IN VITRO INDUCTION OF THE ACROSOME REACTION IN LOCAL EGYPTIAN RAMS SPERMATOZOA BY CALCIUM IONOPHORE (A23187)

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SUMMARY

The main objective of this work was to develop an efficient in vitro test (acrosome reaction, AR) to predict fertility in rams spermatozoa in the Egyptian local rams by using Ca^{++}ionophore. Semen was collected for 5 successive weeks from four Rahmani and four Ossimi Egyptian local rams. Fresh collected semen was initially evaluated. Calcium ionophore was used to stimulate the sperm to undergo acrosome reaction (AR). Results showed that differences in all initial semen characteristic values were not significant among Rahmani rams. The same results were obtained in Ossimi rams, except mass motility (88.75%, P<0.05). After Induction of AR, results showed in Rahmani and Ossimi breeds that live-reacted spermatozoa% (LR) was the highest (34.25 and 34.50%, P<0.05) in ram No. 1 of both breeds when compared to other rams in both breeds, respectively. Conception rate (CR) after natural mating revealed individual differences among rams within each breed, but the differences were significant (P<0.05) within Ossimi breed only. Ram No. 1 of both breeds showed the highest CR compared to other rams of the same breed. However, the mean of CR was insignificantly higher in Rahmani than Ossimi breed (89.19 vs. 81.08%). There was positive correlation between percentage of live-reacted spermatozoa and CR in Rahmani breed (r=0.64, P<0.01) and in Ossimi breed (r=0.96, P<0.01). In addition, there was a significant correlation between the percentage of mass motility and CR (r=0.70, P<0.01) in Ossimi breed. It could be concluded that lamb rams fertility of local sheep can be predicted using acrosome reaction test with Ca^{++}ionophore.

Keywords: Ram, Rahmani, Ossimi, breed,semen, acrosome reaction, calcium ionophore

INTRODUCTION

The capacity to fertilize the ovum and to sustain embryogenesis is the only reliable test of the functional integrity of a semen sample (Watson, 1979; Uçar and Parkinson 2003 and Ashmawy *et al*., 2013). However, the assessment of fertilizing capacity in vivo is costly, time-consuming and laborious. Likewise, the value of routine semen analysis in the prediction of fertility is both subjective and poorly predicts fertility (Rodriguez-Mártinez *et al.,* 1997).

Therefore, over the years, several alternative methods of assessing semen have been sought to predict male fertility (Larsson and Rodrıguez-Martınez, 2000), Standard semen analysis assays include the assessment of sperm morphology (Barth, 1992), sperm motility (Holt *et al.,* 1997), presence of intact acrosomes (Correa *et al.,* 1997) and membrane integrity (Pérez *et al.,* 1997). Significant correlations between motility and field fertility have been found by some workers (Kjaestad *et al.,* 1993 and Correa *et al.,* 1997), while not proved by others (Graham *et al.,* 1980 and Januskauskas *et al.,* 1996).

The tests of sperm penetration assay (Gadea *et al.,* 1998), zona binding (Gadea *et al.,* 1998 and Zhang *et al.,* 1998), hemi-zona assays (Fazeli *et al.,* 1995) and several forms of acrosome reaction assay (Whitfield and Parkinson, 1995 and Januskauskas *et al.,* 1996) provided information about the ability of spermatozoa to interact with the oocyte, whereas some significant correlation, between them and *in vivo* fertility, has been found in several species (pigs and cattle: Fazeli *et al.,* 1995 & 1997).

In vitro methods for analysing the functionality of spermatozoa, namely the *in vitro* induction of AR and the *in vitro* assessment of ability of spermatozoa to undergo AR, are increasingly and widely used as means for determining the fertility of sires (Parkinson, 1996), rams (Uçar and Parkinson, 2003) and bucks (Ashmawy *et al*., 2013).

Calcium ionophore-induced acrosome reactions were also studied in rams (Shams-Borhan and Harrison, 1981; Watson *et al*., 1991; Garde *et al*., 1992 and Uçar and Parkinson, 2003). Therefore, the objective of this work was to evaluate the potential of ram lambs' spermatozoa, of Rahmani and Ossimi Egyptian local sheep breeds, for acrosome reaction by Ca^{++} ionophore as an early test for predicting rams fertility.

MATERIALS AND METHODS

The experimental work was conducted at Sakha Experimental Station, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The research station is located in the Northern part of the Nile Delta (latitude 31^{o}N and longitude 31^{o}E) in Kafrelsheikh Governorate, Egypt.

Animals:

Eight lamb rams of Egyptian local sheep breeds (4 Rahmani and 4 Ossimi) with an average age 20.9±0.61 and 21.2±0.16 months, and live body weight 41.12±4.57 and 36.71±3.89 kg, respectively, were used for semen collection. Rams were fed concentrate feed mixture (CFM) containing 15% crude protein (CP) and 70% total digestible nutrients (TDN), plus 1.25 kg rice straw during the experimental period. Ingredients of the CFM included 45.3% corn grains, 11% undecorticated cotton seed, 12% soybean meal, 29% wheat bran, 1.8% limestone, 0.22% sodium bicarbonate, 0.4% common salt and 0.28% mineral mixture. The lambs had free access to water all the day.

Semen Collection and Initial Assessment:

Semen ejaculate was collected once a week from each of the eight rams by artificial vagina (using heated teaser ewe) for five weeks during the period from August to September (20 ejaculates from each breed). Immediately after semen collection, each ejaculate was assessed for mass motility (% of spermatozoa exhibiting progressive motility) at a total magnification of 200X. The volume of each ejaculate was recorded and the concentration of spermatozoa was determined with a Neubauer haemocytometer. Smears were prepared using nigrosin/eosin staining for determining the percentages of live and abnormal spermatozoa.

Induction of Acrosome Reactions:

Calcium ionophore A23187 (Sigma Chemical Co., St Louis, MO, USA) stock solution was prepared as 6 mmol/l stock in dimethylsulphoxide were frozen at -20°C. Before use this was thawed, diluted 1:10 with modified Tyrode's medium (TALP) described by (Parrish et al, 1988). It contained 100 mM NaCI, 3.1 mM KCI, 0.4 mM MgC12, 2 mM CaC12, 0.3 mM KH2PO4, 25 mM NaHCO3, 10 mM Na-Hepes, 1mM Pyruvic acid sodium salt, 21.6 mM Na-lactate, 6mg/mL BSA (Fraction V), and l0 µg/mL phenol red. The 'Tyrode's medium was buffered with 1N NaOH/HCl to pH 7.4, and had a final osmolarity of 290-300 mOsmol/kg. The media stock was prepared every second week and was sterilized by Millipore filtration (0.22-µm pore size).

Semen samples were supplemented with 10 ml of modified Tyrode's medium (TALP) and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the sperm pellet was re-suspended in the same media. This step was repeated three times to remove the whole seminal plasma.

The suspension was then further diluted in TALP to a final concentration of 50 × 106 sperm/ml, then divided into two groups (as treated and control group). In treated group, spermatozoa were capacitated with 5 µl/ml of A23187 diluted stock was added to it (3 µmol/l final concentration of calcium ionophore A23187, Sigma), while in the control group DMSO was added without any supplement, that was found to be exerting no effect (data not shown). Each sample was covered by 1 ml of mineral oil, and all samples were placed into water bath for 30 min at 37°C. Aliquots of about 200 µl were removed from each tube (as treated and control group) and stained with the Dual Stain, according to Didion *et al.* (1989) after 30 min. incubation.

***Evaluation of Acrosome Reactions*:**

To determine the variability and acrosome reacted spermatozoa (under bright flied microscope, (×400 and ×1000) by counting about 200 spermatozoa per slide and examined. They were classified into four categories as: 1) live-reacted spermatozoa with intact acrosome (LR) (Post-acrosome region, PAR white and acrosome bright Pink/purle), 2) live-unreacted spermatozoa with detached acrosome (LU) (PAR white and acrosome white), 3) dead-reacted spermatozoa with intact acrosome (DR) (PAR blue or dark blue and acrosome dark pink /purple) and 4) dead-unreacted spermatozoa with detached acrosome (DU) (PAR blue and acrosome white/gray white). Both categories 1 and 2 were considered as true acrosome reaction, while categories 3 and 4 were served as false acrosome reaction.

Fertility Trial

A total of 74 clinically healthy non-lactating ewes (37 Rahmani and 37 Ossimi breed) were naturally mated with the pervious rams during breeding season in September (from the 1st to the end of September). Each ram of each breed was used for mating 8-10 ewes. Fertility, determined as conception rate (proportion of ewes mated to those conceived). The pregnancy was diagnosed using ultrasonography on day 56 post-mating according to Martinez *et al.* (1998).

Statistical Analysis:

The data obtained were statistically analyzed using General Liner Models (GLM) procedures in SPSS statistical package programme (2008). Duncan Multiple Range Test was used to compare differences between the means. Chi-square test was used to determine the differences in conception rate.

RESULTS AND DISCUSSION

Physical Semen Characteristics (Initial Assessment):

Physical Semen characteristics of Rahmani and Ossimi rams (individuals and means) are shown in Table (1). Based on the individual values, the differences in all physical semen characteristics were not significant in Rahmani rams. The same result was obtained for Ossimi rams, except percentage of mass motility showing significant differences ($P<0.05$) among Ossimi rams.

Means of all physical semen characteristics were higher in Ossimi than Rahmani breed, but the differences were not significant, except the percentage of sperm viability, being significantly ($P<0.05$) higher in Ossimi than Rahmani breed.

Table 1. Physical semen characteristics of Rahmani and Ossimi rams

Breed	Ram number	Semen volume (ml)	Mass motility (%)	Live sperm (%)	Abnormal sperm (%)	Sperm concentration ($x10^9$/ml)
Rahmani	1	0.78±0.11^{a}	85.00±2.89^{a}	91.0±0.41^{a}	2.25±0.25^{a}	2.25± 0.31^{a}
	2	0.85±0.06^{a}	81.25±1.25^{a}	89.5±2.47^{a}	1.75±0.25^{a}	2.14±0.14^{a}
	3	0.70±0.06^{a}	80.00±2.04^{a}	87.5±2.02^{a}	2.25±0.25^{a}	1.93±0.30^{a}
	4	0.83±0.10^{a}	81.25±1.25^{a}	92.0±1.78^{a}	1.75±0.63^{a}	1.71±0.06^{a}
	Mean	**0.79±0.04^{A}**	**81.88±1.01^{A}**	**90.0±0.93^{B}**	**2.00±0.18^{A}**	**2.00±0.12^{A}**
Ossimi	1	0.85±0.05^{a}	88.75±1.25^{a}	94.7±0.25^{a}	2.25±0.25^{a}	2.12±0.03^{a}
	2	0.83±0.06^{a}	83.75±2.39^{ab}	96.0±1.08^{a}	1.50±0.29^{a}	2.25±0.19^{a}
	3	0.90±0.11^{a}	82.50± 1.44^{b}	91.7±1.70^{a}	2.00±0.41^{a}	2.14±0.24^{a}
	4	0.85±0.09^{a}	81.25±1.25^{b}	92.0±1.68^{a}	2.25± 0.48^{a}	2.01±0.09^{a}
	Mean	**0.86±0.04^{A}**	**84.06±1.04^{A}**	**93.6±0.75^{A}**	**2.00±0.18^{A}**	**2.13±0.08^{A}**

Values presented in percentages or means ± SEM.
Values with different superscripts letters (a, b) within the same column for each breed differ significantly (P<0.05).
*Values with different superscripts letters (**A**, **B**) between breeds differ significantly (P<0.05).*

Table 2. Percentage of different categories of acrosome reaction of semen from Rahmani and Ossimi rams.

Breed	Ram number	Category of acrosome reaction (%)			
		Live-reacted (LR)	Live-unreacted (LU)	Dead-reacted (DR)	Dead-unreacted (DU)
Rahmani	1	34.25±2.56^{a}	44.7±1.25^{a}	6.75±0.63^{a}	14.25±1.18^{b}
	2	28.75±1.44^{b}	46.5±1.55^{a}	6.50±1.19^{a}	18.25±1.11^{a}
	3	29.25±0.63^{ab}	46.5±1.32^{a}	7.00±0.41^{a}	17.25±1.44^{ab}
	4	29.25±0.85^{ab}	46.0±0.91^{a}	6.00±0.41^{a}	18.75±1.03^{a}
	Mean	**30.38±0.91^{A}**	**45.9±0.601^{A}**	**6.56±0.34^{A}**	**17.13±0.70^{A}**
Ossimi	1	34.50±1.19^{a}	43.50±1.04^{c}	6.0±0.71^{ab}	16.0±0.41^{b}
	2	30.25±1.11^{b}	44.00±1.29^{c}	7.5±0.65^{a}	18.2±1.11^{ab}
	3	26.75±0.85^{c}	48.50±1.04^{b}	6.2±0.25^{ab}	18.5±0.29^{ab}
	4	22.50±1.19^{d}	52.50±0.50^{a}	5.5±0.29^{b}	19.5±1.04^{a}
	Mean	**28.50±1.24^{A}**	**47.13±1.05^{A}**	**6.3±0.30^{A}**	**18.1±0.49^{A}**

Values presented in means±SEM.
Values with different superscripts letters (a, b, c, d) in the same column for each breed differ significantly (P<0.05).
*Values with different superscripts letters (**A**, **B**) between breeds differ significantly (P<0.05).*

Acrosme Reaction:

Percentage of spermatozoa at different categories of acrosome reaction per individuals and mean of Rahmani and Ossimi semen treated with calcium ionophore are presented in Table (2). Based on the individual values within each breed, the percentage of live-reacted spermatozoa (LR) differed significantly (P<0.05), the highest individual values were 34.25 and 34.50% for Rahmani and Ossimi rams, respectively. Percentage of dead unreacted spermatozoa (DU) differed significantly (P<0.05), the lowest values were 14.25 and 16.0% in rams No. 1 of each Rahmani and Ossimi breeds compared to other rams, respectively. In general, the differences in the mean of all categories of acrosome reaction were not significant between Ossimi and Rahmani breeds.

The present results agree with the previous literature confirming that acrosome reaction test is a stable parameter of sperm function and is useful to predict fertilization success (Maji *et al.,* 2010). Based on the results obtained in term of individual variation in the proportion of spermatozoa undergoing acrosome reaction and those reported by Ucar and Parkinson (2003), calcium ionophore (A23187) can be used to assess the acrosome reaction of ovine spermatozoa *in vitro*.

In addition, Smith and Murray (1996) found differences in fertilizing ability not only among individual rams, but also among ejaculates of the same ram. Attempts to correlate results of acrosome reaction test and *in vivo* fertility using ram semen have given contradictory results. In this respect, Choudhry *et al.* (1995) found a correlation between the number of penetrated spermatozoa per zona-free hamster oocyte and *in vivo* fertility. Although, Codde and Berger (1995) were unable to demonstrate a correlation between *in vivo* fertility and the ability of spermatozoa to bind to or penetrate zona pellucida, while there were significant differences between rams among these parameters *in vitro*.

Fertility Trail

Results of conception rate (CR) after natural mating (Table 3) revealed individual differences among rams within each breed, but the differences were significant (P<0.05) within Ossimi individuals only. Ram No. 1 in each of Rahmani and Ossimi showed the highest CR as compared to other rams, being 90 and 100%, respectively. However, the means of CR were insignificantly higher in Rahmani than Ossimi breeds (89.19 *vs*. 81.08%, respectively).

Table 3. Conception rate of ewes mated naturally by Rahmani and Ossimi rams

Breed	Ram number	Mated ewes (n)	Pregnant ewes (n)	Conception rate* (%)
Rahmani	1	10	9	90.00[a]
	2	9	8	88.89[a]
	3	9	8	88.89[a]
	4	9	8	88.89[a]
	Mean	**37**	**33**	**89.19[A]**
Ossimi	1	8	8	100[a]
	2	10	8	80.00[b]
	3	9	7	77.78[b]
	4	10	7	70.00[b]
	Mean	**37**	**30**	**81.08[B]**

** Conception rate was estimated ultrasonographically 56 days after mating.*
Values presented in percentages.
Values with different superscripts letters (a, b) within the same column for each breed differ significantly (P<0.05).
*Values with different superscripts letters (**A, B**) between breeds differ significantly (P<0.05).*

Table 4. Partial correlations between each of the percentage of mass motility, live sperm, sperm concentration, live-reacted spermatozoa and conception rate

Item	Mass motility (%)	Live sperm (%)	Sperm concentration	Live-reacted spermatozoa (%)
CR of Rahmani rams	0.46	0.16	0.31	0.64**
CR of Ossimi rams	0.70**	0.33	0.08	0.86**

*** Significant at P<0.01.*

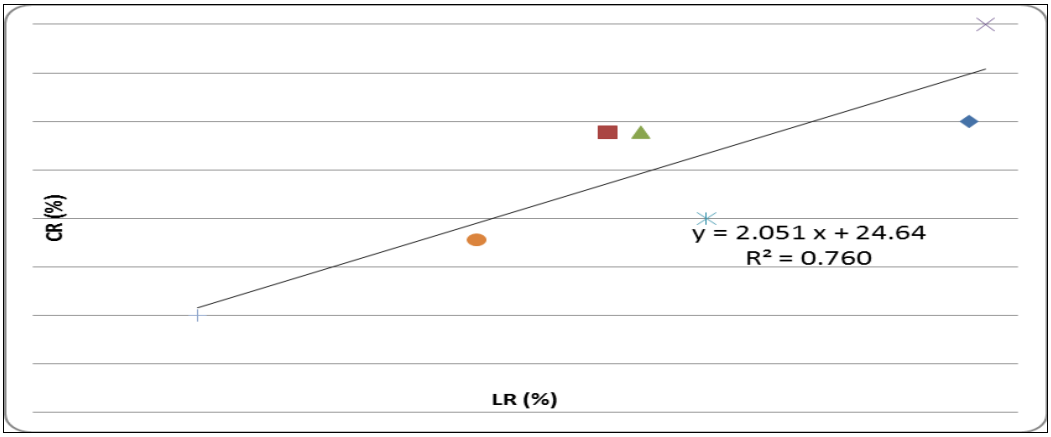

Fig. 1. Regression equation for dependant conception rate on the live-reacted spermatozoa in rams

The present results concerning the CR were in association with the percentage of live-reacted (LR) spermatozoa in both breeds. Results of the partial correlation (Table 4) indicated significant (P<0.01) positive correlation between percentage of live-reacted spermatozoa and the CR in Rahmani (r=0.64, P<0.01) and in Ossimi (r=0.96, P<0.01). Also, there was a significant correlation between the percentage of mass motility and CR (r=0.70, P<0.01) in Ossimi rams.

In addition, the relationship between CR and percentage of LR spermatozoa could be indicated in the coming regression equation for dependent conception rate on the live-reacted spermatozoa, as illustrated in figure (1):

Conception rate (%) = 2.051 x live-reacted spermatozoa (%) + 24.64.

While the adjusted R-square (R^2) was 0.76, such results facilitate the prediction of fertility of rams using acrosome reaction test using calcium ionophore.

As the result obtained by Lenz *et al.* (1988), it was reported that acrosome reaction technique could be used to predict the relative fertility of bulls. In addition, Costa *et al.* (2010) stated that the induction of the acrosome reaction test is a valuable tool to predict the fertilization in cattle. Accordingly, in conjunction with our present results, ram fertility could be predicted using acrosome reaction test with Ca++ ionophore to determine the percentage of live-

reacted spermatozoa. The results obtained from the current study imply that *in vitro* induction of the AR provides a sufficiently accurate model of the events during fertilization *in vivo* that allow estimating of fertility of spermatozoa to be made in rams.

REFERENCES

Ashmawy, T. A. M., E. O. H. Saifelnasr, M. E. El-Gendy, Eitedal H. Elsayed, M. E. Shamiah, 2013. Predicting fertility of goat bucks by inducing acrosome reaction in sperm with calcium ionophore A23187. J. Agric. Res. Kafr El-Sheikh Univ., 39(1): 27-36.

Barth, A. D., 1992. The relationship between sperm abnormalities and fertility. Proceedings of the 14th Technical Conference on Artificial Inseminationand Reproduction. pp.47–63.

Choudhry, T. M., T. Berger and M. Dally, 1995. In vitro fertility evaluation of cryopreserved ram semen and its correlation with relative in vivo fertility. Theriogenology, 43: 1195–1200.

Codde, J. M. and T. Berger, 1995. In vivo fertility of rams in relation to sperm-zona pellucida binding and sperm-zona pellucida penetration of ovine oocytes. Theriogenology, 44: 901–906.

Correa, J. R., M. M. Pace and P. M. Zavos, 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.

Costa, M. Z., L. Z. Oliveira, M.V. Resende, A. C. Lucio, A. P. Perini, M. C. V. Miguel and V. F. M. H. Lima, 2010. Induction of the acrosome reaction test to in vitro estimate embryo production in Nelore cattle. Arq. Bras. Med. Vet. Zootec., 62(4): 771-777.

Didion, B. A., J. R. Dobrinsky, J. R. Giles and C. N. Graves, 1989. Staining procedure to detect viability and the true acrosome reaction in spermatozoa of various species. Gamete Research, 22: 51-57.

Fazeli, A. R., C. Holt, W. Steenweg, M. M. Bevers, W. V. Holt and B. Colenbrander, 1995. Development of a sperm hemizona binding assay for boar semen. Theriogenology, 44: 17–27.

Fazeli, A. R., B. R. Zhang, W. Steenweg, B. Larsson, M. M. Bevers, J. van der Broek, H. Rodriguez-Martinez and B. Colenbrander, 1997. Relationship between sperm–zona pellucida binding assays and the 56-day nonreturn rate of cattle inseminated with frozen–thawed bull semen Theriogenology, 48: 853–863.

Garde, J. J., C. G. Artiga, A. Gutiérrez and I. Vázquez, (1992): A simple staining method for evaluating the acrosomal status of ram spermatozoa. 12th Int. Congr. Anim. Reprod., 1: 464–466.

Gadea, J., C. Matás and X. Lucas, 1998. Prediction of porcine semen fertility by homologous in vitro penetration (hIVP) assay. Animal Reproduction Science, 56: 95–108.

Graham, E. F., M. K. L. Schmehl, B. K. Evenson and D. S. Nelson, 1980. Problems with laboratory assays. Proceedings of the 8th Technical Conference on Artificial Insemination and Reproduction, National Association of Animal Breeders, Wisconsin. 1–8.

Holt, C, W. V. Holt, H. D. M. Moore, H. C. B. Reed and R. M. Curnock, 1997. Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials. Journal of Andrology, 18: 312–323.

Januskauskas, A., L. Söderquist, M. C. Haard, N. Lundeheim and H. Rodriguez- Martinez, 1996. Influence of sperm number per straw on the postthaw postthaw sperm viability and fertility of Swedish Red and White AI bulls Acta Veterinaria Scandinavica, 37:461–470.

Kjaestad, H., E. Ropstad and K. A. Berg, 1993. Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. Acta Veterinaria Scandinavica, 34: 299–303.

Larsson, B. and H. Rodrıguez-Martınez, 2000. Can we use in vitro fertilization tests to predict semen fertility? Animal Reproduction Science, 60–61: 327–336.

Lenz, R.W., J. L. Martin; M. E. Bellin and R. L. Ax, 1988. Predicting fertility of dairy bulls by inducing acrosome reactions in sperm with chondroitin sulfates. J. Dairy Sci., 71(4): 1073-1077.

Maji, S., U. Datta and M.L. Hembram, 2010. Cell surface changes associated with in vitro capacitation and acrosome reaction of goat epididymal sperm by a marine bio-active compound from the snail Telescopium telescopium. Veterinarski Arhiv., 80(5): 561-570.

Martinez, M. F., P. Bosch and R. A. Bosch, 1998. Determination of early pregnancy and embryonic growth in goats by transrectal ultrasound scanning. Theriogenology, 49:1555-1565.

Parkinson, T. J. (1996): Fertility and infertility in male animals. In: Arthur, G. H., Noakes, D. E., Pearson, H. and Parkinson, T. J. (eds) Veterinary Reproduction and Obstetrics. 7th edn. W. B. Saunders Co. Ltd., London, England. pp. 572–633.

Pérez, L. J., A . Valcárcel, M. A. de las Heras and H. Baldassarre, 1997. Comparative study of four techniques for evaluation of sperm quality in ovine and bovine frozen–thawed samples. Reproduction in Domestic Animals, 32: 157–160.

Rodriguez-Mártinez, H., B. Larsson, B. R. Zhang and L. Söderquist, 1997: In vitro assessment of

viability and fertilizing capacity of bull spermatozoa. J. Reprod. Dev., 43, 1–11.

Shams-Borhan, G. and R. A. P. Harrison, 1981. Production, characterisation, and use of ionophore- induced, calcium-dependent acrosome reaction in ram spermatozoa. Gamete Res., 4: 407–432.

Smith, J. F. and G. R. Murray, 1996. Use of bovine oocytes for the evaluation of ram semen. Proc. N. Z. Soc. Anim. Prod., 56: 304–306.

SPSS, 2008. Statistical Package for the social sciences. Release 16, SPSS Inc, Chicago, USA.

Ucar, O. and T. J. Parkinson, 2003. In vitro induction of the acrosome reaction in ovine spermatozoa by calcium ionophore A23187. Acta Veterinaria Hungarica, 51(1): 103–109.

Watson, P. F., (1979): The preservation of semen in mammals. In: Finn, C. A. (ed.) Oxford Reviews of Reproductive Biology. Vol. 1. Oxford Univ. Press, GB. pp. 283–350.

Watson, P. F., P. S. Jones and J. M. Plummer, 1991. A quantitative comparison of the spontaneous and ionophore-induced acrosome reaction in ejaculated ram spermatozoa: the effects of temperature, time and individual. Anim. Reprod. Sci., 24: 93–108.

Whitfield, C. H. and T. J. Parkinson, 1995. Assessment of the fertilizing potential of frozen bovine spermatozoa by in vitro induction of acrosome reactions with calcium ionophore (A23187). Theriogenology, 44: 413-422.

Zhang, B.R., B. Larsson, N. Lundeheim and H. Rodriguez-Martinez, 1998. Sperm characteristics and zona pellucida binding in relation to field fertility of frozen–thawed semen from dairy AI bulls. International Journal of Andrology, 21: 207–216.

إحداث تفاعل الأكروسوم للحيوانات المنوية مختبريا في ذكور الاغنام المحلية المصرية باستخدام الكالسيوم أيونوفور (A23187)

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أجريت هذه الدراسة بهدف إيجاد اختبار معملي للتنبؤ المبكر بالخصوبة لذكور الاغنام المحلية المصرية باستخدام الكالسيوم أيونوفور (A23187) لإحداث تفاعل الأكروسوم للحيوانات المنوية. تم جمع السائل المنوي لمدة ٥ أسابيع متتالية من أربعة ذكور رحماني وأربعة أوسيمي. ثم تم تقييم السائل المنوي الطازج التي تم جمعها في البداية. وقد استخدم الكالسيوم أيونوفور لتحفيز الحيوانات المنوية لإحداث تفاعل الأكروسوم للحيوانات المنوية.

أظهرت النتائج عدم وجود اختلافات معنوية في كل القيم للصفات الطبيعية للسائل المنوي لذكور الاغنام الرحماني. كذلك تم الحصول علي نفس النتائج من ذكور الأوسيمي باستثناء الحركة الكلية (88.75%, P<0.05). بعد إحداث تفاعل الأكروسوم لذكور الاغنام الرحماني والاوسيمي أظهرت النتائج في ذكور الرحماني والأوسيمي أن تفاعل الحيوانات المنوية الحية كانت الأعلى (34.25 and 34.50%, P<0.05) في الذكر رقم ١ في السلالاتين مقارنة بالذكور الأخرى في السلالاتين، وأظهرت نتائج نسبة الخصوبة فروق فردية بين الذكور داخل كل سلالة ولكن كان الاختلاف معنوي (P<0.05)بين ذكور الاوسيمي فقط.

أظهرت النتائج أن الكبش رقم واحد في كلتا السلالاتين كان الأعلى في نسبة الخصوبة. كانت الخصوبة في ذكور الرحماني أعلي من الأوسيمي (89.19 vs. 81.08%) ولكن هذه الزيادة لم تكون معنوية. كانت هناك علاقة إيجابية بين نسبة الحيوانات المنوية الحية التي احدثت تفاعل الأكروسوم ونسبة الخصوبة في ذكور الرحماني (r=0.64, P<0.01) وذكور الاوسيمي (r=0.96, P<0.01). بالإضافة إلى ذلك، كان هناك ارتباط معنوي بين نسبة الحركة الكلية ونسبة الخصوبة (r=0.70, P<0.01) في ذكور الاوسيمي . ويمكن أن نخلص نتائج الدراسة أنه يمكن التنبؤ بخصوبة ذكور الأغنام المحلية باستخدام اختبار تفاعل الأكروسوم معمليا باستخدام الكالسيوم ايونوفور .