Comparison of ICSI results in a group of patients with and without oral L-carnitine, acetyl-L-carnitine and nutrients supplementation

Mounir Ajina[1]
Henda Mustapha[1]
Slah Tagorti[1]
Mohamed Ashraf Virmani[2]

[1] Unit of Reproductive Medicine, University Hospital F Hached, Sousse, Tunisia
[2] Research and Development, Alfasigma HealthScience BV, Utrecht, The Netherlands

Address for correspondence:
Mounir Ajina
Unit of Reproductive Medicine,
University Hospital F Hached
Sousse, Tunisia
E-mail: mounir.ajina@rns.tn

Summary

Epidemiological, preclinical and clinical studies show that specific metabolic compounds and nutrients play an important role in oocyte and embryo development. Metabolic compounds like L-carnitine and acetyl-L-carnitine play a role in fertility related processes by facilitating LH levels and oocyte development. However, the effects of using these substrates and nutrients to improve ICSI outcomes are not clear. The aim of our work was to evaluate the effect of these carnitines, N-acetyl cysteine and other specific vitamins and minerals on ICSI outcomes in couples with infertility. Infertile couples (n=56) were enrolled and followed over a period of one year. The couples had already done a cycle of ICSI without any treatment and were than treated with the supplement for two months followed by the next ICSI cycle. The group of women underwent hormonal treatment followed by follicular aspiration, intra-cytoplasmic spermatozoa injection and embryo transfer. The comparison of the results between the two cycles of ICSI with and without supplement, showed a significant improvement in the number of mature oocytes (5.2 ± 3.2 *vs* 7.3 ± 4.4, p=0.04), the level of embryos, type I (40% ± 10.5 *vs* 65% ± 12.2, p=0.02), blastocyst levels (22% ± 10.5 *vs* 33% ± 7.5, p=0.02) and the top quality blastocyst rate (25.3% ± 10.8 *vs* 45.2% ± 10.6, p=0.01) following supplementation. The biological pregnancy rate also increased from 17% ± 2.1 to 22% ± 2.3 with p=0.07, and this attained significance if we only looked at the values for the group of women <35 years. The treatment duration of two months and group number was probably not sufficient to significantly improve the ICSI results in women >35 years. The results show that supplementation improves many biological parameters as well as results of ICSI and could be beneficial in the overall rate of pregnancy in couples with infertility. Further studies are necessary to examine the role in ICSI as well as studies of longer duration and with larger and more homogeneous population size to show the role of specific nutrients in fertility.

KEY WORDS: ICSI, embryo quality, oocyte, acetyl-L-carnitine, nutrient supplementation.

Introduction

Since the introduction of the IVF technique for infertility in 1978 the pregnancy success rates have increased. Many factors have contributed to this progress such as improved IVF laborato-

ry techniques and in particular intracytoplasmic sperm injection (ICSI). There are various studies ongoing on ways to improve and maximize success rates with ICSI (1). For ICSI an important issue is the reproductive health of the men and/or the women and this has to be taken into account when considering the chance of a successful pregnancy. The overall reproductive health is dependent on a number of factors such as age, general health (obesity, stress), underlying disease as well as environmental factors such as nutrition, pollutants and stress. Limiting or improving some of these factors by for example, stopping smoking, drinking and starting to exercise, together with optimal nutrition, may improve the chances of ICSI as well as IVF and natural pregnancy. There are studies suggesting that oocyte quality may be improved by nutrients (2-4). This improvement could be in part related to the attenuation of toxic effects either directly on the sperm and the oocyte but also indirect effects on the endocrine system and other systems involved in successful reproduction, such as libido, erectile function, fallopian tube motility and uterus function (5, 6). In couples where there is male factor infertility, ICSI can still give good results with pregnancy rates up to 52% and this Figure maybe further improved if both the partners improve their reproductive health (7). Over the past decade, environmental factors have been shown to induce epigenetic changes in oocytes and embryos, which in turn contribute to altered developmental potential (8). Many animal and human studies show that specific metabolic compounds and nutrients play an important role in oocyte and embryo development. Nutrients such as vitamin C are important in human fertilization (8-11). Studies also show the beneficial effects of vitamin C and vitamin E supplementation in the prevention of the luteal phase deficiency and increase of the pregnancy rate (9, 10). Antioxidants such as vitamins A, C and E may play a role in compensating for the oxidative burst during early pregnancy since their deficiency was shown to be involved in the pathogenesis of recurrent pregnancy loss (8). Oral supplementation of the drug pentoxifylline during the IVF cycle can improve estradiol levels, endometrial thickness, and embryonic implantation (12). Improved pregnancy rates have been reported for a combined treatment of pentoxifylline and vitamin E for six months in patients scheduled for IVF with oocyte donation and having a thin endometrium (13).

The role of mitochondria, the energy generating organelles in all cells, is also becoming important for fertility since they are essential for oocyte maturation, fertilization and embryonic development (14). Studies are suggesting important roles for mitochondrial nutrients and for mitochondrial replacement as therapy especially in age-related decline in oocyte quality (15). The mitochondriotropic compounds L-carnitine and acetyl-L-carnitine are known to facilitate fertility (16). In *in vitro* studies, the addition of L-carnitine and acetyl-L-carnitine to the culture media not only improved oocyte chromosomal structure and reduced embryo apoptosis (17), but also improved blastocyst development rate (4, 18). In addition, oocytes isolated from the follicles treated with L-carnitine had greater rates of maturity (metaphase II/MII), higher fertilization rates and improved blastocyst development (4, 19).

In this study, we examined the role of oral supplementation of L-carnitine, acetyl-L-carnitine, N-acetyl cysteine and other specific vitamins and minerals on ICSI outcomes in couples with infertility.

Patients and methods

Study design and Patients

This was a comparative prospective study carried out on 56 infertile women who consulted the ART Reproductive Center, University hospital, Sousse, Tunisia, between January and August 2016. All women were the partners of men who had failed to conceive after 1 year of unprotected regular sexual report. This study was conducted according to the guidelines established for research on human subjects (Ethical committee, University hospital, Farhat Hached, Sousse).

Inclusion criteria were: non-smokers, non-alcoholic women and not using any medication, man age <50 years, without sexual disorder, and without oral L-carnitine, acetyl-L-carnitine and nutrients supplementation in the 6 months preceding the ICSI cycle. Couple in good health by means of their medical histories and clinical examination.

Exclusion criteria were: women with severe endometriosis, female age >40 years, men with significant bacteriospermia ($\geq 10^3$ colony-forming units/ml), elevated seminal leukocyte concentration (>1X 10^6 cells /ml), cryptozoospermia and azoospermia.

In their history, each couple had an ICSI cycle without oral supplementation. The second ICSI attempt was performed immediately after the oral supplementation treatment; each woman received daily two sachets of oral metabolic nutrients supplementation during the two months before the ICSI cycle. The average duration per couple between the two cycles of ICSI was 6 ± 2 months and the duration of the whole study was two years.

Preparation

Each sachet of the supplement (Proxeed Women) contained: L-carnitine 500 mg, acetyl-L-carnitine 250 mg, L-arginine 500 mg, vitamin C 90 mg, N-acetyl cysteine 50 mg, vitamin E 30 mg, iron 7 mg, pantothenic acid (vitamin B5) 6 mg, zinc 5 mg, vitamin B6 (pyridoxine HCl) 2 mg, copper 0.5 mg, beta carotene (provitamin A) 8 mg=800 µg RE, folic acid 200 µg, vitamin D3 5 µg, selenium 27.5 µg, vitamin B12 (cyanocobalamin) 2.5 µg and was provided by Sigma-Tau Health science BV, Utrecht, The Netherlands.

Sperm Preparation

All semen samples were collected by masturbation after a period of three to five days of sexual abstinence and analyzed for basic sperm parameters according to World Health Organization recommendations (WHO, 2010). A sperm-washing procedure was performed after centrifugation on a two-layer density (90% and 45%) gradient (spermGrad™, Vitrolife, Sweden). Sperm concentration and motility were assessed after selection.

Ovarian Stimulation and Oocyte Retrieval

Controlled ovarian stimulation was achieved using standard protocols including either long or short stimulation with agonist or antagonist. The protocols for inducing ovarian stimulation did not significantly differ between the two attempts. Ovulation was triggered by administration of 5000 to 10,000 IU of human chorionic gonadotrophin (hCG) when at least two follicles reached 18 mm in maximal diameter associated with a consistent rise in serum estradiol concentration. Transvaginal oocyte retrieval was scheduled 36 hours after hCG injection and followed by standard ICSI procedures.

ICSI Procedure

ICSI was routinely performed on each metaphase II stage oocyte. On day 1 (16 - 20 h after injection), fertilization was assessed by the presence of two pronuclei (2 PN) and two polar bodies (G-IVF™ PLUS, Vitrolife, Sweden). On day 2-3 and day 5-6 each embryo was graded according to traditional morphological criteria (20), including size and shape of blastomeres and degree of fragmentation. One to two best-scoring embryos were usually transferred to the patient's uterus, blastocyst stage culture was programmed systematically when more than four type I embryos were obtained at the cleavage stage. Vitrification was done only at the blastocyst stage and supernumerary blastocysts. The luteal phase was supported with 400 mg/day of intravaginal progesterone. Pregnancy test was done fourteen days after embryo transfer.

The sperm preparation and embryo culture were performed under the same conditions between the two cycles of ICSI.

Statistical Analysis

Statistical Analysis was performed using SPSS10 (SPSS, Inc., Chicago, IL, USA). The comparisons between controls and patients were calculated using Mann-Whitney U-test. A statistical difference was considered as significant when the p-value was $p<0.05$.

Results

Study subjects

In the study population, the average age of women and men was 33 ± 2.3 and 35 ± 5.6 years respectively, the average duration of infertility was 4 ± 1.2 years, infertility was male in 69% (severe or extreme oligoasthenoteratozoospermia) and female or mixed in 31% (endometriosis, tubal infertility and polycystic ovary). The type of infertility was primary in the majority of cases (89%).

The sperm analysis showed the following averages: volume 2.3±1.6 ml, sperm count (millions/ml) 14.5 ± 12.2, motility 12.1 ± 10.2 (%), morphology 18.4 ± 8.2 (%) and vitality 22.8 ± 14.6 (%). The comparison of sperm parameters between the two ICSI cycles found no significant difference.

We studied the effects of oral supplementation on the monitoring cycle and the ICSI outcomes. Several parameters during the monitoring cycle were evaluated including the number of gonadotropin ampoules, number of antral follicles on human chorionic gonadotropin (hCG) day, estradiol on hCG day and thickness of the endometrium on hCG day (Table 1). Globally the stimulation protocol did not change much between the two cycles, and in the first cycle, 30% of the patients benefited from agonist long protocol, 60% agonist short protocol and 10% the antagonist protocol.

The results evidenced that the oral treatment with supplementation for two months prior to the ICSI cycle did not affect the monitoring cycle parameters (Table 1).

In relation to the ICSI, a number of parameters, including the number of oocytes, number of mature oocytes, fertilization rate, cleavage rate day 2, type I embryos rate day 2, number of embryos transferred, blastocyst rate, type I blastocyst rate, biological pregnancy rate and clinical pregnancy rate concerning fresh embryos transferred were examined (Table 2).

The effects on outcomes in women by age

The supplementation treatment significantly improved certain ICSI results: the number of matured oocytes (5.2 ± 3.2 *vs* 7.3 ± 4.4, p=0.04), type I embryos day 2 (40% ± 10.5 *vs* 65% ± 12.2 p=0.02), blastocyst rate (22% ± 10.5 *vs* 33% ± 7.5, p=0.02) and type I blastocyst rate (25.3% ± 10.8 *vs* 45.2% ± 10.6, p=0.01) (Table 2).

The oral supplementation had no significant effect on the parameters of monitoring cycle regardless of the age of the woman. The results showed that the supplementation significantly improved certain ICSI parameters in women <35 years such as the number of mature oocytes (6.2 ± 4.5 *vs* 7.8 ± 5.3, p=0.01), blastocyst rate (28% ± 10.5 *vs* 48% ± 12.2, p=0.02) and type I blastocyst rate (35.3% ± 15.8 *vs* 55.2% ± 10.6, p=0.01). Although the parameters showed trend towards improvement with the oral supplementation these did not attain significance for women >35 years of age.

The outcomes of normal responders

The normal responders (n=35) were selected according to a normal anti Müllerian Hormone (AMH) value (2.5-6 ng/ml) at the previous ICSI

Table 1. Effects of oral supplementation on the monitoring cycle parameters.

Parameters (mean SEM)	Before treatment (n=56)	After treatment with oral supplementation (n=56)	p value
No of Gonadotropin ampoules	29.5 ± 3.2	28.3 ± 2.8	0.51
No of antral follicles (>16 mm) on hCG day	8.6 ± 4.5	9.2 ± 3.5	0.36
E2 on HCG day	1045 ± 542	1154 ± 456	0.64
Thickness of the endometrium on hCG Day (mm)	8.2 ± 2.4	8.7 ± 2.1	0.31

Table 2. Effects of oral supplementation on the ICSI outcomes.

Parameters (mean SEM)	Before treatment (n=56)	After treatment with oral supplementation (n=56)	p valu
No of oocytes	7.5% (308) ± 5.2	8.6% (336) ± 6.2	0.30
No of oocytes Injected	5.2% (224) ± 3.2	7.3% (280) ± 4.4	0.04
Fertilization rate	55% (123) ± 15.5	62% (168) ± 11.2	0.10
Cleavage rate day 2	58% (71) ± 10.5	63% (102) ± 13.2	0.63
Good embryos rate day 2	40% (28) ± 10.5	65% (65) ± 12.2	0.02
No of embryos transferred/couple	1.5% (62) ± 0.5	1.6% (75) ± 1.2	0.84
Total number of embryos obtained day 2	71	102	0.003
Blastocyst rate	22% (13) ± 10.5	33% (28) ± 7.5	0.02
Top quality blastocyst Rate	25.3% (4) ± 10.8	45.2% (12) ± 10.6	0.01
Biological pregnancy rate	17% (8) ± 2.1	22% (13) ± 2.3	0.07
Clinical pregnancy rate	15.6% (7) ± 2.7	20% (11) ± 2.2	0.08
Implantation rate	10.6% (8) ± 1.3	13.6% (13) ± 1.6	0.20

cycle, the ovarian response was >5 oocytes. In the normal responders, the oral supplementation did not significantly improve the parameters of the monitoring cycle. However for ICSI a significant improvement was noted in the number of mature oocytes (6.4 ± 4.5 *vs* 8.2 ± 5.3, p=0.01), blastocyst rate (26.5% ± 11.6 *vs* 45% ± 14.2, p=0.032), type I blastocyst rate (35.3% ± 10.3 *vs* 59.2% ± 10.6, p=0.021) and biological pregnancy rate (18% ± 1.5 *vs* 22.8% ± 2.2, p=0.05).

The effects in poor responders

The poor responders (n=12) were selected according to the consensus of ESHRE, Bologna (21). In the poor responders, the oral supplementation did not significantly improve the parameters of the monitoring cycle and the ICSI outcomes except for type I blastocyst rate (p=0.05).

The outcomes of couples with male infertility

Among couples with male infertility, the oral supplementation of women led to improved: number of mature oocytes (6.2 ± 3.5 *vs* 8.4 ± 3.3, p=0.03), top quality blastocyst rate (35.3% ± 8.5 *vs* 43.2% ± 10.6, p=0.021) and biological pregnancy rate (18.2% ± 1.5 *vs* 23.8% ± 2.2, p=0.048).

The outcomes of couples with female infertility

In couples with female infertility (n=17) oral supplementation of women had no significant effects on the monitoring cycle and the ICSI results for the two-month treatment period.

Discussion

The study by Patrizio (22) showed that only 5% of oocytes can produce a baby in an assisted reproductive technology cycle. The factors known to affect the success of IVF are still not completely known and undermine achievement of pregnancy. One of the know factors is the women's age and this can influence the ovarian response to stimulation, number of oocytes, oocyte quality, fertilization rate, and the number and quality of embryos. This and other factors such as nutrition may also affect the chance of successful ICSI. The present study evaluated the effect of metabolic substrates and other specific vitamins and minerals on the ICSI outcomes in couples with infertility. The monitoring cycle parameters were not affected but some of the ICSI parameters were significantly improved after oral supplementation of L-carni-

tine, acetyl-L-carnitine, N-acetyl cysteine and nutrients. Significantly improvements were seen in the number of mature oocytes, the level of type I embryos, the blastocyst rate, and the type I blastocyst rate.

The positive effects of carnitine have been reported before in two separate studies (17, 18). These studies showed that the *in vitro* addition of L-carnitine to the culture media not only improved oocyte chromosomal structure and reduced embryo apoptosis (17), but also improved blastocyst development rate (18). In addition, oocytes isolated from the follicles treated with L-carnitine had greater rates of maturity (metaphase II/MII), higher fertilization rates and improved blastocyst development (19).

Effect of age

Since age is a known factor, the effects of supplementation were also analyzed on the monitoring cycle and on ICSI outcomes of the women by age. Previous studies showed that the age of the patient does not influence the rate of estradiol, FSH and the size of the endometrium on the day of onset in IVF (23). However, additional treatment with micronutrients, starting three months before IVF cycles, protects the follicular microenvironment from oxidative stress, and has been shown to increase the number of good quality oocytes recovered at the pickup of women aged >39 years (24). In this study, treatment of the patients of all ages with oral metabolic and nutrient supplementation had no significant effect on the parameters of the monitoring cycle. The present study evidenced that in women under 35 years of age, oral supplementation significantly improved some ICSI outcomes, such as number of mature oocytes, type I blastocyst rate and the biological pregnancy rate. In women >35 years of age the oral supplementation there was a trend towards improvement but did not attain significance with the ICSI results. This could be due to various reasons and it is possible that we need more numbers in the group and also these women may require a treatment period >2 months to see an effect or have other underlying factors not as yet understood.

The dependence of the ICSI results on the age of the patients has been shown before (25, 26). Studies showed that the increased intakes of beta-carotene, vitamin C, and vitamin E from dietary supplements were associated with shorter time to pregnancy among women with BMI <25 kg/m2 with increasing vitamin C, among women with BMI R25 kg/m2 with increasing beta-carotene, among women <35 years with increasing beta-carotene and vitamin C, and among women R35 years with increasing vitamin E (27).

The effects in normal and poor responders

In our study, most of the patients were aged less than 35 years (30/56) and the majority were normal responders (35/56). Hence the interest of oral supplementation for normal responders before the ICSI cycle. In the present study, the normal responders treated with oral supplementation of L-carnitine, acetyl-L-carnitine and nutrients significantly improved results of ICSI especially in patients less than 35 years of age. This effect could be due to an improvement in the underlying metabolism and antioxidant status. Lower total antioxidant status is observed in peritoneal fluid of women with idiopathic infertility compared to fertile controls (28). However, in a previous study the use of oral antioxidants in the form of a combination of multivitamins and minerals (amino acid chelated) did not improve oocyte quality and pregnancy rates in women with unexplained infertility undergoing IVF/ICSI treatment (29).

The management of poor responders is a significant challenge in assisted reproduction (21). The results showed that in this study the only parameter that improved significantly in the poor responders after treatment with oral supplementation was the blastocyst quality rate ($p=0.05$). This would suggest that oral supplementation with specific compounds may positively modulate the blastocyst stage after even in the poor responders. Indeed, in many preclinical studies the addition of L-carnitine has been found to normalize the alterations in oocyte mitochondria and markers of oxidative stress and improve blastocyst development (30-37). Other studies have shown that oral melatonin

supplementation can have a beneficial effect on the improvement of oocyte and embryo development in patients with low fertilization rate and poor oocyte quality (38). In poor responders, treatment with myo-inositol resulted in increased fertilization rate, implantation rate, grade 1 embryos rate and pregnancy rate, but without reaching a statistical significance (39).

The effects on couples with male and female infertility

The statistical results observed in couples with male infertility were almost the same as the group of normal responders or the group of patients under 35 years of age. In the couples with male infertility, the oral supplementation of women improved the number of mature oocytes, top quality blastocyst rate and biological pregnancy rate. These results reinforce the idea of giving women specific oral supplementation before ICSI even when infertility is male factor. It is possible that by improving oocyte quality or some other unknown factor the possibility of a successful fertilization is improved. This may be especially important when sperm quality is compromised. Thus, for ICSI the selected spermatozoon despite having a morphologically-normal appearance may carry a greater risk of having DNA damage, especially since natural spermatozoa selection has been bypassed during the ICSI procedure. Furthermore, ICSI is usually the technique of choice when spermatozoa quality is poor (40). In infertile men with prior failed IVF/ICSI, coenzyme Q10 supplementation increased fertilization rates in the subsequent cycle (41). Further studies would be useful to study the treatment with supplementation on the male partner but also on treatment of both the couple prior to ICSI.

The supplementation of women with female infertility had no significant effects on the monitoring cycle and the ICSI results for the two-month treatment period in this study. In this case, it may be necessary to increase the supplementation treatment period to 4 or 6 months and to look for any other underlying issues to obtain positive results. Underlying issues like the tubal and peritoneal microenvironments are known to influence fertilization and early embryonic development. Elevated concentrations of ROS in these environments may have detrimental effects on gamete and embryos both in the fallopian tube and the peritoneal cavity (42). Özkaya et al showed that follicular fluid zinc and selenium, and serum zinc, copper, and selenium levels were lower in patients with IVF than in control. However, follicular fluid copper, selenium, and aluminum levels, and serum copper, zinc, selenium, aluminum, and magnesium levels were higher in IVF+ vitamin+ mineral group when compared to the IVF group (43). These minerals have also been reported to influence oocyte quality (4). In a study by Ismail et al, combined L-carnitine and clomiphene citrate significantly improved both ovulation and cumulative pregnancy rates in patients with clomiphene-resistant polycystic ovary syndrome (44). Thus, carnitines and other components in the supplement may be positively influencing the oocyte quality and other underlying factors that are yet unknown. This may be especially important in women with underlying metabolic disturbances and in their male partners with poor sperm when the improved oocyte quality may compensate for some of the dysfunctions in the poor-quality sperm.

Conclusion

The ICSI results showed that some parameters were significantly improved after oral supplementation of L-carnitine, acetyl-L-carnitine, N-acetyl cysteine and the nutrients during the two months preceding the ICSI cycle. This effect was especially evident in young women and normal responder. The treatment duration of two months was probably not sufficient to allow for a significant improvement in the ICSI results in women >35 years and in women from couples with female infertility. Significant improvements were noted in the number of mature oocytes, the type I embryos rate, the blastocyst rate and the type I blastocyst rate. In the poor responders, oral supplementation significant improved the type I blastocyst rate

hence the interest to culture up to the blastocyst stage after oral supplementation. Further studies are necessary to examine the role of supplementation of the couple in ICSI. Studies of longer duration and with larger and more homogeneous population size, examining role of specific nutrients in fertility would also be useful.

Acknowledgements

This work was carried out with the help of AlfaSigma laboratories.

Conflict of interests

Prof M.A.Virmani works with the AlfaSigma laboratories. The other two authors have no conflict of interest in this study.

References

1. Jeve YB, Potdar N, Blower JA, Gelbaya T. Strategies to improve fertilization rates with assisted conception: A systematic review. Hum Fertil (Camb). 2017 May 26:1-19.
2. Papaleo E, Potenza MT, Brigante C, De Michele E, Pellegrino M, Candiani M. Nutrients and infertility: An alternative perspective. Eur Rev Med Pharmacol Sci. 2011 May;15(5):515-517.
3. Gu L, Liu H, Gu X, Boots C, Moley KH, Wang Q. Metabolic control of oocyte development: linking maternal nutrition and reproductive outcomes. Cell Mol Life Sci. 2015 Jan;72(2):251-271.
4. Virmani MA, Zerelli S, Vitullo P, Cossetti C. Effect of nutrients on ovulation and oocytes quality in mice. Giorn It Ost Gin. 2015;37(4):212-214.
5. Virmani MA, Angeli A. Nutritional strategies to improve reproductive health and pregnancy. Vitafood. Palexpo, Geneva, Switzerland. 2015 May;5-7.
6. Genazzani AD, Despini G, Czyzyk A, Podfigurna A, Simoncini T, Meczekalski B. Modulatory effects of L-carnitine plus L-acetyl-carnitine on neuroendocrine control of hypothalamic functions in functional hypothalamic amenorrhea (FHA). Gynecol Endocrinol. 2017 Dec;33(12):963-967.
7. Schlegel PN, Girardi SK. Clinical review 87: In vitro fertilization for male factor infertility. J Clin Endocrinol Metab. 1997 Mar;82(3):709-716.
8. Mier-Cabrera J, Genera-Garcıa M, De la Jara-Dıaz J, Perichart-Perera O, Vadillo-Ortega F and Hernandez-Guerrero C. Effect of vitamins C and E supplementation on peripheral oxidative stress markers and pregnancy rate in women with endometriosis. Int J Gynaecol Obstet. 2008 Mar;100(3):252-256.
9. Henmi H, Endo T, Kitajima Y, et al. Effects of ascorbic acid supplementation on serum progesterone levels in patients with a luteal phase defect. Fertil Steril. 2003;(80):459-446.
10. Crha I, Hruba D, Ventruba P, et al. Ascorbic acid and infertility treatment. Cent Eur J Public Health. 2003;(11):63-67.
11. Kazemi A, Ramezanzadeh F, Nasr-Esfahani MH. The relations between dietary antioxidant vitamins intake and oxidative stress in follicular fluid and ART outcomes. Iran J Reprod Med. 2015 Sep;13(9):533-540.
12. Chen X, Chen Sl. Successful pregnancy in recurrent thin endometrium with new uses for an old drug. J IVF Reprod Med Genet. 2013;1:2.
13. Ledee-Bataille N, Olivennes F, Lefaix JL, et al. Combined treatment by pentoxifylline and tocopherol for recipient women with a thin endometrium enrolled in an oocyte donation programme. Hum Reprod. 2002;(17)1249-1253.
14. Babayev E, Seli E. Oocyte mitochondrial function and reproduction. Curr Opin Obstet Gynecol. 2015 Jun;27(3): 175-181.
15. Oktay K, Baltaci V, Sonmezer M, Turan V, Unsal E, Baltaci A, Aktuna S, Moy F. Oogonial precursor cell-derived autologous mitochondria injection to improve outcomes in women with multiple IVF failures due to low oocyte quality: A clinical translation. Reprod Sci. 2015 Dec;22(12):1612-1617.
16. Virmani A, Diedenhofen A, Zerelli S. Mitochondriotropic compounds in energy, oxidative stress and inflammation: Role in reproductive health, fertility and successful pregnancy. Giorn It Ost Gin. 2014;36(1):293-296.
17. Mansour G, Abdelrazik H, Sharma RK, Radwan E, Falcone T, Agarwal A. L carnitine supplementation reduces oocyte cytoskeleton damage and embryo apoptosis induced by incubation in peritoneal fluid from patients with endometriosis. Fertil Steril. 2009;(91):2079-2086.
18. Abdelrazik H, Sharma R, Mahfouz R, Agarwal A. L-carnitine decreases DNA damage and improves the in vitro blastocyst development rate in mouse embryos. Fertil Steril. 2009;(91)589-596.
19. Dunning KR, Akison LK, Russel DL, Norman RJ, Robker RL. Increased beta-oxidation and improved oocyte developmental competence in response to l-carnitine during ovarian in vitro follicle development in mice. Biol Reprod. 2011;(85):548-555.
20. Kay E and Brian D. In-vitro fertilization, Cambridge University Press; 2nd Edition. 2003.
21. Ferraretti AP, La Marca A, Fauser BCJM, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of “poor response” to ovarian stimulation for in vitro fertilization: The Bologna criteria, Human Reproduction. 2011;(26)7:1616-1162.
22. Patrizio P, Sakkas D. From oocyte to baby: A clinical evaluation of the biological efficiency of in vitro fertilization. Fertil Steril. 2009;91(4):1061-1066.
23. Wu YX, Liu JQ, An G, Liu HY. Influence of male age on the pregnancy outcomes of IVF. 2015;21(6):527-531.
24. Luddi A, Capaldo A, Focarelli R, Gori M, Morgante G, Piomboni P, De Leo V. Antioxidants reduce oxidative stress in follicular fluid of aged women undergoing IVF. Reprod Biol Endocrinol. 2016 Sep 7;14(1):57.
25. Belaisch J, Devaux A, Ayel JP, de Mouzon J. La femme de 40 ans et plus en FIV et en ICSI: données FIVNAT. Gynécologie Obstétrique & Fertilité. 2004;(39)730-736.

26. Rossin B, Pouly, JL, Belaisch-Allart J, de Mouzon J, La stimulation de l'ovulation pour fe'condation in vitro, en France: choix et résultats en fonction des protocoles et des gonadotrophines. Gynecologie Obstetrique & Fertilité. 2009;(37):864-872.
27. Ruder EH, Hartman TJ, Reindollar RH, and Goldman M.B. Female dietary antioxidant intake and time to pregnancy among couples treated for unexplained infertility. Fertil Steril. 2014;(101):759-766.
28. Polak G, Koziol-Montewka M, Gogacz M, Blaszkowska I, Kotarski J. Total antioxidant status of peritoneal fluid in infertile women. Eur J Obstet Gynecol Reprod Biol. 2001;(94):261-263.
29. Youssef MFM, Abdelmoty HI, Elashmwi H, Abduljawad EM, El Ghamary N, Magdy A, Mohsen MN, Abdellar MA, Abdel Bar M, Gouda H, Sayed Al AM, Raslan A, Youssef D, Sherif NA and Ismail AI. Oral antioxidants supplementation for women with unexplained infertility undergoing ICSI/IVF: Randomized controlled trial. Human Fertility. Early Online. 2014;1-5.
30. Miyamoto K, Sato EF, Kasahara E, Jikumaru M, Hiramoto K, Tabata H, Katsuragi M, Odo S, Utsumi K, Inoue M. Effect of oxidative stress during repeated ovulation on the structure and functions of the ovary, oocytes, and their mitochondria. Free Radic Biol Med. 2010;(49):674-681.
31. Abdelrazik H, et al. A L-carnitine decreases DNA damage and improves the in vitro blastocyst development rate in mouse. Fertil Steril. 2009 Feb;91(2):589-596. Doi: 10.1016/j.
32. Reader KL, et al. Effects of acetyl-L-carnitine on lamb oocyte blastocyst rate, ultrastructure, and mitochondrial DNA copy number. Theriogenology. 2015 Jun;83(9): 1484-1492.
33. You J, et al. L-Carnitine treatment during oocyte maturation improve in vitro development of cloned pig embryos by influencing intracellular glutathione synthesis and embryonic gene expression. Theriogenology. 2012 Jul 15;78(2):235-243.
34. Wu GQ, et al. L-Carnitine enhances oocyte maturation and development of parthenogenetic embryos in pigs. 2011 September 15;768(5):785-793.
35. Somfai T, et al. Enhancement of lipid metabolism with L-carnitine during in vitro maturation improves nuclear maturation and cleavage ability of follicular porcine oocytes. Reproduction, Fertility and Development. 2011;23 (7):912-920.
36. Fathi M, El-Shahat KH. L-Carnitine enhances oocyte maturation and improves in vitro development of embryos in dromedary camels (Camelus dromedaries). Theriogenology. 2017 Aug 4;104:18-22.
37. Zare Z, et al. Effect of L-carnitine supplementation on maturation and early embryo development of immature mouse oocytes selected by brilliant cresyle blue staining. J Assist Reprod Genet. 2015 Apr;32(4):635-643.
38. Nishihara T, Hashimoto S, Ito K, Nakaoka Y, Matsumoto K, Hosoi Y, and Morimoto Y. Oral melatonin supplementation improves oocyte and embryo quality, in women undergoing in vitro fertilization-embryo transfer. Gynecol Endocrinol. 2014;(30)5:359-362.
39. Caprio F, D'Eufemia MD, Trotta C, Campitiello MR, Ianniello R, Mele D, Colacurci N. Myo-inositol therapy for poor-responders during IVF: A prospective controlled observational trial, J Ovarian Res. 2015 Jun 12;8:37.
40. Gandini L, Lombardo F, Paoli D, Caponecchia L, Familiari G, Verlengia C, Dondero F, Lenzi A. Study of apoptotic DNA fragmentation in human spermatozoa. Hum Reprod. 2000;(15):830-839.
41. Lewin A, Lavon H. The effect of coenzyme Q10 on sperm motility and function. Mol Aspects Med. 1997;18(Suppl): 213-219.
42. Agarwal A, Durairajanayagam D and Plessis S.S. Utility of antioxidants during assisted reproductive techniques: an evidence based review. Reprod Biol Endocrinol. 2014 Nov 24;12:112. Doi: 10.1186/1477-7827-12-112.
43. Özkaya MO, Nazıroğlu M, Barak C, Berkkanoglu M. Effects of multivitamin/mineral supplementation on trace element levels in serum and follicular fluid of women undergoing in vitro fertilization (IVF). Biol Trace Elem Res. 2011;(139):1-9.
44. Ismail AM, Hamed AH, Saso S. Adding L-carnitine to clomiphene resistant PCOS women improves the quality of ovulation and the pregnancy rate. A randomized clinical trial. Eur J Obstet Gynecol Reprod Biol. 2014 Sep;180: 148-152.