

Effect of Fertility Blend® Administration on the Epididymal Sperm Function Parameters of Vasectomized Mice: Physiological and Genetical Study as Model for Obstructive Azoospermic Men

تأثير اعطاء Fertility Blend® على المعايير الوظيفية للنطف البربخية للفئران مقطوعة الاسهر: دراسة فسلجية وراثية كموديل للرجال المصابين بالانسدادية

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Abstract

Male Fertility Blend® is a new nutrient supplements containing many constituents especially a plant called *Dong quai* (*Angelia Sinensis*). The plant extract has been used to facilitate the sperm function parameters. However, the studies concern on its effects on epididymal sperms of vasectomized males and obstructive azoospermia are very rare. Thus, this investigation was designed to elucidate the role of Male Fertility Blend® (MFB) formula on the *in vivo* epididymal sperm characters and DNA fragmentations of vasectomized male mice as a model for man. In this study, the orally administration of 3.4 µg/ml MFB was used for vasectomized and non-vasectomized mice along 35 days. The results revealed a significant ($p \leq 0.05$) increment in certain sperm function parameters of vasectomized mice by using fertility Blend® than that of not treated by this supplement. The percentage of progressive and unprogressive active sperm motility using MFB was significantly ($P \leq 0.05$) increased compared with non-treated group. It was concluded that the MFB formula containing different sources of energy and variety of factors that sustain the epididymal sperm of healthy and vasectomized mice. Therefore this supplement can be utilized for males complaining from obstructive azoospermia and other factors of infertility.

Keywords: Fertility Blend® for men, Caudal epididymal sperm, vasectomy, DNA fragmentation

المخلص

يعد مقوي الخصوبة للرجال MFB من مجهزةات الغذائية الجديدة والتي تحتوي على مواد مختلفة وخاصة مسحوق دونك ايكوي الذي يساعد على حركة النطف ونوعيتها وعددها. مع ذلك حسب المعلومات المتوفرة لم نجد بحوث مهتمة بتأثير هذا المجهز الغذائي على النطف البربخية في الذكور مقطوعي الاسهر او المصابين بالانسدادية. لذلك صممت الدراسة لمعرفة تأثير المجهز الغذائي على معايير النطف البربخية وهدم الدنا في الذكور مقطوعة الاسهر كموديل للانسان. في هذه الدراسة استخدم مقوي الخصوبة للذكور MFB بجرعة (3.4 µg/ml) وتم اعطاها فمويا لمدة 35 يوم لفئران مقطوعة الاسهر واخرى سليمة. بينت النتائج بوجود تحسن معنوي في صفات النطف للفئران مقطوعة الاسهر المعالجة بالمجهز الغذائي MFB بالمقارنة بالفئران مقطوعة الاسهر وغير المعالجة. وكانت نسبة الحركة التقدمية وغير التقدمية قد ازدادت بشكل معنوي في الفئران مقطوعة الاسهر والمعالجة مقارنة بغير المعالجة. وطبقا لنتائج هذه الدراسة، فان البحث اظهر بان المجهز الغذائي MBF يحتوي على عوامل متعددة ومصادر للطاقة تساعد في تغذية النطف البربخية في الفئران السليمة ومقطوعة الاسهر. يمكن الاستفادة من نتائج الدراسة للرجال المصابين بالانسدادية وعوامل عقم الرجال الاخرى.

الكلمات الدالة: مقوي الخصوبة للرجال، نطف بربخية، قطع الاسهر، تجزؤ الدنا

Introduction

Male Fertility Blend® is a new formula of mixed constituents especially a plant called *Dong quai*. This extract has been well known to facilitate the sperm movement, and concentration [1]. Fertility Blend™ for men is a scientifically validated herbal that enhances fertility health by improving sperm characters. It contains L-carnitine (LC) is which is an amino acid that considered as critical to the formation of healthy and improve semen quality [2]. However there is rare studies concern the role of this new nutrient supplements on certain epididymal sperms function parameters and its DNA fragmentation of men

complaining from obstructive azoospermia. Thus the goal of recent study is to found out the effect of Fertility Blend[®] treatment on the epididymal sperms and DNA fragmentation of vasectomized mice as a model for men complaining from abstractive azoospermia.

Materials and Methods

The current research was done following the acceptance of Scientific Board and the Scientific Research Ethic Board of the Biotechnology Research Center in February 2016.

1. Experimental animals: Twenty eight fertilized male Balb/C- mice of 8-12 weeks age old and 25-35 gm weight were allocated from the Animal House at Biotechnology Research Center /AL-Nahrain University and involved the study through the period from April to August, 2016. The mice were managed in air conditioned room with 21-26 °C and the photoperiod of 12_±2 hours. The tap water and mice pellets were freely offered for the mice groups.

2. Vasectomy

Fourteen mature males' mice were anesthetized by using 0.1ml (10mg/ml) of pentobarbital sodium .Then the scrotum was opened from the intermediate line. The vas deferens was sutured from the two sides using microsurgical set. The area between the sutures was cut by scissors. Then the open area was closed directly from the side of scrotum by simple continuous suture. Antibiotic powder was added locally, leaving the animal individually in the cage for at least 14 days as recommended by [3].

3. Preparation of male Fertility Blend[®] (MFB) solution

The Fertility Blend[®] stock solution was prepared by measuring 3.4 mg of MFB using electrical balance and dissolved in liter of distilled water .Each animal was orally administrated 3.4µg/ml /day for 35 days for 7 healthy fertilized mice and 7 vasectomized mice. The other 14 male mice were allocated for control group

(seven animals for each healthy and vasectomized) were orally administrated MFB –free DW only for the same period.

3-Isolation of Epididymal sperm

- All mice groups were sacrificed by cervical dislocation.The epididymal region was isolated and then the caudal part was obtained and placed on the Falcon dish filled with warmed Hams F-12 (one ml) for washing. The isolated caudal epididymal region was cut off many (200) times by microsurgical scissors to have the spermatozoa. Then the sample was left in the incubator (Mettler Company, Germany) for 10 minutes to allow the spermatozoa to swim up and then isolated. The isolated spermatozoa were kept in the incubator for at least 30 minutes. Certain sperm characters namely: Sperm concentration (million/ml), the percentage of active Sperm motility and the percentage of morphologically normal sperm were accounted as recommended by [3, 4].The sperm motility was measured as recommended by the manual of WHO 2010.

4. Test of DNA fragmentation

The acridine orange fluorescence test was performed for all groups to determine the DNA fragmentation according to the method of [6].The stock solution of Acridine orange was added to 40 ml of (0.1 M citric acid) and 2.5 ml of (0.3 M) Na₂HPO₄.7H₂O. Then pH adjusted to (2.5) before staining. Then Carnoy's solution was prepared which is a fixative solution consists of three parts of Methanol, (BDH, England); and one part of Glacial Acetic acid (GCC, U.K.). Carnoy's solution provides a better predictive value for sperm DNA damage by using AO dye [5,6].

Statistical Analysis

The data were statistically analyzed using SPSS.21. Version for the vasectomized mice treated by Male Fertility Blend[®] and non-vasectomized groups. The results of certain sperm parameters were expressed as mean ± standard error and analyzed by using analysis of variance (ANOVA) when the F value researched the significant level, least significant test was performed. DNA fragmentation was accounted by using Chi square test [7].

Results and Disscution

The data of certain sperm characters were shown in Table (1). A highly significant ($p \leq 0.01$) improvement was observed in sperm concentration (million/ml) of healthy mice treated with MFB (45.48 ± 1.32) compared to healthy non treated mice (35.7 ± 0.22), vasectomized not treated and vasectomized males treated with MFB (12.04 ± 0.42 and 22.44 ± 0.52 , respectively). Moreover, the mean of sperm

concentration was significantly ($p \leq 0.01$) increased in vasectomized mice treated with MFB compared to vasectomized mice not treated with MFB.

The percentage of sperm motility of vasectomized mice treated (62.23 ± 1.11) and not treated (44.50 ± 2.52) with MBF was significantly ($p \leq 0.05$) lower than healthy mice treated (90.74 ± 1.78) and not treated (88.03 ± 0.80) with MFB. However the vasectomized group that treated with MBF showed a significant improvement in sperm motility compared to vasectomized male without any treatment.

There was a significant ($P \leq 0.05$) increase in the mean of morphologically normal sperm (MNS) in healthy mice treated with MFB ($83.33\% \pm 0.46$) and healthy non treated mice ($85.77\% \pm 2.02$) compared to vasectomized mice treated ($78.66\% \pm 1.20$) and not treated with MFB ($55\% \pm 2.23$). Moreover, there was a significant ($p \leq 0.05$) increase in MNS of vasectomized treated with MFB compared to vasectomized not treated as shown in Table (1).

-DNA fragmentation results

Figure (1) showed that the vasectomized not treated (29.77 ± 0.44) and vasectomized males treated (20.36 ± 0.65) with MFB have significantly ($P \leq 0.05$) higher DNA fragmentation in the epididymal sperms than that of healthy males (10.55 ± 0.86) and healthy males treated with MFB (12.60 ± 0.73).

At the same time the vasectomized males treated with MFB have significantly ($P \leq 0.05$) lower DNA fragmentation than that of the vasectomized not treated mice.

Table (1): Certain sperm function parameters following *in vivo* administration of male Fertility Blend® (MFB) for 35 days in adult healthy and vasectomized male mice.

Groups	Certain sperm function parameters		
	Sperm conc. (sperm X 10 ⁶ /ml)	Sperm motility (%)	Morphologically normal sperm (%)
healthy group without MBF	35.7 ± 0.22b	88.03 ± 0.80a	83.33 ± 0.46a
healthy with MBF	45.48 ± 1.32a	90.74 ± 1.78a	85.37 ± 2.02a
vasectomy without MBF	12.04 ± 0.42c	44.50 ± 2.52c	55.00 ± 2.23c
vasectomy with MBF	22.44 ± 0.52d	62.23 ± 1.11b	78.66 ± 1.20b

-Values are presented as Means ± SE (n=7 mice/group).

-The different small letters denote significant differences between groups ($P \leq 0.05$).

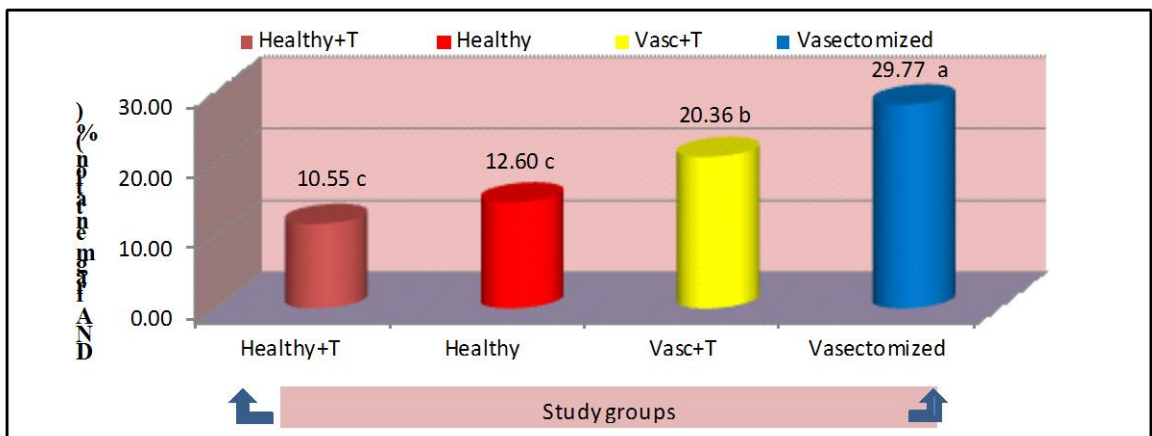


Fig. (1): DNA fragmentation of epididymal sperms in all groups on this study.

Different superscripts mean significantly different at ($P \leq 0.05$).

Healthy+T=Healthy mice treated with MFB. Vasc+T=vasectomized mice treated with MFB.

Dicussion

The result of this research noticed that, gavages of fertility Blend® to different male mice groups had been improved for certain sperm parameters. The differences in sperm concentration and active sperm movement between vasectomized mice that treated with fertility Blend® and vasectomized mice not treated may be due to the contents of fertility Blend® of different components such as, L- carnitine. The LC is known to act as antioxidant, and can protect sperm plasma membrane from the impact of high level of production of unsaturated fatty acid content [8].The production of free radicals e.g. superoxide and hydroxide, can suppressed mitochondrial energy availability and decrease the percentage of active sperm motility. It has been reported that LC improves sperm movement by interfere with the pathway of free fatty acid oxidation [9]. Therefore low levels of carnitine may reduce fatty acid concentrations within the mitochondria, resulted in decrement of energy production and impaired sperm motility [10].

Collectively, the new nutrient supplement MFB containing other components namely; green tea, selenium vitamins C and E, and Dong Quai extract. In addition to B vitamins (i.e.B6, B12 and folate) that found to play a role in fertility capacity by increase the percentage of sperm motility and normal morphology [11]. The decrease in sperm concentration and active sperm motility may result from a deficiency of vitamin B12 in the nutrition ,therefore, the orally administration of MFB for 35 days may enhanced certain sperm function parameters by vitamin B12 found in its constituent [12]

Furthermore, zinc (Zn) levels are one of this supplement components .The zinc known to have important role in fertilizability. It has been noticed that decreased Zn concentration in subfertile men will negatively effects on the number of sperm in the semen sample ,therefore the optimum dose of zinc may be overwhelm the fertility problem in oligozoospermic men [13]. Also ,the MFB is containing a gradient from vitamin E that is helpful for the sperm function as antioxidant and vitamin E has been shown to prevent the chain production from the free radical which damage the cell membranes [14]. *In vitro* study reported that vitamin E administration can improve sperm fusogenic capability to fertilize the ovum *in vitro* [15]. The nutrient supplementation contained vitamin C. It has been recorded that Vitamin C can positively effects the sperm function parameters of smoker infertile couples [16].

The other possibility of positive effects of MBF administration in this study leading to increase active sperm progressive movement is adding gradients of Selenium and green tea. These components are acting as antioxidant and the sperm flagella integrity is thought to be stabilized by both effects. It has been found that daily administration of nutrients that containing selenium may enhance the progressive forward movement of asthenozoospermic men [17].

In this current work, the epididymal sperm of vasectomized mice may highly sensitive from oxidative stress that induced from either environmental toxicants and/or aging of sperm in the caudal part [18]. But administration of MFB that containing the Dong quai extract may sustain the epididymal sperm to overcome this stress by its containing ferulic acid which known as powerful antioxidant leading to prove epididymal sperm function parameters too[19]. Thus, It is concluded from the present research that daily addition of MFB in the diet of vasectomized mice is so important to enhance epididymal sperms characters.

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