Egypt. J. Anim. Porod. 23, No. 1-2 pp. 151-158(1983)

Mineral Changes in Ram Spermatozoa as Affected by Freezing and Various Extenders

R.S. Farag, S.T. El-Aassar, H.El-Okash, A.A. Mohamed and A.A. El Sharabassy[c]

Biochemistry Department, Faculty of Agriculture, Cairo University, Biochemistry Department, Faculty of Medicine, Zagazig University and Animal Production Department, Faculty of Agriculture, Al-Azhar University.

THE protective effect of egg yolk citrate (EYC),egg yolk skimmed milk (EYSM) and egg yolk lactose (EYL)extenders on the element content of Ossimi and Rahmani spermatozoa against freezing injury was evaluated. Atomic absorption spectrophotometer was used for the determination of Zn, Ca, Mg, K, Fe and Cu in fresh, equilibrated and frozen ram spetmatozoa. In fresh spermatozoal samples, the differences in the concentrations of Ca, Mn and K, Mg, Cu were highly significant and significant between the two breeds, respectively. On the contrary, the differences in Zn and Fe amounts between the two breeds were not significant. Variable changes in the mineral content of equilibrated and frozen spermatozoa were occurred for both breeds. These changes indicated that the spermatozoal se-sitivity for cooling and freezing processes depended upon ram species. Little changes occurred in the element content due to freezing of spermatozoa using EYL extender and is recommended as a protective medium for freezing ram semen.

The elements constitute a relatively small amounts of the sperm cells. Howevers they are essential for many vital processes. For instance, potassium serve, in connection with sodium and chloride in the maintenance of the normal osmotic pressure and acid-base balance in the animal body (West *et al.*, 1967). Calcium in connection with other divalent metal ions occurring in semen has a significant regulation of sperm adenylate cyclase activity which influences sperm metabolism and motility (Braun, 1975). Also, Zn, Cu, Mn and Fe ions are vital for many enzymes (West *et al.*, 1967).

The harmful effect of freezing can be minimized by the presence of certain protective substances in the extenders such as sugars (Nagase, *et al.*, 1964), skimmed milk (Perry, 1965), lecithin (Lanz *et al.*, 1965) and glycerol (Pursel *et al.*, 1978). The aim of the present work was to study the mineral distribution of fresh, equilibrated and frozen spermatozoa of Ossimi and Rahmani breeds diluted with egg yolk citrate (EYC), egg yolk skimmed milk (EYSM) and egg lactose (EYL) extenders. The efficiency of these extenders as protective agents on the metal content of the spermatozoa against freezing injury was also evaluated.

Material and Methods

Animals

Six Ossimi and six Rahmani rams, of about 2-3 years of age and fed on a standard ration consisting of concentrates and elephant grass were used in the present study.

Semen collection and handling

Semen samples were collected twice per week from each ram between 8 and 9 a.m. using artificial vagina. The ejaculates of each breed were pooled together and maintained at 37 C until semen dilution.

Semen dilution and freezing

The sample of pooled ejaculates of every breed was diluted in a ration of 1:6 immediately after examination by EYC, EYSM and EYL extenders. Each extender comprises of two equal parts, i.e., the non-glycerized and glycerlized portions as shown in Table 1. The diluted semen was sucked immediately into 0·5 ml French straws inside the refrigerator.. The straws were plugged with a $\frac{1}{2}$ cm thick layer of polyvinyl alcohol powder then put into a water bath maintained at 4-5 C and kept in the refrigerator till the equilibration process was ended (2hr). At the end of the equilibration period, the dried straws were exposed to the nitrogen vapour at about 4 cm distance above the surface of the liquid nitrogen for 7 min then dipped into the liquid nitrogen and left there for 5 min.

TABLE 1. **Ingrediants of egg yolk citrate (EYC), egg yolk skimmed milk (EYSM) and egg yolk lactose (EYL) extenders.**

EYC		EYSM		EYL	
part A	part B	part A	part B	part A	part B
20 ml egg yolk	17 ml egg yolk	10 ml egg yolk	8.5 ml egg yolk	20 ml egg yolk	20 ml egg yolk
80 ml sodium	68 ml sodium	90 ml reconstituted	76 ml reconstituted	80 ml lactose	70.6 ml lactose
citrate.$2H_2O$	citrate. $2H_2O$	skimmed milk powder	skimmed milk powder	solution	solution
(2.9%,w/v)	(2.9%,w/v) 15 ml glycerol	(9%,w/v)	(9%,w/v) 15 ml glycerol		9.4 ml glycerol

Every extender contained 500 ug streptomycin calcium chloride and 500 I.U. penicillin sodium per ml of extender as antibacterial agents.

Separation of spermatozoa from semen samples

Spermatozoa of fresh, equilibrated and frozen semen were separated by centrifugation at 3000 rpm for 15 min then washed twice with sodium citrate (2.9%, v/v).

Element analysis

A Pye Unicam atomic absorption spectrophotometer model SP 1900 equipped with a boiling air-acetylene gas mixture was used for metal determination. Hollow cathode current, slit sittings, wavelengths and gases flow rates were set according to the manufactory suggestions. A known weight of the washed spermatozoa was dried overnight at 105 C, ashed at 525 C and the ash was dissolved in IN HCl. Standard stock solutions (1000 ppm) for K, Ca, Mg, Zn, Cu, Mn, and Fe were prepared from their metals or salts using deionized water. Series of 5 to 10 working standard solutions with differen concentrations were prepared daily from the stock solution. Mean reading values for samples or standards were calculated and the quantity of each element was read from the corresponding standard curve. Element content was expressed as mg/100 g dry weight.

Statistical analysis

The influence of equilibration and freezing processes on some metals of ram spermatozoa of Ossimi and Rahmani breeds using various extenders was statistically evaluated using anlysis of variance according to Snedecor and Cochran (1973).

Results and Discussion

Results obtained with the atomic absorption spectrophotometer are shown in Tables 2 and 3. Ossimi and Rahmani spermatozoal samples were especially rich in k, contained moderate amounts of Mg and Ca and small amounts of Cu, Mn, Fe and Zn. The contents of the different metals in Ossimi fresh samples were in the decreasing order: K > Mg > Ca > Cu > Mn > Zn > Fe. In Rahmani fresh samples the order was: K > Mg > Ca > Zn > Fe > Cu > Mn. The contents of Mn, Cu and K were about 2.24, 1.47 and 1.15 times, respectively as high in Ossimi fresh spermatozoa as in Rahmani fresh spermatozoa. On the contrary, the amounts of Zn, Ca and Mg in Rahmani fresh spermatozoa were about 1.29, 1.26 and 1.20 times as great as that in Ossimi fresh spermatozoa. Iron concentration was nearly the same in spermatozoa of both breeds. Statistical analysis showed that there were significant variations between the two breeds in Cu, K, Mg and Ca, Mn at $P > 0.05$ and $P > 0.01$, respectively. If these variations are sufficiently constant in areas where diet and other conditions differ, they might possibly be useful in differetitation between spermatozoa of different breeds.

TABLE 2. **The mean concentration (mg/100g dry weight), standard error and test of significance (DMRT) of some matals in fresh, equilibrated and frosen Ossimi and Rahmani ran,s spermatosoa.**

Treatment	Zn mean ± SE DMRT	Ca mean ± SE DMRT	Mn mean ± SE DMRT	Mg mean ± SE DMRT	K mean ± SE DMRT	Fe mean ± SE SMRT	Cu mean ± SE DMRT
Ossimi xam's spermatosoa							
Undiluted (fresh)	4.44 ± 0.68	14.92 ± 0.39	9.36 ± 0.28	23.45 ± 0.98	250.60± 7.75	4.09 ± 0.07	5.95 ± 0.42
Equilibrated							
a. Egg yolk oitrate	4.89 ± 0.48a	16.31 ± 1.30a	5.12 ± 0.55 a	21.86 ± 1.70a	225.26±11.82a	3.95 ± 0.12 a	5.93 ± 0.29a
b. Egg yolk skimmed milk	4.67 ± 0.30 a	6.25 ± 0.82a	5.14 ± 0.51 a	22.33 ± 1.33a	227.28±2.59 a	3.97 ± 0.08 a	5.94 ± 0.08a
c. Egg yolk lactose	4.54 ± 0.21 a	15.51 ± 0.45a	5.34 ± 0.24 a	22.13 ± 0.25a	244.19±1.06 a	3.99 ± 0.08 a	5.93 ± 0.55a
Frosen thawed							
a. Egg yolk citrate	5.92 ± 0.37 a	24.85 ± 0.11a	4.38 ± 0.82 a	14.16 ± 0.19b	189.19±5.80 b	3.05 ± 0.12 b	3.91 ± 0.53b
b. Egg yolk skimmed milk	5.69 ± 0.54 a	22.65 ± 0.61b	4.74 ± 0.54 a	18.96± 0.42a	196.30± 2.2b	3.70 ± 0.18 b	5.77± 0.15a
c. Egg yolk lactose	5.07 ± 0.36 a	18.13 ± 0.38c	5.12 ± 0.28	20.32 ± 0.44a	215.43± 0.94a	3.86 ± 0.08 a	5.88 ± 0.25a
Rahmani ram's spermatosoa							
Undiluted (fresh)	5.74 ± 0.66	18.82 ± 0.41	2.39 ± 0.25	28.04 ± 1.15	217.77± 4.65	4.21 ± 0.31	4.05 ± 0.50
Equilibrated							
a. Egg yolk citrate	5.78 ± 0.14 a	20.04 ± 1.02a	2.35 ± 0.11a	26.90 ± 0.53a	179.32±2.61 b	4.17 ± 0.41 a	3.90 ± 0.12a
b. Egg yolk skimmde milk	5.92 ± 0.57 a	20.03 ± 0.79a	2.38 ± 0.20a	27.24 ± 0.18a	181.61 ±2.76b	4.19 ± 0.23 a	3.96 ± 0.49a
c. Egg yolk lactose	5.79 ± 0.14 a	19.45 ± 0.18a	2.29 ± 0.30a	27.44 ± 0.34a	205.84±2.22 a	4.20 ± 0.29 a	4.02 ± 0.06a
Frosen thawed							
a. Egg yolk citrate	7.28 ± 0.51 b	31.23 ± 0.68a	1.98 ± 0.16a	15.58 ± 0.30c	129.91± 2.68b	3.87 ± 0.18 a	0.46 ± 0.02b
b. Egg skimmed milk	9.39 ± 0.17 a	30.67 ± 0.36a	2.10 ± 0.41a	18.36 ± 0.28b	139.66± 2.00b	4.02 ± 0.11 a	2.37 ± 0.20a
c. Egg yolk lactose	8.32 ± 0.18 b	27.47 ± 1.64a	2.07 ± 0.38a	25.35 ± 0.33a	180.63± 0.69a	4.12 ± 0.28 a	2.20 ± 0.37a

In any category, any two treatments having at least one letter in common does not differ significantly, otherwise they do.

TABLE 2. **The mean concentration (mg/100g dry weight), standard error and test of significance (DMRT) of some matals in fresh, equilibrated and frosen Ossimi and Rahmani ran,s spermatosoa.**

Treatment	Zn mean ± SE DMRT	Ca mean ± SE DMRT	Mn mean ± SE DMRT	Mg mean ± SE DMRT	K mean ± SE DMRT	Fe mean ± SE SMRT	Cu mean ± SE DMRT
			Ossimi xam's spermatosoa				
Undiluted (fresh)	4.44 ± 0.68	14.92 ± 0.39	9.36 ± 0.28	23.45 ± 0.98	250.60± 7.75	4.09 ± 0.07	5.95 ± 0.42
Equilibrated							
a. Egg yolk oitrate	4.89 ± 0.48a	16.31 ± 1.30a	5.12 ± 0.55 a	21.86 ± 1.70a	225.26±11.82a	3.95 ± 0.12 a	5.93 ± 0.29a
b. Egg yolk skimmed milk	4.67 ± 0.30 a	6.25 ± 0.82a	5.14 ± 0.51 a	22.33 ± 1.33a	227.28±2.59 a	3.97 ± 0.08 a	5.94 ± 0.08a
c. Egg yolk lactose	4.54 ± 0.21 a	15.51 ± 0.45a	5.34 ± 0.24 a	22.13 ± 0.25a	244.19±1.06 a	3.99 ± 0.08 a	5.93 ± 0.55a
Frosen thawed							
a. Egg yolk citrate	5.92 ± 0.37 a	24.85 ± 0.11a	4.38 ± 0.82 a	14.16 ± 0.19b	189.19±5.80 b	3.05 ± 0.12 b	3.91 ± 0.53b
b. Egg yolk skimmed milk	5.69 ± 0.54 a	22.65 ± 0.61b	4.74 ± 0.54 a	18.96± 0.42a	196.30± 2.2b	3.70 ± 0.18 b	5.77± 0.15a
c. Egg yolk lactose	5.07 ± 0.36 a	18.13 ± 0.38c	5.12 ± 0.28	20.32 ± 0.44a	215.43± 0.94a	3.86 ± 0.08 a	5.88 ± 0.25a
			Rahmani ram's spermatosoa				
Undiluted (fresh)	5.74 ± 0.66	18.82 ± 0.41	2.39 ± 0.25	28.04 ± 1.15	217.77± 4.65	4.21 ± 0.31	4.05 ± 0.50
Equilibrated							
a. Egg yolk citrate	5.78 ± 0.14 a	20.04 ± 1.02a	2.35 ± 0.11a	26.90 ± 0.53a	179.32±2.61 b	4.17 ± 0.41 a	3.90 ± 0.12a
b. Egg yolk skimmde milk	5.92 ± 0.57 a	20.03 ± 0.79a	2.38 ± 0.20a	27.24 ± 0.18a	181.61 ±2.76b	4.19 ± 0.23 a	3.96 ± 0.49a
c. Egg yolk lactose	5.79 ± 0.14 a	19.45 ± 0.18a	2.29 ± 0.30a	27.44 ± 0.34a	205.84±2.22 a	4.20 ± 0.29 a	4.02 ± 0.06a
Frosen thawed							
a, Egg yolk citrate	7.28 ± 0.51 b	31.23 ± 0.68a	1.98 ± 0.16a	15.58 ± 0.30c	129.91± 2.68b	3.87 ± 0.18 a	0.46 ± 0.02b
b. Egg skimmed milk	9.39 ± 0.17 a	30.67 ± 0.36a	2.10 ± 0.41a	18.36 ± 0.28b	139.66± 2.00b	4.02 ± 0.11 a	2.37 ± 0.20a
c. Egg yolk lactose	8.32 ± 0.18 b	27.47 ± 1.64a	2.07 ± 0.38a	25.35 ± 0.33a	180.63± 0.69a	4.12 ± 0.28 a	2.20 ± 0.37a

In any category, any two treatments having at least one letter in common does not differ significantly, otherwise they do.

TABLE 3. **Percent change of some metals as affected by freezing and type of extender measured as deviation from fresh semen values.**

Extender	Breed	Percentage Change						
		Zn	Ca	Mn	Mg	K	Fe	Cu
		Deviation from fresh semen values						
Egg yolk citrate	Ossimi	+33.33	+66.55	—18.28	—39.62	—24.51	—25.43	—34.28
	Rahmani	+26.83	+65.94	—17.15	—44.44	—40.34	— 8.08	—88.64
Egg yolk skimmed milk	Ossimi	+28.15	+51.81	—11.57	—19.15	—21.67	— 9.54	— 3.02
	Rahmani	+63.59	+62.96	—12.13	—34.52	—35.87	— 4.51	—41.48
Egg yolk lactose	Ossimi	+14.19	+21.51	— 4.48	—13.35	—14.03	— 5.62	— 1.18
	Rahmani	+44.95	+45.96	—13.39	—16.73	—17.05	— 2.14	—45.68
		Deviation from equilibrated semen values						
Egg yolk citrate	Ossimi	+21.06	+52.36	—14.45	—35.22	—16.01	—22.78	—34.06
	Rahmani	+25.95	+55.84	—15.74	—42.08	—27.55	— 7.19	—88.21
Egg yolk skimmed milk	Ossimi	+21.84	+39.38	— 7.78	—15.09	—13.63	— 6.80	— 2.86
	Rahmani	+58.61	+53.12	—11.76	—32.60	—23.10	— 4.06	—40.15
Egg yolk lactose	Ossimi	+11.67	+16.89	— 4.12	— 8.18	—11.78	— 3.26	— 0.84
	Rahmani	+39.36	+41.23	— 9.61	— 7.62	—12.25	— 1.90	—45.27

Effect of equilibration and freezing

There was an influx of Zn and Ca and an efflux of Mn, Mg, K, Fe and Cu after equilibration and freezing. The influx and efflux of various elements were more obvious in frozen samples. Similar results were reported in boar, bull and ram spermatozoa by Quinn and White (1966) and Pursel *et al.* (1969). The influx of Zn was not significant after equilibration by using the three extenders in both breeds. Frozen Ossimi spermatozoa showed the same trend. In frozen Rahmani spermatozoa, the influx of Zn was greater by using EYSM extender than that of EYC or EYL extenders. The authors found that freezing Rahmani spermatozoa greatly increased coiled tail percentage and at the same time increased the influx of Zn. Hence, there is a correlation between Zn influx and coiled tail percentage. A similar relationship was achieved by Blom and Wolstrup (1976).

The calcium content of equilibrated spermatozoa of both breeds did not differ significantly than the fresh spermatozoa using various extenders. After freezing, there was a highly significant difference ($P<0.01$) in the influx of Ossimi spermatozoa between the three extenders. However, the influx of Ca was lowest using EYL extender compared to other ones. In Rahmani spermatozoa, the three extenders caused almost the same rate of Ca influx. This phenomenon was greater in Rahmani spermatozoa than in Ossimi spermatozoa. In this respect, White and Wales (1960) found that Ca accumulation within the sperm cell on cold shocking and was largely prevented by lecithin or glycerol. These findings coincided with our results concerning Ca influx as a result of freezing.

The amount of Mn was the same in fresh, equilibrated and frozen spermatozoa of Ossimi and Rahmani breeds. Therefore, no efflux or influx of Mn has taken place from the spermatozoa. Also, no significant differences were found between EYC, EYSM and EYL extenders for spermatozoal Mg content after equilibration for both breeds. After freezing of Ossimi spermatozoa, Mg significantly influxed only by using EYC extender. With frozen Rahmani spermatozoa, there were significant differences ($P<0.01$) between various extenders. EYL extender exhibited the least Mg efflux from the spermatozoa in comparison with other extenders.

The results of K indicated that the three extenders had the same effect on the efflux of K in fresh and equilibrated Ossimi spermatozoa. Concerning equilibrated Rahmani spermatozoa, EYL extender was the best one in reducing the efflux of K from the spermatozoa, followed by EYC and EYSM extenders. Equilibrated Ossimi and Rahmani spermatozoa in various extenders had approximately the same Fe concentration as that in fresh spermatozoa. The same conclusion was found by frozen Rahmani spermatozoa. There were no significant changes between EYC and EYSM extenders for frozen Ossimi spermatozoa. While EYL extender differed significantly ($P<0.01$) than the other two extenders. This would means that EYL extender is the most efficient one in protecting the sperm against loss of Fe during freezing.

Statistical information concerning Cu content of equilibrated Ossimi and Rahmani spermatozoa indicated that the efflux of Cu was similar for the three extenders. In frozen samples, both EYL and EYSM extenders were equal in the Cu efflux from the spermatozoa. On the other hand, EYC extender greatly increased the efflux rate of Cu which reached 34.28 mg% and 88.64 mg% in Ossimi and Rahmani breeds, respectively.

Generally speaking, the element study indicated that the equilibration process in EYC, EYSM and EYL extenders had no significant influence on spermatozoal influx or efflux of Zn, Ca, Mn, Fe and Cu in both breeds. Concerning K, its efflux was not significant in Ossimi breed by using the various extenders. In Rahmani breed, the efflux of K was not significant in EYL extender and was highly significant in EYC and EYSM extenders. Variable changes in the element content of frozen spermatozoa were observed in both breeds. The percentage change due to freezing in the element content of spermatozoa with EYL extender was the lowest compared with the changes that took place with the other extenders (Table 4). The results of this study suggest

the use of EYL extender as an efficient medium for freezing ram semen. Also the metal analysis indicated that the percentage change in elements of frozen Ossimi spermatozoa was lower in comparison with Rahmani spermatozoa. Hence, Ossimi spermatozoa are much better in tolerating the freezing effect.

Acknowledgments ; Thanks are accorded to the U.S.A. Agricultural Research Service for the financiance authorized by the Public Law 480, Project No. FG-EG 155.

References

Blom, E. and Wolstrup, C. (1976) Zinc as possible causal factor in the sterilizing sperm tail defect in Jersy bulls. *VIII th Int. Cong. Anim. Reprod. and A.I.* Cracow, July 12-16.

Braun, T. (1975) The effect of divalent cations on bovine spermatozoal adenylate cyclase activity. *J. Cyc. Nucl. Res.* **1**, 271.

Lanz, R.N., Pickett, B.W. and Komarck, K.J. (1965) Effect of lipid additives on pre-and post-freeze survival of bovine spermatozoa. *J. Dairy Sci.* **48**, 1622.

Nagase, H., Niwa, T., Yamashita, S. and Iric, S. (1964) Deep freezing bull semen in concentrated pellet form. 11. Protective action of sugars. *Proc. 5th Int Congr Anim. Reprod.* (Trento). **4**, 498.

Perry, E.J. (1965) "*The Artificial Insemination of Farm Animals*". First Indian Edition, Oxford & Ibh Publishing Co. Calcutta, Bombay, New Delhi.

Pursel, V.G., Schulman, L.L. and Johnson, L.A. (1978) Effect of glycerol concentration on frozen boar sperm. *Theriogenology* **9**, 305-312.

Puselr, V.G., Johnson, L.A. and Gerrits, R.J. (1969) Effect of cold shock and freezing on actions of boar spermatozoa. *J. Anim. Sci.* **29**, 196.

Quinn, P.J. and White, I.G. (1966) The effect of cold shock and deep freezing on the concentration of major cations in spermatozoa. *J. Reprod. Ferti*. **12**, 263.

West, E.S., Todd, W.R., Mason, H.S. and Van Bruggen, J.T. (1967) Textbook of Biochemistry. The MacMillan Company, New York, Collier-MacMillan Ltd, London.

White, I.G. and Wales, R.G. (1960) The susceptibility of spermatozoa to cold shock. *Ints. J. Fert.* **5**, 195-201

Snedecor, G.W. and Cochran, W.G. (1973) "*Statistical Methods*", 6th ed., Iowa State University Press, Ames, Iowa, U.S.A.

دراسة تأثير التبريد والتجميد على المحتوى العنصرى للحيوانات المنوية لكباش الأاسيمى والرحمانى

رضوان صدقي فرج ، شاكر طلخان الاعصر ، حسن عبد الجواد ، على عباس محمد وعادل أحمد الشرباصي

أقسام الكيمياء الحيوية بكليات الزراعة والطب بجامعتى القاهرة والزقازيق وقسم الانتاج الحيوانى بكلية الزراعة ـ جامعة الأزهر

درس تأثير التبريد والتجميد على المحتوى العنصرى للحيوانات المنوية لكباش اوسيمى ورحمانى الموجودة فى ثلاثة أوساط وهى صفار البيض والسترات ، صفار البيض واللبن الفرز المجفف ، صفار البيض واللاكتوز ٠

وقد استخدم جهاز الامتصاص الذرى لتقدير بعض العناصر الهامة مثل: الزنك ـ الكالسيوم ـ المغنسيوم ـ المنجنيز ـ البوتاسيوم ـ الحديد والنحاس للحيوانات المنوية الطازجة والمتزنة والمتجمدة وقد وجد فرق عال المعنوية فى تركيز الكالسيوم والمنجنيز وفرق معنوى فى تركيز المغنسيوم والنحاس فى الحيوانات المنوية الطازجة مابين سلالتى الكباش ٠ ومن ناحية اخرى لم يظهر فرق معنوى فى تركيز الزنك والحديد فى كلا النوعين وبالتالى يمكن الاستفادة من هذه الدراسة فى معرفة اصل الحيوانات المنوية عن طريق تحليل مكوناتها من العناصر ٠

دل التحليل الاحصائى على عدم وجود فرق معنوى فى دخول الزنك والكالسيوم وخروج المنجنيز والمغنسيوم والحديد والنحاس خلال اغشية الحيوانات المنوية وفى حالة البوتاسيوم لم يظهر فرق معنوى بين خروجة من الحيوانات المنوية للاوسيمى باستخدام الثلاثة اوساط ولكن فى حالة الرحمانى لم يظهر فرق معنوى لخروج البوتاسيوم فقط باستعمال صفار البيض واللاكتوز ، ولكن ظهر فرق عال المعنوية باستخدام الاوساط الاخرى ٠

ظهرت اختلافات متباينة فى المحتوى المعدنى نتيجة لتجميد الحيوانات المنوية لسلالتى الكباش وأن الحيوانات المنوية للاوسيمى اكثر تحملا للتبريد والتجميد عنها فى الرحمانى ٠ وتبين من هذه الدراسة انه يفضل استخدام الوسط المكون من صفار البيض واللاكتوز لحفظ الحيوانات المنوية لحين الحاجة اليها للتلقيح الصناعى نظرا لحدوث تغير بسيط فى المحتوى المعدنى لها بالتبريد والتجميد ٠