spermatozoa were placed in a 15 ml tube in bath water at 37°C. Composition

of extender used in semen dilution is presented in the following table according to Aminu Deen *et al.* (2003):

Buffer*	Amount	Extender	Amount
Tris	30.28 g	Buffer	80 ml
Fructose	12.5 g	Egg yolk	20 ml
Citric acid	16.7 g	Benzyl penicillin	1000 IU/ml
Caffeine	0.039 g	Streptomycin sulfate	1000 µg/ml
Distilled water add to	1000 ml		

*** The buffer was autoclaved at 1.1 kg/cm2 pressure for 30 min, cooled and refrigerated until used.**

In the laboratory, spermatozoa was recovered at 6, 12 and 24h after animal slaughtering from the testes stored at 25 or 5°C. Thereafter, samples of diluted epididymal spermatozoa were evaluated to determine the progressive motility, livability, abnormality and hypo-osmotic swollen test.

The percentage of progressive sperm motility in each semen sample was determined using research microscope supplied with hot stage adjusted to 37°C according to Rao and Hart (1948). Percentage of sperm livability was determined in a smear from diluted semen stained by eosin (1.67%) and nigrosin (10%) mixture stain (Hackett and Macpherson, 1965). During the examination of live/dead sperm at a high power magnitude (x 400), the percentage of morphological abnormalities of spermatozoa was also determined according to classification adopted by Blom (1983).

The response of camel epididymal spermatozoa to HOS-test was assessed using solution prepared with fructose (1.25%) and Na-citrate (2.9%) in distilled water (2 times) to give osmolarity of 300 mOsm/l using a freezing-point depression osmometer (Osmett A, Model 5002, Fisher Svientific, Pittsbury, PA, USA). Then, distilled water was added to reach osmolarity level to (50, 100, 150 and 200 mOsm/l) using osmometer. One drop of diluted semen was added to one ml of the hypo-osmotic solution with osmolarity of 50, 100, 150, 200 and 300 mOsm/l into 15 ml tube and the mixture was immediately examined in semen incubated for 10, 20 and 30 min at 37°C in water bath. A semen smear from the mixture was made and dried at the same temperature. The slides were stained with eosin-nigrosine mixture stain.

All prepared slides were examined and numbers of spermatozoa with curled tail were determined using research microscope at higher magnification (x 400). Hundred spermatozoa per slide were counted and percentage of spermatozoa having curled tails was calculated.

Statistical analysis of the obtained data were conducted using General Linear Model of SAS (1996), while differences among the treatment mean were preformed using Duncan Range Test (Duncan, 1955). The percentages values were adjust to arcsine transformed before performing the analysis of variance. Means were presented after being recalculated from transformed values to percentages.

RESULTS

Effect of testis storage period:

Epididymal sperm characteristics:

Results presented in Table (1) show that only progressive motility of epididymal spermatozoa stored at 25 °C significantly ($P<0.05$) decreased from 61.67 to 43.33%, while sperm livability insignificantly decreased and sperm abnormality increased by increasing storage period from 6 to 12h.

At 5°C, only epididymal sperm abnormality percentage was affected significantly ($P<0.05$) by increasing storage period from 6 to 24 h (14 to 25%), while, both percentages of motility and livability of epididymal spermatozoa decreased but differences were not significant.

Table (1): Sperm characteristics of camel testes stored at 25 or 5°C for different storage periods.

Storage period (h)*	Sperm characteristics (%)		
	Motility	Livability	Abnormality
At 25°C:			
6	61.67± 6.01[a]	54.33±2.96	19.0±5.51
12	43.33± 3.33[b]	51.67±1.67	20.0±2.89
24	00.0	00.0	00.0
At 5°C:			
6	71.00± 6.07	73.33±2.97	14.00±1.39[b]
12	61.67± 7.84	72.00±3.83	15.20±1.79[b]
24	58.75±6.78	70.00±3.32	25.00±1.55[a]

[a] and [b]: Means denoted within the same column with different superscripts are significantly different at $P<0.05$. * Storage period of camel testes at 25°C for 6 and 12 h.

It is of interest to note that the observed reduction rate in sperm motility percentage by increasing storage period at 5°C was the highest between 6 and 12 h and the lowest between 12 and 24 h. The opposite trend was true for sperm abnormality. However, reduction rate in sperm livability was nearly similar at all storage period.

It is of interest to observe that percentages of motility and livability were higher and percentage of abnormality was lower for epididymal spermatozoa recovered from testes stored at 5°C than at 25°C (Table 1).

Hypo-osmotic swollen test (HOS-test):

Effect of storage period:

Analysis of variance showed that the percentage of curled spermatozoa was not significant affected after storing camel testes at 25 or 5°C. It is worthy noting that camel spermatozoa recovered from camel epididymis stored at 25°C for 6 or 12 hours showed similar response to HOS-test, being 46.33 and 46.87%, respectively. Inspite the insignificant effect of storage period at 5°C, percentage of curled spermatozoa showed marked decrease from 51.93 up to 49.92% by increasing storage period from 6 up to 24 hours (Table 2).

Analysis of variance showed that the effects of osmolarity level on percentage of curled spermatozoa was significant at 25 or 5°C ($P<0.01$, Table 2).

Table (2): Percentage of curled spermatozoa at different osmolarity levels and testing times.

Item	Curled spermatozoa (%)	
	At 25°C	At 5°C
Testis storage period (h) :		
6 h	46.33±2.88	51.93±0.866
12 h	46.87±3.36	50.64±1.118
24 h	0.00±0.00	49.92±0.968
Osmolarity level (mOsmol/l):		
300	20.10± 1.50^{d}	19.23±1.277^{e}
200	30.72±2.40^{d}	27.14±1.277^{d}
150	49.50± 2.67^{c}	59.48±1.277^{c}
100	62.94±2.31^{b}	71.36±1.277^{b}
50	69.78±1.73^{a}	76.95±1.277^{a}
Testing time (min):		
10	42.10±3.47^{b}	47.49±0.989^{c}
20	46.63±3.79^{ab}	50.62±0.989^{b}
30	51.10±3.96^{a}	54.38±0.989^{a}

$^{a, b,...d}$: Means denoted within the same column with different superscripts for each factor are significantly different at P<0.05).

Camel spermatozoa stored either at 25 or 5°C showed significant (P<0.05) reduction by decreasing osmolarity level from 20.10 or 19.13% at 300 mOsmol/l to 69.78 or 76.95% at 50 mOsmol/l. The highest response was observed by decreasing osmolarity level from 200 to 150 mOsmol/l, while the lowest response was found between 100 and 50 mOsmol/l (Table 2).

The response of camel spermatozoa to HOS-test significantly increased by increasing testing time fro 10 up to 30 min for epididymal spermatozoa recovered from testes stored at both 25 or 5°C (Table 2).

The interaction between storage period and osmolarity level on percentage of curled spermatozoa were not significant at for testes stored at 25 or 5°C.

It is worthy noting that the response of camel spermatozoa was higher when stored at 5 than at 25°C as affected by testis storage period, osmolarity level and testing time.

DISCUSSION

Sperm characteristics

In nearly agreement with present results, Zeidan *et al.* (2006) showed that sperm motility of dromedary camel was 60.75% in semen extended with fructose-Na-citrate solution at 37°C for 60 min. In addition, Martinez-Pastor *et al.* (2005) demonstrated that the quality of epididymal spermatozoa recovered from deer testes decreased with postmortem time. Such decrease was different for semen characteristics. Who added also, motility was the most affected one, and membrane and acrosomal integrity seemed to endure better the postmortem conditions. However, Soler *et al.* (2005) found that the motility of deer spermatozoa stored in the epididymis for up to 96 h did not differ significantly.

In accordance with the marked reduction in sperm characteristics of camel with increasing storage period in this study, Martinez-Pastor *et al.* (2005) recorded acceptable sperm characteristics could still be found after several days of refrigeration in term of sperm motility and acrosomal integrity. Moreover, results of Saenz (2007) indicated no significant differences in sperm parameters of deer epididymal spermatozoa after cooling postmortem testes for 22 hours.

The percentages of sperm motility and livability in this study are lower than those obtained by Wani (2008), who found that the initial motility of epididymal dromedary camel spermatozoa was 85.0% in Tris–egg yolk extenders. In addition, Tajik and Hassan-Nejad Lamsoo (2008), showed that the proportions of live spermatozoa in right dromedary camel testicle were 76.8, 86.9 and 88.8% for epididymal caput, corpus and cauda, respectively, and were not significantly different. In the left testicle, the corresponding values were 85.3, 83.1 and 88.4% for the same parts. The proportions of live sperm in breeding and non-breeding seasons were not significantly different.

It is worthy noting that the present sperm characteristics were almost better when epididymal spermatozoa were collected from camel testis stored at 5°C for 6 or 12 hours than 25°C, while all spermatozoa lost their response (dead spermatozoa) when camel testes were stored at 25 °C for 24 hours. In this respect, early researches indicated that epididymal spermatozoa are less susceptible to cold shock (Lasley and Bogart 1944 and Bialy and Smith 1959) and proteins in seminal plasma reversed cold shock damage on ram spermatozoa (Barrios *et al.*, 2000). In ram, Kaabi *et al.* (2003) found that epididymal spermatozoa stored at 5°C showed better sperm motility and a lower percentage of abnormal forms than epididymes stored at 25°C after 24 and 48 h.

The obtained acceptable results from camel testes stored at cool temperature (5°C) indicated the possibility of using camel epididymal spermatozoa for *in vitro* fertilization inspite of the differences between epididymal and ejaculated spermatozoa. Sperm characteristics from paired epididymal caudae of a bull are not fully comparable, except for the percentage live spermatozoa (Goovaerts *et al.*, 2006). However, motility rates as well as fertilization rates of epididymal spermatozoa were superior to those of ejaculated semen. Meanwhile, Tebet *et al.* (2006) found no significant differences between the electro-ejaculated and epididymal fresh or frozen-thawed cat spermatozoa for sperm motility, sperm progressive status, plasma membrane integrity and sperm morphology.

Curling percentages

Osmotic shock phenomenon caused by the exposure of spermatozoa to different conditions after a time of hypertonic exposure is characterized by increased coiling of the sperm tail, which results in loss of progressive motility (Zavos, 1983 and Mehrez, 2001). For this reason, spermatozoa should be diluted slowly to allow for gradual osmotic adjustment to take place between the intra- and extra-cellular compartments, thus preventing or minimizing the osmotic shock phenomenon occurring (Zavos, 1992). As stated by Correa *et al.* (1997) in bull spermatozoa, the present results indicated differences in response of camel epididymal spermatozoa to

osmotic shock were affected by osmolarity level and incubation time. Also, the response of epididymal spermatozoa to HOS-test obtained in this study was reported in ejaculated spermatozoa of dromedary camel by Zeidan *et al.* (2006). The later authors showed that the percentages of spermatozoa with coiled spermatozoa were significantly ($P<0.01$) lower in the extended semen with fructose-Na-citrate solution at osmolarity levels of 200 and 300 than 50 and 100 mOsmol/L, during incubation at 37°C for up to 60 minutes. They also added, the incubation of extended camel semen at 37°C for up to 60 minutes at different osmolarity levels significantly ($P<0.01$) increased the percentages of coiled spermatozoa. The maximum reactivity of camel spermatozoa to HOS- test significantly ($P< 0.01$) reached at 30 minutes of incubation at 37°C. The present results regarding the effect of level of osmolarity and incubation time on epididymal spermatozoa camel indicated similar responses as mammalian spermatozoa to hypo-osmotic swelling test (HOS-test) were reported in bovine (Rodringuez *et al.*, 1994 and Zaneveld and Jeyendran, 1990), ram (Moussa, 1999), man (Jeyendren *et al.*, 1984), horse (Caiza *et al.*, 1997), buffaloes (Dandoush, 2002, El-Sherbieny, 2004 and Ahmad, 2008) and camel (Zeidan *et al.*, 2006). A high correlation between HOS-test and sperm motility or live spermatozoa was recorded and was mainly attributed to that motility depends partly on membrane transport (membrane integrity) and other biochemical activity (Jeyendren *et al.*, 1984). It is of interest to observe that the response to HOS-test was higher for spermatozoa obtained from testes stored at cool temperature than those stored at 25°C, regardless storage period, osmolarity level and incubation time. These findings were also observed in buffalo spermatozoa (Ahmad, 2008 and El-Nagar, 2008).

The current study indicated the possibility of recovering epididymal spermatozoa with acceptaple percentages of motility, livability and membrane integrity from testes stored at 5°C for 24 hours collected from slaughtered camels.

REFERENCES

Abdoon, A. S. S. (2001). Factors affecting follicular population, oocyte yield and quality in camels (*Camelus dromedarius*) ovary with special reference to maturation time *in vitro*. Anim. Reprod. Sci., 66: 71-79.

Ahmad, I. A. E. (2008). Effect of adding ascorbic acid on semen quality of Egyptian buffalo bull. M. Sc. Thesis Fac. of Agric., Mansoura Univ., Egypt.

Ali, A. and Abdel-Razek, A. KH. (2001). Comparison of number and quality of oocytes in the Egyptian buffaloes (*Bubalus bubalis*), cows (*Bos taurus*) and camels (*Camelus dromedarius*). Assiut Vet. Med. J., 45: 317-325.

Aminu Deen, Sumant, V. and Sahani, M.S. (2003). Semen collection, cryopreservation and artificial insemination in the dromedary camel. Animal Reproduction Science, 77: 223-233

Barrios, B; Perez-Pe, R; Gallego, M; Tato, A; Osada, J; Muino-Blanco, T. and Cebrian-Perez, J. A. (2000). Seminal plasma proteins revert the cold-shock damage on ram sperm membrane. Biol. Reprod. 63: 1531-1537.

Bialy, G. and Smith, V. R. (1959). Cold shock of epididymal spermatozoa. J. Dairy Sci., 42: 2002.

Blash, S; Melican, D. and Gavin, W. (2000). Cryopreservation of epididymal sperm obtained at necropsy from goats. Theriogenology, 54: 699-905.

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

Caiza, F. L; Rigau, T; Bonet, S; Miro, J; Briz, M. and Rodriguez-Gil, J. E. (1997): Subjecting horse spermatozoa to hypo-osmotic incubation effects of equine. Theriogenology, 47: 765-784.

Correa, J. R.; Pace, M. M. and Zavos, P.M. (1997). Relationships among frozen-thawed sperm characteristics assessed via the routine semen analysis, sperm functional tests and fertility of bulls in an artificial insemination program. Theriogenology, 48: 721-731.

Dandoush, S. I .S. (2002). Comparative studies on methods of evaluating semen quality in farm animals. M. Sc. Thesis, Fac. of Agric. Mansoura Univ., Egypt.

Drouineaud, V; Sagot, P. Faivre, L; Michel, F. and Jimenez, C. (2003). Birth after intracytoplasmic injection of epididymal sperm from a man with congenital bilateral absence of the vas deferens who had a robertsonian translocation. Fertil. Steril., 79: 1649-1651.

Duncan, D.B. (1955). Multiple Range and Multiple "F" Test. Biometrics, 11: 1-42.

Edwards, R. G. (1970). Fertilization of human eggs *in vitro*. Proc 9th Int'l Embryological Conference, (Moscow). Plenum Press: New York. 44-50.

El-Nagar, H. A. A. (2008). Studies on frozen buffalo bull semen. Ph.D.Thesis Agric. Sci. Faculty Agric., Al-Azhar. Univ., Egypt.

El-Sherbieny, M. A. (2004). Physiological study on farm animals. Ph. D. Sc. Thesis Fac. of Agric. Mansoura Univ., Egypt.

Goovaerts, I. G. F; Hoflack, G. G; Van Soomb, A; Dewulf, J; Nichi, M; de Kruif, A. and Bols, P. E. J. (2006). Evaluation of epididymal semen quality using the Hamilton–Thorne analyser indicates variation between the two caudae epididymides of the same bull. Theriogenology, 66: 323-330.

Goto, K; Kinoshita, A; Takuma, Y. and Ogawa, K. (1990). Fertilization of bovine oocytes by the injection of immobilised, killed spermatozoa. Vet. Rec., 127: 517-520.

Hackett, A. J. and Macpherson, J. W. (1965). Some staining procedures for spermatozoa. A review. Can. Vet. J., 5: 55.

Hirabayashi, M; Kato, M; Aoto, T; Sekimoto, A; Ueda, M; Miyoshi, I; Kasai, N. and Hochi, S. (2002). Offspring derived from intracytoplasmic injection of transgenic rat sperm. Trans. Res., 11: 221-238.

Hori, T; Hagiuda, K; Kawakami, E. and Tsutsui, T. (2005). Unilateral intrauterine insemination with prostatic fluid-sensitized frozen caudal epididymal sperm in beagle dogs. Theriogenology, 63: 1573-1583.

Jeyendren, 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.

Kaabi, M; Paz, P; Alvarez, M; Anel, E; Boixo, J.C; Rouissi, H; Herraez, P. and Anel, L. (2003). Effect of epididymis handling conditions on the quality of ram spermatozoa recovered post-mortem. Theriogenology, 60: 1249-1259.

Kolbe, T. and Holtz, W. (1999). Intracytoplasmic injection (ICSI) of *in vivo* or *in vitro* matured oocytes with fresh ejaculated or frozen-thawed epididymal spermatozoa and additional calcium-ionophore activation in the pig. Theriogenology, 52: 671- 682.

Lasley, J. F. and Bogart, R. (1944). A comparative study of epididymal and ejaculated spermatozoa of the boar. J. Anim. Sci., 3: 360-370.

Mahmoud, K. GH. M; El-Shahat, K. H. and El-Nattat, W. S. (2003). Chromosome configuration during *in vitro* maturation of dromedary camel oocytes. Vet. Med. J., Giza, 51: 411-420.

Martinez-Pastor, F; Guerra, C; Kaabi, M; Diaz, A. R; Anel, E; Herraez, P; De Paz, P. and Anel, L. (2005). Decay of sperm obtained from epididymes of wild ruminants depending on postmortem time. Theriogenology, 63: 24-40.

Mehrez, A. F. (2001). Study on viability and membrane integrity of cryopreserved spermatozoa of friesian bull under different thawing temperature. J. Agric. Sci. Mansoura Univ., 26: 6735.

Moussa, I. A. (1999). Evaluation of nagdi rams spermatozoa using hypo-osmotic test. Zag. Vet. J. 27: 26.

Probst, S. and Rath, D. (2003). Production of piglets using intracytoplasmic sperm injection (ICSI) with flowcytometrically sorted boar semen and artificially activated oocytes. Theriogenology, 59: 961-973.

Rao, C. K. and Hart, G.H. (1948). Morphology of bovine spermatozoa. Am. J. Vet. Res., IX: 117–124.

Rodringuez, J. E.; Montserrat, A. and Rigau, T. (1994). Effect of hypo-osmotic incubation on acrosome and tail structure on canine spermatozoa. Theriogenology, 42: 815.

Saenz, J. R. (2007). Cryopreservation of White-Tail Deer Epididymal Sperm for Artificial Insemination. M.Sc. Thesis, Faculty of the Louisiana State University and Agricultural and Mechanical College.

SAS (1996). SAS/Stat. User's Guide Static's, Ver., 6.06 4th Ed. SAS Institute Inc. Cary, NC.

Soler, A.J; Esteso, M.C; Fernandez-Santos, M.R. and Garde, J.J. (2005). Characteristics of Iberian red deer (*Cervus elaphus hispanicus*) spermatozoa cryopreserved after storage at 5-8°C in the epididymis for several days. Theriogenology, 64: 1503-1517.

Tajik, P. and Hassan-Nejad Lamsoo, M. R. (2008). Assessment of epididymal sperm obtained from dromedary camel. Iranian Journal of Veterinary Research, Shiraz University, 9: 46-50

Tebet J. M; Martins, M. I. M; Chirinea, V. H; Souza,F. F; Campagnol, D. and Lopes, M. D. (2006). Cryopreservation effects on domestic cat epididymal versus electroejaculated spermatozoa. Theriogenology, 66: 1629–1632.

Torner, H; Heleil, B; Alm, H; Ghoneim, I.M; Srsen V; Kantiz, W; Tuchscherer, A. and Fattouh, E.M. (2003). Changes in cumulus-oocyte complexes of pregnant and non-pregnant camels (*Camelus dromedarius*) during maturation *in vitro*. Theriogenology, 60: 977-987.

Wani, N. A. (2008). *In vitro* embryo production in camel (*Camelus dromedarius*) from *in vitro* matured oocyte fertilized with epididymal spermatozoa stored at 4°c. Animal Reproduction Science, In press.

Zaneveld, L. J. D. and Jeyendran, R. S. (1990). Hypo-osmotic swelling test. In: Handbook of the Laboratory Diagnosis and Treatment of Infertility. Keel B.A., Webster B.W.(eds.). Boca Raton, CRC Press.

Zavos, P. M. (1983). Opisthosmotic shock of frozen – thawed human spermatozoa. Infertility, 5: 247-255.

Zavos, P. M. (1992). Preparation of human frozen – thawed seminal specimens using the spermprep filtration method : Improvements over the conventional swim-up method. Fertility and Sterility, 57: 1326 – 1330.

Zeidan, A. E. B; Ahmadi E. A. A; Alfuraiji M. M. and Daader, A. H. (2006). Evaluation of the functional integrity of the dromedary camel spermatozoa membrane using hypoosmotic swelling test. Proc. of the International Scientific Conference on Camels, 10-12May, Qassim University, K.S.A.

بعض الخصائص الطبيعية والأستجابه لمستويات أسموزيه منخفضة للحيوانات المنوية المستردة من مخاصي الجَمال المخزنه على ٢٥ أو ٥ درجه مئويه.

عبد الخالق السيد عبد الخالق*، مصطفي عبد الحليم الحرايري*، شريف مغاوري شاميه، محمد عوض أبوالحمد** و وائل أحمد خليل*.**

*** قسم إنتاج الحيوان – كلية الزراعة – جامعة المنصورة**

**** معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية**

تهدف هذه الدراسة إلى معرفة أثر فترة التخزين (٦، ١٢ و ٢٤ ساعة) لمخاصى الجمال وحيد السنام على درجة حرارة ٥ أو ٢٥ °م على الخصائص الطبيعية للحيوانات المنوية المستردة من بربخ الخصية ومدى استجابة الحيوانات المنوية لاختبار (HOST-test). تم جمع مخاصى الجمال من السلخانات المحلية ووضعت في أكياس بلاستيك ثم في ترمس على درجة حرارة ٥ أو ٢٥ °م ونقلت إلى المعمل. حيث تم استرداد الحيوانات المنوية من ذيل البربخ بطريقة الشفط.

أظهرت النتائج أن نسبة الحركة التقدمية كانت (٦١,٦مقابل ٤٣,٣ %) وكانت نسبة الحيوانات المنوية الحية (٥٤,٣ مقابل ٥١,٦%) بينما كانت نسبة الشواذ (١٩,٠ مقابل ٢٠,٠%) للحيوانات المنوية المستردة من الخصية المخزنة علي ٢٥°م لمدة ٦ ساعات و١٢ ساعة علي التوالي. تناقصت نسبة كل من (الحركة التقدمية والحيوانات المنوية الحية) بزيادة فترة التخزين من ٦ إلي ٢٤ ساعة وذلك عند الحفظ علي درجة ٥°م، بينما زادت نسبة الشواذ من ١٤% بعد ٦ ساعات إلي ٢٥% بعد ٢٤ ساعة من التخزين.كانت نسبة الحيوانات المنوية ذات الذيل الملتوي ٤٦,٣ و٤٦,٨% للحيوانات المنوية المستردة من الخصية المخزنة علي ٢٥°م لمدة ٦ و١٢ ساعة علي التوالي. بينما علي ٥°م تناقصت نسبة الحيوانات المنوية ذات الذيل الملتوي من ٥١,٩ إلي ٤٩,٩% بزيادة فترات التخزين من ٦ وحتي ٢٤ ساعة. زادت نسبة الحيوانات المنوية ذات الذيل الملتوي والمستردة من الخصية المخزنة علي ٢٥°م من ٢٠,١% علي ٣٠٠ مللي إزمول/لتر إلي ٦٩,٧٨% علي ٥٠ مللي إزمول/لتر. بينما علي ٥°م كانت نسبة الحيوانات المنوية ذات الذيل الملتوي ١٩,٢% علي ٣٠٠ مللي إزمول/لتر و ٦٧,٩% علي ٥٠ مللي إزمول/لتر. زادت نسبة الحيوانات المنوية ذات الذيل الملتوي زيادة معنوية بزيادة وقت الإختبار من ١٠ وحتي ٣٠ دقيقة للحيوانات المنوية المستردة علي ٥ °م وأيضا للحيوانات المنوية المستردة علي ٢٥°م. تشير نتائج هذا البحث إلي إمكانية إسترداد الحيوانات المنوية من ذيل البربخ في خصية الجمال مع نسبه عالية من الحركة التقدمية والحيوانات المنوية الحية وسلامة أغشية الحيوانات المنوية وذلك عند تخزين الخصية علي ٥°م حتى ٢٤ ساعه بعد الذبح.