Effect of Honey on Mice in Vitro Fertilization (IVF)

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ABSTRACT

Traditionally, honey is frequently consumed by the local population as a nutrient, as well as for the enhancement of fertility and vitality. This study concerning the effects of honey on sperms motility, abnormality, dead sperm, and in vitro fertilization, after 35 days of honey administration orally. 18 adult male mice were divided into one control and two treated groups containing 6 mice in each group. Treated groups received honey in two different doses i.e 1.2 and 1.8 g/kg body weight, the results showed significant ($P<0.05$) increase in oocyte maturation, fertilization by in vitro fertilization (IVF) and cleavage of oocyte by IVF between the three groups. Also there is significant increase in sperms motility. Decreased percentage of dead sperms between control and the treated groups and significant decrease in abnormalities of sperms in mice between the three groups.

Keywords: Honey, IVF, Sperm.

تأثير العسل على الاخصاب الخارجي في الفئران

الخلاصة

تقليدياً يعتبر العسل من المواد الغذائية الاستهلاكية للسكان المحليين وكذلك يعتبر من محسنات الخصوبة والفعالية. يتناول البحث دراسة تأثير العسل على حركة الحيوانات المنوية, والحيوانات المنوية المشوهه والميتة وعلى الاخصاب الخارجي بعد تجريع العسل عن طريق الفم لمدة 35 يوم. تمت الدراسة على 18 ذكر فئران بالغ وقسمت الى ثلاث مجاميع المجموعه الاولى هي مجموعة السيطرة ومجموعتين تم تجريعهم العسل وتحتوي كل مجموعة على ستة ذكور فئران. المجموعتين التي تم تجريعها بالعسل بتركيزين مختلفين الاول 1.2 والثانية 1.8 غم /كغم من وزن الجسم, واظهرت النتائج ان هناك فروقات معنوية لزياده نسية نضج البويضات والاخصاب عن طريق الاخصاب الخارجي وكذلك انقسام الاجنة التي لقحت بالاخصاب الخارجي بين المجاميع الثلاثة. وكذلك هناك زيادة معنوية في حركة الحيوانات المنوية. والتقليل من نسية الحيوانات المنوية الميتة بين مجموعة السيطرة والمجاميع المعاملة. وهناك نقصان مميز في نسبة تشوهات الحيوانات المنوية للفئران بين المجاميع الثلاثة.

INTRODUCTION

Honey is a candidate of being a reproductive health protection substance. It has been reported that honey contains moisture, sugars such as glucose and fructose [1], enzymes such as catalase and glutathione reductase [2], trace

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essential elements such as iron, copper, zinc and calcium, vitamins such as vitamin A, C and E [3] as well as some flavonoids and phenolic acids [4].

The decline in male reproductive health and fertility for the past 30 years has been linked to environmental toxicants and xenobiotics [5]. The medical history collected information on risk factors that may contribute to poor semen quality. This included testicular torsion surgery, vasectomy and vasectomy reversal, prostatectomy, varicocelectomy, orchiodopexy, cystic fibrosis, diabetes, infection of seminal vesicles, prostatitis, epididymitis, cancer, radiation therapy, chemotherapy, injured testicles with/without accompanying increase or decrease of testis size, and mumps before or after puberty [6]. One of the toxicants that have detrimental effects on male reproductive function is cigarette smoke which has been reported to be associated with abnormalities in male reproductive function such as decreased sperm count and motility [7], increased percentage of abnormal sperms [8] and sperm chromatin damage [9].

To date, whether honey has any effect on sperm and *in vitro* fertilization testicular functions is yet to be reported and there is a lack of data concerning the medicinal use of honey on reproductive performance and testicular dysfunction. The present study aimed to determine the effects of 35 days oral administration of mountain honey on sperm quality and *in vitro* fertilization of mice.

MATERIALS AND METHODS

Administration Doses

The doses prepared from original mountain honey with concentrations of 1.2 g/kg body weight and 1.8 g/kg body weight. These concentrations orally administrated daily for 35 days [10].

Treatment of animals

Eighteen adult male mice (30-36 gm) were purchased from Biotechnology Research Centre-Al-Nahrain University and maintained on a 14:10-hour light dark cycle in the animal house control and treated mice were provided with feed and water, there were no differences in feed intake.

Males were randomly divided into 3 groups, each composed of 6 mice. The first group was treated with 1.2 g/kg body weight; the second group was treated with 1.8 g/kg body weight orally administered daily for 35 days and the third group was given normal saline as a control group.

Female were treated with super ovulation regimen with injections of 5 IU of eCG and 5 IU of hCG given 48 h apart. Oviducts were removed 14–15 h after the injection of hCG and placed in phosphate buffered saline (For 1 liter of 1X PBS, prepare as follows: Start with 800 ml of distilled water, add 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na2HPO4 and 0.24 g of KH_2PO_4 adjust the pH to 7.4 with HCl, add distilled water to a total volume of 1 liter. Dispense the solution into aliquots and sterilize by autoclaving (20 min, 121°C, liquid cycle). Store at room temperature), in a Petri dish containing 1ml PBS, then the oviducts were isolated, Cumulus oocyte complex was released from the ampullary region of each oviduct into oil by rupturing the oviduct with the aid of a 25-gauge needle. The oviduct was discarded and the cumulus-oocyte complex moved into the fertilization drop. For IVF, the cumulus-oocyte complexes were released from the oviducts into 0.1% bovine testicular hyaluronidase (300 USP units/mg) in Hepes-buffer Earle's medium to disperse cumulus cells. The cumulus-free oocytes were washed with Hepes- buffer Earle's medium and used immediately for IVF [11].

Microscopic examination for sperm and oocytes

Sperms were assessed according to world health organization Laboratory manual [12] for motility, percentage of dead/live sperm and abnormalities. Sperm were collected from cauda epididymis into TCM-199 medium. The sperm suspension was incubated for 1 hour at 37°C under 5% CO_2. The sperm parameters were assessed for sperm count, viability and morphology with 5 replicates for each mouse. Sperm morphology and viability were stained with eosin stain and observed under light microscope.

To measure sperm concentration, motility dead/live sperm and abnormalities, aliquots of semen samples were placed into a counting chamber. A minimum of 200 sperm from at least four different fields were analyzed from each specimen. We defined % motile sperm as WHO grade `a' sperm (rapidly progressive with a velocity ≥25 mm/s at 37°C) plus `b' grade sperm (slow/sluggish progressive with a velocity ≥5 mm/s but <25 mm/s).

The oocytes are then examined under the inverted microscope to assess the maturation stage by observing the presence of a germinal vesicle, germinal vesicle breakdown, and the extruded first polar body. Metaphase II oocytes are identified by the presence of the extruded first polar body.

In vitro fertilization (IVF)

The method for sperm capacitation and IVF using TCM-199 medium [13]. Briefly, 200-µl drops of TCM-199 medium (fertilization drops) were overlaid with mineral oil in a plastic culture dish (diameter, 60 mm) and equilibrated overnight at 37ºC in a humidified atmosphere of 5% CO_2 in air. The volume of sperm suspension added to the fertilization drop was dependent on the concentration of spermatozoa after dispersion in the capacitation drop. Generally, 10 µl of sperm suspension from the capacitation drop were added to each fertilization drop to give the final concentrations of approximately 2×10^6 sperm/ml. The contents of four oviducts were released into each fertilization drop. After gamete incubation for 4 h, the oocytes were washed several times with Hepes- buffer Earle's medium followed by at least one wash with buffer Earle's medium. Only morphologically normal oocytes were selected for culture.

Embryo Culture

After IVF, the oocytes were placed in 50-µl drops of TCM-199 medium pre-equilibrated overnight with humidified 5% CO_2 in air. The culture drops were contained in plastic culture dishes and overlaid with mineral oil. The number of embryos (fertilized) was recorded after 24 h in culture [11].

Statistical analysis

Statistical analysis was performed to compare two different groups by using Chi-square and ANOVA-test. Statistical significance was determined at $P<0.05$ [14].

RESULTS AND DISCUSSION

In this study we examined the effect of honey supplementation on spermatic, sperm parameters and in vitro fertilization following 35 day of honey administration. The results, according to sperm motility and dead sperm, showed that there is a significant differences ($P<0.05$) between the control and the two other groups. But according to the sperm abnormality there is a significant differences ($P<0.05$) between the three groups Table (1), Figure (1) and this result disagree with Yamamoto *et al.* [15] who found that there were no significant

differences between control and honey treated groups for all the parameters such as spermatid count and sperm parameters a higher percentage of abnormal sperm and lower percentage of motile sperm. But agree with Syazana *et al.* [16], who found that sperm count of treated group was significantly higher than control group ($p<0.05$). Based on sperm morphology, treated group showed significantly higher percentage of normal sperm as compared to control group. Lower percentage of abnormal sperm were also observed in treated group as compared to control group. Also agree with a higher sperm count was observed in Mohamed *et al.* [17] the study following the oral administration of Malaysian honey. A significantly higher epididymal sperm count was also found in adult rats following the daily treatment of 5% Palestinian honey for 20 days [18].

Table (1) Effect of honey on sperm motility, dead sperm and sperm abnormalities after 35 days from treatment in mice.

Group	Sperm motility % (μ±SE)	Dead sperm % (μ±SE)	Sperm abnormalities % (μ±SE)
Control	A 67.66±10.72	A 21.64±3.84	A 23.61±4.84
Treated with Honey (1.2 g/kg daily)	B 77.47±11.29	B 16.26±3.66	B 17.22±4.02
Treated with Honey (1.8 g/kg daily)	B 81.03±10.94	B 14.38±4.71	C 12.17±3.26

Differences A, B, C are significant ($P<0.05$) to compared rows

A B

Figure (1) Showed normal sperms (A), and abnormal sperm With Coiled tail (X40).

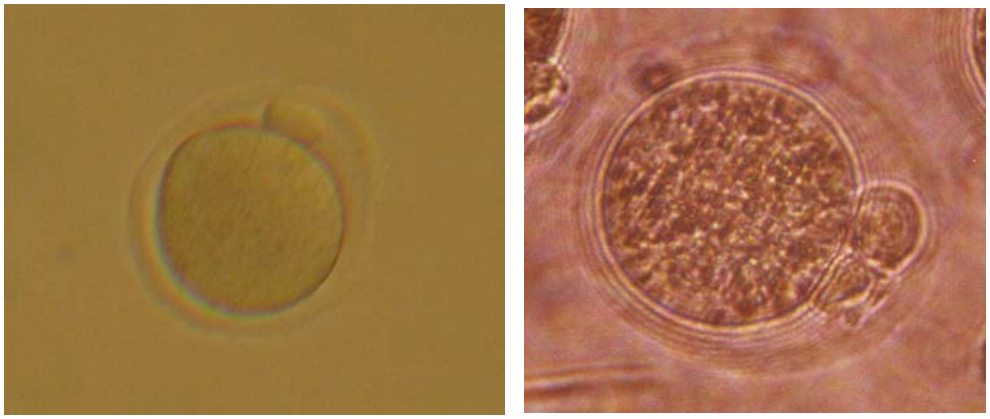
In case of IVF and embryo culture there is a significant increase in the percentage of matured oocytes, oocyte fertilized by IVF and the oocytes cleaved by IVF between the control and the other treated groups Table (2), Figure (2 &3).

Traditionally, honey is frequently consumed by the local population as a nutrient, as well as for the enhancement of fertility and vitality [19]. This study agrees with Abdel hafiz and Muhammad [20] who observed in vitro that diluted

Egyptian bee honey and royal jelly had an enhancing effect on sperm motility, particularly in subnormal samples. Also found significantly increase testosterone level, body weight, relative weight of testis, relative weight of epididymis semen characteristics and seminal plasma enzymes and decreased the levels of free radicals and lactate dehydrogenase [21].

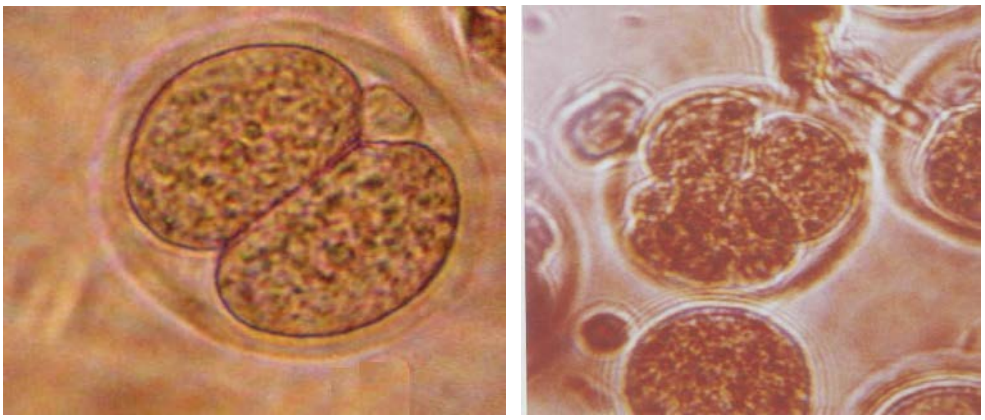
Table (2) Effect of honey on maturation, fertilization and cleavage by IVF After 35 days from treatment in mice.

Group	NO. of Incubated oocytes	NO. and % of maturation oocytes	NO. and % of oocyte fertilized by IVF	NO. and % of oocyte cleavage by IVF
Control	58	32, 55.17%	14, 43.75%	6, 42.85 %
Treated with Honey (1.2 g/kg daily)	55	34, 61.81%	19, 55.88%	12, 63.15 %
Treated with Honey (1.8 g/kg daily)	60	35, 58.33 %	23, 65.71 %	15, 65.21 %



A B

Figure (2) matured oocyte (A) and fertilized oocyte (B) (X 10).



A

B

Figure (3) cleaved oocyte by IVF. (A) 2 cells embryo,

(B) 4 cells embryo. (X 10).

Moreover, there is a strong correlation between plasma oestrogen level and testicular blood flow in male mammals suggesting that oestrogen may play a role in testicular perfusion. Indeed, the presence of oestrogen receptors and aromatase, an enzyme that transforms androgens into oestrogens, in germ cells of the testis might suggest that locally produced oestrogen may also involved in spermatogenesis. Consequently, it is also possible that honey could ameliorate the toxic effect on testicular function partly by improving testicular blood flow and spermatogenesis via the oestrogenic activity and this requires further study [22]. Till now the mechanisms of action of honey on testes and reproductive performance is not known and need further study in this field.

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