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INJECTION EFFECT OF GIBBERELLIC AND/ OR BORIC ACID TO RABBITS ON RESPONSE OF SPERMATOZOA TO HYPO-OSMOTIC SWELLING TEST

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ABSTRACT

A total number of forty eight sexually mature New-Zealand White (NZW) rabbit bucks of nine months age were used in this experiment. The experiment was designed to evaluate grade and percentages of progressive sperm motility, in addition to percentages of spermatozoa with swelling heads and coiled tails ejaculated by bucks injected with Gibberellic and/ or Boric acid as responded to hypo-osmotic solution, during incubation at 37 ^{o}C for up to 60 minutes. Animals were divided into four experimental comparable groups (12 in each). Rabbits in 1st group were subcutaneously injected with 1 ml saline solution (NaCL) and served as a control group, while the rabbits in 2nd, 3rd and 4th groups were injected subcutaneously with 1 mg Gibberellic acid, 2.5 mg Boric acid and mixture of 1 mg Gibberellic and 2.5 mg Boric acids, respectively. Rabbit injection started 6 weeks before semen collection and during experimental period, weekly.

The results obtained from this study indicated that, sperm motility in solutions with different osmolarities (300 or 100 mOsmol/ L) were significantly ($P \leq 0.01$) higher, while the percentages of spermatozoa with coiled tails or swollen heads were significantly ($P \leq 0.01$) lower at level of 300 mOsmol/ L (normal osmolarity) than 100 mOsmol/ L (osmotic stress), during incubation at 37 ^{o}C for up to 60 minutes in all studied rabbit semen groups.

Advancement of incubation time up to 60 minutes of rabbit semen in solutions with different osmolarities decreased and increased significantly ($P \leq 0.01$) {grade & advanced sperm motility} and {spermatozoa with coiled tails & swollen heads}, respectively, of all studied rabbit semen groups. Values of each of progressive sperm grades & percentages and percentages of spermatozoa with coiled tails or swollen heads in both osmolarities used (300 and 100 mOsmol/ L) were significantly ($P \leq 0.01$) arranged descendingly as recorded by semen of rabbit injected with mixture of Gibberellic + Boric acids followed by Boric acid, Gibberellic acid then saline solution (NaCL) control, respectively.

It be concluded that, injecting rabbit bucks with Gibberellic and Boric acids improved their semen quality, during semen conservation at incubation condition.

Keywords: Rabbit, semen, Gibberellic, Boric, incubation, HOS-test.

INTRODUCTION

Gibberellic acid is essential acid regulates various physiological processes (Alkhiat *et al.*, 1981). Gawienowski and Chatterijee (1980) showed that Gibberellic acid is consedered as estrogenic hormone-like action that resulted in improvement in libido and semen quality of males and increasing maternal ability of females. Kamel *et al.* (2009) used Gibberellic acid at 400 µg /kg body weight/ week and revealed stimulating and supporting spermatogenesis and sex accessory gland. It and causes an improvement in

sperm enzymatic activity represented in significantly decrease in alanin aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes and significantly increase in alkaline phosphatase activity (ALP) and acid phosphatase activity (ACP) enzymes in seminal plasma. The last author recorded a significant increase in serum testosterone hormone concentration due to Gibberellic Acid.

Heindel *et al.* (1994) and Catherine *et al.* (1996) recorded a significantly increase in litter size and weight at birth and at weaning, milk quality and quantity and pre-weaning mortality rates of rabbit does due to 125 µg/kg/day Boric Acid treatment. They added, Boric Acid display an essential role in regulating osmotic pressure and PH of seminal plasma resulted in improving fertilizing ability of males.

If artificial insemination is applied in a Rabbitries, it is considered that one single buck is affecting the fertility and prolificacy of about one hundred does (Alvarino, 2000). Consequently, reliable evaluation of semen and fertilizing ability of bucks is of great importance for successfulness of AI technique.

No one single and simple parameter for semen evaluation can be used as reliable predictor of sperm fertilizing ability (Daader and Seleem, 2005). The hypo-osmotic swelling test (Hos-test) was used to evaluate response to hypo - osmotic condition in human spermatozoa (Jeyendran *et. al.*, 1984), rabbit spermatozoa (Seleem, 2003, Daader and Seleem, 2005 and Zeidan *et. al.*, 2005). Some studies have shown that HOS-test is more reliable in assessing the outcome of in vitro fertilization than other semen parameters (Zaneveld *et. al.*, 1990).

The present work was planned to evaluate the response of spermatozoa of New-Zealand White rabbit bucks injected with Gibberellic and/ or Boric acids to hypo-osmotic swelling test (HOS-test).

MATERIALS AND METHODS

The field part of the present study was carried out in an industrial Rabbitry, Sakarah city, Giza province, Egypt. The laboratory work was conducted in Animal Production Research Institute, Agriculture Research Center, Giza, Egypt. The study lasted for four months.

Forty eight sexually mature New-Zealand White (NZW) rabbit bucks of nine months age and 3115 ± 35 gm body weight were used in the present work. All animals were fed ad libitum a commercial pelleted diet according to NRC (1994) recommendations. The ingredients and chemical composition of the pelleted ration fed to rabbits, during the experimental period was as shown in Table 1**.**

Animals were healthy and clinically free of external and internal parasites. The animals were kept under the same managerial and hygienic conditions and raised in wire batteries with wire-netted windows on their sides for providing natural ventilation. The windows were oriented with an elevation of 2 meters from the floor. The floor was made from ceramic plates and has moderately slope (from the middle to both sides) to facilitate water drainage

towards a large longitudinal gutter outside the Rabbitry. The fresh tap water was automatically available all the time by stainless steel nipples in each cage.

Table 1. The ingredients and chemical composition of the pellet ration fed to rabbits, during the **experimental period.**

Ingredients	**(%)**	**Vitamins & Minerals premix per Kilogram.**	
Clover hay	40.50	Vit.A (IU)	10000
Wheat bran	25.00	Vit.D3 (IU)	9000
Yellow corn	14.00	Vit.E (IU)	10000
Soybean meal (44%)	11.00	Vit.K (IU)	3
Molasses	3.00	Vit.B1 (IU)	2
Vinass	3.00	Vit.B2 (IU)	6
Bone meal	1.75	Vit.B6 (IU)	2
Lime stone	0.70	Biotin (mg)	0.2
Sodium chloride	0.55	Choline (mg)	1200
Vitamins & Mineral Premix	0.35	Niacine (mg)	40
DL-Methionine	0.15	Zn. (mg)	60
Total	**100**	Cu. (mg)	0.1
Calculated chemical composition **		Mn. (mg)	85
Crude protein (CP)%	18.00	Fe. (mg)	75
Ether extract (EE)%	3.00	Folic acid (mg)	5
Crude fiber (CF)%	14.00	Pantothenic acid (mg)	20
Digestible energy (Kcal/Kg)	2720.00		

**** Calculated according to NRC (1994) for rabbits.**

The present work was planned to estimate the response of spermatozoa of New-Zealand White (NZW) ejaculated by bucks injected with Gibberellic and/ or Boric acid to hypo-osmotic shock test (HOS-test), during incubation at 37 ^{o}C for up to 60 minutes. The hypo-osmotic shock (swollen) test (HOS-test) represented by each of grade and percentages of progressive sperm motility, in addition to percentages of spermatozoa with swelling heads and coiled tails. Animals were divided into four experimental comparable groups (12 in each). Rabbits in 1st group were subcutaneously injected with 1 ml saline solution (NaCL) 0.9% and served as a control group, while the rabbits in 2nd, 3rd and 4th groups were injected subcutaneously with 1 mg Gibberellic acid, 2.5 mg Boric acid and mixture of 1 mg Gibberellic and 2.5 mg Boric acids, respectively. The volume of each injecting dose was 1 ml at all parameters studied. Rabbit injection started 6 weeks before semen collection and during experimental period, weekly.

Semen samples were collected and diluted with saline solution (sodium chloride, NaCl 0.9 gm, egg yolk 5 ml, 50000 IU sodium penicillin and 50000 µg streptomycin sulphate/ 100 ml sterilized distilled water) to produce a final dilution rate of 1 semen: 10 diluent, and at osmolarities 100 or 300 mOsmol/ L (Daader and Seleem, 2005 and Zeidan *et. al.*, 2005) .The diluted semen in different osmolarities were incubated at 37^{o}C for up to 60 minutes. The final osmolarity of the test solutions measured by freezing point

depression was modified from 300 mOsmol/ L. (normal osmolarity) to 100 mOsmol/ L (hypo-osmolarity) via serial dilutions to obtain hypo-osmotic solution.

Grades and percentages of advanced sperm motility and spermatozoa with coiled tail and/ or swollen head were estimated at different intervals (0, 15, 30, 45, and 60 minutes) of incubation at 37°C. Grade of the progressive sperm motility was graded as follows: Grade 1, oscillating movement but stationary, Grade 2, slow movement with no fixed direction, Grade 3, slow progressive movement and Grade 4, fast progressive movement. Sperm swelling was assessed by placing 15µl of well-mixed sample on a warm slide (37°C). Slides were stained with eosin-nigrosin mixture stain and covered with a cover glass before being observed under a phase contrast microscope at x1000. Two hundred spermatozoa per slide were counted and the percentage of swelling/coiling was determined (number of spermatozoa with swollen/coiled tails divided by the total number of spermatozoa counted multiplied by 100). The proportion of coiled/swollen spermatozoa from a control sample (300mOsmol/L) was subtracted from the calculations (Vazquez *et al.*, 1997). The response of spermatozoa to hypo-osmotic shock test was carried out as studied by Marwa (2002), Seleem (2003), Daader and Seleem (2005) and Zeidan *et. al.*, (2005).

Data were statistically analyzed by analysis of variance according to Snedecor and Cochran (1967). Percentage values were transformed to arcsine before being statistically analyzed. Duncan·s multiple range test (Duncan, 1955) was used to test the significance of the differences between means. Kindling rates were analyzed using the Contingency Tables according to Everitt (1977).

RESULTS AND DISCUSSION

Progressive sperm motility:

Data presented in Tables 2 & 3 showed that, the highest progressive sperm motility grades and percentages in both osmolarities used (300 and 100 mOsmol/ L), during incubation at 37°C for up to 60 minutes were recorded for semen ejaculated from bucks injected with mixture of Gibberellic + Boric acids followed by Boric acid, Gibberellic acid then saline solution (NaCL) control, respectively. The effect of rabbit injection with Gibberellic and Boric acids on progressive sperm motility grades and percentages was highly significant ($P \leq 0.01$)

Grades and percentages of progressive sperm motility of the four semen used were significantly ($P \leq 0.01$) lower as represented to hypo-osmotic solution (100 mOsmol/ L) than normal osmotic solution (300 mOsmol/ L). These results are in agreement with those obtained by Marwa (2002); Seleem (2003); Daader and Seleem (2005) and Zeidan *et. al.* (2005). Similar results were recorded previously by Jeyendran *et. al.* (1984) in man; Correa and Zavos (1994) in bull and Moussa (1999) in ram spermatozoa.

Table 2. Grades of progressive sperm motility of NZW rabbit semen ejaculated by bucks injected with Gibberellic and/ or Boric acid as responded to hypo-osmotic solution, during incubation at 37 ºC for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsmol/L)	Semen ejaculated by injected bucks with acids				Means ± SE
		(NaCL) Control	1 mg Gibberellic acid	2.5 mg Boric acid	Gibberellic and Boric acid	
0	300	3.87±0.14	4.17 ± 0.25	4.61 ± 0.29	4.75 ± 0.26	4.35± 0.24
	100	3.54±0.17	3.89 ± 0.28	4.52 ± 0.28	4.61 ± 0.24	4.14± 0.19
Means ± SE		3.71±0.12 [b]	4.03± 0.21 [b]	4.57± 0.19 [a]	4.68± 0.23 [a]	
15	300	3.79±0.16	4.12 ± 0.21	4.54 ± 0.32	4.72± 0.24	4.29± 0.18[A]
	100	2.77±0.15	3.37 ± 0.12	4.29 ± 0.17	4.50± 0.14	3.73± 0.16[B]
Means ± SE		3.28±0.11 [c]	3.75± 0.14 [b]	4.42± 0.22 [a]	4.61± 0.17 [a]	
30	300	3.65±0.19	4.01 ± 0.19	4.47 ± 0.24	4.68±0.29	4.20± 0.21[A]
	100	1.79±0.15	3.28 ± 0.14	3.95 ± 0.16	4.46± 0.25	3.37± 0.18[B]
Means ± SE		2.72±0.13 [c]	3.65± 0.12 [b]	4.21± 0.17 [a]	4.57± 0.22 [a]	
45	300	3.39±0.17	3.81 ± 0.15	4.31 ± 0.21	4.60 ± 0.22	4.03±0.16[A]
	100	0.66±0.11	2.07± 0.09	3.11 ± 0.09	3.86± 0.11	2.43± 0.8[B]
Means ± SE		2.03±0.09 [d]	2.94± 0.10 [c]	3.71± 0.13 [b]	4.23± 0.13 [a]	
60	300	3.11±0.14	3.64 ± 0.12	4.18 ± 0.13	4.54 ± 0.18	3.87±0.12[A]
	100	0.00±0.00	0.54± 0.03	1.89 ± 0.06	2.79± 0.08	1.31±0.0.5[B]
Means ± SE		1.56±0.06 [d]	2.09± 0.07 [c]	3.04± 0.08 [b]	3.67± 0.08 [a]	
Storagability (%)	300	80.36±2.17	87.29±2.42	90.67±2.95	95.58±3.01	88.48±2.84 [A]
	100	00.00±0.00	13.88±0.76	41.81±0.94	60.52±1.26	29.05±0.79 [B]
Means ± SE		40.18±1.64 [d]	50.59±1.90 [c]	66.24±1.94 [b]	78.05±2.00 [a]	

Means within the same row (a, b, ...) or within the same column (A, B) bearing different letter superscripts are significantly (P ≤ 0.05) different.

Table 3. Percentages of progressive sperm motility of NZW rabbit semen ejaculated by bucks injected with Gibberellic and/ or Boric acid as responded to hypo-osmotic solution, during incubation at 37 ºC for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsmol/L)	Semen ejaculated by injected bucks with acids				Means ± SE
		(NaCL) Control	1 mg Gibberellic acid	2.5 mg Boric acid	Gibberellic and Boric acid	
0	300	61.28±1.53	69.37 ± 1.42	75.29 ± 1.88	78.61 ± 2.01	71.14± 1.49
	100	58.52±1.67	67.51 ± 1.24	73.75 ± 1.93	78.56 ± 2.11	69.59± 1.82
Means ± SE		59.90±1.55 [d]	68.44± 1.19 [c]	74.52± 1.75 [b]	78.59± 1.82 [a]	
15	300	59.37±1.26	68.41 ± 1.33	74.78 ± 1.92	78.35± 1.67	70.23±1.61[A]
	100	43.27±1.42	57.34 ± 1.41	67.01 ± 1.52	74.33 ± 1.81	60.49±1.49[B]
Means ± SE		51.32±1.32 [d]	62.88± 1.28 [c]	70.90± 1.58 [b]	76.34± 1.73 [a]	
30	300	57.11±1.18	66.57 ± 1.35	73.25 ± 1.66	77.41 ± 1.94	68.59±1.47[A]
	100	23.14±0.83	42.55 ± 0.94	57.31 ± 1.31	68.09 ± 1.26	47.77±0.99[B]
Means ± SE		40.13±0.94 [d]	54.56± 0.82 [c]	65.28± 0.52 [b]	72.75± 1.07 [a]	
45	300	54.06±1.07	64.03 ± 1.27	71.27 ± 1.39	75.91 ± 1.27	66.32±1.26[A]
	100	04.12±0.07	43.46 ± 1.01	54.99 ± 1.14	63.92 ± 1.33	41.62±1.11[B]
Means ± SE		29.09±0.82 [d]	53.75± 0.93 [c]	63.13± 1.01 [b]	69.92± 1.12 [a]	
60	300	49.47±0.79	60.17 ± 1.27	68.34 ± 1.17	73.87 ± 1.25	62.96±1.03[A]
	100	00.00±0.00	18.29 ± 0.15	34.22 ± 0.71	48.86 ± 0.84	25.34± 0.37[B]
Means ± SE		24.74±0.31	39.28± 0.69 [c]	51.28± 0.83 [b]	61.37± 0.91 [a]	
Storagability **(%)**	300	80.73±2.17	86.74±2.42	90.77±2.95	93.97±3.01	88.05±2.84 [A]
	100	00.00±0.00	27.09±0.76	46.40±0.94	62.19±1.26	33.92±0.79 [B]
Means ± SE		40.37±1.64 [d]	56.92±1.90 [c]	68.59±1.94 [b]	78.08±2.00 [a]	

Means within the same row (a, b…etc) or within the same column (A, B, C) bearing different letter superscripts are significantly (P ≤0.05) different.

The advancement of incubation time at 37^0C for up to 60 minutes decreased significantly (P ≤ 0.01) the grades and percentages of progressive sperm motility in all rabbit semen studied and in both osmolarities used. These results are comparable with those obtained by Marwa (2002); Seleem (2003); Daader and Seleem (2005) and Zeidan *et. al.* (2005). Similar trend was recorded previously by Kumi-Diaka (1993) in canine; Correa and Zavos (1995) in bovine; Vazquez *et. al.* (1997) in boar and Moussa (1999) in ram spermatozoa.

Percentages of spermatozoa storagability were arranged in descending order as ejaculated by bucks injected with mixture of Gibberellic + Boric acids followed by Boric acid, Gibberellic acid then saline solution (NaCL) control, respectively. The effect of treatment on spermatozoa storagability was highly significant (P ≤ 0.01).

It is interested to notice that, the grades and percentages of progressive motility of spermatozoa in solution at 300 mlosmol/ liter (normal osmolarity) were superior (P ≤ 0.01) than those in solution at 100 mlosmol/ liter (hypo-osmotic stress), during different periods of semen preservation at incubation condition (15, 30, 45 and 60 minutes).

Spermatozoa with swelling head or coiled tails:

Data presented in Tables 4 & 5 showed that, osmolarity tested solution (100 mOsmol/ L, hypo-osmotic pressure) recorded the highest percentages of spermatozoa with swollen heads or coiled tails, during incubation at 37^0C for up to 60 minutes due to semen of rabbit injected with mixture of Gibberellic + Boric acids followed by Boric acid, Gibberellic acid then, saline solution (NaCL) control, respectively. The adverse results were obtained by using normal osmolarity solution (300 mOsmol/ L)

Percentages of swelling spermatozoa and spermatozoa with coiled tails of the four rabbit semen used were significantly (P ≤ 0.01) higher as responded to hypo-osmotic solution (100 mOsmol/ L) than normal osmotic solution (300 mOsmol/L). These results are in agreement with those obtained by Marwa (2002); Seleem (2003); Daader and Seleem (2005) and Zeidan *et. al.* (2005). Similar results were recorded previously by Jeyendran *et. al.* (1984) in man; Correa and Zavos (1994) in bull and Moussa (1999) in ram spermatozoa. These results may be due to that spermatozoa exhibited morphologic changes which were evidenced by coiling of the tail, when subjected to HOS-test as shown in Figure 1 (Zeidan *et. al.*, 2005).

The advancement of incubation time at 37^0C for up to 60 minutes increased significantly (P≤0.01) the percentages of swelling spermatozoa and spermatozoa with coiled tails in all rabbit semen studied and in both osmolarities used. These results are in agreement with those obtained by Marwa (2002); Seleem (2003); Daader and Seleem (2005) and Zeidan *et. al.* (2005). Similar trend was recorded previously by Kumi-Diaka (1993) in canine; Correa and Zavos (1995) in bovine; Vazquez *et al.* (1997) in boar and Moussa (1999) in ram spermatozoa.

Regarding different periods of semen preservation at incubation condition (15, 30, 45 and 60 minutes), percentages of spermatozoa with coiled tails or swollen heads in solution at 300 mlosmol/ liter (normal

osmolarity) were significantly (P ≤ 0.01) lower than those in solution at 100 mlosmol/ liter (hypo-osmotic stress).

The response of spermatozoa to hypo-osmotic solution may be due to the transportation of the physical and biochemical compounds across the sperm-cell membrane which plays an essential role in biochemical process and consequently sperm viability and fertilizing capabilities (Zavos, 1983). Seleem (2003); Daader and Seleem (2005) and Zeidan *et. al.* (2005) added that, the abrupt decrease in osmotic pressure may cause malfunction in physiological processes of spermatozoa.

Table 4. Percentages of spermatozoa with swelling heads of NZW rabbit semen ejaculated by bucks injected with Gibberellic and/ or Boric acid acid as responded to hypo-osmotic solution, during incubation at 37 ºC for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsmol/L)	Semen ejaculated by injected bucks with acids				Means ± SE
		(NaCL) Control	1 mg Gibberellic acid	2.5 mg Boric acid	Gibberellic and Boric acid	
0	300	10.11 ± 0.52	09.92 ± 0.47	09.83± 0.51	07.42±0.34	9.32±0.34
	100	07.53±0.44	10.01 ±0.57	10.12 ± 0.43	10.29 ± 0.52	9.49± 0.36
Means ± SE		O8.82±0.50 [b]	09.97±0.41 [a]	09.98±0.31 [a]	08.86±0.29 [b]	
15	300	10.25± 0.39	10.06 ± 0.52	10.01 ± 0.51	07.48±0.31	9.45± 0.41
	100	7.75±0.47	10.39 ± 0.54	10.64 ±0.38	10.86±0.55	9.91± 0.46
Means ± SE		09.00±0.56 [b]	10.23±0.53 [a]	10.33±0.50 [a]	09.17±0.37 [b]	
30	300	10.69 ± 0.55	10.41 ±0.55	10.29 ±0.47	07.59±0.42	9.75± 0.45[B]
	100	10.81±0.53	15.63 ±1.14	21.12 ±1.27	23.17 ± 1.33	17.68± 0.99[A]
Means ± SE		10.75±0.45 [c]	13.02±0.52 [b]	15.71±0.64 [a]	15.38±0.61 [a]	
45	300	11.18±0,57	10.75±0.61	10.58 ± 0.48	07.75±0.59	10.07± 0.55[B]
	100	15.94±1.26	25.57±1.74	33.19 ±1.38	37.81±1.86	28.13±1.07[A]
Means ± SE		13.56±0.82 [c]	18.16±0.71 [b]	21.89±0.53 [a]	22.78±0.73 [a]	
60	300	11.61± 0.71	11.13 ±0.56	10.91±0.56	07.92±0.64	10.39±0.59[B]
	100	23.01±1.57	37.24 ±2.14	48.29±2.96	55.16 ± 3.18	40.93±2.06[A]

Means within the same row (a, b, c) or within the same column (A, B, C, D) bearing different letter superscripts are significantly (P ≤ 0.05 or 0.01) different.

It's apparent clearly from these results that, the reproductive efficiency of Rabbit bucks injected with mixture of (1 mg Gibberellic + 2.5 mg Boric acids) is better than those injected with only 2.5 mg Boric or 1 mg Gibberellic acids then saline solution, respectively, under Egyptian sub-tropical condition.

It could be concluded that, the response of rabbit spermatozoa to hypo-osmotic swelling test (HOS-test) was good indicator for reproductive capability of rabbit bucks as the hypo-osmotic condition, that spermatozoa are subjected is considered as stressful factor, under which spermatozoa that show better results of motility or survivability can be used as a good indicator of fertilizing ability. In such a case it is not necessary to carry out more than one test for better evaluation of spermatozoa.

In conclusion, reproductive performance of rabbit bucks injected with mixture of (1 mg Gibberellic + 2.5 mg Boric acids) were superior than those

injected with only 2.5 mg Boric or 1 mg Gibberellic acids then saline solution, respectively under Egyptian environmental conditions.

Table 5. Percentages of spermatozoa with coiled tails of NZW rabbit semen ejaculated by bucks injected with Gibberellic and/ or Boric acid as responded to hypo-osmotic solution, during incubation at 37 ºC for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsmol/L)	Semen ejaculated by injected bucks with acids				Means ± SE
		(NaCL) Control	1 mg Gibberellic acid	2.5 mg Boric acid	Gibberellic and Boric acid	
0	300	07.94± 0.35	05.59 ± 0.29	04.67 ± 0.22	04.01±0.24	5.55± 0.13^{B}
	100	07.11±0.16	09.72 ± 0.27	12.67 ± 0.36	17.91± 0.28	11.85± 0.17^{A}
Means ± SE		7.53±0.11 ^{d}	7.66±0.15 ^{c}	8.67± 0.21 ^{b}	10.96±0.21 ^{a}	
15	300	08.21±0.33	05.78 ± 0.33	04.71 ± 0.27	04.07±0.23	5.69± 0.15^{B}
	100	12.37±0.41	16.74 ± 0.47	21.54 ± 0.92	28.77±0.86	19.86± 0.21^{A}
Means ± SE		10.29±0.17 ^{d}	11.26±0.22 ^{c}	13.13±0.41 ^{b}	16.42±0.72 ^{a}	
30	300	08.54 ± 0.34	05.92 ± 0.37	04.87±0.24	04.18±0.27	5.88± 0.24^{B}
	100	22.24±0.57	28.61±0.91	35.62±1.24	43.84±1.69	32.58± 0.83^{A}
Means ± SE		15.39±0.34 ^{d}	17.27±0.28 ^{c}	20.25±0.42 ^{b}	24.01±0.71 ^{a}	
45	300	08.75± 0.37	06.03 ± 0.42	05.06 ± 0.31	04.34±0.24	06.05± 0.22^{B}
	100	34.37±1.19	43.44 ± 1.54	52.75 ± 1.63	61.01± 1.65	47.89± 0.94^{A}
Means ± SE		21.56±0.73 ^{d}	24.74±0.81 ^{c}	28.91±0.92 ^{b}	32.68±0.84 ^{a}	
60	300	09.06±0.42	06.31±0.38	05.29 ±0.28	04.51±0.22	6.29±0.20^{B}
	100	39.26±1.19	61.57±1.83	70.24±2.10	82.22±2.79	63.32±1.01^{A}
Means ± SE		24.16±0.51 ^{d}	33.94±0.68 ^{c}	37.77±1.14 ^{b}	43.37±1.37 ^{a}	

Means within the same row (a, b, c, d) or within the same column (A, B, C, D, E) bearing different letter superscripts are significantly (P ≤ 0.05 or 0.01) different.

Figure 1. Micrograph of the response of NZW rabbit spermatozoa to the hypo-osmotic swelling test HOS-Test (black arrows indicated spermatozoa with coiled tails).

REFERENCES

Alkhiat, A.A.; H. Morsy; E. Shehata and A. Abdelatif (1981). Veterinary pharmacology and toxicology. Ministry of High Education and Scientific Research., Iraq.

Catherine, J.; C. Price, Melissa; B. Marr, Chritina; J. Myers; C. Seely; J.J. Heindel and Bernard, A. Schwetzt (1996). The developmental Toxicity of Boric Acid in Rabbits Fundamental and Applied Toxicology (Toxicol. Sci.), 34:176-187.

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

Daader, A.H. and T.S.T. Seleem (2005). Response of spermatozoa of different breeds of rabbits to Hypo-Osmotic Swelling test. The 4th Inter. Con. on Rabbit Prod. in Hot Clim., Sharm El-Sheikh, Egypt,177 – 181.

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

Everitt, B.S. (1977). The analysis of contingency tables. Monographs of Applied Probability and statistics. PP, 38-66 London, Chopman Hall.

Gawienowski, A.M. and D. Chatterijee (1980). Effect of prostaglandin inhibitor on the uterotrophic response of estradiol and gibberellic acid. Life Sciences, 27:1393-1396.

Heindel, J.J.; C.J. Price; and B.A. Schwetz (1994). Developmental toxicity of boric acid in mice, rats and rabbits. Environ. Health Perspect. 102(Suppl. 7), 107-112.

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

Kamel, K.I.; A.E. El-Komy, and M.E. El-Sbeiy (2009). The Androgenic Action of Gibberellic Acid (GA_3) on Reproductive Performance of New Zealand White Rabbit Bucks. World J. of Agric. Sci., 5(1):40-48.

Kumi-Diaka, J. (1993). Subjecting canine semen to the hypo-osmotic test. Theriogenology, 391: 1279-1289.

Marwa, E.H. (2002). Physiological studies on storage of rabbit semen. M. Sci. Thesis, Fac. Agric., Zagazig Univ., Zagazig, Egypt.

Moussa, L.A. (1999). Evaluation of Nagdi rams spermatozoa using hypo-osmotic test. Zagazig Veterinary Journal, 27:26-33.

NRC (1994). Nutrient Requirement of Rabbits. National Academy of Science, Washington, DC. USA.

Seleem,T.S.T. (2003).Studies on productive and physiological characteristics in rabbits under different managerial conditions. Ph.D.Thesis, Fac. of Agric., Zagazig Univ., Zagazig, Egypt.

Snedecor, G.W. and W.G., Cochran (1967). Statistical Methods. 2nd Ed. Iowa Univ. Press. Ames, Iowa.

Vazquez. J.M.; Martinez, E.A.; Martinez, p.; Garcia-Artiga, C. and Roca, J. (1997). Hypo-osmotic swelling of boar spermatozoa compared to other methods for analyzing the sperm membrane. Theriogenology, 47:913-922.

Zaneveld, L.J.D.; R.S. Jeyendran; P. Krajeski; K. Coetzee; T.F. Kruger and L.J. Lombard (1990). Hypoosmotic Swelling Test. In: Acosta A.A. Swanson RJ. Ackerman S.B, Knuger TF, Van Zyl JA, Menkveld R (eds). Human Spermatozoa in Assisted Reproduction Baltimore: Williams and Wilkins, pp 223-227.

Zeidan, A.E.B; A.H. Daader; L.B. Bahgat and E.A.A. Ahmadi (2005). The functional integrity and response of rabbit spermatozoa membrane to the hypoosmotic swelling test. The 4th Inter. Con. on Rabbit Prod. in Hot Clim., Sharm El-Sheikh, Egypt,153 – 159.

تـأثير حقـن الأرانـب بحامضـى الجبريليـك أو/ والبوريـك علـى اسـتجابة الحيوانـات المنوية لاختبار منخفض الأسموزية

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أستخدم فى هذه الدراسة عدد 48 ذكر أرنب نيوزيلندى أبيض ناضـج جنسياً علـى عمر 9 أشهر. صممت الدراسة لتقييم الدرجـه والنسبة المئوية للحركـة التقدميـة للحيوانـات المنويـة بالإضـافة إلى النسبة المئوية للحيوانات المنوية ذات الذيول الملتوية أو الرؤوس المنتفخة والمقذوفة من الذكور المحقونه بحامضى الجبريليك و/ أو البوريك، والمحفوظه على فى مخفف منخفض الإسموزية وعلى درجة حرارة التحضين 37 ˚م لمدة 60 دقيقة.

قسمت الحيوانات المستخدمة فى التجربة إلى أربعة مجاميع تجريبيـة متجانسة (12 أرنـب فى كل مجموعة). تم حقن الأرانب فى المجموعة الأولـى تحت الجلد 1 مل محلول ملحـى (كلوريد الصوديوم) وحفظت كمجموعة مقارنه، أما الأرانب فى المجاميع التجريبية الثانية، والثالثة، والرابعة فتم حقنها تحت الجلد 1ملجم حامض الجبريليك، و2,5 ملجم حامض البوريـك، و خليط من (1ملجم حامض الجبريليك +2,5 ملجم حامض البوريك)، على الترتيب.تم حقن الأرانب إسبوعياً وقبل جمع السائل المنوى بستة أسابيع وإستمر الحقن خلال فترات إجراء التجربة.

أوضحت النتائج المتحصل عليه من هذه الدراسة أن حركة الحيوانات المنوية فى المحاليل مختلفة الإسموزية (300 أو 100 مل أسمول/ لتر) كانت أعلـى معنوياً (علـى مستوى 5 أو 1%)، بينمـا سـجلت النسـبة المئويـة للحيوانـات للمنويـة ذات الـذيول الملتويـة أو الـرؤوس المنتفخـة إنخفاضـاً معنوياً (علـى مستوى 5 أو 1%) فـى والمحفوظـة فـى المخفف 300 مـل أسمول/ لتر (الإسموزية الطبيعية)، مقارنة بتلك المحفوظة فى المخفف 100 مل أسمول/ لتر (إجهاد أسموزى) خـلال فتـرات الحفظ على درجة 37 ˚م، ولمدة 60 دقيقة فى كل المجاميع التجريبية المدروسة.

تقدم زمن حفظ السائل المنوى المخفف على درجة حـرارة التحضـين حتـى 60 دقيقـة وفـى المحاليل الإسموزية المختلفة (300 أو 100 مل أسمول/ لتر) طبيعى أو منخفض الإسموزية، علـى الترتيب، أدى إلى إنخفاض معنوى (على مستوى 5 أو 1%) فى جودة وخصـائص السـائل المنـوى، فى كل المجاميع التجريبية المدروسة. الدرجة والنسبة المئوية للحركة التقدمية للحيوانات المنويـة فـى كلا المحاليل الإسموزية المختلفة والمستخدمة (300 أو 100 مل أسمول/ لتر) كانـت مرتبـة معنويـاً (على مستوى 5 أو 1%) ترتيباً تنازلياً، بينما النسب المئوية للحيوانات المنويـة ذات الـذيول الملتويـة أو الـرؤوس المنتفخـة كانـت مرتبـة معنويـاً (علـى مستوى 5 أو 1%) ترتيبـاً تصـاعدياً، مـن الـذكور

المعاملة على النحو التالى: خليط من حامضى الجبريليك و البوريك، ثم حامض البوريك، ثم حامض الجبريليك، ويليهم الذكور المحقونة بالمحلول الملحى (مجموعة المقارنة)، على الترتيب.

من نتائج هذه الدراسة نستخلص أن حقن ذكور الأرانب بحامضى الجبريليك والبوريك حسن من جودة وخصائص السائل المنوى، خلال الحفظ على درجة حرارة التحضين

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