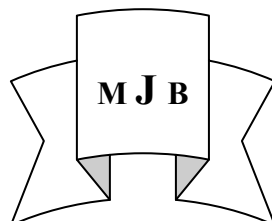


The Effect of Recurrent Use of Clomiphene Citrate in the Treatment of Infertility on the Level of Serum Total Protein, Total Cholesterol and Triglyceride

Buthena Abass Frhan

Dept. of Biochemistry, College of Medicine, University of Al-Qadisiah, Iraq.



Abstract

For the last forty years, the first line of treatment for anovulation in infertile women has been clomiphene citrate; the choice of clomiphene was appropriate because the drug was highly effective in inducing ovulation in selected patients with the advantage of being orally administered, relatively safe and inexpensive. In contrast alternative treatment usually involved parenteral gonadotropins that were significantly more complicated and uncomfortable to administer. However clomiphene was also found to have adverse effects, especially in the form of common antiestrogenic, endometrial and cervical mucous changes. *The aim of this study* is to identify whether there is a relation between recurrent administration of clomiphene (as ovulation induction) and serum concentration of total protein, total cholesterol and triglyceride

Thirty women were involved in this study, twenty with infertility and ten as control group. The twenty infertile female patients undergo ovulation induction by clomiphene citrate as treatment to their condition.

The results show a significant difference in the serum level of total protein, total cholesterol and triglyceride between infertile patients (primary and secondary) and control group in the third month of clomiphene treatment. The total protein was significantly higher in the control group than infertile patients; this is the same for total cholesterol while for triglyceride it was significantly higher in infertile patients than in the control group.

الخلاصة

يعتبر الكلوميديد اول خط في علاج حالات العقم الناتجة من قلة الاباضه لفعاليته العاليه في تحفيز الاباضه وسهولة استخدامه وتوفره لكن الكلوميديد لا يعتبر البرنامج الوحيد فهناك العديد من برامج التحفيز ولكنها اكثر تعقيدا . كان الهدف من الدراسة هو معرفة التأثيرات الناتجة من الاستخدام المتكرر لبرنامج التحفيز على الحالة البنائية العامة ممثلة بنسب البروتين والدهون نتيجة للاختلال بمستوى الهرمونات التناسلية.

أشتركت في هذا البحث ثلاثون امرأة ،عشرون يعانين من حالة العقم وعشر في حاله صحيه طبيعيه تم استخدام الكلوميديد للتحفيز ، أوضحت النتائج أختلاف بنسب البروتين و الدهون لدى المريضات بالعقم عن النساء غير المريضات وبدلاله أحصائيه معنويه (٠,٠٥) حيث كان البروتين عاليا لدى النساء غير المريضات وكذلك بالنسبه للكلوليستيرول ولكن نسبة الكليسيرين كانت عاليه لدى المريضات وبدلاله أحصائيه معنويه (٠,٠٥).

Introduction

The ovarian cortex at puberty contains hundreds of thousands of primordial follicles[1]. In response to unknown signal, independent of gonadotropins, a hundreds of primordial follicles are recruited to grow[2]. During this early follicle development, the oocyte

enlarges and the granulosa cells proliferate to form a preantral follicle. Over 3-6 months, the follicle develops follicles stimulating hormone receptors in the granulosa cells and luteinizing hormone receptors in the theca cells, and the follicle forms a fluid filled space called an antrum[1]. At this stage, antral follicles become acutely

dependent on follicle stimulating hormone for further development [1]. In natural cycle just before menses, falling estrogen levels result in withdrawal of negative feedback centrally leading to increased gonadotropins levels [3]. Follicle stimulating hormone stimulates granulosa cell proliferation and differentiation, with the development of more follicle stimulating hormone receptors and production of aromatase [4]. Luteinizing hormone stimulates androstenedione production by theca cells that diffuses into the granulosa cell providing substrate for estrogen secretion[5]. Follicle stimulating hormone concentration must exceed a certain level (FSH threshold) before follicular development will proceed[6]. The duration of this period in which the threshold is exceeded (the FSH window) is limited in the normal cycle by a gradual decrease in follicle stimulating hormone (FSH) occurring in the early midfollicular phase as a response to negative feedback mechanism from rising estrogen levels produced by the larger follicles[7]. Smaller follicles, with fewer FSH receptors, are no longer stimulated to grow by FSH levels below the FSH threshold and undergo atresia. Therefore, generally only one follicle reaches the stage of ovulation each cycle[8].

Administration of exogenous FSH prolongs the time FSH levels are above the FSH threshold and extends the FSH window. This allows multiple ovulation by rescuing smaller follicles that would otherwise have undergone atresia[9]. This will lead to increasing levels of estradiol produced by the mature preovulatory follicle[10]. This sequence of events is orchestrated by the interaction of local ovarian factors and endocrine factors from the pituitary and hypothalamus. The World Health Organization (WHO) classify

the anovulation in to three group(11). Women in WHO group II are not estrogen deficient, their FSH and prolactin levels are normal[11]. They typically experience oligomenorrhea, but they may have anovulatory cycles or amenorrhea with bleeding in response to progestin challenge. This is the most common type of anovulation and includes women with polycystic ovary syndrome(PCOS)(12). For this group, oral agents such as clomiphene citrate are useful for ovulation induction[13]. Chemically clomiphene citrate (CC) is nonsteroidal triphenylethylene derivative that exhibits both estrogen agonist and antagonist properties, that is to say selective estrogen receptor modulating activity[14]. *The mechanism of action of CC* binds to estrogen receptors(ERs) throughout the body due to it is structural similarity to estrogen. CC binding to ERs occur for an extended period of time, weeks rather than hours as with natural estrogen. Such extended binding ultimately depletes ER concentrations by interfering with the normal process of ER replenishment [15]. The antiestrogenic effect on the hypothalamus, and the pituitary, is believed to be the main mechanism of action for ovarian stimulation. Depletion of hypothalamic ERs prevents correct interpretation of circulating estrogen levels; estrogen concentrations are falsely perceived as low leading to reduced estrogen-negative feedback on gonadotropins releasing hormone (GnRH) production and subsequent increased gonadotropins follicle stimulating hormone (FSH) & luteinizing hormone (LH) secretion[16]. The rise of FSH promotes growth of ovarian follicles and ovulation in anovulatory women. It is believed that the hypothalamus is the main site of action because in normally ovulatory women, CC treatment was found to increase GnRH

pulse frequency[17]. However, actions at the pituitary level may also be involved because CC treatment increased pulse amplitude but not frequency in anovulatory women with PCOS in whom the GnRH pulse frequency is already abnormally high[18]. During CC treatment, levels of both LH and FSH undergo a prolonged rise, compared with a natural cycle, because of the prolonged ER depletion in the brain[19].

Supraphysiological levels of estrogen can occur without central suppression of FSH because the normal ER mediated feedback mechanism is blocked as a result of prolonged ER depletion, this perturbation of normal negative feedback is reflected in the FSH window being extended, leading to multiple follicle growth and a higher levels of estrogen hormone [20,21].

Subjects and Methods

Thirty women were involved in this study. Their age ranged from 23 to 33 years, their weight range between 70-75 Kg, 10 female from the thirty were with primary infertility, the other 10 were with secondary infertility and the last 10 were control group.

Two blood samples were taken each month for three months the first blood sample was taken in the second day of the cycle to measure the concentration of estrogen hormone, the second blood sample were taken in the tenth day of the cycle to measure estrogen concentration, serum total protein, serum total cholesterol and triglyceride.

Enzyme linked fluorescent assay techniques were used to determine serum concentration of estrogen hormone(Biomerieux kit,France). In this study the minividas instrument was used in the assay. it is principle is to combines an enzyme immunoassay

sandwich method with a final fluorescent detection.

Serum total cholesterol concentration was measured by enzyme colorimetric testing (Biomerieux Kit, France) and serum triglyceride (TGL) was measured by enzyme colorimetric testing Kit (Biomerieux Kit, France). Serum total protein was also measured by colorimetric biuret method (Spinreact Girona Spain Kit).

Ovarian stimulation was induced by clomiphene citrate (clomid, Merrel company, England) 100 mg/day starting from cycle day 2 and for 5 days given to the infertile group (primary and secondary). Comparisons between groups are assessed using student (t-test), the results were considered significant when ($P<0,05$).

Results

The serum concentration of estrogen hormone in the first month in infertile women was not significantly different from the control group when measured in the second day of the cycle as shown in fig (1). This is true also for the second month of treatment fig (2), while in the third month the control group shows a significantly higher ($P<0.05$) estrogen hormone level than women with primary and secondary infertility fig(3).

The serum concentration of estrogen hormone in the tenth day of the cycle shows a significant rise ($P<0.05$) in the infertility groups than control group as expected due to the induction treatment by clomiphene citrate in the first, second and third month as shown in table (1).

The measurement of the serum concentration of total protein in the first month of treatment shows no significant differ between infertile groups and control group fig(4), similar results in the second month fig (5), while in the third month there was

a significant higher level of total protein in the control group ($P<0.05$) than infertile groups fig (6) as shown in table (2).

Serum total cholesterol concentration was not significantly differ between control and infertile groups (primary and secondary) in first and second month of treatment fig (7) and fig (8). While in the third month it was significantly higher in the control

group ($P<0.05$)fig (9) also shown in table (2).

Serum concentration of triglyceride again showed no significant difference between infertile and control group in the first and second month fig (10) and (11) respectively, while in the third month it was significantly higher in the infertile patients more than control group fig (12) as shown also in table (2).

Table 1 Shows the(mean $\pm$ SD) of estrogen hormone concentration in the three month after treatment for the three groups

	Serum estrogen level in control group	Serum estrogen level in primary infertility group	Serum estrogen level in secondary infertility group	$P < 0.05$
First month of treatment	241.7 $\pm$ 40.7	341.9 $\pm$ 14.5	342.5 $\pm$ 9.9	S
Second month of treatment	226.6 $\pm$ 10.7	368 $\pm$ 6.7	369 $\pm$ 4.1	S
Third month of treatment	224.4 $\pm$ 12.7	446.6 $\pm$ 20.2	437.9 $\pm$ 17.4	S

Table 2 Shows the (mean $\pm$ SD) of serum total protein, triglyceride and total cholesterol in the three month of treatment in the Three groups

	Primary infertility			Secondary infertility			Control group		
	Total protein	triglyceride	total cholesterol	Total protein	triglyceride	total cholesterol	Total protein	triglyceride	total cholesterol
First month	7.34 $\pm$ 0.5	117.9 $\pm$ 5.3	181.1 $\pm$ 6.5	7.16 $\pm$ 0.45	124.5 $\pm$ 3.5	1186.6 $\pm$ 5.2	6.98 $\pm$ 0.48	118.8 $\pm$ 8.1	175.5 $\pm$ 10.4
Second month	6.77 $\pm$ 0.37	162.5 $\pm$ 15.4	169.3 $\pm$ 7.2	7 $\pm$ 0.49	174.3 $\pm$ 8.7	168.7 $\pm$ 7	6.9 $\pm$ 0.48	118.1 $\pm$ 8.3	176.5 $\pm$ 10
Third month	4.87 $\pm$ 0.34	218.3 $\pm$ 9.8	117.5 $\pm$ 10.2	4.14 $\pm$ 0.47	245.3 $\pm$ 16.8	124.3 $\pm$ 7.2	6.79 $\pm$ 0.41	119 $\pm$ 7.2	178.7 $\pm$ 9.9

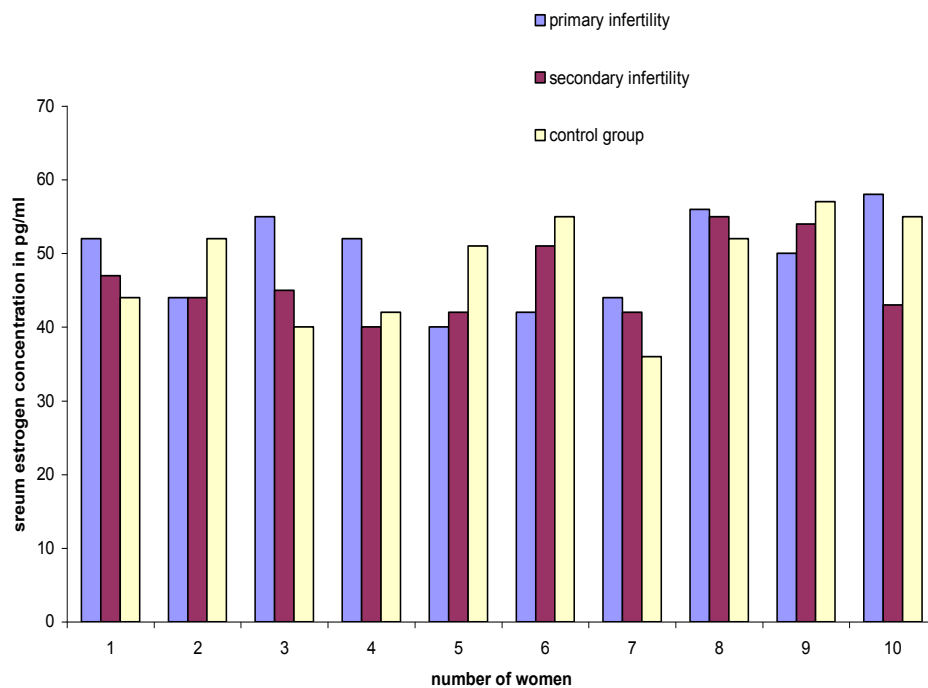


Figure 1 Show the level of serum estrogen in the first month of treatment second day of cycle no significant difference between control and infertility group.

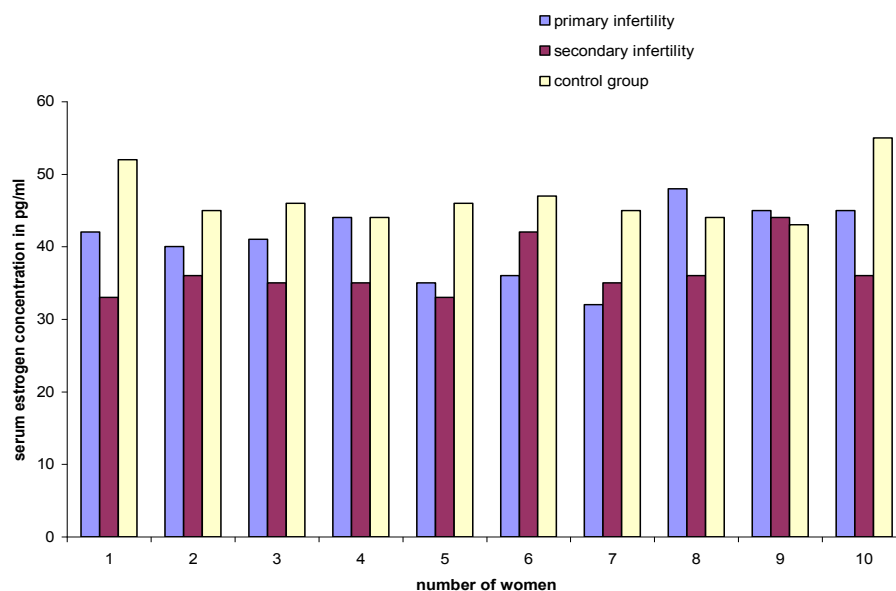


Figure 2 Show the level of serum estrogen in the second month of treatment second day of cycle no significant difference between control and infertility groups.

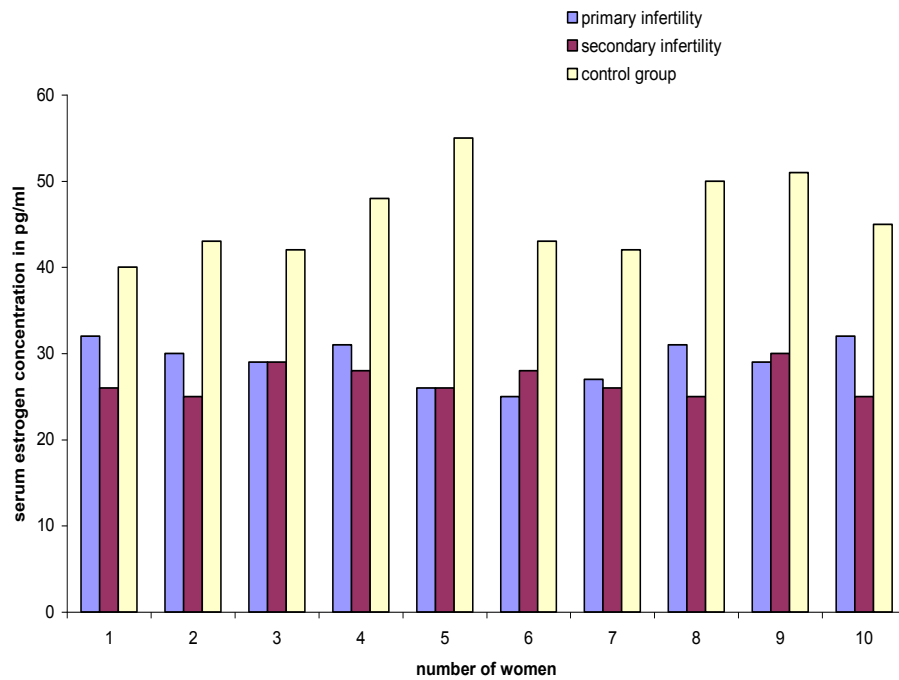


Figure 3 Show the level of serum estrogen in the third month of treatment second day of the cycle it was significantly higher in control group than infertility groups.

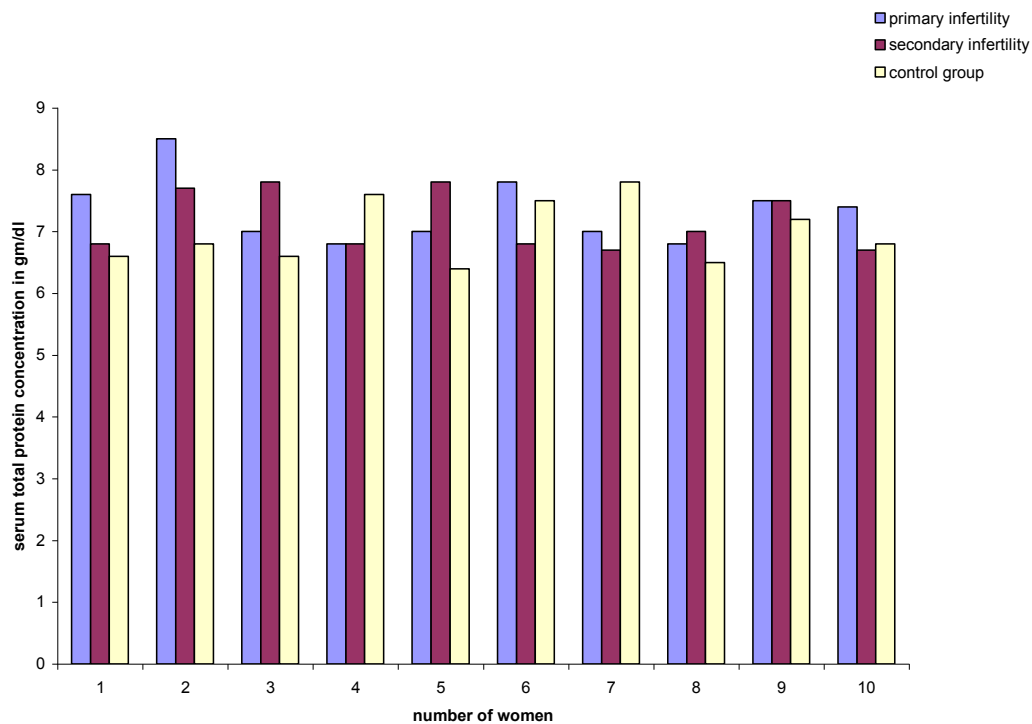


Figure 4 Show the level of serum total protein in the first month of treatment no significant difference between control and infertility groups.

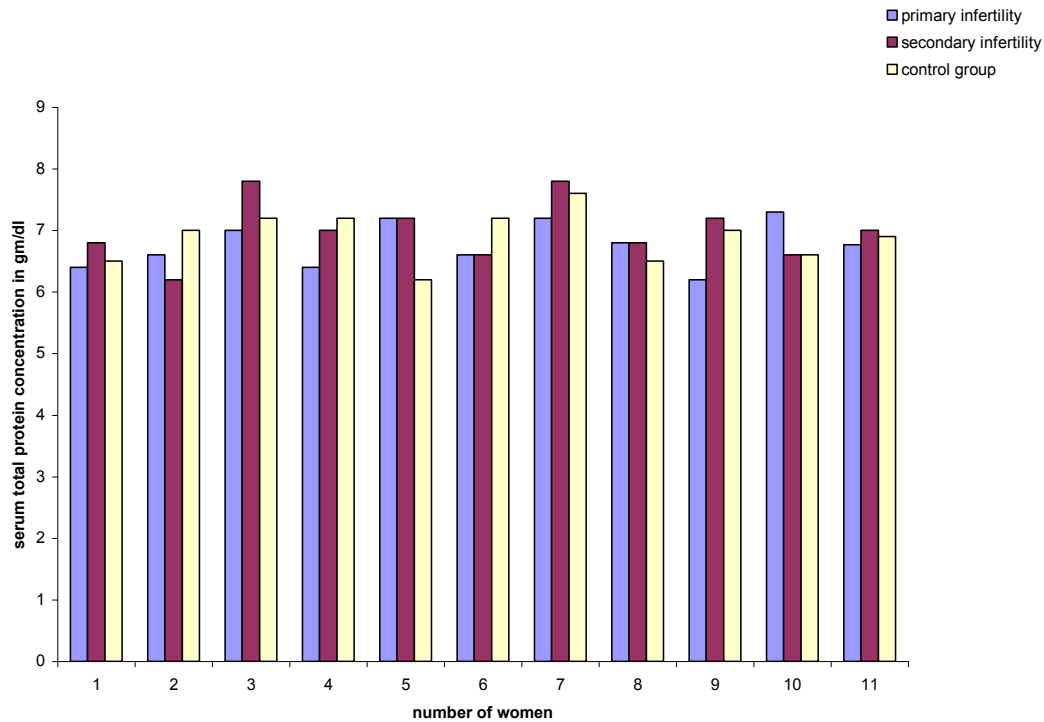


Figure 5 Show the level of serum total protein in the second month of treatment no significant difference between control and infertility groups.

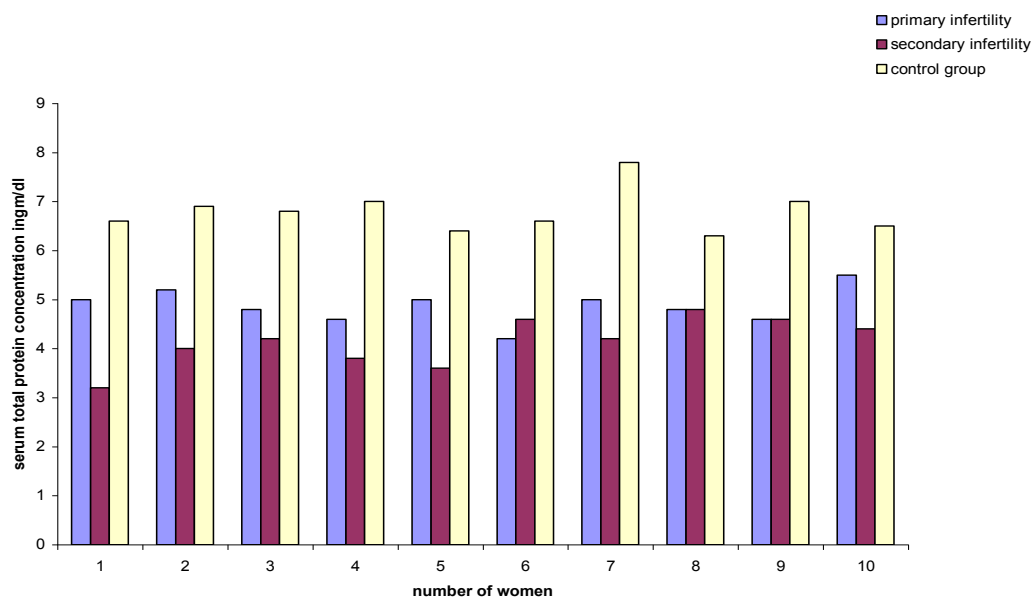


Figure 6 Show the level of serum total protein in the third month of treatment it was significantly higher in control group than infertility groups.

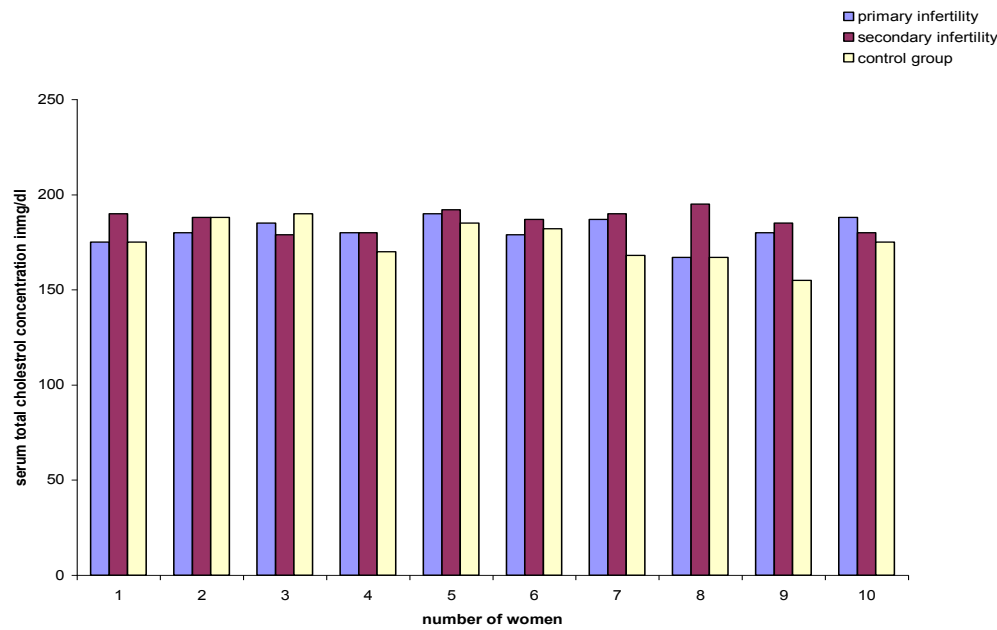


Figure 7 Show the level of serum total cholesterol in first month of treatment no significant difference between control and infertility groups.

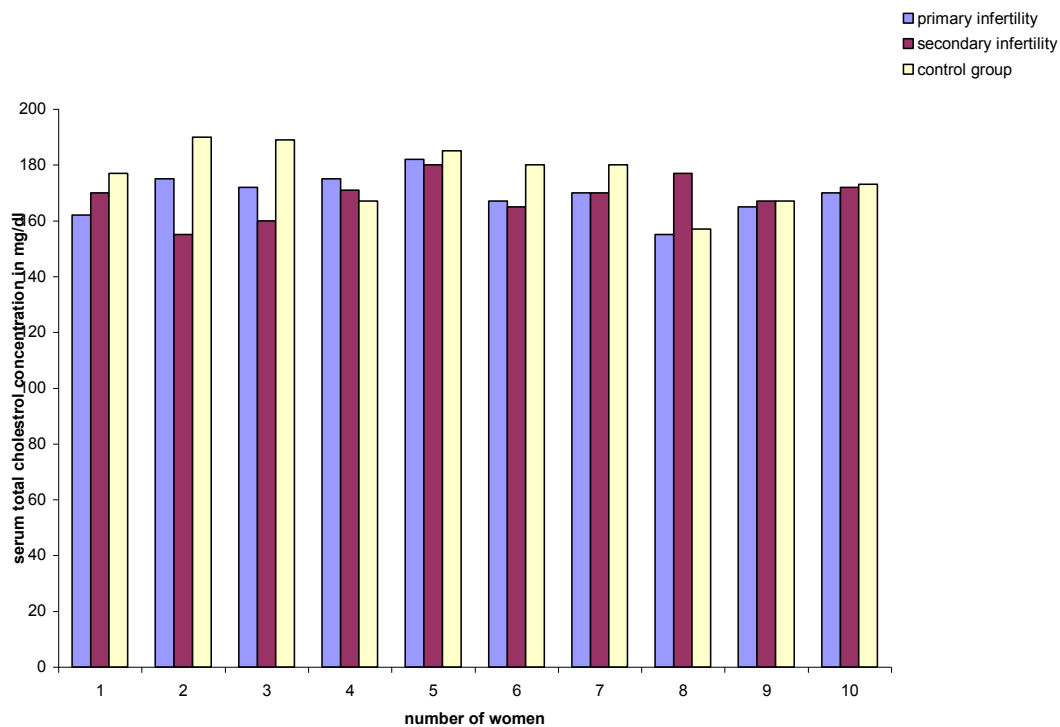


Figure 8 Show the level of serum total cholesterol in second month of treatment no significant difference between control and infertility groups.

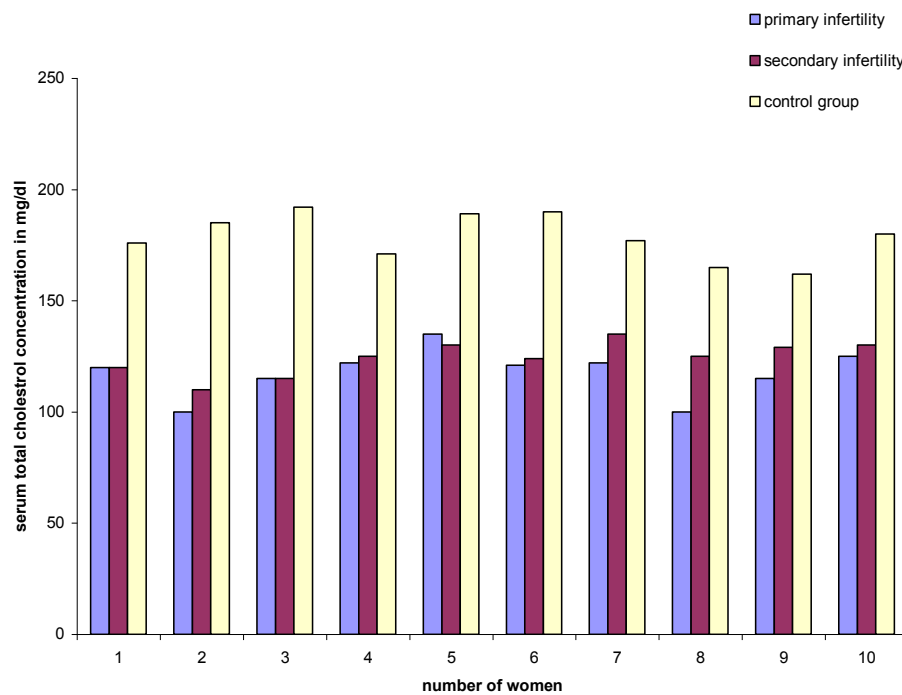


Figure 9 Show the level of serum total cholesterol in third month of treatment it was significantly higher in control group.

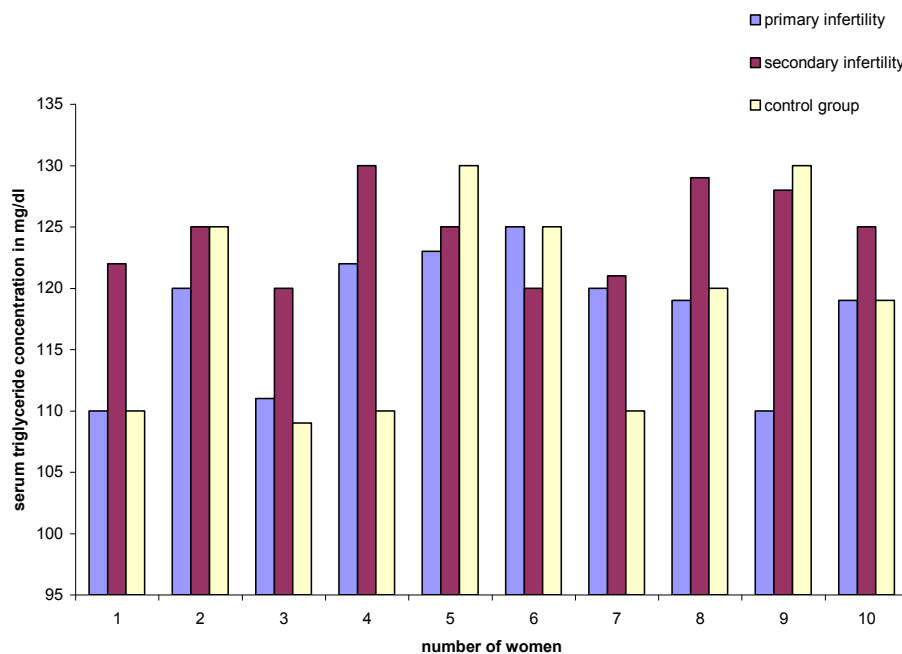


Figure 10 Show the level of serum triglyceride in the first month of treatment no significant difference between control and infertility groups.

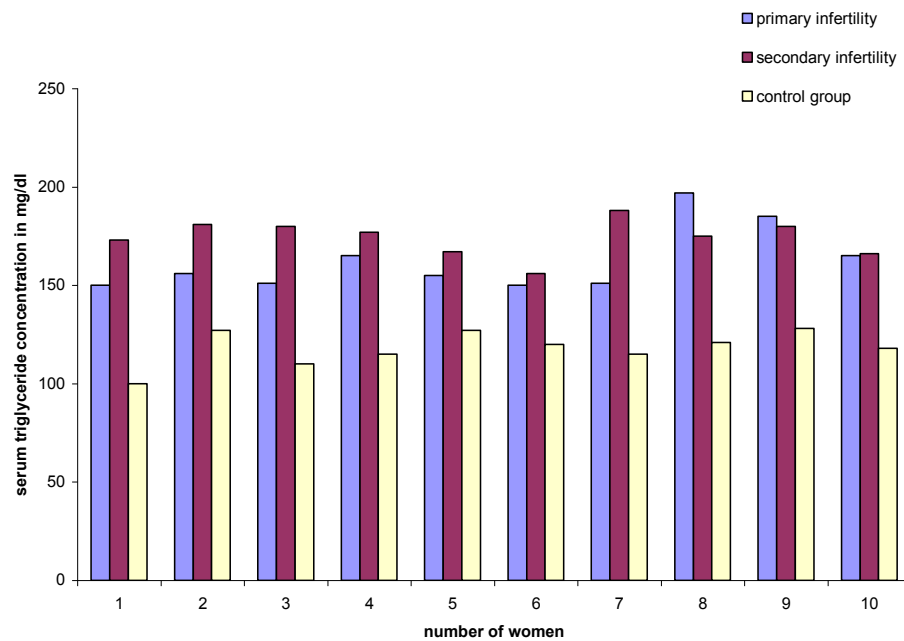


Figure 11 Show the level of serum triglyceride in the second month of treatment no significant difference between control and infertility groups.

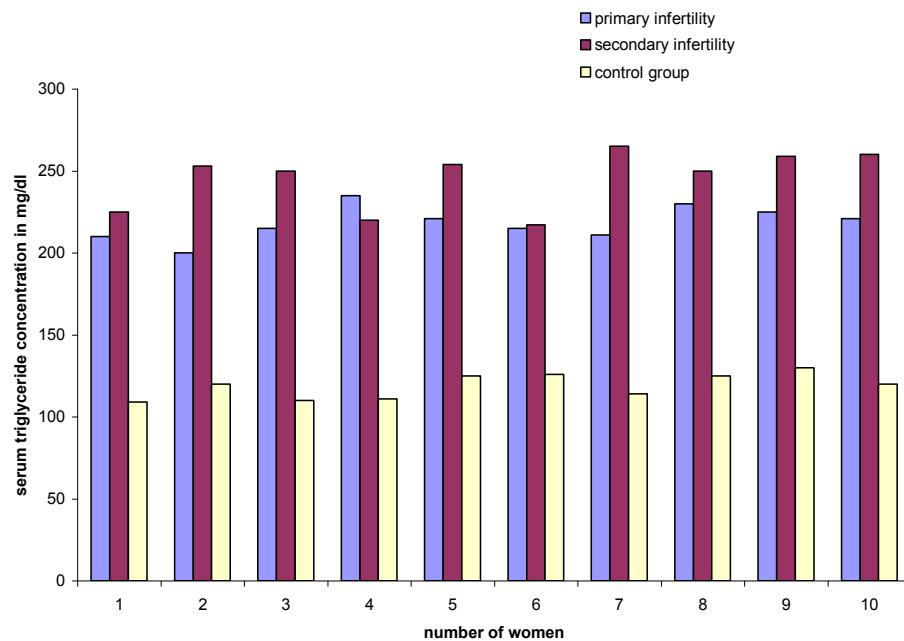


Figure 12 Show the level of triglyceride in the third month of treatment it was significantly higher in infertility groups than control group.

Discussion

In this study the recurrent increase in the levels of gonadotropins more than normal concentration due to the induction programs by clomiphene citrate is found to have a serious effect on the protein causing a low level of serum protein specially after the third month of treatment. The reason for these changes could be related to the general effect of gonadotropins on metabolic pathways either directly or through the effect on other hormones specially estrogen.

Many studies confirm that regulation of the proliferation, cyto-differentiation and atresia associated with folliculogenesis involves complex interactions between a host of factors originating from within the follicle itself and gonadotropins from the pituitary [22,23].

Some studies established the presence of a functional bone morphogenetic protein (BMP) system in the ovary; these BMP enhanced and attenuated the stimulatory action of follicle stimulating hormone on estradiol and progesterone production respectively [24,25].

The increase in estrogen levels (as a result to the increase in number of mature follicle) is possible to cause the increment in the triglyceride serum concentration that could lead to hypertriglyceridemia that may cause later the development of cardiovascular disease in women as confirmed by some studies [26,27].

In this study the use of induction leads to more mature follicle that means extraordinary levels of estrogen the use of induction for three month lead to increase of triglyceride levels specially in the third month.

Several studies have shown an association between the increase in estrogen level and hypocholesterolemia [28,29], However some other studies confirm no relation between estrogen

increase and cholesterol serum level [30,31].

In this study cholesterol was found to be decreased with increasing level of estrogen hormone specially after the use of clomiphene citrate for the third month of clomid treatment.

Some studies show that estrogen increase the rate of LDL catabolism suggesting the importance of estrogen in regulation of cholesterol. Studies on rats shows that pharmacological doses of estrogen mediated uptake of LDL and decreased plasma LDL concentration [32-34].

In this study the use of induction program by clomiphene citrate result in endogenous increase of estrogen level to an abnormal concentration this was associated with decreased serum level of cholesterol.

So the use of clomid is useful to stimulate follicular maturation but the recurrent administration of induction program could lead to a very serious hormonal changes that might effect the general metabolic pathways it need further studies to identify the possible ways to avoid those complication.

References

- 1-Gougeon A 2000 :Ovarian follicular growth in humans: Ovarian ageing and population of growing follicle. *Maturitas* 30:137-142.
- 2-Brown JB 2002:Pituitary control of ovarian function- concepts derived from gonadotropins therapy. *Aust N Z J Obstet Gynaecol* 18: 46-54.
- 3-Le Nestours E, Marraoui J, Lahlou N, Roger M, de Ziegler D, Bouchard P 2002:Role of estradiol in the rise in follicle stimulating hormone levels during the luteal follicular transition. *J Clin Endocrinol Metab* 77:439-442.
- 4-Marsters P, Kendall NR, Campbell BK 2003 Temporal relationships between FSH induced differentiation of bovine granulosa cells maintained in

- serum-free culture. Mol cell Endocrinol 203:117-127.
- 5-Sullivan M W, Stewart AKers A, Krasnow JS, Berga SL, Zeleznik AJ 2000: Ovarian response in women to recombinant follicle stimulating hormone and luteinizing hormone(LH) a role for LH in the final stages of follicular maturation. J Clin Endocrinol Metab 84:228-232.
- 6-Gougeon A 2000 Dynamics of follicular growth in the human a model from preliminary results. Human Reprod 1: 81-87.
- 7-Fauser BCJM, van Heusden AM 2000: Manipulation of human ovarian function: physiological concepts and clinical sequences. Endocr Rev 18-71-106.
- 8-Zeleznik AJ, Hillier SG 2003: The role of gonadotropins in the selection of the preovulatory follicle. Clin Obstet Gynecol 27:927-940.
- 9-Shima K, Kitayama S, Nakano R 2000: Gonadotropins binding site in human ovarian follicles and corpora lutea during the menstrual cycle. Obstet Gynecol 69:800-806.
- 10-Liu JH, Yen SS 2002: Induction of midcycle gonadotropins surge by ovarian steroid in women a critical evaluation. J Clin Endocrinol Metab 57:797-802.
- 11-Finkelstein JS, Badger TM, O Dea LS, Spratt DI, Crowley WF 2002: Effect of decreasing the frequency of gonadotropins- releasing hormone stimulation on gonadotropins secretion in gonadotropins releasing hormone deficient men and perfused rat pituitary cells. J Clin Invest 81: 1725-1733.
- 12-Armstrong DT, Dorrington JH 2000: Estrogen biosynthesis in the ovaries and testes Adv Sex Horm Res 3:217-258.
- 13-Mikkelsen TJ, Kroboth PD, Cameron WJ 2000: Single dose pharmacokinetics of clomiphene citrate in normal volunteers. Fertil Steril 46:392
- 14-Young SL, Opsahl MS, Fritz MA 2002: Serum concentrations of enclomiphene and infertile women. Fertil Steril 7:639-644.
- 15-Kurl RN, Morris ID 2000: Differential depletion of cytoplasmic high affinity estrogen receptors after the in vivo administration of the antiestrogens, clomiphene MER- and tamoxifen. Br Pharmacol 62:487-493.
- 16-Kerin JF, Liu JH, Phillipou G, Yen SS 2000: Evidence for a hypothalamic site of action of clomiphene citrate in women. J Clin Endocrinol Metab 61: 265-268.
- 17-Kettel LM, Roseff SJ, Berga SL, Mortola JF, Yen SS 2000: Hypothalamic pituitary ovarian response to clomiphene citrate in women with polycystic ovary syndrome. Fertil Steril 59:532-538.
- 18-Fisher SA, Reid RL, Van Vugt DA, Casper RF 2002: A randomized double blind comparison of the effects of clomiphene citrate and the AI letrozole on ovulatory function in normal women. Fertil Steril 78:280-285.
- 19-Fish B, Margara RA, Winston RM, Hillier SG 2000: Cellular basis of luteal steroidogenesis in the human ovary. Endocrinol 122:303-311.
- 20-Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC 2002: Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligomenorrheic infertility. J Clin Endocrinol Metab 84:1617-1622.
- 21-Capelo FO, Kumar A, Steinkampf MP, Azziz R 2003: Laparoscopic evaluation following failure to achieve pregnancy after ovulation induction with clomiphene citrate. Fertil Steril 80:1450-1453.
- 22-Hirshfield AN 2001: Comparison of granulosa cell proliferation in small follicles of hypophysectomized,

- prepubertal, and mature rats. Biol Reprod 32:979-987.
- 23-Gougeon A 2002: Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 17:121-155.
- 24-Woo-Sik Lee, Fumio Otsuka, R. Kelly Moore, and Shunichi Shimasaki 2002: Effect of bone morphogenetic protein-7 on folliculogenesis and ovulation in the rat, Biology of reproduction 65, 994-999.
- 25-Hirshfield AN, Midgley AR. Morphometric analysis of follicular development in the rat. Biol Reprod 2000; 19:597-605.
- 26-Wakatsuki A, Sagara Y 2002:Effect of continuous medroxyprogesterone acetate on lipoprotein metabolism in postmenopausal women receiving estrogen, Maturitas;25:35-44.
- 27-Ettinger B, Friedman GD, Bush T, Quesenberry CP Jr 2002. Reduced mortality associated with long term postmenopausal estrogen study, obstet Gynecol; 87:6-12.
- 28-Tall AR 2000:Plasma cholesterol ester transfer protein, J lipid Res; 34 1255-74.
- 29-Wakatsuki A, Ikenoue N, Okatani Y, Izumiya C 2002: Lipid transfer reaction and lipid composition of low density lipoprotein particles in postmenopausal women receiving estrogen, Obstet Gynecol;94:492-7.
- 30-Lagrost L, Gambert P, Lallemand C 2000: Combined effects of lipid transfers and lipolysis on gradient gel patterns of human plasma LDL. Arterioscler thromb; 14:1327-36.
- 31-Gambert P, Bouzenard-Gambert C, Athias A, Farnier M, Lallemand C 2000: Human low density lipoprotein sub fractions separated by gradient gel electrophoresis: composition, distribution, and alterations induced by cholesteryl ester transfer protein. J Lipid Res 31:1199-210.
- 32-Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM 2000: Low density lipoprotein subclass patterns and the risk of myocardial infarction. JAMA 260:1917-21.
- 33-Wakatsuki A, Ikenoue N, Sagara Y 2000: Effect of estrogen on the size of low density lipoprotein particles in postmenopausal women. Obstet Gynecol, 90:22-5.
- 34-Wakatsuki A, Ikenoue N, Sagara Y 2000: Estrogen induced small low density lipoprotein particles in postmenopausal women. Obstet Gynecol, 91:234-40.