WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 11, 280-286. Research Article ISSN 2277– 7105

MICE EPIDIDYMAL SPERMS ACTIVATION *IN VITRO* BY MALE FERTILITY BLEND® MEDIUM: MODEL FOR MAMMALS

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Article Received on
10 Sep. 2016,

Revised on 30 Sep. 2016,
Accepted on 20 Oct. 2016

DOI: 10.20959/wjpr201611-7299

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ABSTRACT

Background: Male Fertility Blend® (MFB) new nutrient supplements have different components especially Dong qui extract which may facilitate the sperm motility, quality and sperm count. However, in our knowledge there is no research concern on the effect of this new nutrient supplements on *in vitro* activation of epididymal sperms. **Objective:** This study was designed to study the effect of male fertility formula on the *in vitro* activated mice epididymal sperm parameters. **Materials and Methods:** In this study, MFB (0.1 mg/ml and 0.15 mg/ml culture medium) was used for *in vitro* direct sperm activation technique. **Results**: This study showed a significant ($p<0.01$) higher improvement in sperm characters by using 0.15% MFB than that of 0.1% MFB medium. The percentage of active sperm motility grade A using medium containing 0.15%MFB was highly significantly ($P<0.01$) increase compared to medium containing 0.1% MFB and medium free MFB. **Conclusion:** According to the results of present study, the investigation showed that the MFB medium contain many factors and energy sources with epididymal sperm of mice. This result can be utilized for IVF program in mammals.

KEYWORDS: Male Fertility Blend®, epididymal sperm, *in vitro* activation.

INTRODUCTION

Infertility affects 15% of all couples. In 39% of these couples, the male generates semen analyzed as abnormal. Spermatogenic failure is one of the important causes of male infertility.[1, 2] Treatments for male infertility range from medications, surgical intervention or intrauterine insemination (IUI) to various forms of assisted reproductive technologies (ART),

such as *in vitro* fertilization (IVF) or intra cytoplasmic sperm injection (ICSI). Depending on the source of the problem, sperm can be taken from the man's ejaculate for use in assisted fertilization procedures.[3] Apart from the above procedures pharmacological intervention is needed, particularly in the cases of Oligozoospermia and idiopathic infertility to improve the quality of semen.[4] Therefore various drugs are used to treat different conditions of male infertility in modern medicine i.e. Antibiotics, Antiphlogistics, kallikrein, corticosteroids, hormone preparations and others, with uncertain results. are popular to treat male infertility despite the lack of scientific experimentation to assess its effectiveness.[3]

Male Fertility Blend® new nutrient supplements have different components especially Dong qui extract which may facilitate the sperm motility, and sperm count.[6] In addition to L-carnitine , Ferulic acid, vitamin C and E, coenzyme CoQ10 , selenium , Zinc and B Vitamins.[7]

However, in our knowledge the studies on such nutrient supplement did not concern its effect on *in vitro* activation of epididymal sperms. Therefore the present investigation was proposed to identify the effect of MFB on the *in vitro* activation of mice epididymal sperm parameters.

MATERIALS AND METHODS

1- Experimental animals: Thirty male Albino – Swiss mice of 8-12 weeks age and 25-35 gm weight were obtained from the Animal House at High Institute of Infertility Diagnosis and Assisted Reproductive Technologies /AL-Nahrain University and involved the study through the period from Nov 2015 to May 2016. The mice were kept in prepared air conditioned room (23-25℃) with a photoperiod of 13±2 hours. The male mice were accommodated in box cage of opaque plastic measuring (29×15×12) cm. The box floor covered with wooden shave and in each cage only four mice were kept. The tap water and diets were freely available for the animals.

2-Preparation of Culture Media

2-1-Preparation Concentration 0.1% of Male Fertility Blend® medium for mice sperm activation *in Vitro:* The Male Fertility Blend® stock solution was prepared by adding (10mg) of MFB to (10ml) Ham_F12 (0.1%). The medium was filtered using Millipore (0.20µM). All prepared medium have been fixed at pH 7.4-7.8 at room temperature. The addition of MFB medium was prepared by adding (0.3) ml of MFB solution to (0.7) ml of Ham_F12 medium in plastic test tube and the ratio was 3:7.

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

The Male Fertility Blend® stock solution was prepared by adding (15mg) of MFB to (10ml) Ham_F12 (0.15%). The medium was filtered using Millipore (0.20µM). The prepared medium was fixed at pH 7.4-7.8 at room temperature. The solution of 0.3ml MFB was adding to (0.7) ml of Ham_F12 medium in plastic test tube too.

3-Sperm collection and activation

- The male mice were sacrificed by cervical dislocation .Epididymal caudal region was isolated and placed on the Falcon dish with 0.5 ml of Ham's F-12 for washing. three media were prepared; 1ml of Ham's F-12 medium alone(control) and 1 ml of Ham-F12 with 0.1% MFB and 1ml of Ham-F12 with 0.15%MFB.Snip was done to the isolated cauda. Then the sperms were taken out, following dispose the cauda epididymis. The collected sperm were incubated for at least 30 minutes in each prepared media. Certain sperm function parameters were measured as described by Al-Dujaily *et al*.[8,9]

Statistical Analysis: The statistical analysis was performed using SPSS.21. version. For the treatment (Male Fertility Blend medium) and for the control (Male Fertility Blend -free Ham's F-12 medium) groups data of mice sperm analysis were expressed as mean ±SE and analyzed by using paired sample t-test.[10]

RESULTS

Certain sperm function parameters following *in vitro* direct activation by a media containing two concentrations of Male Fertility Blend® (MFB)

The results of certain sperm function parameters of sperms obtained from caudal epididymal region and activated *in vitro* by direct activation technique were shown in (table 1). There was a highly significant ($p<0.01$) decrease in the mean of sperm concentration (m/ml) by using medium containing 0,1%MFB (36.42 ± 2.81) and 0.15% MFB (41.00 ± 2.84) compared to MFB-free medium(45.71 ± 3.07). At the same time the mean of sperm concentration was significantly ($p<0.01$) higher by using 0.15% MFB than that of 0.1% MFB medium. The percentage of active sperm motility grade A using medium containing 0.15%MFB(26.03 ± 0.61)was highly significantly($P<0.01$) improved compared to medium containing 0.1%MFB (20.06 ± 0.64) and medium free MFB(12.00 ± 0.45). There was a significant($p<0.01$) increase in active sperm motility grade A by using medium containing 0.1% MFB compared to MFB –free medium.

The percentage of sperm motility grade B revealed a highly significant ($p<0.01$) increment by using a medium containing 0.15% MFB compared to other media. At the same time, activation of epididymal sperm *in vitro* by using medium containing 0.1% MFB was significantly($p<0.01$) higher than that of using MFB-free medium**.**

The percentage of morphologically normal sperm (MNS) was highly significantly ($P<0.01$) different by using a medium containing 0.15% MFB (71.32 ± 1.49) and 0.1% MFB (65.64 ± 1.59) compared to MFB –free medium(61.42 ± 1.59) as shown in table -1.Moreover,there was a highly significant ($p<0.01$) increase in MNS when using a medium containing 0.15% MFB compared to 0.1% MFB.

Table 1: Certain sperm function parameters following *in vitro* direct activation by a media containing two concentrations of male Fertility Blend® (MFB)

Certain sperm function parameters	Media used	Mean ± SE	P-value
Sperm concentration (million/ml)	**MFB –free medium (Control)**	45.71 ± 3.07	-
	Treated group with Fertility Blend 0.1%	36.42 ± 2.81	P<0.01 HS
	Treated group with Fertility Blend 0.15%	41.00 ± 2.84	P<0.01 HS
Sperm motility Grade A (%)	**MFB –free medium (Control)**	12.00 ± 0.45	-
	Treated group with Fertility Blend 0.1%	20.06 ± 0.64	P<0.01 HS
	Treated group with Fertility Blend 0.15%	26.03 ± 0.61	P<0.01 HS
Sperm motility grade B (%)	**MFB –free medium (Control)**	35.29 ± 0.97	-
	Treated group with Fertility Blend 0.1%	45.87 ± 1.07	P<0.01 HS
	Treated group with Fertility Blend 0.15%	56.25 ± 1.01	P<0.01 HS
Morphologically normal sperms (%)	**MFB –free medium (Control)**	61.42 ± 1.59	-
	Treated group with Fertility Blend 0.1%	65.64 ± 1.59	P<0.01 HS
	Treated group with Fertility Blend 0.15%	71.32 ± 1.49	P<0.01 HS

DISCUSSION

The current study found that, *in vitro* activation of caudal epididymal sperms in culture containing 0.1% and 0.15% MFB-Hams-F12 medium for 30 minute resulted in a significant increase in the percentages of sperm motility and grade activity of forward movement (grade A and grade B). The differences in sperm concentration and sperm motility between medium containing 0.1% and 0.15% MFB medium and MFB –free medium may be explained by the booster effect of MFB medium contents herbal/nutritional blend namely;, L-carnitine, vitamins C and E, green tea, selenium and the constituent of Dong Quai extract. In addition to that, ingredients of zinc and B vitamins (B6, B12 and folate) may improve male fertility health by improving sperm quality and motility.[11]

The L-carnitine (LC), as anti-oxidant, may protect sperm plasma membrane with high level of unsaturated fatty acid content[12] as free radicals can decreases mitochondrial energy availability and impairs sperm motility. L carnitine increases sperm motility by interfere in the pathway of fatty acid metabolism. Mitochondria in the middle piece of the sperm are also involved in fatty acid metabolism.[13] It has been noticed that low levels of carnitine reduce fatty acid concentrations within the mitochondria, leading to decreased energy production and potential alterations in sperm motility.[14] On the other hand, zinc levels are important in fertility capacity. Generally lower Zn level in infertile men will diminished sperm count and several studies have found supplemental zinc may prove helpful in treating male infertility.[15]

The other components of MFB that found in the medium used is the Vitamin E. This Vitamin is well-documented as antioxidant and has been shown to inhibit free radical- induced damage to sensitive cell membranes.[16] Vitamin E improved sperm function in the zona binding assay, therefore enhancing the ability of the sperm to penetrate the egg *in vitro*.[17]

The other possibility of increase sperm motility and forward movement is the existence of Selenium and green tea in the medium. Both are powerful antioxidants and is thought to stabiles the integrity of the sperm flagella. A male fertility dietary supplement that contains selenium may increase sperm motility.[18]

Vitamin C can help to remove lead from the body and is particularly important if the diet is low in fresh fruit and vegetables. Supplementation of vitamin C has reportedly improved the sperm quality of smokers.[19]

The medium containing ferulic acid an antioxidant found in Dong quai extract that which is part of MFB composition. This acid improves sperm quality too.[20] Sperm are highly susceptible to free radical or oxidative damage from environmental toxicants and natural aging.[20] in addition to B vitamins (B6, B12) are involved in cell maturation and DNA synthesis. A deficiency of vitamin B12 in the nutrition is associated with decreased sperm count and motility, therefore, for enhanced certain sperm function parameters vitamin B12 is essential.[21] Dong Quai a traditional Chinese fertility herb that is believed to help the balance of estrogen levels and improve the chances of embryos for implantation.[22] The current study concluded that MFB medium is an effective culture can be used to enhance certain epididymal sperms function parameters. Further studies are suggested to found out its potential on IVF procedure.

REFERENCES

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet, 1978; 12; 2(8085): 366.
2. Jungwirth A, Diemer T, Dohle GR, et al. Guidelines on Male Infertility. European Association of Urology, 2015.
3. Raju B, Krishna M, Siva R, et al. Natural Compounds to Treat Male Infertility. Newsletter. Pharmacology online. 2009; 2: 240-251.
4. Li H. More attention should be paid to the treatment of male infertility with drugs testosterone: to use it or not? Asian J Androl. 2014; 16(2): 270–273.
5. Wang C, Swerdloff S. Treatment of male infertility. Up To Date. http://www.uptodate.com/contents/treatment-of-male-infertility-beyond-the-basics/abstract/ 2015.
6. Schuster DB. Tribulus terrestris: Fertility herb for both men and women. The Natural Fertility Company.2015. http\www. Natural Fertility Info.com.
7. Aileen ST. Method and composition for improving fertility health in female and male animals and human. United States Patent. https://www.google.ch/patents/US6497885, 2002.
8. Al-Dujaily SS Sachid M and Al-Faisal A M. Effect of cryopreservation on DNA normality of mice epididymal sperms following in vitro preparation with pentoxifylline, and Glycyrrhiza glabra. Iraqi J. Embryos. Infert. Res. 2013; 3(5): 24-30.
9. AL-Dujaily SS, AL-Janabi AS and Nori M. Effect of Glycyrrhiza extract on in vitro sperm activation of asthenospermic patients. J. Babylon Uni. 2006; 11(3): 477-483.
10. Barton B, Peat J .Medical Statistics: A Guide to SPSS, Data Analysis and Critical Appraisal, 2nd Edition. September 2014.
11. Rodriguez H. Which Vitamins are Good for Fertility?. 2015 The Natural Fertility Company. http://natural-fertility-info.com/vitamins-good-for-fertility.
12. Solarska K, Lewińska A and Karowicz-Bilińska A, et al. The antioxidant properties of carnitine in vitro. Cell Mol Biol J. 2010; 15: 90-97.
13. Dokmeci D. Oxidative stress, male infertility and the role of carnitines. Folia Med. 2005; (47): 26-30.
14. Sinclair S. Male Infertility: Nutritional and environmental considerations .Alternative Medicine Review, 2000; 5(1): 28-38.
15. Madding CI, Jacob M, Ramsay VP, Sokol RZ. Serum and semen zinc levels in normozoospermic and oligozoospermic men. Ann Nutr Metab, 1986; 30: 213-218.

16. Aitken RJ, Clarkson JS, Hargreave TB, et al. Analysis of the relationship between defective sperm function and the generation of reactive oxygen species in cases of oligozoospermia. J Androl, 1989; 10: 214-220.
17. Kessopoulou E, Powers HJ, Sharma KK, et al. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated with male infertility. Fertil Steril, 1995; 64: 825-831.
18. Moslemi M. Selenium – Vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. Int J Gen Med. 2011; 4: 99-104.
19. Colagar AH, Marzony ET. Ascorbic Acid in human seminal plasma: determination and its relationship to sperm quality. J Clin Biochem Nutr. 2009; 45(2): 164-9. 124.
20. Sandler B. The treatment of oligospermia with stilbestrol and vitamin B. Fertil Steril. 1961; 12: 469-73.
21. Moriyama H. Studies on the usefulness of a long-term, high-dose treatment of methylcobalamin in patients with oligozoospermia. Hinyokika-Kiyo. 1987; 33(1): 151-156.
22. Menu Docshop. Conception Tips. .http://www .docshop .com / education/fertility/getting-pregnant/pregnancy-tips. 2015.