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STUDY ON THE EFFECT OF ROYAL JELLY OF BEES (*APIS MELLIFERA*) ON THE MORPHOLOGY AND SPERM FUNCTION PARAMETERS IN MICE (*SWISS ALBINO*)

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ABSTRACT

The objective of this study was to investigate the effect of Royal jelly RJ on morphology and motility of mice sperms. Sperms were collected from the cauda region of the epididymis of each 10 mice from the treatment and control groups. Direct activation techniques and evaluation of sperm morphology were carried out. Dhino microscope was used for sperm measurement. The inspection was carried out in Salamatic laboratory for pathological analysis in 2015.The result revealed that all of the sperm function parameters registered significant activation ($P < 0.05$) in the treatment group. There was a significant increase in both the percentage of the sperm motility grade A and the progressive motility (A+B) of the treatment group. Spermatozoon with different lengths were noticed. The study explains that this difference may be due to the presence of different sperm developmental stages and not to the increase in the number and sizes of mitochondria in the mid-peace during activation or not to the sliding movement of microtubules in the axon of spermatozoon. These findings provide evidence that RJ can play an important role only in improving the male mice motility and which may open the way for further researches to demonstrate the possibility of using RJ in artificial insemination in field animals.

Key words**:** Apis mellifera, Bees mice, Sperm morphology royal jelly.

INTRODUCTION

Royal jelly is a substance that is secreted from worker honey bees. It is the main food source for only the first 3 days of worker larva. One larva that is to be the Queen bee is fed only RJ its entire life, so that bee worker alives only for 6 months while Queen alives for 2-7 years. This exclusive feeding triggers the full development of her ovaries which is needed to lay the millions of eggs she will lay in her lifetime. This may be related to the extremely high nutritional contents of the RJ. (Mishima *et al.*, 2005), (Narita *et al.*, 2006), (Okuda *et al.*, 1988), and (Taylor, 2004).

Royal jelly is rich in amino acids, lipids, simple sugars (Monosaccharides), some enzymes, antibacterial and antibiotic components, vitamins such as pantothenic acid (Vitamin B_5), vitamin B_6 (pyridoxine), high levels of vitamins D and E. It contains ample levels of iron and calcium, fatty acids and most importantly, proteins. RJ also contains acetylcholine, which is needed to transmit nerve messages from cell to cell (Viuda-Martos *et al.*, 2008). Also the objective of this study is to examine the *in vitro* effect of RJ on morphology and motility of sperms. Similar literature on this rasped were not available. The aim of this study is to investigate the effect of (RJ) in morphology and activity of sperms in mice.

MATERIALS AND METHODS

Experimental animals:

Twenty mature apparently healthy male mice (Balb/C St Can BR Strain) 8-12 weeks old were purchased from the higher institute for the diagnosis of infertility & techniques of assisted production of Al-Nahrain university in 2015. Mice were divided into control and treatment groups.

1- Sperms Collection: Animals were sacrificed by cervical dislocation, The caudal region of the epididymis were removed aseptically and placed immediatly into 1ml of in vitro fertilization (IVF) medium in a 35-mm culture dish. The collected pieces of epididymis were minced by using forceps and scissors. The sperms were allowed to disperse by gently shaking the dish by hand for 3 to 5 minutes at room temperature. The sperms suspension was divided into two parts.

2- *In vitro* Sperm Activation Technique: Direct Activation Technique was used where the sperms were allowed to swim-up through the medium for at least 60 minutes at 37℃ by 5% CO2 incubation (Cross and Overstreet 1987). This technique for sperm activation is characterized by direct effect of the culture medium on sperm parameters (Fig.1). Then the sperms were counted.

3- Royall Jelly preparation: Royal jelly was used as 10% concentration, was prepared by adding 0.1ml of RJ suspension to 0.9 ml of mouse IVF media (Gain medium., FertiPro NV, Industriepark Noord 32,8730 Beernem, Belgium v.C1). The 10% solution was filtered with pore size 0.45μm and 0.22 μm, and then pH was adjusted to reach 7.2-7.4 with HCl one molar (CYBOW 10, DFI Co., Ltd. Korea). Then activate the sperms using Direct Activation Technique that described previously.

4-Sperm concentration, motility, grade of activity and normal morphology: The motility of spermatozoa were graded according to (Mishima *et al.* 2005): A-Rapid linear progressive motility, B-Rapid nonlinear or linear non rapid progressive motility, C-Non progressive motility (localized) and D- Immotile**.**

5-Evaluation of sperm morphology: Sperms recovered from the treatment and control groups were fixed in zinker's fluid. The Nigrosin-Eosin stain was used for examining the sperm morphology. Live sperms appear does not take the Eosin stain and looks colorless under light microscope; whereas dead sperms take up eosin and appear pinkish in color (Graham, 2004). The lengths of sperms from each of the treatment and control were examined and measured by Digital microscope Dinocapture 2.0 (Made in Taiwan) (Check *et al.,* 1992).

RESULTS

***In vitro* sperm activation technique:**

(Table 1) revealed that the mean sperm concentration ($x10^6$ sperm/ml) following direct activation with 10% R.J-IVF medium was significantly ($P<0.05$) higher than before activation. Active sperm motility (grade A and grade B) was significantly ($P<0.05$) increased. The percentage of morphologically normal sperms following the addition of 10% R.J-IVF medium showed no significant ($P< 0.05$) difference when compared with the R.J-free IVF medium.

The morphological results: With the digital Dino microscope, the sperm appears to consist of only two portions: The head and tail. The measurement of the different parts of

spermatozoa was difficult as the head of mice sperm was irregular and characterized by severe polymorphism. Although, the morphological study couldn't detect any apparent increase in the head of treated spermatozoa, we could observe a non significant total elongation in the length of treated mice sperm (71±0.98) compared to control ones (67±1.07) (Table 2).

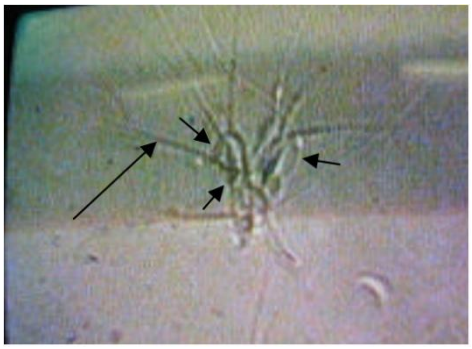

Fig. 1: Sperms before activation. Head of sperm (short arrows), Tail of sperm (long arrow). x400. Eosin-Nigrosin stain.

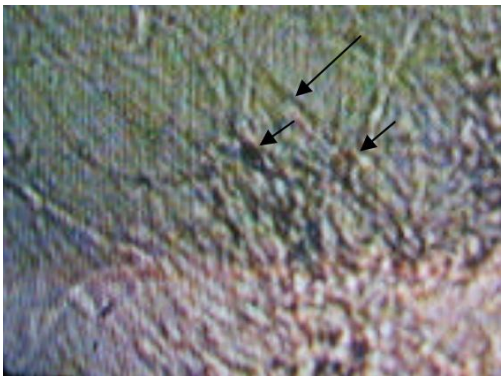

Fig. 2: Sperms after activation. Head of sperm (short arrows). Tail of sperm (Long arrow). x400. Eosin-Nigrosin stain.

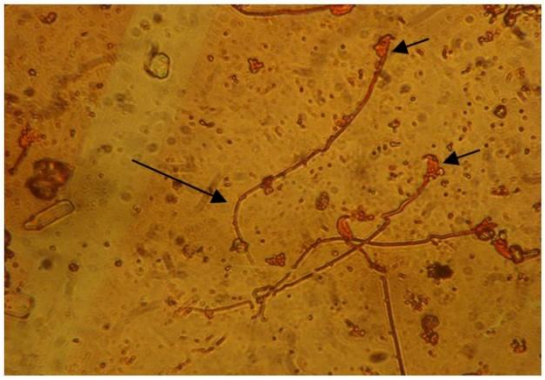

Fig. 3: Sperms after activation. Head of the sperm (short arrows). Tail of the sperm (long arrow). x400. Eosin stain.

Table (1): Comparison between control and treatment groups with 10% R.J-IVF medium on certain sperm function parameters following *in vitro* direct activation. (Mean SE)

***In vitro* sperm activation**			
Grouping with and without R.J.		**After 1 hour incubation Mean± SE**	**Significance**
Sperm concentration (10^6/ml)	Without R.J	25.22±1.405	S
	With R.J	36.32±4.323	
Sperm motility grade A (%)	Without R.J	12.40±2.315	S
	With R.J	18.73±0.918	
Sperm motility grade B (%)	Without R.J	21.33±2.622	S
	With R.J	30.73±2.472	
Sperm motility Grade C (%) (Localized)	Without RJ	26.14±0.05	S
	With RJ	36.21±0.123	
Sperm motility Grade D (%) (Immotile)	Without RJ	40.13±0.04	S
	With RJ	14.33±0.984	
Progressive motility (A+B)%	Without R.J	33.73±4.362	S
	With R.J	49.07±3.065	
Morphologically normal sperms(%)	Without R.J	30.43±4.410	NS
	With R.J	31.93±3.084	

Table (2). Morphometric measurement (μm) of the spermatozoon with and without adding RJ.

	Head	Tail & middle piece	Total	
Without RJ	4±0.17	63±1.56	67±1.07	NS
With RJ	4±0.42	67±1.92	71±0.98	NS

DISCUSSION

In the current study, the results referred to the improvement of sperm motility in the treatment group. This is confirmed by (Rodriguez, 2007) who reported that (RJ) increase libido and supports egg and sperm health. This may be related to the components of the RJ present in the activation fluid (Svoboda *et al.* 1986). The presence of different lengths of the sperms taken from the treatment may be attributed to the different developmental stages of spermiogenesis and not due to the proliferation of mitochondria and proteinaceous materials present in the mid-piece and principal part of the tail respectively (Ganong, 2005) or the slide movement of microtubules (Samuelson, 2007). This is confirmed by the finding of (Noguchi and Koizumi, 2011). This could also be in agreement with (Lercker *et al.*, 1982) who stated that mitochondria aggregate around the proximal part of each flagellum, forming a thickened region known as the middle piece, the region where the ATP for flagellar movements of spermatozoa is generated. However the present findings were within the limits of the spermatozoon length of domestic species (Samuelson, 2007). Further studies under electron microscope might shade more light on the effect of RJ on the mitochondria or other structures that are involved in the sperm motility. The current study shows that increased sperm length is unlikely to be driven by selection for increased swimming speed, and that the relative lengths of a sperm's constituent parts, rather than their absolute lengths are likely to be the target of selection. All else being equal, we suggest that a simple measurement of the ratio of head to tail length should be used to assess the possible link between morphology and speed. However, this is mostly likely to be the case for external fertilizers in which females have relatively limited opportunity to influence a sperm's motility. In this study, there was an enhancement in certain sperm function parameters, and that was attributed firstly to the direct activation technique with IVF medium. The medium provided the same culture components which found in the female genital tract and that will trigger the sperm hyperactivity motility (Mishima *et al.*, 2005). The technique sustains the epididymal sperms to get rid of the decapacitating factors in the seminal plasma and makes the sperm ready for successful fertilization in *vitro* (Tournaye *et al.*, 2003). Secondly, adding of RJ to the culture media enhances different sperm function parameters following 60 minutes of activation, mainly sperm concentration, total sperm motility percentage and grade activity of forward progressive movement.

The results of the present study found that, in *vitro* activation of caudal epididymal sperms by direct activation technique with 10% RJ-IVF medium resulted in a significant increase in the concentration of the recovered spermatozoa by swim-out after 60 minutes of incubation. The differences in sperm concentration between RJ-free IVF and 10% RJ-IVF medium may be explained by the booster effect of R.J one epididymal sperms to move out and to release from the epididymal tissue.

Moreover, culturing of the semen sample with 10% RJ-IVF medium result a significant increase in the percentages of sperm motility and grade activity of forward movement to reach in the last one to 50% (Grade A+Grade B) of the semen sample.

Results of this study also showed that, there was no significant difference in the morphologically normal sperms between the treated and control groups, and the mean was near 30% as an average. These results were compatible with the improvements in sperm concentration and grade activity of forward movement; because it was very difficult to make any enhancement on either sperm parameters when the semen sample considered morphologically abnormal (Coetzee *et al.*, 1998).

It was concluded from the present study that the addition of RJ to the culture media of sperm can enhance the sperm quality in mice. This result can be utilized for other mammalian IVF programs.

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دراسة تأثير الغذاء الملكي لنمل العسل *Apis mellifera* على شكلياء ومعايير وظيفة نطف ذكور الفأر السويسري *Swiss albin*

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الخلاصة

صممت هذه الدراسة للمقارنة بين نتائج متغيرات النطف قبل وبعد التنشيط بالغذاء الملكي للنحل. إستخدم في هذه التجربة عشرون من ذكور الفئران البالغة (عشرة لكل من مجموعتي التجربة والسيطرة) والتي جمعت من بيت الحيوان في المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب بجامعة النهرين. أجريت التجربة والتحليلات في مختبر سلامتك للتحليلات المرضية ببغداد - الرصافة ٢٠١٥. أخذت النطف من ذيل البربخ. تم تقييم تركيز النطف، والنسبة المئوية للنطف المتحركة، والنسبة المئوية لنشاط النطف، والنسبة المئوية لشكلياء النطف الطبيعي. تم تسجيل الأبعاد الشكليائية للنطفة في الحالتين باستخدام الميكروسكوب نوع داينو. تم تحليل النتائج إحصائيا. أظهرت نتائج الدراسة الحالية تحسن معنوي ($p<0.05$) في متغيرات النطفة في مجموعة المعاملة بعد التنشيط (خارج الجسم) بمادة الغذاء الملكي مقارنة بمجموعة السيطرة حيث حصلت زيادة معنوية ($P<0.05$) بعد تنشيط النطف لكل من الحركة التقدمية A للنطف والحركة التقدمية الكلية (B+A) للنطف. أظهرت الدراسة وجود أطوال مختلفة للنطف في مجموعتي السيطرة و التجربة وزيادة غير معنوية في أطوال النطف لمجموعة التجربة، تعزي الدراسة هذه الزيادة إلى المراحل التطورية المختلفة لنشأة النطف وليس بسبب إزدياد أعداد وأحجام المتقدرات الموجودة في المنطقة الوسطية للنطفة بعد التنشيط أو بسبب حركة ألنبيبات الدقيقة ألإنزلاقية الموجودة في محور النطفة. ترى الدراسة أن إضافة الغذاء الملكي الى الوسط الزرعي للنطف يحفز حركتها وقد يؤدي إلى زيادة نشاطها، وإمكانية إستخدامه لزيادة نشاط النطف في التلقيح الاصطناعي في الحيوانات الحقلية.