Embryonic development after 48 hours in vitro fertilization with mice epididymal sperms activated in vitro by male Fertility Blend ® medium

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ABSTRACT

Background: The new nutritional supplement Male Fertility Blend® (MFB) has a diversity of ingredients, counting Dong qui excerpt, which may improve sperm motility, superiority, and quantity. The new nutritional supplement Male Fertility Blend® (MFB) has a diversity of elements, including Dong qui excerpt, which may improve sperm motility, quality, and quantity.

Materials and Methods: In this study, the in vitro direct sperm activation technique was carried out using MFB (0.1percent mg/ml and0.15 percent mg/mlculture medium). Three groups of mature female mice were created; the first group received insemination using epididymal sperm that needed been started by adding0.1 percent MFB to the culture medium; the second group received insemination using epididymal sperm that had been started by adding0.15 percent MFB to the medium; and the third group received insemination expending epididymal sperm that needed been activated byMFB-Free medium (control group). The degree of fertilization and developing growth were together taken into explanation.

Results: According to this education, employing a average containing0.15 percent MFB (65.80 percent) compared to a medium containing0.1 percent MFB (54.04 percent) and a medium containing free-MFB increased the rate of 3-4 cell stage after 48 hours of insemination significantly (P 0.05). (44.44 percentage).

Conclusion: The inspection exposed that theMFB medium comprises several basics and energy bases that allow the development and typical advance of primary cleavage, rendering to the answers of the current study.

Keywords: Primary development, Sperm, Conception, grading.

Introduction: The first in vitro fertilization child, Louise Brown, was born in 1978. Since then, people with a variety of reproductive issues have been able to have children thanks to this surgery, which has seen increased success rates. The true of the first in vitro-conceived child marked the beginning of a completely new era in the field of infertility therapy. The first in vitro fertilization child, Louise Brown, was born in 1978. Since then, people with a variety of reproductive issues have been able to have children thanks to this surgery, which has seen

increased success rates. The birth of the first in vitro-conceived child marked the beginning of a completely new age in the field of infertility therapy[1]. 15% of all couples struggle with sterility. In39% of these pair, the male crops abnormally assessed sperm. Single of the major causes of male infertility is spermatogenic failure[2]. Male infertility can be treated with a variety of assisted reproductive technologies (ART), including intracytoplasmic sperm injection (ICSI), intrauterine insemination (IUI), and prescription drugs (ICSI). For assisted reproductive techniques, sperm can be extracted from the man's ejaculate contingent on the cause of the issue [3]. In addition to the aforementioned methods, pharmacological intervention is necessary, especially in cases of oligospermia and idiopathic infertility, to enhance the quality of semen [4]. Therefore, a variety of medications, including antibiotics,antiphlogistics,kallikrein,corticosteroids,hormone preparations, and others, are used to treat various diseases of male sterility in modern medicine, with varying degrees of success. Male infertility is a common condition that is treated using herbal medicines from naturopathy (Ayurveda, Siddha, Unani, and Chinese traditional medicine), despite the lack of scientific research to determine the efficacy of these treatments [3]. Infertility can result from a variety of circumstances. Issues with the testicles cause30 to40 percent of cases (of which about15 to25 percentage are unpaid to hereditary causes). While10 to20 percent are the result of a route obstruction. However, 1 to 2 percent of instances are brought on by issues with the hypothalamus or pituitary glands. Even after an evaluation, the last 40 to 50 percent of instances lack an identifiable reason [5]. The new nutritional supplements Male Fertility Blend® comprise a change of ingredients, especially Dong qui excerpt, which might improve spermmotility andcount [6]. A nutritional and herbal supplement for men called Fertility BlendTM improves sperm quality and motility to increase fertility health (spontaneous wave). It has been demonstrated that the amino acid L-carnitine is essential for the development of strong, active sperm.[7]. Dong quai contains the antioxidant ferulic acid, which has also been demonstrated to enhance the quality of sperm. Selenium, coenzyme CoQ10, and the anti-oxidants vitamin C and E enhance reproductive health in general. The male reproductive system requires zincandB vitamins, particularlyB6, B12,and folate, for healthy hormone absorption, sperm production,and motility [7].

The FoodandDrug Management hasn't however reviewed these assertions. This article is not meant to be used in the diagnosis, treatment, or prevention of any disease. No preservatives, colors, or flavors that are artificial. However, to the best of our knowledge, neither the investigations on this nutrient supplement nor its effects on the in vitro activation of epididymal sperm or on embryonic development addressed these issues.

Materialsand Methods

1-Preparation ofCulture Media

1-1-Preparation Concentration0.1%of Male Fertility Blend®medium for micesperm activation*inVitro.*

In order to create the Male Fertility Blend® (MFB) stock solution, 10 mg of MFB were added to 10 ml of HamF12 (0.1 percent). Millipore(0.20 M) filters were used to strainer the medium. The pH of every prepared medium has been set at7.4 to 7.8 at room temperature. In a plastic test tube, the MFB addition was made by fraternization(0.3)ml of MFB solution with (0.7)ml of Ham F12medium in a 3:7ratio.

1-2-Preparation Concentration 0.15% of Male Fertility Blend® medium for mice sperm activation *in Vitro.*

In order to create the Male Fertility Blend® (MFB) stock solution, 10ml of Ham F12 was mixed with 15mg of MFB (0.15percent). Millipore(0.20 M) filterswere used to filterthe medium.The pH of every prepared medium has been set at 7.4 to 7.8 at room temperature. In a plastic test tube, the MFBaddition was made by mixing(0.3)ml of MFB solution with (0.7)ml of Ham F12 medium in a 3:7ratio.

2-Sperm collection and activation

2-1-I*n vitro s*perm directactivation technique:

1-Malemice are sacrificed 13 hours after female mice receive HCG.

2- 0.5 ml of Ham'sF-12 was placed on the Falcon dish with the epididymal caudal area segregated for washing.
3-Two media—1 ml of Hams F-12 medium alone, 1 ml of Ham-F12 with 10% MFB, and 1 ml of Ham-F12with 15%MFB were created.
4- After cutting the cauda, the sperms were removed, and the cauda epididymis was discarded.
5-Each prepared media was incubated with the collected sperm for at least 30 minutes. Since there is no seminal fluid present, the sperm are merely cellular, and the culture medium serves to capacitate them.

Sperm remained incredibly active, highly motile,and spinning in a straight line. counted [8].

2-2-Sperm parameters assessment:

2-2-1-Sperm concentration: A cover slip (22x22 mm) was placed over a drop of 10 µl of spermatozoa suspension with the culture media and placed on a microscopic slide.

The unkind number of spermatozoa in four high control microscopic arenas at a magnification of 100 was used to calculate the concentration of spermatozoa(106/ mL) (400x).This amount has been increased by a million [9].

Sperm concentration= No.spermatozoa x the multiplication factor (million)

2-2-2-Sperm motility

The statistics of gradually motile and immotilesperms were counted after 100 sperms on a simple slide were examined. The formula below was used to compute the sperm motility percentage:

$$\text{Sperm Motility \%} = \frac{\text{No. Motile sperm}}{\text{Total No.Sperms}} \times 100$$

Sperm motility remained evaluated by grouping the sperm intofour categories: linear -and rapid progressive (A)25 m/sec.
nonlinear linear or nonlinear linear quick (B) -5–24 m/sec.
-Non-progressive (C) at a speed of 5 m/s.
- Immotile(D) [10].

The amount of forward-moving spermatozoa(grades A+B), which should make up around 50%of the total sperm count , or the part of rapidly moving spermatozoa(grade A), which should make up about25% of the entire sperm count,were used to calculate the sperm motility.

2-2-3-Sperm morphology

Using the similar prepared slides for sperm motility,spermatozoa remained analyzed for usual and abnormal morphology. At least 100spermatozoa were tested,and the formula below was applied

$$\text{Morphologically normal sperms\%} = \frac{\text{No.Normal sperms}}{\text{Total No.Sperms}} \times 100$$

3-Oocytes collection:

The technique for collecting oocytes was carried out in sterile conditions, with surgical tools autoclave sterilized and a sterile operation site protected by a laminar air flow hood. 13–14 hours after receiving an HCG injection, a cervical dislocation scarified a female mouse. Following a longitudinal abdominal surgical incision,the ovary,fallopian tube,and brief part of the uterus were detached. To get rid of any leftover blood and tissue, wash the genitalia with regular saline. After that, use an insulin syringe and a needle with a gauge of 30 to extract the adipose tissue. To separate the fallopian tube from the ovary, the genitalia were located in a Petri dish with Ham'sF-12medium.The following techniques were used to extract the oocytes: Fallopian tube flushing, fallopian tube slicing, identifying small oocytes,and sliding.

4-*In vitro fertilization*:

1-An aliquot ofcapacitated sperms was ready to be additional to each well of a 4- well dish after the oocytes had been incubated for 1-2 hours.

2- There are 4 oocytes in each well, and each well has 0.7ml of Ham'sF-12medium. There is 0.2 ml of paraffin oil in each well.

3- Mature oocytes were inseminated using 1-2105/ml of sperm that had been cultured with HamsF-12alone, HamsF-12containing0.1 percent MFB,and other HamsF-12 containing 0.15percent MFB .
4- Overnight, fertilization dishes were incubated at 37°C, 5% CO_2, and 95% humidity.

5- To get rid of extra sperm and debris, the oocytes were washed through several drops of Ham's F-12 medium "in wash dish" after 18 hours of incubation.

6- The fertilized oocytes were then incubated once again for an overnight period after being examined under a dissecting microscope to determine the gradation of fertilization and oocyte mitosis and maturing [11].

5-Early embryonic development rate:

By counting the amount of zygotes, two-cell phase, and three- to four-cell phase embryos24 hours after impregnation, the early embryonic cleavage rate was calculated. Transferring embryos at the two-celland three- tofour-cell stages to dishes for embryo culture in order to culture them at subsequent stages in Hams-F12media and scheming the embryonic progress rate using the formulation:

No.embryos of each stage
Embryonic Development Rate= --------------------------------------- x 100
Total No.of embryos

Statistical Analysis:

SPSS.21, the numerical analysis software,was used for all numerical analysis. The results of the mice sperm analysis for the treatment (Male Fertility Blend medium) and the control (Male Fertility Blend-free Ham's F-12 medium) groups were reported as means standard errors and evaluated using paired sample t-tests. After 48 hours of insemination, early embryo score and the pace of embryonic development were compared between the treatment and control groups using the Chi-square test. In this search, a P-value of 0.05was regarded as important [12].

Results

1. Embryonic development after 48hours:

Out of 225 fertilized oocytes, 92 reached the 2-cell stage when grown in a medium containing free MFB (control group) after 48 hours following artificial insemination. Another 100 reached the 3-cell stage, and 33 reached the 5-8 cell stage. The number of embryos was 80 at the 2-cell stage out of 272 after the same length of insemination and incubation in medium containing 0.1 mg MFB (treated group), 147 at the 3-4 cells stage, and 45 at the 5-8 cells stage. 22 out of 231 embryos in a medium containing 0.15 mg MFB had two cells, 152 had three to four cells, and 57 had five to eight cells. In the 2-cell stage, the statistical analysis revealed a difference between amedium containing freeMFB (control group)and amedium containing0.1mg MFB (treated group) (P 0.012). And in the 2-cell stage, there were highly significant differences (P 0.01) between amedium containing freeMFB (control group)and a media containing0.15mg MFB (treated group). There were significant differences between a medium containing free MFB (control group) and a media containing 0.1 mg MFB (treatment group) in the 3–4 cell stage (P = 0.036). A medium containing freeMFB (control groupand a medium containing0.15mg MFB (treatment group) both showed a highly significant (p0.01) improvement. In terms of the embryonic development stage 5-8 cell, there were extremely significant differences (P0.01) between the media containing free MFB (control group)and the medium containing0.1mg MFB (treatment collection), as well as substantial differences (P0.008) between the two (treated group), as showen in (Table 1).

Table 1:- Evaluation of embryonic development rate after 48hours of*in vitro* fertilization procedure between treated media and control medium.

Embryonic stage	Grouping with and withoutMFB medium	Embryonic Development		P-value
		NO	%	
Total number of 2-cell stage of embryos	Control group (Free MFB)N=225	92	40.88	-
	Treated group	80	29.41	0.012

	With0.1mg MFB N=272			
	Treated group With0.15mgMFB N=231	**22**	**9.52**	**P<0.01**
Total number of 3-4 cell stage of embryo	**Control group (Free MFB) N= 225**	**100**	**44.44**	**-**
	Treated group With 0.1 mg MFB N= 272	**147**	**54.04**	**0.036**
	Treated group With 0.15 mgMFB N= 231	**152**	**65.80**	**P<0.01**
Total number of 5-8 cells stage of embryos	**Control group (Free MFB) N= 225**	**33**	**14.66**	**-**
	Treated group With0.1 mg MFB N= 272	**45**	**16.54**	**0.008**
	Treated group with 0.15 mg MFB N= 231	**57**	**24.67**	**P< 0.01**

2. Embryos grading total: The embryonic viability was evaluated using the embryological criterion as a starting point.

2.1. Two-cell stage embryos after 48hours of IVF: There were 53 embryos at the 2-cell stage after 48 hours of mature oocytes being inseminated by sperms that were educated and incubated in MFB- free medium. These embryos stayed divided into four categories based on their morphological criteria: grade A (16.98%), grade B (22.64%), grade C (26.41%), and grade D (33.96%).

There were 40 embryos at the 2-cell stage during the same insemination period that were activated in vitro by a medium containing 0.1 mg MFB and scored as grade A (27.5 percent), grade B (37.5 percent), grade C (22.5 percent), and grade D (12.5 percent) embryos.

The total number of embryos that developed in the medium containing 0.15 mg MFB was 18, and they were divided into grade A (33.33 percent), grade B (38.88 percent), grade C (16.66 percent), and grade D (11.11 percent) embryos at the 2-cell stage.

The statistical analysis revealed a rise in grade A and grade B levels of normal embryonic development that was statistically significant (P=0.007). Additionally, there remained a substantial (P= 0.007) decline in the amount of grade C and grade B embryos in the MFB-free medium related to the medium containing0.1percent MFB, as shown in table (2).

While there was a highly significant (P0.01) drop in the number of grade C and grade D embryos compared to the MFB-free medium, there was a highly significant (P0.01) increase in the number of grade A and grade b embryos that were developing normally, as shown in Table (2).

2.2. Three–Four cells stage embryos after 48hours: There were 90 embryos at the 3-4 cell stage that were graded as grade A (17.77 percent), grade B (24.44 percent), grade C (34.44 percent), and grade D (23.33 percent) according to their morphological criterion after 48 hours of successfully completing IVF for

mature oocytes by epididymal sperms that were learned and incubated in the control standard.
There stayed 124embryos at the 3- to 4-cell stage during the same insemination period that were graded as grade A (33.06 percent), grade B (30.64 percent), grade C (20.96 percent), and grade D (15.32 percent) embryos in the standard containing0.1mg MFB.
The full number of embryos that settled in the medium containing 0.15 mg MFB was 162, and they were divided into embryos of grades A (40.74 percent), B (30.24 percent), C (18.51 percent), and D (10.49 percent).
The statistical analysis revealed a rise in grade A and grade B levels of normal embryonic development that was extremely significant (P 0.01). Additionally, there was a highly significant (P0.01) reduction in the number of grade C and grade D embryos in the MFB-free medium compared to the medium containing 0.1 mg and 0.15 mg of MFB. As showed in (Table 2).

2.3 Five– Eight cell stage embryos after 48 hours:

There were 29 embryos at the 5-8 cell stage that were graded according to their morphological criteria as gradeA (31.03 percent), gradeB (34.48 percent), gradeC (10.34 percent), and as gradeD (24.13 percent) embryos after 48 hours of mature oocytes being inseminated by sperms that were learned and hatched in the control standard.

There existed 57embryos at the 5-8 cell stage that were scored as grade A (33.33 percent), grade B (35.08 percent), grade C (21.05 percent), and grade D (10.52 percent) embryos throughout the same age of IVF in a medium having 0.1 mg MFB.

The total number of embryos grown in the medium containing0.15 mg MFB was 70 at the 5-8 stage, and they were divided into grade A(38.57 percent), grade B(41.42 percent), grade C(12.85 percent), and grade D(7.14 percent) embryos.
The results revealed a highly significant (P0.01) difference between the medium containing0.1 mg MFB and the MFB- free medium in the number of embryos that developed normally in grades A and B and a highly significant (P0.01) drop in the number of embryos that did so in grades C and D. (Table 2).
Compared to the MFB-free medium, there was a large (P=0.003) drop in the amount of grade C and grade D embryos while there was a significant (P=0.003) rise in the number of grade A and grade B embryos that were developing normall, as exposed in Table(2).

Table 2: Evaluation of embryo categorizing score among treated medium and control standard after 48hours of reproduction by *in vitro* fertilization procedure.

Embryos Stages	Medium used	No. Developed Embryos	Embryonic grading score				P-value
			A	B	C	D	
2Cell Stage	Control group (Free MFB)	53	9 16.98 %	12 22.64 %	14 26.41%	18 33.96 %	-
	Treated group With 0.1mg MFB	40	11 27.5%	15 37.5%	9 22.5%	5 12.5 %	0.007 S
	Treated group	18	6 33.33%	7 38.88%	3 16.66%	2 11.11 %	P<0.01 HS

	With 0.15mg MFB						
3-4Cell Stage	**Control group (FreeMFB)**	**90**	**16 17.77%**	**22 24.44%**	**31 34.44%**	**21 23.33 %**	**-**
	Treated group With 0.1mg MFB	**124**	**41 33.06%**	**38 30.64%**	**26 20.96%**	**19 15.32 %**	**P<0.01 HS**
	Treated group With 0.15mg MFB	**162**	**66 40.74%**	**49 30.24%**	**30 18.51%**	**17 10.49**	**P<0.01 HS**
5-8 Cell Stage	**Control group (Free MFB)**	**29**	**9 31.03%**	**10 34.48%**	**3 10.34%**	**7 24.13 %**	**-**
	Treated group With 0.1mg MFB	**57**	**19 33.33%**	**20 35.08%**	**12 21.05%**	**6 10.52 %**	**P<0.01 HS**
	Treated group With 0.15mg MFB	**70**	**27 38.57%**	**29 41.42%**	**9 12.85%**	**5 7.14 %**	**0.003 S**

Discussion

Embryonic development:

After reproduction and incubation with two meditations of MFB medium for 24 and 48 hours, the existing study demonstrated a significant advance in EDand embryo quality. The number of embryos in grades Aand B at the two-cell, three-cell, and five- to eight-cell stages increased.

The research has demonstrated that the treated group's embryos produced higher-quality offspring than the control group. As the cutting stages advanced to added advanced phases, the quality and amount of embryos also significantly declined.

Therefore, short culture settings initially jeopardized theoutcome of the experimental and medical IVF,resulting in impeded embryo development and a subsequent loss of viability [13,14]. However, any changes to the in vitro culture environment can have a significant impact on how well the resultant embryos perform when measured [13]. The following active components may enhance the helpful things of MFB in this learning: Dong Quai, a conventional Chinese fertility herb, is said to increase the likelihood of embryo implantation [15].

Oxidative stress can impact oocytes and embryos during oocyte culture and embryonic development whether it is produced endogenously or exogenously. As aresult, metabolic byproducts of sperm accumulation in

the epididymal space in IVFculture media may stand the endogenous andexogenous sites of oxidative stress formation, destructive oocytes and preimplantation embryos[16]. High ROS levels in day one culture media have been reported to be linked to delayed embryonic development, high levels of fragmentation, and the production of morphologically aberrant embryos during prolonged culture [17].

Therefore, the MFB medium's antioxidant system, which includes a variety of vitamins and minerals like vitamin C, vitamin E, selenium, and zinc, may be essential for the conversion of ROS to H2O [18,19]. Because vitamin C and vitamin E are antioxidants that break chains, they prevent the peroxidative process from spreading, which results in more normal embryos than in control media.

The embryos' capacity for development was enhanced by vitamin E, which prevented oxidative damage [20]. The likelihood of IVF operations succeeding has been reported to rise with higher vitamin E intake [15]. It has been demonstrated that vitamin E, a well-known antioxidant, can prevent sensitive cell membrane damage brought on by free radicals [21].

The increase in the rate of ED, however, may be connected to the Zn, selenium,and L-carnitine detected in the0.1 percent and0.15 percent MFB.Primary stages of embryonic growth were impacted by zinc,but not later ones[22]. Mammal teratogenicity of zinc absence has been confirmed [23]. Antioxidant selenium can lower the chance of birth irregularities. L-carnitine also enlarged the cleavage and blastocyst rates, although it had no discernible impact on the total amount of blastocyst cells[24]. L-carnitine keeps antioxidant things that help uphold the mitochondrial membrane then shield DNA fromROS-caused damage. [25].

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