Oocyte Quality and Embryonic Development after Oral Administration of Speramax® in Female Mice as Experimental Model for Mammals

Saad S. Al-Dujaily, Hiba S. Hamza

High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University,Baghdad-IRAQ

Abstract:

Background:

Speramax® was found to play an important role in sperm function characters and males reproductive performance with no studies on its effects on the oocyte maturation and embryonic development in females.

Objective:

The goal of the present work was to examine the effect of Speramax ® on oocyte maturation ,ova quality , embryonic development and live birth using mice as a model for mammals .

Materials and Methods:

In this study, Speramax ® was administrated orally for 1, 2 and 4 weeks . One hundred and ten female mice were randomly divided into four groups , the first group was superovulated(SUO) while the second group was treated by Speramax® with SUO and the third group was spontaneously ovulated (SPO) and treated with Speramax ® and the forth group was spontaneously ovulated (SPO) and served as control group .Another forty- eight female mice were used for the determination of the number of live births .

Results: The results indicated that treatment with Speramax® showed a positive effect on oocytes maturation *in vivo.* There was a highly significance ($p< 0.0001$) improvement in the number of mature oocytes following treatment with Speramax® in SPO and SUO mice compared with SPO and SUO mice not treated with Speramax® . The percentage of embryonic development after 24 and 48 hours of mating in treated groups with Speramax® was significantly ($p< 0.05$) higher than SPO and SUO mice .The study showed that the quantity and quality of embryos obtained from the treated groups were superior to that of the untreated group .

Conclusions:

It was concluded that the Speramax® greatly improved oocyte maturation, early embryonic development and embryo grading quality embryos with an increase in the numbers of mice live births.

Key words: Speramax® , oocyte quality, embryonic development.

Introduction:

Fertility is defined as the capacity to reproduce or the state of being fertile [1] while Infertility is a relatively common problem that affects couples worldwide. The quality of oocytes plays a key role in a proper embryo development. In humans, oocytes of poor quality may be the cause of women infertility and an important obstacle in successful *in vitro* fertilization (IVF) [2]. 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 in fertilization, but also in subsequent development [3].

The quality of the oocytes is determined not only by the nuclear and mitochondrial genome, but the microenvironment provided by the ovary and the pre-ovulatory follicle [3]. On the other hand, there are so many medicines used to improve male fertility .Speramax ® is a new medicine containing a number of different vitamins and L-carnitine , all involved in cell metabolism and used for men. The L- carnitine is involved in fatty acid oxidation . The vitamins act as antioxidants as well as anabolism of the body[4] .

For treatment of male infertility, Speramax ® with its content of L-carnitine, Zinc & vitamin E increases sperms quality. L-carnitine, Vitamin E and selenium increases the sperm motility. Zinc and Folic acid increases sperm count [4] .However, to our knowledge there are no studies concerning the effect of Speramax ® on female fertility potential.Therefore ,the present study was designed to investigate the effect of Speramax ® on oocyte maturation, ova quality embryonic development and live birth in female mice.

Materials and Methods:

This study was carried out at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies /AL-Nahrain University. Thirty male and One hundred fifty eight female Albino – Swiss mice of 8-12 weeks age and 25-35 gm weight were obtained from the Animal House of the Institute and used in this investigation. Each cage contains four animals with tap water and diet freely available for the animals. The isolated females were kept in separate cages to make sure there is no meeting between them happened and no pregnancy taking place by natural intercourse. The animals were examined weekly. Abnormal and sick mice were excluded from the experiment. The animals and cages were cleaned and sterilized with 70% ethyl alcohol once a week regularly.

Methods:

Female fertility mice were divided into three main groups. Group one for spontaneously Speramax® , group two for treatment with Speramax® with superovulation and group three for superovulation.

Superovulation induction:

Superovulation was induced by IP injection of 7.5 I.U. of PMSG(Folligon® ,Holland), followed by IP injection of 7.5 I.U. of hCG(Pregnyl®,Serono Company) ,48 hours later. Oocytes were recovered 13 hours post-HCG.

Mating of the animals

After isolation of the sexually mature females which at the estrus stage by examing the vaginal smears under light microscope. The isolated females were placed in breeding cages (2 females with one mature male) and left overnight.

Early in the next morning, copulation was confirmed by observing, the

presence of the vaginal plug or the sperms microscopically using vaginal swabs.

In this work the gestational day zero was defined as the day when spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug .

Evaluation and grading system of embryos:

Fertilized ova were diagnosed by observing of two pronuclei and two polar bodies. The morphology of 2 to 8 blastomere embryos was divided to in 4 grades and was done according to the criteria of Khalil and Anvari [5].

Statistical Analysis:

Statistical analysis was performed using version 16 .0 Minitab statistical program. For the treatment (speramax®) and for the control group data of mice maturation oocyte , embryonic development ,and early embryo scoring after 24 and
48 hours of insemination. Chi- square test was used to compare values .P-value<0.05 was considered significant [6].

Results:

1. Oocytes collection:

The number of mature oocytes collected from 50 female mice was (664) out of (972) oocytes. They were divided into three groups through the periods 1,2,4 weeks to account the oocyte maturation.

2.oocyte maturation : Table 1 shows the number of mature and immature oocytes. A high significant ($p < 0.0001$) difference in the number of mature and immature oocytes is found in two groups treated with Speramax®. There is no significant ($p>0.05$) difference in the number of mature and immature oocytes when the female mice superovulated only for one week.

Table 1: Maturation status of oocytes obtained from spontaneously ovulated (SPO)and superovulated(SUO) female mice after one week of treatment with Speramax®.

Mice group	Oocyte maturation no.				P value
	Total no.	Oocyte		Mature %	
		Mature	Immature		
SPO	62	40	20	65.65	0.012
SPO with Speramax®	70	68	2	97.14	0.0001
SUO with Speramax®	129	104	25	80.6	0.0001
SUO	100	52	48	52	0.693

Chi-square test at 0.05 level of significance

The number of mature oocytes in SPO mice , SPO mice treated with Speramax® and SUO mice treated with Speramax® shows highly significant ($p<0.01$, $p < 0.0001$ and $p< 0.0001$ respectively) difference compared to the number of immature oocyte in the corresponding group following two weeks of treatment. Whereas the number of mature and immature oocytes in SUO mice did not show significant($p>0.05$) different between them ,as shown in table (2).

Table 2: Maturation status of oocytes obtained from spontaneously ovulated (SPO)and superovulated(SUO) female mice following two weeks of treatment with Speramax®.

Mice groups	Oocyte maturation no			Mature %	P value
	Total no.	Oocyte			
		Mature	Immature		
SPO	60	40	20	66.6	0.010
SPO with Speramax®	75	69	6	78.6	0.0001
SUO with Speramax®	145	110	35	87.4	0.0001
SUO	115	62	53	53.1	0.617

Chi- square test at 0.05 level of significance

The results of oocyte maturation of SPO and SUO female mice treated with Speramax® are shown in Table 3.There is a significant difference between the number of mature and immature oocytes in SPO ,SPO with Speramax® and SUO with Speramax® groups. No significant($p>0.05$) difference is observed in the number of mature oocytes compared to immature oocyte in SUO group (Table 3).

Table 3: Maturation status of oocytes obtained from spontaneously ovulated (SPO)and superovulated(SUO) female mice after 4 weeks of treatment with Speramax®.

Mice group	Oocyte maturation no.				Prob.
	Total no.	Oocyte		Mature %	
		Mature	Immature		
SPO with					
Speramax®					
SUO with Speramax®					
SUO					

The square test at 0.05 level of significance

3.Embryonic Development

3.1.Embryonic Development after 24 hours of mating

Table 4 illustrates the effect of oral administration of speramax® for one ,two and four weeks on embryonic development after 24 hrs of mating. A significant ($P<0.001$) improvement was shown in the total number of developed embryos at 3-4 cells stage of SPO and SUO mice treated with Speramax® (46% and 42.15 % respectively)and SUO (40.96%)compared to the SPO mice not treated with Speramax® (32.14%) after one week . There is a significant ($P<0.0001$) improvement in the total number of developed embryos at 3-4 cells stage of in SUO and SPO mice treated with Speramax® (46.017% and 41.66% respectively) and SUO (45.45% compared to the SPO mice not treated with Speramax® (42.88%) after two weeks .After 4 weeks of treatment, there is a significant ($P<0.0001$) improvement in the total number of developed embryos at 3-4 cells stage of SPO and SUO mice treated with Speramax® (44.18% and 34.06% respectively) and SUO (41.02%) compared to the SPO mice not treated with Speramax® (27.27%).

3.2 Embryonic Development after 48 hours

Table 5 shows significant ($P<0.001$) differences between treatments in the total number of two cells, three-four cell and five –eight cells stages of embryos after 48 hour compared to SPO. Also, there are variations in the Embryonic development between treatments. The best is with SUO (24.09%) at two cells stage, SUO mice treated with Speramax® (30.39%) at 3-4 cells stage and (58.82%) 5-8 cells stage after one week .

A significant ($P<0.0001$) differences is observed between group SPO treated with Speramax® and SPO group in the total number of two cells, three-four cell and five –eight cells stages of embryos after 48 hour.Moreover, there are variations in the ED between treatments used was best SPO and SUO mice treated with Speramax® (8.33% ,7.96 respectively) at two cells stage, SPO mice treated with Speramax® ,SUO (35% ,31.81% respectively) at 3-4 cells and 5-8 cells stages of embryos after two weeks . A significant($P<0.0001$) differences is observed between group SPO treated with Speramax® and SPO group in the total number of two cells, three-four cell and five –eight cells stages of embryos after 48 hour. Also, there are variations in the Embryonic development between treatments. The best is with SPO mice treated with Speramax® at two cells, 3-4 cells and 5-8 cells stages of embryos in one month.

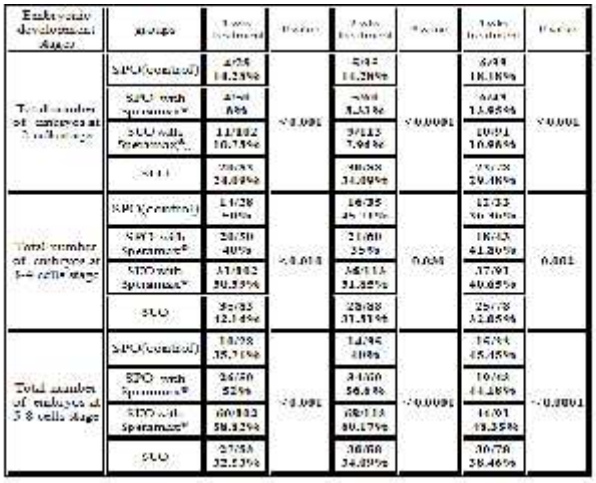
Discussion:

1. Oocytes Maturation:

The present study demonstrates that Speramax® has a positive effect on

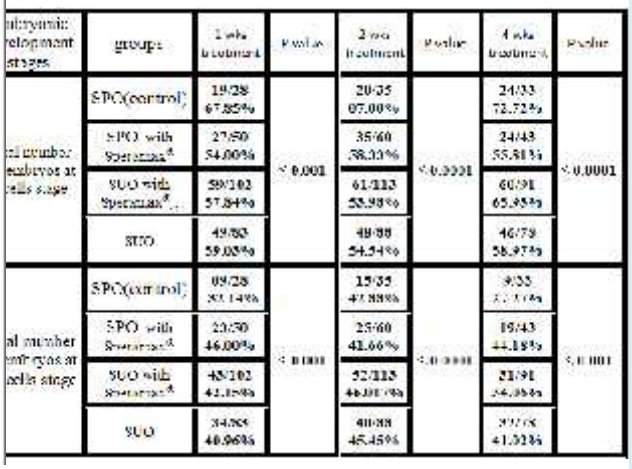
maturation of the oocytes *in vivo* in SPO and SUO groups after one, two and four weeks .

Table 4 : Embryonic development after 24 hours of mating in female mice spontaneously ovulated(SPO) and superovulated (SUO) treated with Speramax® for one , two and fourth weeks .



maturation and then the consequence of fusogenic process and embryonic development. An externally derived calcium is required for oocyte maturation in the hamster [11] and other mammals [12, 13-15].

Table 5 : Embryonic development after 48 hours of mating in female mice spontaneously ovulated(SPO) and superovulated(SUO) treated by Speramax® for one ,two and fourth weeks.



Successful oocyte maturation causes the oocyte to undergo normal fertilization and embryonic development, involves not only nuclear maturation, but also cytoplasmic maturation which comprises events that are poorly understood [7].
In the present study, Speramax® may cover the basic metabolic needs of the oocytes. Specifically it contains vitamin E , folic acid ,calcium, selenium ,zinc oxide and L- Carnitine which are important as antioxidant factors . These powerful antioxidants can play a critical role in oocyte maturation. The follicular fluid (FF) is rich with vitamin E. The environment of the FF is thought to play a critical role in oocyte maturation and the eventual development of an embryo [8] .It has been reported that calcium plays appositive role in oocyte maturation. The intracellular calcium oscillation is required for spontaneous maturation of mouse oocytes [9, 10]. Therefore, Speramax® used in present study may trigger the Ca^{2+}channals to increase the Ca^{2+} influx leading to increase the percentage of ova

2.Embryonic Development:

The positive effects of Speramax® on embryonic development and quality that recognized in this study may be augmented by a number of active ingredients found in the Speramax® like: Folic acid , L-Carnitine , vitamin E ,zinc oxide and selenium. Each of which exerts different improvements on oocytes and early developed embryos .It has been observed that L-Carnitine may play a principal role on embryonic development at early cleavage stages where L-carnitine-mediated β-oxidation of fatty acids plays an essential energy source for the metabolism of oocytes and embryos [16-19]. The effect of L-carnitine as well known antioxidant on preimplantation ED suggests its role in oocytes and early developed embryos [20].

On the other hand, upregulation of β-oxidation during oocyte maturation by L-carnitine increased oocyte developmental competence as manifested by the increased rate of

cleavage to 2-cells stage [21].The improvement of oocytes maturation and embryo development by supplementation of LC found in the speramax® may be resulted from the utilization of lipid via β-oxidation to generate ATP which is necessary for the resumption of meiosis and cytoplasmic maturation [22]. It has been reported that supplementation of LC during oocyte maturation significantly increased β-oxidation , and improved FR [23].

Studies on infertile women revealed that preconception folic acid supplementation increased folate levels and decreased homocysteine levels in follicular fluid [24], and was related to better embryo quality and chance of pregnancy[25].

Folate is important for oocyte quality and maturation, implantation, placenta - ion, fetal growth and organ development [26].It is necessary for energy production and healthy cell division, and it is also important for the formation of the red blood cells [27]. Folate is considered to be important for oocyte quality and maturation as well as for implantation and normal continuation of pregnancy [28].Although infertility treatment is one factor associated with high folic acid supplement intake [29].

The positive effect of Speramax® on oocyte maturation and ED may be explained on the basis of the presence of non -enzymatic antioxidants such as, vitamin E, selenium, zinc, which all have the capability to control ROS production [30,31,32].

It has been recorded that the administration of Fertility Blend, a product containing vitamins E, folate, zinc, and selenium(a supplement similar to Speramax®), to female patients ,5 out of 15 patients receiving this product became pregnant after 5 months of treatment compared to none of the 15 patients receiving placebo, and no major side effects noted with this supplement[33].Furthermore, zinc plays a role in sexual development, ovulation and the menstrual cycle. Both folate and zinc have antioxidant properties that counteract reactive oxygen species [26].

The significantly higher quality of ovarian follicles, oocytes and embryos in study group supplemented with selenium may support the findings of the present study. Selenium was demonstrated to reduce the production of ROS, increase total antioxidant content and glutathione production [34].

It is concluded that Speramax® greatly improve oocyte maturation, early embryonic development and embryo grading quality in mice in addition to the increase in the live birth number.

References

1.Gurunath S, Pandian Z, Anderson R, *et al*. Defining infertility-a systematic review of prevalence studies. Hum Reprod. 2011; 17 (5): 575.

2-Gaëlle Marteil, Laurent Richard-Parpaillon. *et al* . Role of oocyte quality in meiotic maturation and embryonic development Reprod. Biol. , review . 2009 ; 9 (3) : 203-224.

3.Laura Rienzi , Ga´bor Vajta and Filippo Ubaldi Predictive value of oocyte morphology in human IVF: a systematic review of the literature Hum. Reprod. Update, 2011; 17 (1) : 34–45.

4. Generated on**:** This page was generated by TSDR on 2014-03-06 14:36:17 EST (Mark: Speramax®).

5. Khalili MA and Anvari M. the effect of *in vitro* culture on cleavage rates and morphology of the *in vivo*– developed embryo in mice .Iran JRepordMed.2007;5:17-22.

6.Minitab, Inc. 2000. Minitab Statistical Software, Release 13 for Windows. State College, PA: Minitab Inc.

7.Eppig, J.F.; Schultz, R.M. and O'Brien, M. Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. Dev. Biol. 2000; 164:1-9.

8. Oyawoye O, Abdel Gadir A, Garner A, *et al* . Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome. Hum Reprod. 2003; 18(11):2270–2274
9.De Felici M, Dolci S, Siracusa G: An increase of intracellular free Ca2+ is essential for spontaneous meiotic resumption by mouse oocytes. *J* Exp Zool 1991, 260:401-405.
10.Carroll J, Swann K: Spontaneous cytosolic calcium oscillations driven by inositol triphosphate occur during *in vitro* maturation of mouse oocyte. J Biol Chem 1992, 267:11196-11210.
11. Racowsky C: The releasing action of calcium upon cyclic AMPdependent meiotic arrest in hamster oocytes. J Exp Zool 1986, 239:263-275
12. Powers RD, Paleos GA: The combined effects of Ca and dibutyryl cyclic AMP on germinal vesicle breakdown in the mouse oocyte. J Reprod Fert 1982, 66:1-8.
13. Preston SL, Parmer TG, Behrman HR: Adenosine reverses Ca dependent inhibition of follicle-stimulating hormone action and induction of maturation in cumulus enclosed rat oocytes. Endocrinology 1987, 120:1356-1364.
14. Goron S, Oron Y, Dekel N: Rat oocyte maturation: role of calcium in hormone action. Mol Cell Endocrinol 1990, 72:131-138.
15. Mattioli M, Barboni B: Signal transduction mechanism for LH in the cumulus-oocyte complex. Mol Cell Endocrinol 2000, 161:19-23.
16. Hillman N, Flynn TJ: The metabolism of endogenous fatty acids by preimplantation mouse embryos developing *in vitro*. J Embryol Exp Morphol 1980, 56:157–168.
17. Downs SM, Mosey JL, Klinger J: Fatty acid oxidation and meiotic resumption in mouse oocytes. Mol Reprod Dev 2009, 76:844–853.
18. Sturmey RG, Reis A, Leese HJ, *et al*. Role of fatty acids in energy provision during oocyte maturation and early embryo development.Reprod Domest Anim 2009, 44:50–58.
19. McKeegan PJ, Sturmey RG: The role of fatty acids in oocyte and early embryo development. Reprod Fert Develop 2011, 24:59–67.
20. Agarwal A , Sajal G and Rakesh S. Oxidative stress and its implications in female infertility – a clinician's perspective Reprod Biol Med. 2005; 11: 641–650.
21. Dunning KR, Cashman K, Russell DL, *et al*. Beta-oxidation is essential for mouse oocyte developmental competence and early embryo development. Biol Reprod 2010, 83:909–918.
22. Ferguson E M and Leese H J. A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo development. Mol Reprod Dev. 2006; 73:1195-1201.
23. Dunning KR, Akison LK, Russel DL, *et al*. Increased betaoxidation and improved oocyte developmental competence in response to l-carnitine during ovarian *in vitro* follicle development in mice. Biol Reprod 2011; 85:548–555.
24. Boxmeer JC, Brouns RM, Lindemans J, *et al*. Preconception folic acid treatment affects the microenvironment of the maturing oocyte in humans. Fertil Steril. Jun 2008;89(6):1766-1770.
25. Boxmeer JC, Macklon NS, Lindemans J, *et al*. IVF outcomes are associated with biomarkers of the homocysteine pathway in monofollicular fluid. Hum Reprod. May 2009;24(5):1059-1066.
26. Ebisch, Thomas, Peters,*et al*, The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility , Human Reproduction Update, 2007; 13(2): 163–174.

27. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr. May 2000;71(5 Suppl):1295S-1303S.
28. Ebisch IM, Thomas CM, Peters WH, *et al*. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod Update. Mar-Apr 2007;13(2):163-174.
29. McGuire M, Cleary B, Sahm L, *et al*. Prevalence and predictors of periconceptional folic acid uptake--prospective cohort study in an Irish urban obstetric population. Hum Reprod. Feb 2010;25(2):535-543.
30. Attaran M, Pasqualotto E, Falcone T, *et al.* The effect of follicular fluid reactive oxygen species on the outcome of *in vitro* fertilization. Int J Fertil Womens Med 2000; 45: 314-20.
31. Perkins A. Endogenous anti-oxidants in pregnancy and preeclampsia. Aust N Z J Obstet Gynaecol 2006; 46: 77-83.
32. Agarwal A. Role of Antioxidants in Treatment of Male Infertility: An Overview of the Literature. Reproductive BioMedicine Online, 2004; 616-627.
33. Westphal LM, Polan ML, Trant AS, *et al*. A Nutritional Supplement for Improving Fertility in Women. J. Reprod Med, 2004; 289-293.
34. Abedelahi A, Salehnia M, Allameh AA, *et al,* Sodium selenite improves the *in vitro* follicular development by reducing the reactive oxygen species level and increasing the total antioxidant capacity and glutathione peroxide activity. Hum Reprod. 2010 ;25(4):977-85.