DIFFERENCE OF ENDOMETRIAL THICKNESS AND VASCULARITY IN WOMEN STIMULATED BY CLOMIPHENE CITRATE WITH AND WITHOUT VITAMIN C AND E

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Abstract

Objective: To observe the effect of vitamin C and E administration on endometrial thickness and vascularity in clomiphene citrate stimulated endometrium
Method: Research design was randomized clinical trial. Both groups were stimulated with clomiphene citrate. Treatment group was given vitamin C and E, while control group was given placebo. Transvaginal ultrasound examination is used to assess endometrial thickness and vascularity (resistance index/RI) in cycle day 2 and 12. The difference between two groups is tested with independent t and Mann-Whitney test.
Result: A total of 38 subjects was included as research subjects. Endometrial thickness in treatment and control group have cycle day 12 means of ±8.89 and ±5.9 mm, respectively. In the first and latter group were found differences of 60.76% and 27.26%, respectively. Mann-Whitney test showed very significant difference $p=0.000$ ($p<0.05$) between endometrial thickness in treatment and control group. Endometrial vascularity between treatment and control group have cycle day 12 RI means of ±0.51 and ±0.52. In the first and latter group is found differences of -13.52% and -11.07%. Independent t test showed no significant difference p=(0.217) between treatment and control group.
Conclusion: Endometrium stimulated with clomiphene citrate and addition of vitamin C and E is thicker compared to control group. No significant difference on endometrial vascularity stimulated by clomiphene citrate with or without vitamin C and E.

Keywords: clomiphene citrate, endometrial receptivity, vitamin C, vitamin E

{**Citation:** Kurniawan E, Djuwantono T, Sabarudin U, Krisnadi SR, Permadi W, Madjid TH. Difference of endometrial thickness and vascularity in women stimulated by clomiphene citrate with and without vitamin C and E. American Journal of Research Communication, 2014, 2(10): 1-10} www.usa-journals.com, ISSN: 2325-4076.

Introduction

The number of infertility patients increasingly rising. Most are caused by ovulation disorders. Ovulation disorders occur in about 15% of couples infertility and become the cause of infertility in about 40% of women.[1] The main therapy for ovulation disorders are by giving antiestrogen clomiphene citrate (CC), but it has adverse effects on the growth of the endometrium. The endometrium is important to accommodate the conception. Many obstetricians provide therapeutic vitamin C and E in an attempt to reduce the side effects of the CC, but the basis of such therapy has not been widely discussed in the scientific thus giving vitamin C and E are still bias.

Several factors that affect pregnancy outcomes in patients with infertility is ovarian reserve, oogenesis pathology, and endometrial receptivity.[2] Endometrial receptivity quality has been studied in several studies investigating the thickness, pattern, volume, and vascularity of the endometrium. Endometrial thickness and vascularity are both needed to give pregnancy outcomes.[3] Good endometrial thickness ranged from 7–16 mm. Endometrial thickness <7 mm will reduce the likelihood of pregnancy with a pregnancy rate of 29.4%, compared with normal endometrial thickness was 35.8%.[4] In the study by Wang et al.[5], the role of endometrial vascularity (spiral arteries) on the pregnancy rate can be evaluated using non-invasive method that does not injure the endometrium, one of them with ultrasound.

Treatment of choice for induction of ovulation in anovulatory women with infertility is commonly with antiestrogen CC.[6] Clomiphene citrate stimulates ovulation by binding to estrogen receptors in various places around the reproductive organs and works as an estrogen agonist or antagonist.[7] There are differences in ovulation rate and pregnancy in women receiving CC, presumably due to prolonged antiestrogenic effect of CC on the receptivity of the endometrium, cervical mucus, uterine, oocyte cells and granulosa blood flow.[8] Clomiphene citrate will indirectly increase estradiol levels through the stimulation of the growth of multiple ovarian follicles. Estradiol induces peroxidase activity in endometrium.[9]

There are studies that suggest endometrial thickness is thinner in women taking CC compared with natural menstrual cycle, and this can affect the successful implantation.[10] Endometrial thickness was significantly thinner in the proliferative phase of CC-treated women than in control group, it was supported by some studies, but not in several studies.[11,12] One of the factors that affect the endometrial thickness and vascularity is estrogen that functions to promote angiogenesis in the endometrium by controlling the expression of vascular endothelial growth factor factor (VEGF).[13]

In a healthy body, reactive oxygen species (ROS) and antioxidants are in balance. When the balance is disrupted towards an excess of ROS, oxidative stress occurs.[14] There is a relationship between oxidative stress and cytokine receptor expression in vascular smooth muscle cells and endometrial cells.[15] ROS production associated with the presence of steroid hormones such as estradiol. Estradiol will increase 200-fold in peroxidase activity, principally due to the infiltration of eosinophils induced by estradiol. Production of hydrogen peroxide (H_2O_2) increased in vitro and in vivo by the presence of estrogen.[9]

Histochemical studies showed the production of H_2O_2 derived from superoxide anion (O_2•) dysmutation derived from the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH), which is located in the apical plasma membrane of endometrial epithelium. Hydrogen peroxide is probably involved in the formation of prostaglandins

bioreactivity.[16] Reactive oxygen species can cause tissue injury. Superoxide dismutase (SOD) works to protect the ROS recovery. Evidence suggests ROS and recovery system plays an important role in the reproductive function of a woman's uterus.[17] ROS are produced in the human endometrium.[18] Endometrium have copper-zinc SOD (Cu, Zn-SOD), located in the cytosol, and manganese SOD (Mn-SOD), located in mitocondria.[18,19] Both SOD enzymatic function as the first step to protect cell from toxic ROS. Reactive oxygen species increase in the secretion of the final phase of the endometrium, before menstruation, and decrease in the decidua in early pregnancy. SOD activity is lower in late secretion phase endometrium, where high activity is found in early pregnancy. These findings imply that SOD has an important role in the stability of the human endometrium and the accumulation of ROS are involved in the endometrium decay.[18]

The body has antioxidant system as a protection against excessive ROS production. When the balance between ROS and prooxidant protection mechanism shift toward excessive levels of ROS, then there will be damage by free radicals (oxidative stress) and lipid peroxidation are involved in the pathophysiology of various clinical circumstances.[20] Oxidative stress will cause damage and decay of the endometrial lining.[21]

The presence of antiestrogen such as CC will cause a decrease in cell viability and induces apoptosis.[22] Giving CC will trigger oxidative stress in the endometrium that interfere the growth and angiogenesis of the endometrium. Antioxidants can prevent and abate the effects of oxidative stress. In the study conducted by Cicek et al[23], it was found that administration of vitamin E improve endometrial growth and angiogenesis through antioxidant and anticoagulant mechanisms. According to a study conducted Al-Katib et al[24], vitamin C can improve endometrial growth through the mechanism of antioxidant and as a cofactor in the synthesis of collagen in the extracellular matrix luteal. Vitamin C and E as an antioxidant selected in this study because both have been long known as an antioxidant that is proven to be safe at a cheap price. This study selects a combination of antioxidant vitamins C and E because when combined will give potentiating effect due to the ability of vitamin C to improve the function of vitamin E (α-tocopherol) by providing an electron donor to vitamin E that will enhance the antioxidant effect. Research conducted by Mier-Cabrera et al[25] showed that vitamin E supplementation increases the growth of the glandular epithelium, blood vessel development and endometrial VEGF expression. It is expected that the provision of the antioxidant vitamins C and E to prevent oxidative stress, which in turn can improve the receptivity endometrium.[22]

Research Methods

This research is an experimental study with a randomized controlled clinical trial design with a double blinded, parallel design. The subjects were all women of reproductive age with primary infertility, wants pregnancy, willing to basic infertility examination. Inclusion criteria were:

1. Age 20-35 years
2. BMI between 18.5-24.9 kg/m^2

3. Ovarian volume <10 cm^3
4. Endometrial thickness on transvaginal ultrasound examination day 2 <6 mm.

Every woman who met the inclusion criteria and are not included in the exclusion criteria, get a detailed explanation of the research procedures and voluntarily signed a waiver willing to participate in the study. Subjects were divided 2 groups and randomization block to make each group had a comparable number of subjects.

Data analysis was performed with the independent t test, if the data are not normally distributed then Mann-Whitney test is used. Data analysis was performed using SPSS for Windows version 18.0 at the 95% confidence level with a value p≤0.05.

Results

This research was conducted during February-May 2014. During the period obtained 38 research subjects who met the inclusion criteria consisted of 19 subjects of research for each research group stimulated with CC were given vitamin C + E and the control group. The study subjects were taken from patients came to Aster Assisted Reproductive Technology Clinic. From the results obtained two groups of age, BMI, number of basal follicles, endometrial thickness and vascularity (RI). All subjects performed the study treatment as specified

Table 1 Subject Characteristics

Characteristic	Treatment				p value*
	CC stimulation + Vit C+E		CC stimulation		
	n=19	(%)	n=19	(%)	
Age (years)					
20–25	4	21.1	3	10.5	**0.239**
26–30	5	26.3	10	52.6	
31–35	10	52.6	7	36.8	
Mean (SD)	29 (3.7)		30 (3.2)		
Median	31		30		
Range	23–35		23–35		
Follicles					
<6	0	0	2	10.5	**0.146**
>6–20	19	100	17	89.5	
Mean (SD)	9 (1.9)		9 (3.0)		
Median	9.0		10.0		
Range	6–12		5–15		
BMI					
<18,5	2	10.5	4	21.1	**0.513**
18,5–24,9	13	68.4	13	68.4	
>24,9	4	21.1	2	10.5	
Mean (SD)	22 (2.68)		21 (2.84)		
Median	22.83		23.04		
Range	17–26		17–25		

* Chi square test

No significant difference for age range of both groups with p=0.239 (p>0.05), number of basal follicles of both groups was p=0.146 (p>0.05) and the BMI range of both research groups p=0.513 (p>0.05) (Table 1).

Table 2 Comparison of Endometrial Thickness Between Treatment and Control Groups

	CC Stimulation + Vit C+E	CC Stimulation	p value*
Day 2			
Mean (SD)	5.5 (0.93)	4.89 (1.39)	
Median	5.9	5.7	
Range	2.9–6.3	2.0–6.2	
Day 12			
Mean (SD)	8.89 (1.77)	5.9 (1.87)	
Median	9.5	5.4	
Range	4.1–10.4	3.2–10.3	
% thickness increase	60.76%	27.26%	0.000

*** Mann-Whitney test**

The mean endometrial thickness on CC stimulation cycle with Vitamin C+E is thicker on the 12th day of menstruation. The treatment group had a mean endometrial thickness ±8.89 mm, whereas the control group had a mean endometrial thickness of ±5.9 mm.

In the treatment group obtained endometrial thickness increased by 60.76% and the control group obtained endometrial thickness increased by 27.26%. There is a difference of 33.5% between the treatment group and the control group. Mann-Whitney test proves there is a very significant difference, p=0.000 (p<0.05) between treatment and control groups. (Table 2).

Table 3 Comparison of Endometrial Vascularity Between Treatment and Control Group (RI)

	CC Stimulation + Vit C+E	CC Stimulation	p value*
Day 2			
Mean (SD)	0.59 (0.04)	0.59 (0.05)	
Median	0.60	0.94	
Range	0.5–1.68	0.8–1.68	
Day 12			
Mean (SD)	0.51 (0.04)	0.52 (0.03)	
Median	0.50	0.52	
Range	0.40–0.60	0.49–0.60	
RI Difference	0.08	0.07	0.309

*** Independence t test**

The mean RI of endometrial vascularity in CC stimulation cycle with vitamin C+E is lower on the 12th day of menstruation. The treatment group had a mean RI ±0.51, whereas the

control group had a mean RI of ±0.52. In the treatment group difference of 0.08 is obtained and the control group gained 0.07 difference. There is a difference of 0.01 between the treatment group and the control group. From the results of independent t test shows no significant difference with p=0.309 (p>0.05) (Table 3).

Discussion

Characteristics of Research Subjects

In this study, the characteristics of the subjects compared consisted of 19 patients were measured age, BMI and the number of basal follicles. The mean age was 29 years in treatment group and the control group was 30 years. The mean number of follicles in treatment group and the control group were 9 units. The mean BMI was 22 in the treatment group and the control group is 21. The two groups was not found significant differences in the test of normality, so that research results can be compared (Table 1).

Endometrial thickness relationship on Clomiphene Citrate Stimulation with or without Vitamin C+E

Endometrial thickness in stimulated CC group with vitamin C+E is thicker than CC alone stimulated group. Clomiphene citrate alone stimulated group had thinner endometrial thickness even some samples showed almost no increase in endometrial thickness. This is consistent with the study conducted by Hayon et al.[22] stating antiestrogen (CC) will decrease cell viability and induce apoptosis, then Amita et al[26] study the molecular mechanisms that states CC inhibiting endometrial epithelial cell proliferation is influenced by antiestrogenic effect of CC on coregulator estrogen receptor α inhibits steroid receptor coactivator-1 in endometrial epithelial cells. One of the alleged causes of thin endometrium in stimulated cycles of CC is due to oxidative stress in vascular smooth muscle cells and endometrial cells. Several other studies also obtain the thickness of the endometrium of women who get CC is thinner than women with menstrual cycle alami.[27]

This study shows that there is a significant increase in endometrial thickness between the 2nd and the 12th day of menstruation. This result are consistent with research conducted by Cicek et al[23] stating vitamin E improves endometrial growth and angiogenesis through mechanisms of antioxidant and anticoagulant, Al-Katib et al[24] stated vitamin C improve endometrial growth through the mechanism of antioxidant and as a cofactor in the synthesis of collagen in the extracellular matrix luteal and Mier-Cabrera et al[25] which states vitamin E increases the growth of the glandular epithelium, blood vessel development and endometrial VEGF expression. Afolabi stated that vitamin C+E repair cell membranes through SOD and also improve endothelial capillary.[28]

Endometrial thickness of the treatment group had a mean of ±8.89 mm and in the control group ±5.9 mm. According to Dickey et al[29], chances of pregnancy will increase when the thickness of the endometrium 9–10 mm or more and no pregnancy occurs when the

endometrial thickness is only 6–8 mm or less before implantation.[30] Clomiphene citrate rate is 60-85%. Thicker endometrial thickness may increase the pregnancy rates of patients who were stimulated by 30-40% .[31] Clomiphene citrate results endometrial thickness thicker allegedly due to oxidative stress caused by estrogen antagonist effects can be combated by antioxidants. Oxidative stress occurs as a result of high levels of estradiol by multifollicles stimulation. Oxidative stress causes damage to cell macromolecules including proteins and DNA that disrupts the endometrium growth.[32] This is according to a study by Amita et al[26] investigating the molecular mechanisms of CC inhibits endometrial epithelial cell proliferation were affected estradiol. Kurosawa et al[33] also reported CC will stimulate estrogen antagonist effects via the estrogen receptor α when the body is in high estradiol levels. High estradiol levels are also caused by the decrease in SHBG capacity due to the CC provision.[34] Sex hormone binding globulin binds free estradiol and maintain free estradiol levels in the control of blood. Decreased SHBG will increase levels of free estradiol in the blood. Vitamin E (tocopherol) may increase serum SHBG levels resulting in lower levels of free estradiol, which in turn reduces the effects of estrogen antagonists indirectly.[35] Vitamin E repair tissue growth and endometrial vascularity therefore angiogenesis and endometrial growth defect can be repaired. Pregnancy rate is only 30-40% with CC stimulation, so that if there is no pregnancy, suspicion arises when stimulation CC made back then would give similar results, so the next option is the stimulation by aromatase inhibitors or gonadotropin.[27] Fecundability of gonadotropin-stimulated cycles is 14%, more than double that is observed in CC-stimulated cycles (6%).[36]

Endometrial Vascularity Relationship on Clomiphene Citrate Stimulation With and Without Vitamin C+E

Resistance index treatment group obtained slightly lower compared with the control group, but with no significant difference. This is consistent with the study conducted by Banerjee et al[15] stating oxidative stress will disrupt vascular smooth muscle cells and endometrial cells, endothelial damage or endometrial decidual cells that causes damage to the molecules in the layer endometrium. Estrogen promotes angiogenesis in the endometrium by controlling the expression factors such as VEGF.[13] Clomiphene citrate disturbs proliferation of endometrial epithelial cells were affected by antiestrogenic effects CC E2 on estrogen receptor α coregulator steroid receptor that inhibits coactivator-1 on epithelial cells steroid receptor coactivator endometrium.[26] Capillary endothelial damage will cause damage to the cell membrane so the RI increase.[37,38] Mier-Cabrera et al[25] stated vitamin E increases the growth of the glandular epithelium, blood vessel development and endometrial VEGF expression. Vitamin E inhibits lipid peroxidation function in cell and membranes organelle, lipoproteins and other tissue.[39] Addition of vitamin E also prevents platelet adhesion in blood vessels that can decrease RI in various organs target.[28]

This study shows there is an increase in vascularity of the endometrium between the 2nd day and 12th day of CC stimulation performed with vitamins C+E compared with those not given vitamin C+E, but not significantly. Significance difference between the studies with other possible variations due to the individual response to the effects of CC as expressed by

Yuval et al.[3] Rostami, Contart, Schild and Zaidi et al[40-43] found no significant difference in RI in pregnant and non-pregnant group.

Conclusion

CC-stimulated endometrial thickness with vitamins C+E are thicker than without vitamin C+E (33.5%). CC-stimulated endometrial vascularity with vitamins C+E are not better than without vitamin C+E (0.01).

References

1. Infertility Examination. Infertility Management Consensus: HIFERI; 2013. p. 18.
2. Kupesic S, Kurjak A. Predictors of IVF outcome by threedimensional ultrasound. Hum Reprod. 2002;17(4):950–5.
3. Yuval Y, Lipitz S, Dor J, Achiron R. The relationships between endometrial thickness, and blood flow and pregnancy rates in in-vitro fertilization. Hum Reprod. 1999;14(4):1067–71.
4. Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, Awartani K. The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer (IVF-ET) outcome. Reprod Biol Endocrinol. 2008;6(1):37.
5. Wang L, Qiao J, Li R, Zhen X, Liu ZH. Role of endometrial blood flow assessment with color Doppler energy in predicting pregnancy outcome of IVF-ET cycles. Reprod Biol Endocrinol. 2010;18(8):122.
6. Miywally MFM, Caper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. Fertil Steril. 2001;75(2):305–9.
7. Adashi EY. Ovulation induction: clomiphene citrate. In: Adashi EY, Rock JA, Rosenwaks Z, editor. Reproductive endocrinology, surgery and technology. Philadelphia: Lippincott-Raven; 1996. p. 1182–206.
8. Gerli S, Gholami H, Manna A, Scotto Di Prega A, Vitiello C, Unfer V. Use of ethinyl estradiol to reverse the antiestrogenic effects of clomiphene citrate in patients undergoing intrauterine insemination: a comparative randomized study. Fertil Steril. 2000;73(1):85–9.
9. Santanam N, Shern-Brewer R, McClatchey R, Castellano PZ, Murphy AA, Voelkel S, et al. Estradiol as an antioxidant: incompatible with its physiological concentrations and function. J Lipid Res. 1998;39(11):2111-8.
10. Nakamura Y, Ono M, Yoshida Y, Sugino N, Ueda K, Kato H. Effects of clomiphene citrate on the endometrial thickness and echogenic pattern of the endometrium. Fertil Steril. 1997;67(2):256–9.
11. Sereepapong W, Suwajanakorn S, Trivatanachat S, Sampatanakul P, Pruksananonda R, Boonk Asemsanti W, et al. Effect of clomiphene citrate on the endometrium of regularly cycling women. Fertil Steril. 2000;73(2):287–91.
12. Dehbashi S, Parsanezhad ME, Alborzi S, Zarei A. Effect of clomiphene citrate on endometrium thickness and echogenic patterns. Int J Gynecol Obstet. 2003;80(1):49–53.

13. Albrecht ED, Babischkin JS, Lidor Y, Anderson LD, Udoff LC, Pepe GJ. Effect of estrogen on angiogenesis in co-cultures of human endometrial cells and microvascular endothelial cells. Hum Reprod. 2003;18(10):2039–47.
14. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2005;3:28.
15. Banerjee S, Smallwood A, Moorhead J, Chambers AE, Papageorghiou A, Campbell S, et al. Placental expression of interferongamma (IFN-gamma) and its receptor (IFN-gamma) R2 fail to switch from early hypoxic to late normotensive development in pre-eclampsia. J Clin Endocrinol Metab. 2005;90(2):944–52.
16. Lamirande E, Gagnon C. Reactive oxygen species (ROS) and reproduction. In: Armstrong D, editor. Free radicals in diagnostic medicine. New York: Plenum Press; 1994. p. 185.
17. Suzuki T, Sugino N, Fukaya T, Sugiyama S, Uda T, Takaya R, et al. Superoxide dismutase in normal cycling human ovaries: immunohistochemical localization and characterization. Fertil Steril. 1999;72(4):720–6.
18. Sugino N, Shimamura K, Takiguchi S, Tamura H, Ono M, Nakata M, et al. Changes in activity of superoxide dismutase in the human endometrium throughout the menstrual cycle and in early pregnancy. Hum Reprod. 1996;11(5):1073–8.
19. Telfer JF, Thomson AJ, Cameron IT, Greer IA, Norman JE. Expression of superoxide dismutase and xanthine oxidase in myometrium, fetal membranes and placenta during normal human pregnancy and parturition. Hum Reprod. 1997;12(10):2306–12.
20. Pohanka M, Bandouchova H, Sobotka J, Sedlackova J, Soukupova I, Pikula J. Ferric reducing antioxidant power and square wave voltametry for assay of low molecular weight antioxidants in blood plasma : performance and comparison of methods. Sensors. 2009;9(11):9094–103.
21. Methods for detection of ROS. In: Agarwal A, Aziz N, Rizk B, editors. Studies on women's health. 1 ed: Springer; 2012. p. 48.
22. Hayon T, Dvilansky A, Oriev L, Nathan I. Non-steroidal antiestrogens induce apoptosis in HL60 and MOLT3 leukemic cells; involvement of reactive oxygen radicals and protein kinase C. Anticancer research. 1999;19(3A):2089–93.
23. Cicek N, Eryilmaz OG, Sarikaya E, Gulerman C, Genc Y. Vitamin E effect on controlled ovarian stimulation of unexplained infertile women. Journal of assisted reproduction and genetics. 2012;29(4):325–8.
24. Al-Katib SR, Al-Kaabi MMH, Al-Jashamy KA. Effects of vitamin C on the endometrial thickness and ovarian hormones of progesterone and estrogen in married and unmarried women. AJCR. 2013;1(8):24–31.
25. Mier-Cabrera J, Genera-Garcia M, De la Jara-Diaz J, Perichart-Perera O, Vadillo-Ortega F, Hernandez-Guerrero C. Effect of vitamins C and E supplementation on peripheral oxidative stress markers and pregnancy rate in women with endometriosis. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2008;100(3):252–6.
26. Amita M, Takahashi T, Tsutsumi S, Ohta T, Takata K, Henmi N, et al. Molecular mechanism of the inhibition of estradiol-induced endometrial epithelial cell proliferation by clomiphene citrate. Endocrinology. 2010;151(1):394–405.
27. Mitwally MF, Casper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. Fertil Steril. 2001;75(2):305–9.
28. Afolabi AO, Olotu OO, Alagbonsi IA. Vitamins E and C alleviate the germ cell loss and oxidative stress in crytorchidism when administered separately but not when combined in rats. ISRN Pharmacol. 2012;2012(843569):1–8.

29. Dickey RP, Olar TT, Taylor SN, Curole DN, Matulich EM. Relationship of endometrial thickness and pattern to fecundity in ovulation induction cycles: effect of clomiphene citrate alone and with human menopausal gonadotropin. Fertil Steril. 1993;59(4):756–60.
30. Isaacs JD Jr., Wells CS, Williams DB, Odem RR, Gast MJ, Strickler RC. Endometrial thickness is a valid monitoring parameter in cycles of ovulation induction with menotropins alone. Fertil Steril. 1996;65(2):262–6.
31. Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. Hum Reprod Update. 1997;3(4):359–65.
32. Kobiela J, Stefaniak T, Krajewski J, Kalinska-Blach B, Zurawa-Janicka D, Lachinski A, et al. Dynamics of estrogen-induced oxidative stress. Acta biochimica Polonica. 2007;54(2):289–95.
33. Kurosawa T, Hiroi H, Momoeda M, Inoue S, Taketani Y. Clomiphene citrate elicits estrogen agonistic/antagonistic effects differentially via estrogen receptors alpha and beta. Endocrine journal. 2010;57(6):517–21.
34. Lobo RA, Gysler M, March CM, Goebelsmann U, Mishell DR, Jr. Clinical and laboratory predictors of clomiphene response. Fertil Steril. 1982;37(2):168–74.
35. Nayeem F, Nagamani M, Anderson KE, Huang Y, Grady JJ, Lu LJ. Dietary beta-tocopherol and linoleic acid, serum insulin, and waist circumference predict circulating sex hormone-binding globulin in premenopausal women. The Journal of nutrition. 2009;139(6):1135–42.
36. Male infertility. In: Fritz MA, Speroff L, editors. Clinical gynecologic endocrinology and infertility. 8 ed. Philadelphia: Lippincott Williams & Wilkins; 2011. p. 1281–4.
37. Laing FC, Frates MC, Benson CB. Ultrasound evaluation during the first trimester of pregnancy. In: Callen PW, editor. Ultrasonography in obstetric and gynecology. Philadelphia: Saunders; 2008. p. 181–224.
38. Jauniaux E, Johns J, Burton J. The role of ultrasound imaging in diagnosing and investigating early pregnancy failure. Ultrasound Obstet Gynecol. 2005;25(6):613–24.
39. Kammerer C, Getoff N. Synergistic effect of dehydroascorbic acid and mixtures with vitamin E and B-carotene on mitomycin C efficiency under irradiation in vitro. In Vivo. 2004;18(6):795–8.
40. Rostami-Hodjegan A, Lennard MS, Tucker GT, Ledger WL. Monitoring plasma concentrations to individualize treatment with clomiphene citrate. Fertil Steril. 2004;81(5):1187–93.
41. Contart P, Baruffi RL, Coelho J, Mauri AL, Petersen C, Franco Junior JG. Power Doppler endometrial evaluation as a method for the prognosis of embryo implantation in an ICSI program. Journal of assisted reproduction and genetics. 2000;17(6):329–34.
42. Schild RL, Knobloch C, Dorn C, Fimmers R, van der Ven H, Hansmann M. Endometrial receptivity in an in vitro fertilization program as assessed by spiral artery blood flow, endometrial thickness, endometrial volume, and uterine artery blood flow. Fertil Steril. 2001;75(2):361–6.
43. Zaidi J, Campbell S, Pittrof R, Tan SL. Endometrial thickness, morphology, vascular penetration and velocimetry in predicting implantation in an in vitro fertilization program. Ultrasound Obstet Gynecol. 1995;6(3):191–8.