Bull. Iraq nat. Hist. Mus. (2017) 14 (3): 223-233 DOI: http://dx.doi.org/10.26842/binhm.7.2017.14.3.0223

EFFECT OF HONEY ON SPERM CHARACTERISTICS AND PREGNANCY RATE IN MICE

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Received Date: 30/11/2016 **Accepted Date:27/4/2017**

ABSTRACT

The aim of the current study is to demonstrate the effect of honey on the sperms characteristics (sperm concentration, sperm motility, grade of activity and sperm normal morphology) as well as pregnancy rate in mice. Sperms were obtained from caudal epididymis of male mice and prepared by adding10% of honey to the IVF medium using direct sperms activation technique for 30 minute incubation period before artificial insemination.

The study revealed a significant ($P < 0.05$) increase in active sperm motility (grade A and grade B) 49% and pregnancy rate 53.3% in female mice artificially inseminated with sperms. On the other hand, there were no significant differences in sperm concentrations and normal sperm morphology. In conclusion, the honey was beneficial in improving male fertility of mice by enhancing sperm motility and pregnancy rate of female mice.

Keywords: Honey, Mice, Pregnancy, Sperm characteristics, Sperm motility.

INTRODUCTION

Honey is a natural product of many floral nectar, its flavors and activity varies based on its origin and processing methods (Mahaneem *et al.*, 2010).Honey has many nutrient sources like those available in fruits which become alkaline in stomach (Bradley, 2010). Honey has been used as nutritive sweetener and a healing agent from ancient times, (Estevinho *et al*., 2008). It contains sugars, trace amount of minerals as well as vitamins (Syazana *et al*., 2011). Recently, many studies have reported the valuable effects of honey on surgical and medical treatments. Honey has been demonstrated to have many biological advantages such as antibacterial, antifungal, antiseptic and anti-inflammatory (Tan et al., 2009) , and it also contains many components thought to serve as antioxidants (Viuda-Martos *et al.*, 2005). It has also wound healing properties, burns and the treatment of diabetic ulcers (Cooper, 2001, Eddy and Gideon, 2005) Histological studies have been reported that application of honey to wounds reduces inflammation in superficial and deep wounds (Postmes *et al*., 1997) as well as in burns (Burlando, 1978).Honey is said to have increased thickness of vaginal epithelium and muscle, without effecting circulating hormones, such as testosterone or gonadotropins (Mahaneem *et al.*, 2008), and is useful to treat vaginal dryness and atrophy in post-menopausal women (Mahaneem *et al.*, 2007). Regarding male reproduction semen quality can be affected by genetic, behavioral, physiological (Skakkebaek *et al.*, 1994) and environmental factors (Sikka and Wang, 2008). Honey contains fructose and glucose which provide body with energy, thereby increasing testosterone and libido (Austin, 2012) and improving sexual

virility (Bradley, 2010). Honey was reported to enhance spermatogenesis in rats if given at the appropriate dose (Mahaneem *et al.,* 2007) and reduce the toxicity of cigarette smoke during spermatogenesis (Mahaneem *et al.,* 2008). Honey affects spermatogenesis by activating sorbitol dehydrogenase (SDH) and inhibiting lactate dehydrogenase (LDH) (Salam *et al.,* 2008). World Health Organization (WHO) sperm analysis criteria (World Health Organization, 1999) stated that concentration of 20_X10 sperms/ml or higher, percentage of normal sperm not lower than 30 and 50% of progressive motile sperm or more within 1hour of ejaculation are compatible with male fertility.

The objective of the current study was to determine the influence of honey on sperm function parameters and pregnancy rate by adding 0.1ml of honey suspension to 0.9ml of sperm's culture media.

MATERIALS AND METHODS

Sperm collection

Twenty mature male mice and 60 female mice (Balb/C St Can BR Strain) of 8-12 weeks old were included in the current study at Al-Iraqia University-College of Medicine from May 2015 to November 2015.

Sperms were pooled from male mice, sacrificed by cervical dislocation. Two caudal epididymis were dissected and minced in1 ml of IVF medium with 30-gauge needle syringe to permit the sperms to swim-out (Pl.1) (Erbach *et al.,* 1994). The spermatozoa suspensions were divided into two parts.

Sperms activation *In vitro*

The first part (control group):

The caudal epididymis sperms were allowed to swim-up through 30 minutes in incubator at 37 ºC in 5%CO_2 with 1 ml of IVF medium. Then the sperms were counted and used to inseminate the control group (Erbach *et al.,* 1994).

Honey-media preparation

Ten percent concentration of honey were prepared by adding 0.1ml of honey suspension to 0.9ml of IVF media, filtered using filters with pore size 0.45µm (Mahaneem *et al.*, 2010), and permit the second part of spermatozoa suspensions to swim-out. Sperms were examined under the high-resolution objective of light microscope to evaluate the final motile sperm concentrations and morphology (Pl. 2) and prepared for inseminated the treated group (Duselis and Vrana, 2007).The motility of each spermatozoon encountered was graded:-

•A- linear and rapid progressive motility.
•B - Rapid non- linear or non- rapid progressive motility.
•C-Localized motility.
•D- Immotile.

Artificial Insemination

The pregnancy rate was gained by dividing the number of pregnant mice on the total number of inseminated mice. There were 30 female mice inseminated by the sperms activated with10% honey-IVF medium (treated group). Artificial Insemination (AI) was performed during estrous phase. A blunt 22-gauge needle syringe (1.5 inch long) was needed to sperm administration. A 120º bend was placed about $3/4^{th}$ the way down the needle. An assistant is

used to hold the female in the suitable position, the needle up to the bend was inserted into the vagina and 0.025-0.05mls of sperms suspensions injected (Pl. 3) (Duselis and Vrana, 2007).

Statistical analysis:

Data of mice sperm fluid analysis for the treated and for the control groups were expressed as mean ±SE and were analyzed using paired sample t-test. While Chi-square test was used to compare the pregnancy rate values from treated and control groups. P-value< 0.05 was considered for significant means (Sorlie, 1995).

RESULTS

The results of sperm characteristics (sperm concentration, sperm motility, grade of activity and sperm normal morphology) following *in vitro* activation and incubation of caudal epididymis region for 30 min using IVF medium with and without 10% honey were observed in table (1).The sperm concentrations ($x10^6$sperm/ml) following direct activation with 10% honey-IVF medium showed no significant difference as compared with the honey-free IVF medium.

Active sperm motility (grade A and grade B) in treated group was 49% significantly higher as compared with the control group. The percentage of normal sperms morphology in treated group was 33%. However, the differences between two groups lacked significance.

The pregnancy rate in treated group was 53.3% (16 pregnant mice out of 30 inseminated mice), while it was 40% (12 pregnant mice out of 30 inseminated mice) for control group. There was significant (P≤0.05) increase in pregnancy rate between two groups (Tab. 1).

Table (1): Comparison between control and treated groups with 10% honey-IVF medium on certain sperm properties and pregnancy rate in mice (Means ± SE).

Grouping with and without honey	*In vitro* sperm activation					Pregnancy rate
	Sperm concentration (10^6 /ml)	Sperm motility grade A (%)	Sperm motility grade B (%)	Progressive motility (A+B)%	Normal morphology (%)	
control group	27.62±5.301	15.30±2.203	21.22±1.201	36.52±1.202	32.72±1.531	12/30 (40%)
treated group	28.22±5.351	20.30±0.021	29.20±2.105	49.50±3.032	33.12±4.103	16/30 (53.3%)
p-value	NS	S	S	S	NS	S

S: significant at 0.05 levels
NS: non-significant at 0.05 levels

DISCUSSION

The present study, showed that the adding of honey at 10% to the culture media improves sperm parameters following 30 minutes, mainly total sperm motility, percentage and grade activity of forward progressive movement. Culturing of the semen sample with 10% honey-IVF medium before insemination result in a significant increase in the percentages of sperm motility and grade activity of forward movement to reach in the last one to 49% (Grade A+ Grade B) of the semen sample. Honey is rich in sugars such as fructose and glucose, in addition to the minerals like potassium, magnesium, calcium, sodium chloride, sulfur, ferrous, zinc, phosphates and vitamins C, B1, B2, B3, B5 and B6. All these substances stimulate sperm motility and the grade activity of forward movement (Estevinho *et al.,* 2008 and Syazana*et al.,* 2011).

Minerals especially Ca+2 are known to inhibit the enzyme phosphate diestrase, and these will prevent cAMP degradation and consequently increase sperm motility (Nassar *et al.,* 1998).

Furthermore, honey contains carbohydrates and also has sugars like: glucose, fructose (Estevinho *et al.,* 2008), and these sugars are considered to be a source of energy for sperm motility. Abdul-Ghani *et al.* (2008) suggested that honey can affect the spermatogenesis by activating testicular marker enzymes like sorbitol dehydrogenase by 31% and inhibited lactate dehydrogenase by 48%, which has been indicated to increase its activity in infertility cases (Eliasson and Virji, 1985). During carbohydrate metabolism, sorbitol dehydrogenase converts sorbitol, the sugar alcohol form of glucose, into fructose (El-Kabbani *et al.,* 2004). Honey is rich in fructose, which is an important marker in the seminal fluid, and provides energy and nutrients for the sperm and maintain perfect alkaline medium for their viability and motility. Honey is full of enzymatic and non-enzymatic antioxidants, and contains pinostrobin, pinocembrin, ascorbic acid, vitamins E, diastase, glucose oxidase (Erejuwa *et al.,* 2012). It acts against lipid peroxidation and oxidative stress by ROS such as, hydrogen peroxide, super oxide, and prevents oxygen contact with unsaturated fatty acids in the sperm plasma membrane (Syazana *et al.,* 2011).

The data of the study also demonstrated that, there is no significant difference in the normal morphological sperms between the treated and control groups, and the mean was close to 33% as an average. These results are consistent with the improvements in sperm grade activity of forward movement; because it is very difficult to make any improvement on either sperm parameters when the semen sample is considered morphologically abnormal (Coetzee *et al.,* 1998).

The results of this study showed a significant increase in the pregnancy rate, and the differences in the means were 13% between the treated and control groups. Hence it is a good percentage of difference in the fields of experimental embryology and ARTs, and there were a lot of factors that might meddle with this observation; the study found that, there is a significant increase in the total active sperm after direct activation with 10% honey-IVF medium; all the female mice in this study were inseminated with the similar sperm concentrations. Therefore, the differences in pregnancy rate here are not due to the improvements in the sperm concentrations but it may belong to the effect of the direct activation technique with 10% honey and to the decrease in the insemination volume (0.025-0.05mls) injected to each female (insemination volume was reduced to overcome with that present). This reduction may decrease the decapacitation factors and contamination from the seminal plasma, such as cellular debris, mycoplasmas, chlamydia, trichomonas, bacteria, and

different blood cells, such as RBCs and WBCs (Jeyendran and Zhang, 2003).The other important factor is sperm motility. The study showed that the pregnancy rate increases with an increasing sperm cell motility. This finding agreed with Kasai *et al.* (2002), who demonstrated that one of crucial parameter that could provide treatment outcome was the percentage of motile sperm after proper preparation.

Other vital parameter is sperm morphology that may assume a part to acquire the high rate of FR. Sperm normal morphology had been proven as a good indication for *in vivo* fertilization (Menkveld *et al.*, 1990; van Zyl *et al.*, 1990) and assisted reproduction (Coetzee *et al.*, 1998). Many studies had reported a significant relationship between sperm ability to penetrate the zonapellucida and its morphology and motility (Liu and Baker, 1992); and also it found a powerful correlation between the acrosome reaction and normal sperm morphology and successful fertilization (Menkveld *et al.*, 2003), and increased fertility percentages consequently.

The direct activation technique didn't provide immotile, dead sperm and residual cytoplasmic droplet washing. Thus, even in the samples with good sperm morphology, there was high level of ROS, and that agreed with Keating *et al.* (1997) who correlated with the extensive production of ROS and presence of a residual cytoplasmic droplet that significantly affected sperm fertilizing ability. Moreover, the cytoplasmic residues cause a higher content of cytoplasmic enzymes, such as glucose- 6-phosphate dehydrogenase or creatine kinase (Gomez *et al.*, 1996), which promote the generation of free radicals in the sperm cells themselves (Aitken *et al.*, 1997).

The other factor that may meddle with the increments in pregnancy rate was the addition of 10% of honey to the insemination medium which provided a wide range of active ingredients that gave a nourishment and/or protection to the oocytes and early cleaved embryos. Honey has antioxidant properties (Viuda-Martos *et al.*, 2005). Antioxidant compounds may counteract the action of ROS on epididymal sperm parameters and therefore on fertilization rate, the balance between ROS generation and antioxidant capacity in the semen plays a crucial role on sperm functions parameter, fertilization and pregnancy procedures (Agarwal *et al.*, 2005).This observation is in Line with Pasqualotto *et al.* (2000), who confirmed that infertile patients did not only have excessive production of ROS, but also have a defect in the antioxidant system.

The present study showed that adding of 10 % honey to the culture media increased semen quality through improving the sperm parameters following 30 minutes of activation, and improving post insemination pregnancy rate. Thus, honey furthermore varies advantages, also has positive effects on the male reproductive system.

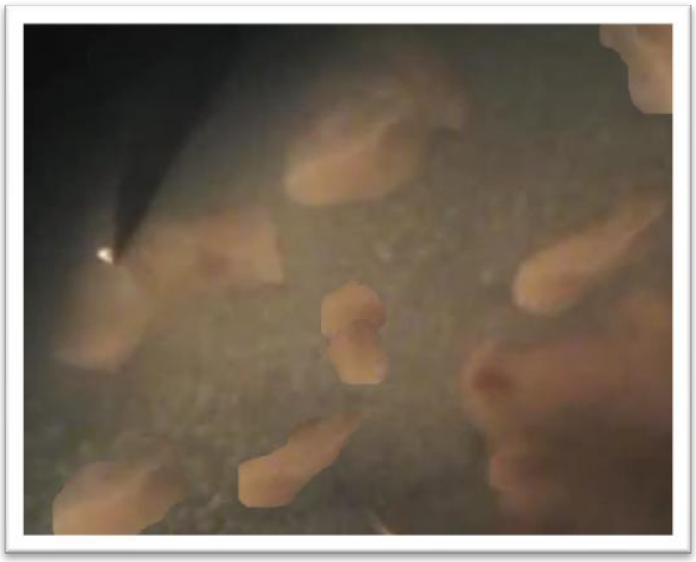

Plate (1): Mincing the caudal epididymis

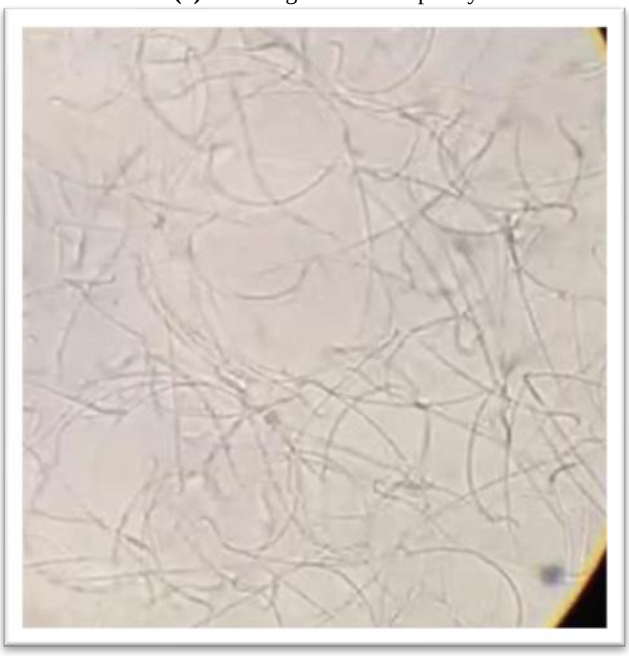

Plate (2): Sperms under a microscope after activation

Plate (3): Artificial Insemination

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Bull. Iraq nat. Hist. Mus.
(2017) 14 (3): 223-233

تأثير العسل في خصائص النطف ومعدل الحمل لدى الفئران

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تأريخ الاستلام: ٢٠١٦,١١,٣٠ تأريخ القبول: ٢٠١٧,٤,٢٧

الخلاصة

أجريت الدراسة بهدف بيان تأثير إضافة العسل بتركيز ١٠% الى الوسط الزرعي الخاص بتنشيط النطف على تركيز النطف ونشاطها والمظهر الخارجي لها وعلى معدل الحمل في اناث الفئران المحقونة بالنطف المنشطة بالطريقة المباشرة، وقد استخدمت الفئران كموديل تجريبي للبائن. تم الحصول على النطف من ذيل البربخ لذكور الفئران واستخدم العسل بتركيز ١٠% والممزوج مع الوسط الزرعيIVF عند تنشيط النطف في الزجاج لمدة نصف ساعة ثم حقن النطف بطريقة التلقيح الاصطناعي.

أظهرت النتائج ان اضافة العسل الى الوسط الزرعي قد أثر تأثيراً إيجابياً على حركة النطف التقدمية (49%) مقارنة مع مجموعة السيطرة. فضلاً عن وجود زيادة معنوية ($P < 0.05$) في معدل الحمل(53.3%) عند أناث الفئران الملقحة بالنطف المنشطة بإضافة العسل الى الوسط الزرعي. فيما اظهرت الدراسة عدم وجود زيادة معنوية في تركيز النطف والمظهر الخارجي لها.

أشارت الدراسة أن مكونات العسل المختلفة وخاصة الكاربوهيدرات والمعادن و الفيتامينات ومضادات الاكسدة. قد أثرت إيجابياً على نتائج مختلف معايير الدراسة لذا يمكن الاستنتاج بأن اضافة العسل للوسط الزرعي الخاص بالنطف قد عزز حركة النطف ونشاطها ومعدل الحمل في الفئران. يمكن الاستفادة من هذه النتائج عند الإعداد لبرامج الاخصاب في اللبائن.