

EFFECT OF FEMALE FERTILITY BLEND® MEDIUM ON OOCYTE QUALITY AND MATURATION STATUS

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ABSTRACT

Background: Female Fertility Blend® is a nutritional supplement for improving fertility in women containing vitamins, minerals, enzymes, amino acids, all may sustain the oocyte quality and ovarian function, at the same time protect oocyte from free radicals damage. **Objective:** The aim of the study is to examine the *in vitro* effect of Female Fertility Blend® (FFB) addition on oocyte maturation and to confirm the effect of Female Fertility Blend® on oocyte quality. **Materials and Methods:** In this study, 0.1ml from FFB concentrations (0.1, 0.15 mg/ml culture medium) was added to 7 ml of Ham_F12 medium for *in*

vitro maturation, where the mature oocytes divided into three groups. The first group the gametes were cultured in Ham_F12 medium alone (FFB-free medium) and considered as control, second group the ova were cultured in 0.1 mg/ml FFB- Ham_F12 culture medium, and the third group treated with 0.15 mg/ml FFB- Ham_F12 culture medium. **Results:** This study showed high significant ($P < 0.001$) differences in maturation rate in both concentration of FFB that added to the medium (0.1, 0.15 mg/ml culture medium). **Conclusions:** It was concluded that the culturing and incubation of oocytes in a medium containing Female Fertility Blend® has a great improvement in oocyte maturation.

KEYWORDS: Female Fertility Blend® medium, oocyte maturation, Hams-F12.

INTRODUCTION

Infertility is an important condition in reproductive medicine.^[1] and is defined as the inability 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]

Female infertility may contribute to 25% of causes of infertility^[4] which may be due to anovulation or structural problems like tubal obstructions or scars of previous operations. Another important problem may cause is endometriosis which is usually more common in women in their mid-twenties and older, especially when postponed childbirth has taken place.^[5]

The quality of oocytes has a direct impact on the fertilization and developmental competence of embryo.^[6] However, oocyte quality is a key limiting factor in female fertility, reflecting the intrinsic developmental potential of an oocyte and has a crucial role not only on fertilization, but also on subsequent development of embryo.^[7]

Using nutritional supplements as a first step in treatment could improve key physiological factors essential to fertility. Different nutrient supplements were produced world wide; one of these supplements is the Fertility Blend[®]. This unique formula is the first fertility product to synergistically combine natural herbal and nutritional ingredients specifically for women's reproductive health. Fertility Blend[®] contains Chaste berry (Vitex) - an herb shown to enhance hormonal balance and ovulation frequency, L-arginine - an amino acid, green tea, vitamin E, selenium, Folic acid, vitamins B6, B12, minerals, iron, zinc and magnesium. However in our knowledge there is no research concern the effect of this new product on oocyte maturation *in vitro*. Therefore the current study was designed to found out the effect of adding Fertility Blend[®] powder in the medium on *in vitro* maturation of mice oocytes.

MATERIALS AND METHODS

1. Housing and management of experimental animals

Forty female and thirty mature Albino – Swiss mice of 8-12 weeks age old and 25-35 gm. weight were obtained from the Animal House at High Institute of Infertility Diagnosis and Assisted Reproductive Technologies /AL-Nahrain University through the period from Oct. 2015 to April 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. Four mice were housed in one cage and the tap water and diet were freely available for the animals.

2. Preparation of Culture Media

2.1. Preparation 0.1% of Female Fertility Blend (FFB) medium for Oocyte quality *In Vitro*

The Female Fertility Blend stock solution was prepared by adding 10 mg of FFB to 10 ml Ham_F12 medium (0.1%), the medium was filtered using Millipore (0.20 μ M). Then 0.1ml from FFB was added to 7 ml of Ham_F12 medium as stock solution, then 0.7 ml was added in each 4_well.

2. 2. Preparation 0.15% of Female Fertility Blend (FFB) medium for Oocyte quality *In Vitro*

The Female Fertility Blend stock solution was prepared by adding 15 mg of F.F.B. to 10 ml Ham_F12 medium (0.15%). The medium was filtered using Millipore (0.20 μ M). Then 0.1ml from FFB was added to 7 ml of Ham_F12 as stock solution, then 0.7 ml was added in each 4_well.

3. Detection of female estrus cycle

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. Estrous cycle of female mice has four phases were detected by using vaginal smears procedure. The smears were performed daily.^[8]

3.5. Oocyte 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 Hamza.^[9] Then the collected oocytes were cultured in Hams –F12 medium in the 5%CO₂ incubator.

3.5.3. Identification of Immature Oocytes

The superovulated oocytes were obtained by flushing the Fallopian tube. In order to determine whether the oocytes were mature or not, a special observation techniques was employed as follows:

3.5.4. Sliding

The cumulus – oocytes complex (COC) was allowed to slide slowly from one side to the other on the bottoms of the Petri dish, while being observed under the microscope. During COC sliding, it was observed clearly whether or not oocytes cytoplasm contains a germinal

vesicle (GV) or if the oocytes have extruded a first polar body (1PB) into the perivitelline space (PVS). If neither GV was seen in the oocytes cytoplasm nor 1PB found in PVS, the oocytes was defined as germinal vesicle breakdown (GVBD) or metaphase-I stages (M-I).^[10]

The maturation of the oocytes was assessed by the level of expansion of the corona-cumulus complex. A tightly packed cumulus and corona radiate layer was classified as "very immature"; this normally corresponds to an oocytes that is at prophase I stages. When the corona radiate was compacted but still distinct from the cumulus, it is usually associated with a metaphase-I (M-I) oocytes with absence of the first polar body; this oocytes was classified as "immature" or "intermediate". A sunburst-like corona radiate and a well-expanded cumulus is generally associated with a metaphase II (MII) oocytes and was classified as "mature" oocytes.^[11]

3.8. 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 oocyte maturation, When P-value reach <0.05 the result was considered significant.^[12]

RESULTS

-Number of mature oocytes by using medium containing female Fertility Blend[®]

Table (1) shown that the number of oocytes collected from the three mice groups was almost the same and there was no significant ($P>0.05$) differences between them (control group=490, treated group using 0.1% FFB medium=509 and treated group using 0.15% FFB medium=495). The number of mature oocytes cultured *in vitro* using FFB-free medium was 317/490 and in mice oocytes cultured in 0.1% FFB medium was 330/509. Whereas the number of mature oocytes of the mice group which cultured in 0.15% FFB medium was 315/495. The statistical analysis revealed no significant ($P>0.05$) differences between the three groups. The same observation was found regarding the number of immature oocytes. as shown in table (1).

Table-1: Number of mature oocytes by using medium containing female Fertility Blend[®]

Parameters	Female Mice groups		
	Control group (Free FFB)	With 0.1 %FFB	With 0.15%FFB
Collected oocyte	490	507	495
No .mature oocyte	317	330 [*]	315 ^{NS}
No. immature oocyte	173	177 ^{NS}	180 ^{**}

NO. Samples =65

- Number of *In vitro* maturation of immature oocytes cultured with and without FFB for 24 hours

Table-2 shown the number of mature oocytes that incubate for 24 hours in three mice groups. There was a highly significant ($P < 0.001$) differences between them (control group $85/173 = 49.13\%$, treated group using 10% FFB medium was $104/177 = 58.75\%$ and treated group using 15% FFB medium was $117/180 = 65\%$).

Table-2: *In vitro* maturation of immature oocytes cultured with and without FFB for 24 hours

Parameters	Female Mice groups			P value
	Control group (FreeFFB)	Treated group with 10% FFB	Treated group with 15% FFB	
No. immature oocyte	173	177	180	$P > 0.05$
No.mature oocyte after 24 hrs	$85/173$ 49.13%	$104/177$ 58.75%	$117/180$ 65%	$P < 0.001$

Person Chi-square test.

$P > 0.05$ non-significant

DISCUSSION

The current study found that the use of FFB has a positive effect on maturation status of the oocytes *in vitro*, by adding two concentrations of FFB (0.1% and 0.15%) to Hams F-12 medium. It has been recorded that the quality of oocyte and its maturation increases the probability of fertilization rate and embryonic development.^[13] 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 process.

The enhancement of oocyte maturation may due to folic acid action which is one of the FFB supplement. Folate (water-soluble vitamin B) is necessary for energy production and healthy cell division^[14] and it has a significant effect on oocyte quality and its maturation, implantation, placenta -ion, fetal growth and organ development.^[15]

L-arginine another component added to the Female Fertility Blend[®], is a basic natural amino acid, l-arginine improved the integrity of CC and may play a role in the nuclear oocyte maturation process.^[16]

Also the supplements consists of zinc which is essential for many biological processes, including proper functioning of gametes, thus it has a significant action in oocyte biology and its maturation.^[17] 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.^[17] Moreover, Many researchers reported that the zinc has a key regulator and completion of the meiosis 1.^[18,19, 20]

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 is Selenium, which is an essential trace element that have antioxidant activity in biological systems and has an effect on maturation process of oocytes.^[21]

Oocyte growth and maturation appears to be affected by nutritional imbalance and conditional of the microenvironment, such as oxidative stress.^[22] Oxygen concentration is higher *in vitro* cultures than *in vivo* conditions and free radicals are produced during aerobic metabolism of cells.^[23] The oxidative stress modification of cell components due to the reactive of oxygen species (ROS) is one of the most potentially damaging processes for cell function and may lead to cell death by necrosis and apoptosis.^[24]

Furthermore, vitamin E is a vital antioxidant for reproduction and fertility and has important role to improve *in vitro* maturation rate of oocyte. This result is in agreement with Farzollahi *et al.*^[25] study. Tareq *et al*^[26] found that the Se and Vitamin E has an important role to control the oxidative stress by their antioxidants properties. Thus in this study the significant improvement of the maturation rate in the treatment groups is may be because of the role of Se and vitamin E where have a role in oocyte development to M II stage.^[26] Another antioxidant is Green tea, which has a function during IVM by protection of oocytes against

oxidative stress which can be affecting the cell membrane and DNA integrity.^[27] It is concluded from the present study that FFB medium have the components that can increase the number of oocytes quality and maturation *in vitro*.

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