Effect of Fertility Blend® Administration on the Oocytes Quality and Embryonic Development using assisted Reproductive Technology in Mice

تأثير اعطاء خليط الخصوبة على نوعية البويضات والتطور الجنيني باستخدام تقنية مساعدة على الانجاب في الفئران

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Abstract

Female Fertility Blend ® (FFB) is one of new nutritional supplement that used to enhance the fertility status in women. This supplement containing vitamins, minerals, enzymes, amino acids, all may improve the oocytes quality and ovarian function; at the same time protect oocytes from free radicals damage. The aim of the study is to examine the *in vivo* effect of FFB on oocyte quality, and *in vitro* fertilization rate (IVFR), and embryonic development (ED) at early cleavage stages using the mice as a model for human being. Therefore, two groups of mature female mice were involved (20 mouse each). The treated group is daily orally administrated by 3.4mg/kg /body weight from FFB for 10 days and the other groups (the control) were treated with FFB- free distilled water only for the same period. Oocytes were collected and an epididymal sperms from mature fertilized male mice are obtained and *in vitro* fertilization (IVF) is done. Following 24 and 48hrs from IVF, the FR and ED rate are recorded. This results showed a significant (P<0.05) differences in fertilization rate and embryonic development when treating the female mice with FFB compared to control group. It is concluded that the FFB treatment has a great improvement in oocytes maturation and *in vitro* fertilization and embryonic development status.

Key Words: female fertility blend, IVF, fertilization rate, embryonic development.

الملخص

يعد خليط خصوبة الاناث Female Fertility Blend ® (FFB) من المجهزات الغذائية الجديدة التي تستخدم في دعم الحاله الاخصابية للنساء. اذ يحتوي على الفيتامينات والمعادن والانزيمات والاحماض الامينية بمجموعها ربما تحسن من نوع البويضات ووظيفة المبايض وفي نفس الوقت تحمي البويضات من التاثيرات الهدامه للجذور الحرة. لذا هدف البحث هو فحص تاثير FFB على نوعية البويضات والاخصاب الخارجي والتطور الجنيني في مرحلة الانقسام المبكر باستخدام الفئران كموديل للانسان. وعليه هيئت اناث الفئران الناضجه وقسمت الى مجموعتين (20 فأر في كل مجموعه). اجري التجريع اليومي لمجموعة المعالجة 3.4 ملغم/كغم/وزن الجسم من FFB ولمدة 10 ايام في حين تم تجريع مجموعة السيطرة ماء مقطر فقط. جمعت البويضات من الاناث المعاملة واناث السيطرة وحصل على النطف البربخة من ذكور ناضجة خصبة واجري الاخصاب في الزجاج. بعد 24 و48 ساعة سجل معدل الاخصاب ومعدل تطور الاجنة. بينت نتائج الدراسة وجود فروقات معنويه (P<0.05) في معدل الاخصاب والتطور الجنيني في المجموعه التي عولجت بـ FFB بالمقارنه مع مجموعة السيطرة. نستنتج من الدراسة الحالية بان المعالجة FFB قد حسنت بشكل كبير من معدل نضوج البويضات والاخصاب وحالة التطور الجنيني.

الكلمات الدالة: خليط خصوبة الاناث، الاخصاب الخارجي،معدل الاخصاب، التطور الجيني

Introduction

Infertility is an important condition in reproductive medicine [1] and is defined as a failure of a couple to achieve of pregnancy after 12 months of regular, unprotected intercourse [2]. Infertility is either primary, when no pregnancy has ever occurred, or secondary, where there has been a previous pregnancy, regardless of the outcome [3].

On the other hand, the medicinal plants were used in most developing countries, as a normative basis for the maintenance of fertility activity. Thus herbs have been used for the treatment of infertility since at least 200AD. Herbal products have the potential to add to existing treatment options. Using nutritional supplements as a first step in treatment could improve key physiological factors essential to fertility [4].

Fertility Blend® for women is a new nutrient supplement described to enhance female hormonal balance and increase the chance of pregnancy. This supplement contains different compounds one of them a plants called Chaste berry (monk's pepper). Also contains L-carnitine and different antioxidant compounds [5].Thus the aim of present work is to study the *in vivo* effect of Fertility Blend® on women ovulation status, oocytes maturation and *in vitro* FR and embryonic development in mice using *in vitro* fertilization procedure.

Materials and Methods

1. Housing and Management of Experimental Animals

Forty mature Albinos – Swiss mice of 8-12 weeks age old and 25-35 gm. weight were obtained from the Animal House of Biotechnology Research Center /Al-Nahrain University through the period from April to July 2016. They were kept in an air conditioned room (25°C) with a photoperiod of 13±2 hours. The animals were housed in box cage of opaque plastic measuring (29×15×12) cm covered its ground with wooden shave. In each cage, four mice were housed and the tap water and diet are freely available for them.

2. Preparation of Female Fertility Blend® (FFB) Solution

The Female Fertility Blend stock solution was prepared by measuring 3.4 mg of FFB using electrical balance and dissolved in one liter of distilled water. Each animal was orally administrated 3.4µg/ml /day for 10 days. The female mice in control group were orally administrated FFB –free DW only.

3. Detection of Female Estrus Cycle and Superovulation

Stages of estrus cycle of female mice were detected and reported using vaginal smears. The smear performed daily between 8:00 am. and 1:00 pm. The female mice were super ovulated by intra-peritoneal injection 7.5 IU of pregnant mare serum gonadotropin (Folligon®, Merck animal Health, Canada) following 48hours 7.5IU of hCG (Pregnyl®**,** Merck&co , USA) was injected intra-peritonally too [6].

4. Oocytes Collection

Under sterile condition which includes surgical instruments sterilized by using autoclave and sterile operation site under the laminar air flow hood, the oocytes collection procedure was done as described by Al-Dujaily and Albrazanchi (1997) [7].Then the collected oocytes were cultured in Ham's –F12 medium in the 5%CO_2 incubator.

5. Identification of Mature Oocytes

The super ovulated oocytes were obtained by flushing the Fallopian tube. The determination of oocytes maturation status was performed as described by Al-Dujaily and Hamza (2014) [8].

6- *In vitro* Fertilization

The male mice were anesthetized by ether and then sacrificed by cervical dislocation and dissect the epididymis. The sperms were obtained by flushing method with 1 ml culture medium (Ham's F-12). After 2-3 hours of oocytes incubation, an aliquot of capacitating sperms are gently added to each well of 4-well dish. Each well contains 4 oocytes flooded with 0.7 ml Ham's F-12 medium. All wells covered with 0.2 ml paraffin oil.

Insemination of mature oocytes was done by adding 1-2×10^5/ml of the incubated sperm to the IVF well. Fertilization dishes were incubated at 37°C, 5% CO_2 and 100% humidity overnight [8]. Fertilization rate and embryonic development was reported following 24 and 48 hours.

Statistical Analysis

A statistical analysis was performed using SPSS (a statistical package of social science, version 21.0 LED technologies, USA). Chi square test was used to compare values of the treatment and the control group at oocytes maturation, FR and embryonic development. When P-value exceed <0.05 the result was considered significant [9].

Results and Discussion

Number of mature oocytes and fertilization rate following IVF of female mice treated with Fertility Blend®

Table (1) shown that the number of oocytes collected from the two mice groups is almost the same and there was no significant (P>0.05) differences between them (control group=490, treated group with FFB = 495). The number of mature oocytes treated *in vivo* by orally administration of FFB-free solution was 317/490 and in mice oocytes treated with FFB solution was 315/495.The statistical analysis revealed no significant (P>0.05) differences between the two groups. The same observation was found regarding the number of immature oocytes. Whereas the fertilization rate was significantly (P<0.05) higher in treated group (241/315, 76.50%) compared to control group (170/317, 53.62 %) as shown in Table (1).

Table (1): Number of mature oocytes and the fertilization rate following IVF of female mice treated with Fertility Blend®

Parameters	Female Mice groups	
	Control group (Free FFB)	Treated group with FFB
Collected oocyte	490	495
No. mature oocyte	317/490	315/495
No. immature oocyte	173/490	180/495
Fertilization Rate	170/317 53.62 %	241/315 76.50% *
*P-value <0.05		

Pearson's Chi-square test

Comparison of early embryonic development rate after 24 hours of *IVF* procedure between oocytes obtained from mice treated by orally administration with FFB and mice treated with FFB-free DW.

In Table (2), there was a significant (P<0.039) increases in the embryonic development rate at 2-cell stage following IVF procedure in treated group (65.14%) compared to control group (57.64%). Whereas the embryonic development rate of 3-4 cell stage in treated group (34.86%) was significantly (P<0.039) lower than that of free-FFB control group (42.36%). No significant (P>0.05) differences was observed in the rate of ED of 3-4 cell stage between treated and control groups after 24hours of IVF procedure as shown in Table (2).

Table (2): Comparison of embryonic development after 24 hours of IVF procedure between oocytes obtained from female mice treated by FFB and mice not treated by FFB

Grouping with and without FFB medium		Embryonic Development		P value
		NO	%	
Total number of 2-cell stage of embryos	Control group (Free FFB)	98/170	57.64	
	FFB Treated group	157/241	65.14	<0.039
Total number of 3-4 cell stage of embryo	Control group (Free FFB)	72/170	42.36	<0.039
	FFB treated group	84/241	34.86	
Pearson's Chi-square test				

Comparison of early embryonic development rate after 48 hours of *IVF* procedure between female mice orally administrated FFB and control group with FFB-free DW.

After 48 hours post insemination by IVF procedure, the number of fertilized oocytes that developed to 2-cell stage from oocytes obtained from free FFB (control group) was 51 embryos out of 170 fertilized oocytes, 88 embryos at 3-4 cells stage and 31 embryos at 5-8 cells stage. While the oocytes that obtained from mice treated with FFB, the number of 2-cell embryos was 157 out of 241, 159 embryos at three to four cells and 67 embryos at five to eight cells stage. The statistical analysis showed a significant differences between the two groups in the total number of 2- cells stage (P<0.01), 3-4 cells stage (P<0.013) and 5-8 cells stage (P<0.039) of embryos after 48 hour of IVF as shown in Table (3)

Table 3: Comparison of early embryonic development rate after 48 hours of *IVF* procedure between the FFB treated group and control group

Grouping with and without FFB medium		Embryonic Development		P -value
		NO	%	
Total number of 2-cell stage of embryos	Control group (Free FFB)	51/170	30.00	
	Treated group with FFB	15/241	6.22	<0.001
Total number of 3-4 cell stage of embryo Total	Control group (Free FFB)	88/170	51.76	<0.013
	Treated group FFB	159/241	65.97	
Total number of 5-8 cells stage of embryos	Control group (Free FFB)	31/170	18.23	
	Treated group FFB	67/241	27.80	<0.039

Pearson's Chi-square test

The current study found that the orally treatment by FFB have tend to increase significantly the positive effect on maturation status of the oocytes *in vivo*. It has been recorded that the quality of oocyte and its maturation increases the probability of fertilization rate and embryonic development [10].The components of Female Fertility Blend ® which contains folic acid, vitamin E, green tea, Chaste berry, L-Arginine, Zinc, Vitamins B6,12, iron, and Selenium were positively interfere with the oocytes maturation and fertilization processes. Therefore increase the periods of orally administration of FFB to 15-29 days may result in a significant increases in oocyte maturation compared to non-treated mice.

The enhancement of non-significant increases in oocyte maturation may due to folic acid action which was one of the FFB supplement. Folate (water-soluble vitamin B) was necessary for energy production and healthy cell division, and it has a significant effect on oocyte quality and its maturation, FR, ED, implantation, placentation, fetal growth and organ development [11]. L-arginine another component was added to the Female Fertility Blend ®, was a basic natural amino acid, L-arginine improved the integrity of cumulus cells (CC) and may play a role in the nuclear oocyte maturation process in addition to helps maintain a healthy uterine lining [12].

Also the supplements consists of zinc which was essential for many biological processes, including proper functioning of gametes, thus it has a significant action in oocyte biology [13] which in turn positively affect the FR and ED. On the other hand, zinc has a role in establishing polarity and proper asymmetric division, zinc also has an essential function in determining oocyte versus polar body cell fate [14]. Moreover, it has been reported that the zinc has a key regulator and completion of the meiosis [15].

It has been reported that oocyte growth and maturation appears to be affected by nutritional imbalance and conditional of the microenvironment, such as oxidative stress [16]. Oxygen concentration was higher *in vitro* cultures than *in vivo* conditions, and free radicals were produced during aerobic metabolism of cells [17].

In this study, the oxidative stress was overcome by using supplements of FFB media with antioxidants in order to enhance oocyte quality. One of the antioxidants that adding to the FFB was Selenium, which was an essential trace element that have antioxidant activity in biological systems and has an effect on maturation process of oocytes[18].

Furthermore, vitamin E was a vital antioxidant for reproduction and fertility, and has important role to improve *in vivo* maturation rate of oocytes, fusogenic process and embryonic cleavage [19]. It has been found that the Se and Vitamin E has an important role to control the oxidative stress by their antioxidants properties [20]. Thus in this study the significant improvement of both the fertilization and ED in the treatment groups is may be because of the role of Se and Vitamin E in oocytes development to M II stage [20]. Another antioxidant was Green tea, which has a function during maturation process by protection of oocytes against oxidative stress which can be affecting the cell membrane and DNA integrity [21]. Consequently, the antioxidants-green tea, vitamin E and selenium, support reproductive health. It was concluded from the present study that FFB have the components that can increases the number of oocytes quality and maturation *in vivo* leading to normal fertilization and embryonic development when treating the females for optimum period.

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