J. Agric. Sci. Mansoura Univ., 31 (11): 6919 - 6929, 2006

EVALUATION AND FREEZING OF BILLY GOAT SEMEN USING UNCONVENTIONAL PACKAGING METHODS

Khalifa, E. I.*; E .S. El-Gohary*; O. A. Salama* and M.M. Awad**

*** Animal Production Res. Inst., Agric. Res. Center, Ministry of Agric., Egypt.**

**** Animal Production Dept., Faculty of Agric., Suez Canal Univ., Ismailia, Egypt.**

ABSTRACT

The experimental work was conducted with two breeds of billy goats Baladi and Zaraibi to evaluate semen characteristics, compare different packaging methods and post thawing sperm recovery during incubation time. Artificial vagina was used to collect twenty ejaculates (ten pooled ejaculates from each breed) to design three experiments for each experimental breed. The ejaculates showed progressive motility over than 80.00 % were pooled and initial pooled ejaculate characteristics were evaluated and extended with Tris-yolk-fructose (TYF) cryoprotective with 6% glycerol. The first experiment was carried out with unfrozen semen to estimate initial semen characteristics (motility, live and normal) after collection, extension and equilibration period at 5^0C for 2 hours. The second experiment was conducted with frozen semen to compare between efficiency of unconventional packaging methods (aluminum sacks and cachet tablet strips) and conventional packaging methods (straws and pellets on paraffin wax) to define the best post-thawing sperm characteristics. The third experiment was to study the effect of incubation time at 37^0C to 3 hrs on post- thawing sperm motility and sperm recovery. The results recorded significant ($p<0.01$) effect between initial sperm characteristics in the first experiment the mean of progressive motility after collection, extension, and equilibration was 89.50 %, 88.50 % and 86.00 % for Baladi bucks however, for Zaraibi bucks was 86.00 %, 82.50 % and 80.50 %, respectively. In the second and third experiments the results showed significant ($p<0.01$) effect between packaging methods and incubation periods on post-thawing sperm characteristics, respectively. The post thawing sperm motility values for Baladi and Zaraibi bucks were respectively with straws (56.00%, 54.50 %) and aluminum sacks (55.00%, 53.50 %) compare to tablets (50.50 %, 47.00 %) and pellets (45.50 %, 42.05 %).The results showed that differences among breeds were not significant.

Keywords: cryopreservation, packaging systems, billy goat semen.

INTRODUCTION

Successful artificial insemination (AI) in goats depends on the method of semen collection, extension media, composition of the cryoprotective sperm material and type of packaging methods for extended semen. AI in goat breeding in Egypt is becoming very important. Natural matting in goat is routinely used in many countries while the use of frozen semen is still limited because the best packaging technique to data of post-thaw survival sperm population was low. The factors affecting post thawing motility and fertilizing capacity of spermatozoa depend on the method of semen collection, preservation technique, cryoprotectant materials and packaging methods. Therefore, the final goal of packaging extended semen is not only to maintain the post thawing sperm motility but also to maintain sperm cells, plasma proteins to survive in the female reproductive tract at the time of fertility,

acrosomal enzymes for penetration of the ovum, capacity of progressives movement and to prevent any damage which reduce life span of spermatozoa (Awad, 1998, Paulenz *et al.*, 2004 and Khalifa, 2005). The packaging method is necessary to protect the sperm cells from the freezing shock and changes in the composition of the diluted media. The present work aimed to preserve Baladi and Zaraibi billy goat semen in unfrozen and frozen form. The first experiment for unfrozen semen was to evaluate initial semen characteristics after collection, extension and equilibration period. Concerning the frozen semen, the second experiment was carried out to compare between conventional packaging methods (straws and paraffin wax pellets) and unconventional packaging methods (aluminum sacks and cachet tablet strips) also, in the third experiment to evaluate post thawing motility and sperm recovery during incubation time at 37^{0}C to 3 hrs for two buck breeds.

MATERIALS AND METHODS

This experimental work was carried out at Suez Canal University, Experimental Animal Research Farm, Faculty of Agriculture, Egypt and El-Serw Animal Production Research Station, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt.

Experimental animals and feeding

In all experiments 4 Baladi goat bucks from Suez Canal University Experimental Animal Research Farm, weighed 38-42 kg and aged 3-4 years, also, 4 Zaraibi goat bucks from El-Serw Experimental Farm Animals, weighed 45-52 kg and aged 3-3.5 years were used. All bucks in experimental work were healthy, free of diseases; the testes were normal, almost equal in size and moved freely within the scrotal pouches. The Baladi bucks in Suez Canal University Farm were fed on rice straw plus concentrate feed mixture (yellow maize 40%, undecorticated cotton seed meal 20%, wheat bran 37%, limestone 2% and commercial salt 1%). However, Zaraibi bucks in El-Serw were fed on rice straw all day plus concentrate feed mixture (35% cotton seed, 22% corn, 33% wheat bran, 4% rice bran, 3% molasses, 2% limestone and 1% salt). Fresh water is presented all day time.

Semen collection and evaluation

Semen was collected into a warmed artificial vagina. Active ejaculates were pooled together for all experimental bucks. Ten pooled ejaculates from each breed were used. The following initial measurements percentage of progressive motile sperm, live sperm %, and normal sperm % were taken from pooled ejaculates immediately after collection.

Semen extension

Egg yolk in semen extension was exposed to heating at 60^{0}C for up 5 minutes in water bath to destroy egg yolk coagulation enzyme according to Roy (1957), Iritani and Nishikawa (1961, 1963), Iritani *et al.* (1961), Leboeuf *et al.* (2000) and Khalifa (2005).

Semen extension was performed in two steps. In the first step, semen diluted 1semen: 4 diluent A from Tris-yolk-fructose (TYF free of cryoprotectant material) at room temperature after collection and still for 30 minuets to adapt with diluent. In the second step, the TYF diluent B contained

double concentration of cryoprotectant material (12 ml glycerol) was added gradually at room temperature to diluent A to give the final extension rate 1 semen: 8 extension (1 semen: 4 diluent A and 4 diluent B) and final cryoprotectant material was 6 % glycerol. Then, extended semen was mixed gently and sperm motility characteristics were estimated.The composition of TYF extender is presented in Table 1.

Packaging methods

Packaging in straws

Before the equilibration period the extended semen was packaging manually filled by suction at room temperature in 0.5 ml French straws and air bubble at the end of the straws was created and plugged with polyvinyl powder for sealing the open end of straws. Filling straws were kept in clean and dry test tubes.

Packaging on paraffin wax

The pellets were done on the surface of paraffin wax block that poured in aluminum box with the layer 1cm depth, then left to reach room temperature and grooves were made by means of an especial catheter immediately in the surface of paraffin wax to full of 0.5 ml extended semen. The extended semen for pellets was kept in dry and clean test tube and dropped in the grooves after equilibration period.

Packaging in cachet tablet strips

Empty cachet tablet strips were used for packaging frozen semen and fill of 0.5 ml extended semen, the frozen semen was considered as tablets. The extended semen for cachet tablets was kept in dry and clean test tube, and then poured in grooves after equilibration period.

Packaging in aluminum sacks

The aluminum sacks were made from foil the dimensions was 2 cm wide x 4 cm length the total volume of extended semen was 0.5 ml in sacks. The sacks were filled before equilibration period. The opening for pouring extended semen in sack was closed by plaiting. The filling straws, aluminum sacks and the two test tubes for pellets and tablets were put in beaker contained water at room temperature. Moreover, test tube with a thermometer was placed in the same beaker to facilitate periodic checking of the temperature.

Equilibration period

The beaker was exposed two types of equilibration periods:

Gradual cooling equilibration period

It was started when the beaker placed into special foam box the crushing ice was surrounded the beaker then, the temperature decreased gradually to reach 5^{0}C during 45-60 minutes.

Refrigerator cooling equilibration period

It was started when the beaker was taken from the foam box immediately and placed into refrigeration at 5^{0}C for 2 hours. The initial sperm characteristics were evaluated after equilibration period.

Freezing and thawing technique

After straws, aluminum sacks and two test tubes containing the extended semen for pellets and tablets were reached 5^{0}C the block of paraffin wax and

cachet tablet sprits filled with extended semen were placed in a horizontal position on the metal rack with straws and aluminum sacks into foam box contained liquid nitrogen and exposed to liquid nitrogen vapor at level 3cm distance above the surface of liquid nitrogen. Thereafter, the foam box containing the liquid nitrogen vapor was covered the packaging were kept at this level for 20 minutes after that dipped in liquid nitrogen. After 5 minutes from dripping straws, aluminum sacks, tablets and pellets were collected and stored in canister below the surface of liquid nitrogen container. The thawing technique for frozen straws and aluminum sacks were withdrawn from container and dipped immediately into water bath at 37^{0}C for 60 seconds. However, pellets and tablets were thawed by taking two pellets and two tablets with forceps and placed in test tube at 37^{0}C for up 60 seconds. After thawing extended semen characteristics (progressive motility %, live sperm %, normal sperm % and sperm recovery %) were estimated.

The creation from data

Sperm recovery was calculated after thawing as the following:

$$\textbf{Sperm recovery \%} = \frac{\textbf{post thawing motility}}{\textbf{Initial motility}} \times 100$$

Incubation period at 37^{0}C for 3hours

Clean and four dry test tubes placed in water bath at 37^{0}C the extended semen from straws, sacks, tablets and pellets were poured in each tube and the incubation period was started the progressive motility and sperm recovery was estimated at zero time for 3hours.

Statistical analysis

Data were statistically analyzed by the analysis of variance according to Snedecor and Cochran (1982). Percentage values were transformed to arcsine values before being statistically analyzed. Duncan Multiple Ranges Test, (Duncan, 1955) was used for test significance of the differences between means.

RESULTS AND DISCUSSION

Initial semen characteristics

Semen characteristics after collection, extension and equilibration are the main parameters used to assess sperm fertilizability of frozen semen. Tables 2 showed the physiological characteristics of Baladi and Zaraibi buck spermatozoa after collection, extension and equilibration period yielded highly significant ($p<0.01$). These results are in agreement with those of Awad (1998) who worked on Boer bucks and found that sperm motility recorded significantly different ($p<0.01$), It was 79% after extension and 76% after equilibration period for TYF extender. The decline in motility after extension and equilibration period could be due to changes in sperm membrane that affected by extension and cryopreservation media includes the plasma membrane, outer acrosomal membrane, the mitochondrial membrane and damaged the motility apparatus of spermatozoa (Ochkur, *et al.*, 1994). Also, Watson (1995) found that changes in active transport permeability of the sperm plasma membrane in the tail region and alteration of the energy availability or damage to the axonemal. However, the maintenance of sperm

motility after equilibration period at 5°C for up 2 hours may be due to the egg yolk was the most effective agent to protect spermatozoa against any shock that preserved life ability of sperm cell. Watson (1976) and Holt *et al.* (1988) found that egg yolk prevented the looping of the tail and protected the motility. It is known that the low density lipoprotein fraction, a high molecular weight component, which can only act at the cell surface against cold shock. The protection is due to the lipids in egg yolk and that emulsification increase its active interaction with the membrane surface (Awad, 1998). Moreover, the active motility after equilibration period at 5°C may be due to penetrate calcium that generate ATP through mitochondrial. These results are in accordance with those obtained by Robetrson and Watson (1986, 1987) who found that membrane permeability was increased by cooling allowing calcium to penetrate the cells.

Semen characteristics after thawing for the conventional and unconventional packaging methods

The packaging methods of extended semen in straws and aluminum sacks appeared to be superior methods than pellets and tablets for post thawing sperm characteristics for Baladi and Zaraibi bucks Table 3. The effect of packaging methods on post thawing characteristics of billy goat spermatozoa were significant ($p<0.01$). These results are in agreement with those of Awad (1989) who found that post thawing motility of ram semen after freezing in straws (32.90%) was superior to freezing than pellets (29.89%). This phenomenon might be due to that the straws act as a coat around the extended semen which gives a gradual cooling and freezing rate when exposed to the vapour of liquid nitrogen this such as aluminum sacks. Also, the foil was kept the cooling at 5°C during equilibration time that beneficial for freezing process. While the pellets or tablets offer less protection to spermatozoa during freezing processes because of their direct contact to the surrounding cold media either paraffin wax or tablet strips. Moreover, it causes faster and less gradual changes in deep freezing temperature in the pellets and tablets than straws and sacks. So that it could be expected that the number of injured spermatozoa by cold shock is higher in pellets and tablets than straws and aluminum sacks (Awad, 1989 and Zeidan, 1995). However, these results disagreed with those of Schmehl *et al.*, (1986) which revealed that freezing of ram semen as pellets on dry ice was superior to freezing in French straws. On the other hand, Ivanova *et al.*, (1997) concluded that the cryopreserved of canine spermatozoa in aluminum tubes results in better semen quality than in pellets and the post thawing motility was 25.90 % for aluminum tubes and 20.45 % for pellets moreover, the post-thaw survival sperm / minutes was 223.64 for aluminum tubes and 190.9 for pellets. The present study showed that the ability of tablets to protect frozen semen was higher than pellets. This phenomenon might be due to that frozen semen setting into tablets grooves until end of freezing process also, the freezing may be happened gradually in tablets because the sheath of grooves surrounded the semen however, pellets were shed directly from the surface of the paraffin wax and became free in liquid nitrogen may be reduced protection and exposed spermatozoa in pellets to freezing shock that caused low sperm characteristics after thawing.

Incubation time at 37^{0}C for different packaging methods

The results for Baladi and Zaraibi bucks showed that, the advancement of thawing incubation up to 3 hours at 37^{0}C, the percentage of post thawing motility (Tables 4) and sperm recovery (Tables 5) of goat spermatozoa decreased significantly ($P<0.01$). The incubation time was better with straws and aluminum sacks than pellets and tablets. These results are in general agreement with those of Awad (1989) who explained that straws gave better post thawing motility than pellets. In the bull semen Zeidan (1995) found that the means of post thawing motility after incubation at 37^{0}C up 2 hours for straws, pellets and tablets was 30.55, 28.60 and 26.10 %. Also, the freezability was 37.35, 34.96 and 32.12 %, respectively. The best values of post thawing motility and sperm recovery for straws, aluminum sacks and tablets during incubation period may be due to that the increasing of spermatozoa protection from freezing shock that causes an increase in sperm metabolic activity compared to pellets. However, decreasing motility during incubation may be due to increasing in sperm metabolic activity and consequently, increase lactic acid production which in turn exerts a toxic effect on the sperm cells (Zeidan, 1995 and Awad, 1998). Moreover, post-thawing motility decrease significantly during incubation because the gradual decline in ability of spermatozoa to generate ATP through mitochondrial respiration as a consequence of mitochondrial aging (Awad, 1998). The toxic effect of membrane bound aromatic amino acid oxidase (AAAO) enzyme released from dead spermatozoa (Vishwanath and Shannon 2000, Marti *et al.*, 2003 and Bag *et al.*, 2004).

Table 1: The composition of TYF extender for the Baladi and Zaraibi billy goats

Ingredients	Basic Dilute. A	Dilute. B
Tris (g)	3.028	3.028
Citric acid (g)	1.675	1.675
Fructose (g)	1.25	1.25
Glycerol (v/v)	-	12
Egg yolk (v/v)	20	20
*Antibiotic (v/v)	0.5	0.5
Distilled water up to/ml	100	100

*Each 0.5 ml of antibiotics contained: 3750 I. U .Penicillin G procaine, 1250 I .U. Penicillin G sodium and 6.25 mg Streptomycin (as Sulfate).

On the other hand, the decreasing in motility during incubation may be related to change in sperm morphology, the sperm has usually containing mitochondria in mid-piece and tail after freezing and thawing, severe dilation or even loss of mitochondria in the mid-piece and tail breakage frequently occurred in the post-thawed sperm these changes might adversely affects function of mitochondria and tails, thus reducing the sperm flagellate movement (Bergeron *et al.*,2002).The loss mitochondria was probably a result of swelling of mid-piece the swelling pushed mitochondria away from the mid-piece or caused structural damage within mitochondria that caused decrease in generation of ATP(Glogowski *et al.*, 2002 and Zhang *et al.*,2003).

Comparison between Baladi and Zaraibi bucks

The overall means between the experimental bucks Baladi and Zaraibi are presented in Table 6. The results showed that the initial characteristics, post thawing semen characteristics for different packaging methods, post thawing motility and sperm recovery during incubation time at 37°C for 3 hours yielded insignificantly different.

Table 2: Means ±S.E of initial semen characteristics for Baladi and Zaraibi billy goats

Animal breed	Item %	Initial semen characteristics		
		collection	extension	equilibration
Baladi	Progressive motility	89.50 [a] ±0.50	88.50 [b] ±0.76	86.00 [c] ±1.00
	Live sperm	88.10 [a] ±1.12	84.00 [b] ±0.54	83.10 [c] ±0.56
	Normal sperm	86.50 [a] ±1.25	82.80 [b] ±0.53	81.90 [c] ±0.65
Zaraibi	Progressive motility	86.00 [a] ±1.250	85.50 [b] ±1.12	83.50 [c] ±1.10
	Live sperm	87.00 [a] ±0.98	84.30 [b] ±0.99	81.60 [c] ±0.85
	Normal sperm	86.30 [a] ±1.25	83.80 [b] ±0.96	80.90 [c] ±0.89

The means values with a, b and c letters in the same raw, appearing significant ($p<0.01$).

Table 3: Means ±S.E of post thawing semen characteristics with different Packaging methods for Baladi and Zaraibi billy goats

Animal breed	Post thawing characteristic %	Packaging method			
		straws	aluminum	tablets	pellets
Baladi	Post thawing	56.00 [a] ±1.25	55.00 [a] ±1.49	50.50 [b] ±1.38	45.50 [c] ±1.17
	Thawing live	53.70 [a] ±2.21	52.10 [a] ±1.70	46.60 [b] ±1.45	41.61 [c] ±1.23
	Thawing normal	67.80 [a] ±2.50	66.70 [a] ±2.71	60.70 [b] ±2.07	54.30 [c] ±1.76
	Sperm recovery	62.55 [a] ±1.24	61.41 [a] ±1.45	55.86 [b] ±1.69	50.82 [c] ±1.18
Zaraibi	Post thawing	54.50 [a] ±1.57	53.50 [a] ±1.30	47.00 [b] ±1.34	42.50 [c] ±2.01
	Thawing live	51.40 [a] ±1.81	50.00 [a] ±1.36	44.40 [b] ±1.35	39.00 [c] ±1.78
	Thawing normal	66.00 [a] ±2.45	64.40 [a] ±2.71	58.50 [b] ±2.44	51.40 [c] ±1.42
	Sperm recovery	63.26 [a] ±1.09	62.17 [a] ±0.96	54.54 [b] ±1.12	49.24 [c] ±1.76

Means with different letters a, b and c in the same raw, differ significantly ($p<0.01$).

Table 4: Percentages ±S.E of post thawing motility for Baladi and Zaraibi goat sperms during incubation time using different packaging methods

Animal breed	Incubation time/ hour	Packaging method			
		straws	aluminum	tablets	pellets
Baladi	0	56.00 [a] ±1.25	55.55 [a] ±2.41	50.00 [b] ±1.29	45.50 [c] ±1.67
	1	48.50 [a] ±1.50	47.00 [a] ±2.00	41.00 [b] ±1.69	37.00 [c] ±1.57
	2	38.00 [a] ±2.13	37.50 [a] ±2.61	32.00 [b] ±2.13	26.50 [c] ±1.83
	3	29.50 [a] ±2.41	28.00 [a] ±2.91	20.50 [b] ±2.63	14.50 [c] ±2.03
Zaraibi	0	54.50 [a] ±1.57	53.50 [a] ±2.41	47.00 [b] ±1.34	42.50 [c] ±2.01
	1	47.00 [a] ±2.13	46.50 [a] ±1.83	41.50 [b] ±1.50	35.00 [c] ±2.47
	2	42.00 [a] ±2.49	39.50 [a] ±2.29	36.50 [b] ±2.69	26.00 [c] ±2.45
	3	36.50 [a] ±2.36	34.50 [a] ±2.83	26.00 [b] ±1.79	18.00 [c] ±2.60

Means with different letters a, b and c in the same row, differ significantly ($p<0.01$).

Table 5: percentages ±S.E of sperm recovery for Baladi and Zaraibi goat sperms during incubation time using different packaging methods

Animal breed	Incubation time/ hour	Packaging method			
		straws	aluminum	tablets	pellets
Baladi	0	62.55 [a] ±1.24	61.51 [a] ±1.49	55.82 [b] ±1.69	50.82 [c] ±1.18
	1	54.73 [a] ±1.31	52.45 [a] ±2.08	45.78 [b] ±1.73	41.31 [c] ±1.61
	2	43.38 [a] ±2.28	41.77 [a] ±2.84	35.72 [b] ±2.32	29.55 [c] ±2.00
	3	32.88 [a] ±2.61	31.18 [a] ±3.18	22.94 [b] ±2.87	16.18 [c] ±2.25
Zaraibi	0	63.26 [a] ±1.09	62.17 [a] ±0.96	54.54 [b] ±1.12	49.24 [c] ±1.76
	1	54.51 [a] ±1.94	53.99 [a] ±1.70	48.16 [b] ±1.25	40.47 [c] ±2.46
	2	48.65 [a] ±2.45	48.81 [a] ±2.32	39.98 [b] ±1.63	29.96 [c] ±2.46
	3	42.24 [a] ±2.30	39.91 [a] ±2.96	30.10 [b] ±1.81	20.67 [c] ±2.78

Means with different letters a, b and c in the same raw, differ significantly ($p<0.01$).

Table 6:Comparison between experimental bucks Baladi and Zaraibi

Item %	Experimental bucks	
	Baladi	Zaraibi
Post thawing motility during incubation	37.91±3.82	39.16±4.32
Sperm recovery during incubation	42.35±4.17	45.23±3.81

Conclusion

The results indicated that aluminum sacks and tablets showed protective effect against mechanical and osmotic damage to spermatozoa during the freezing and thawing process. Therefore, the study demonstrated that aluminum sacks and tablets are beneficial to protect frozen semen and economic for cryopreservation cost.

REFERENCES

Awad, M. M. (1989). Studies on freezing ram semen by two different methods. M. Sc. Thesis. Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

Awad, M. M. (1998). Comparative studies of some treatments on the survival and fertilizing ability of sheep and goat spermatozoa .Ph .D. Thesis, Fac., Agric., Suez Canal Univ., Egypt.

Bag, S., Joshi, A., Naqvi, S. M. K. and Mittal, J. P. (2004). Effect of post-thaw incubation on sperm kinematics and acrosomal integrity of ram spermatozoa cryopreserved in medium-sized French straws. Theriogenology 62: 415-424.

Bergeron, A. V. Eberg, G., Proulx, D. and Bailey, J. L. (2002). Comparison of extenders, dilution ratio and theophylline addition on the function of cryopreserved walleye semen. Theriogenology 57: 1061-1071.

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

Glogowski, J., Kolman, R., Szczepkowski, M., Horvath, A., Urbany, B. and Sieczynski, P. (2002). Fertilization rate of Siberian sturgeon (*Acipenser baeri, Brandt*) milt cryopreserved with methanol. Aquaculture 211:367-373.

Holt, W.V., Morris, G.J., Coulson, G., and North, R.D. (1988). Directobservation of cold-shock effects in ram spermatozoa with the use of programmable cryomicroscope .J. Exp. Zool.246, 305-14.

Iritani, A. and Nishikawa, Y. (1961) .Studies on the egg-yolk coagulating factors in goat semen: II Properties of the coagulating factor and influential conditions for coagulation. In: Proc. Silver Jubilee Lab. Anim. Husbandry, Kyoto University, pp 97-104.

Iritani, A. and Nishikawa, Y. (1963).Studies on egg-coagulating enzyme in goat semen: IV On the position of yolk constituents attacked by the coagulating enzyme. Jpn. J. Anim. Reprod. 8(4), 113-117.

Iritani, A., Nishikawa, U. and Fukuhare, R. (1961).Studies on the egg-yolk coagulating factor in goat sperm: I. localization of coagulating factors and decline of pH following coagulating. In: Proc. Silver Jubilee Lab. Anim. Husbandry, Kyoto University, pp 89-96.

Ivanova-Kicheva, M.G., Bobadov, N and Somlev, B. (1997). Cryopreservation of canine semen in pellets and in 5-ml aluminum tubes using three extenders. Theriogenology 48: 1343-1349.

Khalifa, E. I. (2005). Effects of some processes of cryopreservation on post-thawing characteristics and fertilizing ability of goat spermatozoa. Ph .D. Thesis, Fac., Agric., Suez Canal Univ., Egypt.

Leboeuf, B., Restall, B. and Salamon, S. (2000). Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci. 62:113-141.

Marti, J. I., Marti, E., Cebrain-Perez, J. A. and Muoino Blonco, T. (2003). Survival rate and antioxidant enzyme activity of ram spermatozoa after dilution with different extenders or selection by a dextran Swim-up procedure. Theriogenology 60: 1025-1037.

Ochkur, S.I., Kopeika, E.F., Surai, P.F., and Grishchenko, V.I. (1994). The influence of cryopreservation on parameters of energetic metabolism and motility of fowl spermatozoa. Institute for Problems of Cryobiology and Cryomedicine of the Ukrainian Academy of Sciences, 23 Pereyaslavskaya Strasse, Kharkov 310015, Ukraine. Cryobiology 31: 3, 239-244; 14.

Paulenz, H., Soderquist, I., Adnoy, T., Nordstoga, A., Gulbrandsen B. and Andersonbery, K. (2004). Fertility results after different thawing procedures for ram semen frozen in minitubes and ministraws. Theriogenology, 61: 1719-1727.

Robetrson, L. and Watson, P.F. (1986). Calcium transport in the diluted or cooled ram semen. J. Reproduction and Fertility 77, 177-185.

Robetrson, L. and Watson, P.F. (1987). The effect of egg yolk on the control of intracellular calcium in ram spermatozoa cooled and stored at 5^0C. Anim. Reprod. Sci. 15, 177-187.

Roy, A. (1957). Egg-yolk coagulating enzyme in the semen and Cowper's gland of the goat. Nature (London) 179,318.

Schmehl, M.K., Vasquez, I.A. and Graham, E.F. (1986). The effect of non-penetrating cryoprotectants added to TEST-yolk-glycerol extender on post-thaw motility of ram spermatozoa. Cryobiology, 23: 512-517.

Snedecor, G.W. and Cochran, W.G. (1982). Statistical methods. 7th Edition. Iowa State. Univ., Press, USA

Vishwanath, R. and Shannon, P. (2000). Storage of bovine semen in liquid and frozen state. Anim. Reprod. Sci., 62: 23-53.

Watson, P.F. (1976). The protection of ram and bull spermatozoa by low density lipoprotein fraction of egg yolk during storage at 5^0C and deep-freezing. J. Thermal Boil.1, 137-141.

Watson, P.F. (1995). Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. Reproduction and Fertility 7: 871-891.

Zeidan, A, EL- S.B. (1995). New aspects in freezing cattle semen. Ph. D. Thesis, Faculty of Agriculture, Zagazig University, Egypt.

Zhang, Y.Z., Zhany, S.C., Liu, X.Z., Xu, Y.Y., Wang, C.L., Sawant, M.S., Li, J. and Chen S.L. (2003). Cryopreservation of flounder (Paralichtys olivacens) sperm with a practial methodology. Theriogenology 60:989-996.

تقييم وتجميد السائل المنوى لذكور الماعز بإستخدام طرق غير تقليدية فى التعبئة

عزا لدين إبراهيم خليفة*، عماد صلاح الجوهرى*، عمر عبد الرحمن سلامه* و محمد محمد عوض**

*** معهد بحوث الإنتاج الحيوانى -مركز البحوث الزراعيه- وزارة الزراعة**

**** قسم الإنتاج الحيوانى- كلية الزراعة بالإسماعيلية- جامعة قناة السويس**

أجريت هذه التجربة على نوعين من ذكور الماعز البلدى و الزرايبى لتقييم صفات السائل المنوى والمقارنة بين طرق التعبئة المختلفة وتقييم حركة الحيوان المنوى بعد الإذابة والاسترداد خلال التحضين. تم تجميع٢٠قذفه من السائل المنوى بإستخدام المهبل الصناعى (١٠ قذفات مختلطة من كل مجموعة) لتصميم ثلاثة تجارب لكل مجموعة تجريبية. القذفة التى أظهرت نشاط أعلي من ٨٠ % للحركة التقدمية خلطت وقدرت الصفات الأولية للقذفات بعد الخلط وخففت بمخفف الترس الذى يحتوى على ٦ % جليسيرول لحماية الحيوان المنوى من التجميد. التجربة الأولى وكانت مع السائل المنوى الغير مجمد لتقييم الصفات الابتدائية (الحركة التقدمية و الحى و الطبيعى) للسائل المنوى بعد الجمع، التخفيف وفترة الاتزان على ٥ م° لمدة ساعتان. التجربة الثانية وهى أجريت مع السائل المنوى المجمد للمقارنة بين كفاءة طرق التعبئة الغير تقليدية (أكياس الألومنيوم و أقراص شرائط البرشام) والنظم التقليدية (القشة و الحبيبات على شمع البرافين) لتحديد أفضل الصفات للحيوان المنوى بعد الإسالة. التجربة الثالثة كانت لدراسة تأثير التحضين على ٣٧ م° لمدة ٣ساعات على حركة واستعادة الحيوان المنوى بعد الإسالة. وقد سجلت النتائج تأثير معنوي (على مستوى احتمال ١ %) بين الصفات الابتدائية للسائل المنوى قبل التجميد فى التجربة الأولى وكان متوسط الحركة التقدمية بعد الجمع، التخفيف و فترة الاتزان ٨٩و٥ % ، ٨٨و٥ % ، ٨٦و٥ % لذكور الماعز البلدى ، بينما لذكور الماعز الزرايبى ٨٦و٠ % ، ٨٢و٥ % ، ٨٠و٥ % على التوالى. فى التجارب الثانية و الثالثة النتائج أوضحت تأثير معنوى (على مستوى احتمال ١ %) بين طرق التعبئة و زمن التحضين على حركة الحيوانات المنوية بعد الإسالة و الاسترداد. وقد لوحظت أعلى النتائج لذكور الماعز البلدى و الزرايبى على التوالى مع القشة (٥٦و. % ، ٥٤و٥ %) و أكياس الألومنيوم (٥٥و. %، ٥٣و٥ %) بالمقارنة بالأقراص (٥٠و٥ %، ٤٧و٠ %) والحبيبات (٤٥و٥ %، ٤٢و٥ %). أوضحت النتائج عدم وجود فروق معنوية بين المجموعتين.