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RESEARCH ARTICLE

THE ROLE OF CLOMIPHENE CITRATE (CLOMID) CHALLENGE TEST AND LETROZOLE (FEMARA) CHALLENGE TEST IN ASSESSMENT OF OVARIAN RESERVE IN INFERTILE PATIENTS

Dr. Suad Abdul Zahra'a Al- Kaseer, Dr. Basima Al- Ghazali and *Dr. Muna Kadhum Farhood

University of Kufa, Iraq, AL-Najaf Health Directorate

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ABSTRACT

The aim of our research is to study the OR in infertile patients by assessing hormonal status including basal FSH, LH, ovarian volume ,ovarian diameter, no. of small antral follicle, endometrial thickness, CCCT and femara challenge test. It was carried in the fertility center of Al-Saddar teaching hospital of Al-Najaf city from January 2009 to August 2009. A comparison was done between three groups: Control group (25 patients) without treatment. CCCT group (25 patients) with induction of ovulation by clomiphene citrate tab. And lastly femara challenge test group treated by letrozole tab. In day 2 of MC history was taken about age, cause of infertility, type of infertility, and duration of it. After history, measurement of hormonal level of FSH, LH and TVUS done to all patients and measured ovarian diameter, ovarian volume, endometrial thickness and no. of small antral follicle. In day 10 of MC all these measurement repeated, divided our studied groups into 2 groups according to FSH level (< 12mIu/ml regarded as normal FSH groups and >12 mIu/ml regarded as abnormal FSH groups) and study the effectiveness of CCCT and femara challenge tests in assessing OR by measurement of ovarian volume, ovarian diameter, no. of antral follicle and endometrial thickness. We conclude that CCCT and femara challenge tests had better predictive value than measurement of basal FSH day 2 in assessment of OR.

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INTRODUCTION

The inability of a couple to conceive, affecting about 10% of couples with a wide range of causes affecting both partners. In the female, failure of ovulation is common, and ovaries may be stimulated to produce ova by giving gonadotrophic hormones or by drugs which stimulate their action in the body. The term "ovarian reserve" refers to a woman's current supply of eggs, and is closely associated with reproductive potential. In general, the greater the number of remaining eggs, the better the chance for conception. Conversely, low ovarian reserve greatly diminishes a patient's chances for conception. The decline in fecundity with female age is a well- known phenomenon for clinicians dealing with sub fertility patients. Diminishing ovarian reserve seems to be the reason for declining fecundity since age is only a rough estimate of ovarian reserve, many tests have been developed to predict ovarian reserve more precisely. This review focuses on these ovarian reserve (OR) tests and their clinical role in predicting response to ovarian and pregnancy chances. According to our analysis, the clomiphene citrate challenge test and femara challenge test have the strongest correlation in predicting OR.

Corresponding Author: Dr. Muna Kadhum Farhood,
University of Kufa, Iraq, AL-Najaf Health Directorate.

In our study, we try to use femara (letrozole) which is aromatase inhibit or instead of clomiphene to assess it's efficacy in testing OR.

Infertility: Is defined as the failure to conceive within one year of un protected regular sexual intercourse. For couples who have had no previous conception, the infertility is defined as primary, while couples who have had a previous conception and have then not conceived again are defined as having secondary infertility, or after 6 months if the women is over 35 years of age. In the general population, conception is expected to occur in 84% of women within 12 months and in 92% within 24 months (Tevelde *et al.,* 2000). Data from population – based studies suggest that 10 -15 % of couples in the western world experience infertility (Templeten *et al.,* 1990, Evers, 2003). Half of them (8%) will subsequently conceive without the need for specialist advice and treatment, of the remaining 8% who require input from fertility clinics, half (4%) comprise couples with primary infertility while the other half have secondary infertility. By convention, infertility is commonly divided into five major categories on basis of aetiopathology, results of investigations and prognosis. The proportion of couples in each group varies from population to population depending on environmental factors, and referral patterns (Stuart cambell and Ashmonga, 2006).

Diagnostic categories in infertility

	Primary	Secondary
An ovulation	20%	15%
Male	25%	20%
Tubal	15%	40%
Endometrosis	10%	5%
Un explained	30%	20%

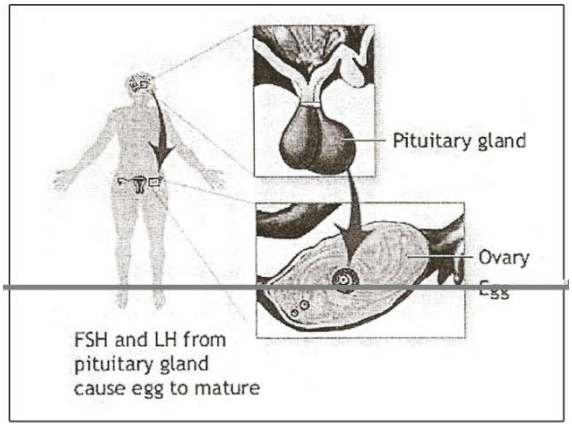
Adapted from: Templeton et al. management of infertility for the MRCOG and beyond 2000 (Keith Edmonds, 2014).

Factors adversely affecting conception rate

Female factors	Male factors	Combined factors
Age > 37 years	Low numbers of motile, healthy sperm	Duration of infertility >2 years
Menstrual FSH level(>10 u/L)	drug intake	No previous conception in current relationship

Female infertility accounts for one third of infertility cases, male infertility for another third, combined male and female infertility for another 15% and the reminder of cases are "unexplained" (Stuart cambell and Ashmonga, 2006).

Pathophysiology of Oogenesis and ovulation



Diagrame shows effect of pituitary hormones (FSH, LH) on follicular maturation

The formation and maturation of an oocyte is known as oogenesis. It starts with the growth of a primordial follicle to form a pre-antral follicle and ends with the final maturation of a pre-ovulation follicle. The formation of pre-antral follicle takes 85 days in a human, while the final maturation stage (the follicular phase of menstrual cycle) from the pre-antral follicle to the pre-ovulatory follicle takes 14 days to complete. An intact hypothalamo-pituitary-ovarian axis is essential for normal ovarian function. Gonadotrophin – releasing hormone (GnRH) is released in a pulsatile manner to control the pituitary and the release of follicle – stimulating hormone (FSH) and lutinizing hormone (LH). The hormones stimulate the development of the follicles, while a mid cycle surge of LH causes rupture of the dominant follicle and release of oocyte (ovulation) (Stuart cambell and Ashmonga, 2006).

Causes of female infertility:

1. **General factors:** Diabetes mellitus, thyroid disorder, adrenal diseases, significant liver, kidney disease, psychological factors, smoking, body mass index – outside the range (20-30) weight (kg)/ height(m2), low coital frequency, inappropriate timing of intercourse to ovulation.
2. **Hypothalamic – pituitary factors:** Hypothalamic dysfunction (Kallman's syndrome, hyperprolactinanemia).
3. **Ovarian factors:** Include polycystic ovarian syndrome, an ovulation, diminished ovarian reserve, premature menopause, menopause, luteal dysfunction, Gonadal dysgenesis (Turner syndrome), ovarian cancer.
4. **Tubal (ectopic)/ peritoneal factors,** Endometeriosis**,** Pelvic adhesions**,** Pelvic inflammatory disease (usually due to Chlamydia), Tubal occlusion
5. **Uterine factors,** Uterine malformations**,** Uterine fibroid (leiomyoma), Asherman's syndrome
6. **Cervical factors,** Cervical stenosis.**,** Antisperm antibodies.**,** Non receptive cervical mucus.
7. **Vaginal factors,** Vaginisms**,** Vaginal abstruction
8. **Genetic factors,** Various intersexed condition such as androgen insensitivity syndrome.

Causes of male infertility:- Pretesticular causes, endocrine problems, i.e Kallmann hyperprolactinemia, hypopituitarism, hypogonadism, due to various causes, psychological factors, drugs, alcohol, testicular factors, genetic defects on the Y chromosome, Y chromosome microdeletions, abnormal set of chromosomes, Klinefelter syndrome, neoplasm. Some causes of male infertility can be determined by analysis of the ejaculate, which contains the sperm. The analysis includes counting the number of sperm and measuring their motility under a microscope. Producing few sperm, oligospermia, or no sperm, azoospermia. A sample of sperm that is normal in number, but shows poor motility, or asthenozoospermia.

Combined infertility: In some cases, both the man and women may be infertile or sub-fertile, and the couples infertility arises from the combination of these conditions.

Un explained infertility: In about 15% of cases, the infertility investigation will show no abnormalities, possible problems could be that the egg is not released at the optimum time for fertilization, that it may not enter the fallopian tube, sperm may not be able to reach the egg, fertilization may fail to occur, transport of the zygote may be disturbed, or implantation fails.

Ovulatory disorders: Absence of ovulation (an ovulation) or infrequent ovulation (oligo-ovulation) is seen in a fifth of all women presenting with infertility.

Types of anovulatory infertility

1. Hypothalamus and pituitary (hypogonadotrophic hypogonadism) (WHO type I): Abnormalities of GnRH agonist secretion are associated with very low levels of oestradiol, FSH, and LH. Kallman's syndrome is a congenital cause of an ovulation characterized by isolated gonadotrophin deficiency and anosmia. Acquired causes include pituitary tumors, pituitary necrosis (Sheehan's syndrome), stress, and excess in weight loss or exercise.
2. Normogonadotrophic hypogonadism: The majority of women with normogonadotrophic an ovulation have PCOS. Other causes include congenital adrenal hyperplasia, adrenal tumors, androgen producing ovarian tumors.

3. Hyper gonadotrophic hypogonadism (WHO III):Amenorrhea with elevated serum FSH and low or undetectable oestrogen levels signify ovarian failure, known causes include turner's syndrome (XO), turner mosaic (Xo, XX,XX) gonadal dysgenesis, autoimmune disorders, irradiation or chemotherapy. In many cases the cause is unknown.

Hyperprolactinaemia: Increased levels of prolactin interfere with normal pulsatile secretion of GnRH, resulting in an ovulation amenorrhea, and occasionally galactorrhea associated with low FSH and oestradiol levels. hyperprolactinaemia is a feature of prolactin producing pituitary adenomas or tumors blocking inhibitory control of the hypothalamus. Other causes include primary hypothyroidism, chronic renal failure, and drugs such as COCP, dopamine depleting agents (reserpine, methyldopa) and dopamine receptor inhibiting agents (metoclopromide and phenothiazine) (Keith Edmonds, 2007).

Physiology of reproductive aging: During fetal life, germ cells rapidly proliferate by mitosis to yield approximately 6-7 million oogonia by 16-20 weeks of pregnancy. From that point forward, the germ cell population begins an inexorable exponential decline via gene-regulated apoptosis. Transformed to oocytes after entering the first meiotic division, the number of germ cells falls to between 1 and 2 million at birth and to about 300000 to 500000 by the onset of puberty. Over the next 35-40years of reproductive life, only about 400-500 oocytes will ovulate; the rest are lost through atresia. During the reproductive years, the rate of follicular depletion is relatively constant and gradual until age 37-38 (when approximately 25000 oocytes remain) and then accelerates over the 10-15 years preceding menopause. At the time of menopause, fewer than 1000 follicles remain (Leon speroff *et al.,* 2005; Gougeon *et al.,* 1994). Menopause occur when the number of remaining follicles fall below a critical threshold (approximately 1000), regardless of age at the time. In the average women, accelerated follicular depletion and declining fertility begin at age 37-38, and menopause follows approximately 13years later (average age 51). However, in epidemiologic studies approximately 10% of women in the general population become menopausal by the age of 45, probably because they were born with a smaller than normal ovarian follicular pool that is functionally depleted at an earlier age (Leon speroff *et al.,* 2005; Tibiletti *et al.,* 1999). As the pace of follicular depletion begins to increase during the later reproductive years, but before any discernible change in menstrual regularity, serum FSH levels begin to increase; LH concentration remain unchanged. This subtle "monotropic" rise in circulating FSH conc could result from age-related changes in the pattern of pulsatile GnRH secretion or from progressing follicular depletion and lower levels of feedback inhibition on pituitary FSH secretion by ovarian hormones. The weight of available evidence strongly supports the second explanation (Leon speroff *et al.,* 2005; Klein *et al.,* 1996). Although slower GnRH pulse frequencies stimulate prefential secretion of FSH over LH, both the frequency and amplitude of LH pulse patterns in younger and older women are similar, even after oophorectomy (Leon speroff *et al.,* 2005; Alexander *et al.,* 1990). Circulating follicular phase inhibin-B levels decrease as or even before FSH conc. begin to increase. later, luteal phase serum inhibin-A levels decline as well (Klein *et al.,* 1996; Welt *et al.,* 1999). Both inhibins selectively inhibit pituitary FSH secretion. Consequently FSH levels rise progressively as inhibin production from an aging follicular pool decreases most noticeably in the early follicular phase. Decreasing inhibin production could reflect a shrinking number of follicles, a reduced functional capacity of older follicles, or both (Leon speroff *et al.,* 2005; Seifer *et al.,* 1996). The observation that pre-ovulatory follicular fluid inhibin concentration are similar in young and older cycling women suggests that the number of remaining follicles is the most important factor (Leon speroff *et al.,* 2005; Klein *et al.,* 1996). Activin, another class of ovarian peptide hormone that stimulates pituitary FSH secretion, may also play a role. Activin is increased in aging cycling women, but the extent to which it may contribute to rising serum FHS levels remain undetermined (Leon speroff *et al.,* 2005; Santoro *et al.,* 1999). Ovarian steroid hormones do not play a role. The initial rise in FSH levels precedes any measurable decrease in estradiol levels, by several years (Leon speroff *et al.,* 2005; Santoro *et al.,* 1996). Follicular phase estrodial levels in older cycling women are generally similar to those in younger women and often even higher. luteal phase progesterone levels in older and younger cycling women are also similar (Leon speroff *et al.,* 2005; Klein *et al.,* 1996). Age increases and FSH levels rise, the follicular phase becomes shorter; LH levels and luteal phase duration remain unchanged. Cycles remain regular, but overall cycle length and cycle variability decrease (Leon speroff *et al.,* 2005; Vollman *et al.,* 1977). As FSH levels increase and the follicular phase becomes shorter, estradiol levels rise earlier, suggesting that higher FSH levels stimulate more rapid follicular development (Leon speroff *et al.,* 2005; Klein *et al.,* 1996). However, careful studies have shown that the earlier acute rise in estradial levels results not from accelerated follicle growth but from advanced follicular development at the beginning of the cycle and earlier selection of the dominant follicle. follicular phase and overall cycle length reach their nadir at approximately age 42. Over the subsequent 8-10 years preceding the menopause average cycle length and variability steadily increase as ovulations become less regular and frequent. Not surprisingly, the age related changes in the endocrine characteristics of the menstrual cycle that result from progressive follicular depletion correlate with a measurable decrease in ovarian volume and in the number of antral follicle observed by transvaginal ultrasonography during early follicular phase (Leon speroff *et al.,* 2005).

Mechanisms of the age related decrease in female fertility: Whereas the number of remaining ovarian follicles steadily declines with increasing age, more rapidly after approximately age 38, observations in stimulated cycles suggest that aging follicles also become progressively less sensitive to gonadotropin stimulation. As age increases, the total dose and duration of treatment reguired to stimulate multiple follicular development increase. Studies of ovarian follicular development and pre-ovulatory follicular fluid hormones in older and younger cycling women do not suggest any age – related decline in follicular function, once growth and development begin. pre-ovulatory follicles in older and younger women are similar in size and inhibin content, and follicular fluids progesterone levels and estrogen /androgen ratios are even higher in older than in younger women (Leon speroff *et al.,* 2005; Iacobs *et al.,* 1990). Older cycling women ovulate as regularly, and more frequently than younger women. Their rising FSH levels apparently compensate quite effectively for any decrease in follicular sensitivity to gonodotropin stimulation. pre-ovulatory follicles in older cycling women get an earlier start, but grow at a normal pace

and reach a normal size ; their follicular fluid characteristics suggest they are also quite healthy. Why then does fertility in women decline progressively with age? The available evidence indicates that both the age – related decline in female fertility and the increase in risk of spontaneous miscarriage can be attributed largely to progressive follicular depletion and a high incidence of abnormalities in aging oocytes (Leon speroff *et al.,* 2005; Pellestor *et al.,* 2003). In sum, accumulated evidence strongly suggests that the primary cause of the age – dependant decrease in fecundability and increase in the incidence of spontaneous miscarriage is an increasing prevalence of aneuploidy in aging oocytes resulting from disordered regulatory mechanisms govering meiotic spindle formation and function. Aging doesn't appear to have any significant adverse effect on the uterus. Although the prevalence of benign uterine pathology (leiomyomas, endometrial polyps, adenomyosis) increase with age (Leon speroff *et al.,* 2005; Pellestor *et al.,* 2003; Nagele *et al.,* 1996). Little evidence exists to indicate it has much overall impact on fertility in women. Age also does not appear to adversely affect endometrial development or function in response to steroid stimulation (Leon speroff *et al.,* 2005; Nagele *et al.,* 1996; Noci *et al.,* 1995).

Background information on aging and infertility: Female age is very important in consideration of probability for getting pregnant. The real issue is egg quantity and quality which translates over to embryo quality after fertilization. As women wait longer to have children, a higher percentage of couples have fertility problems because of the quality of eggs, and other issue that affect fertility and are more common in older women.

Finite supply of eggs: Even before birth, women's eggs begin to diminish in number. During the 20th gestation week, a female embryo contains about seven million eggs. At birth, the number of eggs has already dropped at about 200000. The number of eggs continues to decline as the women ages. Until no eggs remain (menopause). The age of male partner does not appear to matter nearly as much. This is related to the fact that all of a woman's eggs are present at birth. They cannot divide or be "resupplied" whereas sperm are produced constantly after puberty in men. Eggs age over time, while new sperm are constantly coming off the production line. Sperm from older men does not usually have a substantially reduced fertilizing potential as compared to sperm from younger men. However, older men often have less interest in frequent intercourse, which can be a factor in chances of conception (Leon speroff *et al.,* 2005; Tietze, 1957; Zorn, 1995).

Ovarian Reserve (OR): is a term that is used to determine the capacity of the ovary to provide eggs that are capable of fertilization resulting in a healthy and successful pregnancy. The determination of the ovarian reserve is important in the treatment of infertility. The ovary is generally thought of as an bank from which the women draws during her reproductive life. While each month one egg is released by ovulation about one thousand additional eggs are lost by atresia. Thus with advanced maternal age the number of eggs that can be successfully recruited for a possible pregnancy declines. Attempts have been made to assess the number of potential useful oocytes in a noninvasive way. The most commonly used test to assess this ovarian reserve is the day 2 or day 3 FSH test, this blood test determines the level of FSH on cycle day 2 or day 3, cycle day 2 or day 3 is chosen because at this time the oestrogen level is expected to be low, a critical feature, as FSH levels are subject to a negative feedback. In a patient with infrequent menstruation, an FSH level could be measured at random and is valid if the oestrogen level is low. Generally FSH levels are expected to be below 10 mIu./ml in women with reproductive potential (levels of 10-15 miu./ml are considered abnormal).(5) The methods for assessing OR are classified into 2 groups:

* Passive and dynamic ovarian reserve testing; The goal of both approaches is to provide information regarding oocyte (egg) quality and quantity.

Passive OR testing: FSH and LH measurement: as a women ages FSH becomes elevated in an attempt to force the aging ovary to respond. However, the exact mechanism responsible for this adaptive response remains unknown. Arise in early follicular phase FSH is also accompanied by decline in oocyte quality and some investigators have linked such FSH elevations to fetal abnormalities. Since FSH has such high predictive value, should FSH always be measured. It is difficult to establish absolute values that define how high an FSH level can be and still achieve pregnancy due to variations in laboratory assessments and treatment methods. Since FSH fluctuates only slightly during cycle days 2 through 5, testing does not have to be done exactly on cycle day 3. More flexible FSH testing may be done over a range of dates. While FSH values may not change significantly from days 2 through 5 within a given cycle, fluctuations of day 3 FSH from cycle to cycle are more important to detect when FSH does fluctuate, subsequent menstrual cycles will likely produce oocytes of varying quality. This principle has emerged as a fundamental belief in human reproduction physiology. Patient with low FSH values (suggesting satisfactions OR) generally show the least fluctuation, while those with elevated FSH levels have broader ranges. Wide FSH fluctuations from month to month present a difficult "moving target" for laboratory assessment. In such cases, it is difficult to precisely estimate OR (Leon speroff *et al.,* 2005; Tietze, 1957; Zorn, 1995). Early follicular phase estradiol levels may provide additional useful information for the evaluation of ovarian reserve. Like FSH, a high cycle day 3 estradiol conc. (greater than 80pg/ml) also predicts low fecundability. Early elevations in serum estrodial reflect the advanced follicular development and early selection of a dominant follicle observed in older cycling women that are driven by rising FSH levels. A premature elevation in estradiol conc. May also tend to suppress the FSH level, making an elevation that might otherwise reveal a low ovarian reserve. Measurement of both FSH and estradiol on cycle day 3 may, thus, help to decrease the incidence of false –negative tests based on measurement of FSH alone. When both FSH and estradiol are elevated on day 3, ovarian response to stimulation is likely to be very poor (Leon speroff *et al.,* 2005; Zorn *et al.,* 1995).

The Clomiphene citrate challenge test: Is a provocative and even more sensitive test of ovarian reserve that probes the endocrine dynamics of the cycle under both basal and stimulated conditions before (cycle day 3 FSH and estradiol) and after (cycle day 10 FSH) treatment with clomiphene citrate (100mg/day, cycle days 5-9) (Leon speroff *et al.,* 2005; Navot *et al.,* 1987). When administered to cycling fertile women younger than age 35, clomiphene typically stimulates a transient increase in gonadotropin levels; LH generally rises more than FSH. However, in women with a low ovarian reserve, that pattern may be reversed; FSH may increase more

than LH, sometimes to frankly elevated concentration. Although the mechanisms responsible are not entirely clear, evidence suggests that the smaller follicular cohorts in aging women produce less inhibin-B and estradiol, resulting in less negative feedback inhibition on Clomiphene induced pituitary FSH release. The Clomiphene challenge test can identify women who might otherwise go unrecognized if evaluated by basal cycle day 3 FSH and estradiol levels alone. The likelihood of successful pregnancy is inversely related to both the cycle day 3 and day 10 FSH levels. More importantly, however, in women with a normal day 3 FSH level, a high day 10 value has the same poor prognosis as an elevated day 3 FSH concentration. The stimulated day 10 estradiol level has no prognostic value. An elevated cycle day 3 FSH level or abnormal Clomiphene challenge test correlates consistently with a poor prognosis for I.V.F. success (less than 10%), regardless of age (Leon speroff *et al.,* 2005; EI- Tonkhy *et al.,* 2002). These and other observations indicate that age and ovarian reserve test results are independent predictor of I.V.F. outcome and, by inference, general fecundability. The prognosis for women with an abnormal OR test is generally poor, even if they are young. In contrast, the prognosis for women with normal test results relates to their age; a normal test result does not improve the poorer, age-specific prognosis for older women (Leon speroff *et al.,* 2005; Scott *et al.,* 1998). Women with low cycle day 3 FSH levels generally exhibit less between cycle variability than women with higher levels. Numerous other methods for measuring OR have been investigated including; ovarian volume and early follicular phase antral follicle counts; basal and clomiphene or exogenous FSH – stimulated inhibin B-levels; response (FSH, estradiol, inhibin-B) to stimulation with a GnRH agonist or human menopausal gonadotropin; and basal and GnRH agonist or gonadotropin stimulated anti mullerian hormone levels (Leon speroff *et al.,* 2005; Tietze, 1957). The number of small antral follicles observed by TVUS examination at the onset of the menstrual cycle reflects the size of the resting follicular pool, and correlates with age and response to gonadotropin stimulation ; observation of 10 or fewer follicles is associated with increased risk of cycle cancellation low basal or stimulated inhibin –B levels suggest a low OR, but studies of their productive value have yielded conflicting result (Leon speroff *et al.,* 2005; Smotrich *et al.,* 1995).

To date, GnRH agonist stimulation test and exogenous FSH OR test, although also predictive do not provide any more sensitive information than the simpler and less costy Clomiphine challenge test, and data derived from measurement of AMH are preliminary. When the Clomiphene challenge test is used as a screening test for infertile women of all ages, the prevalence of abnormal tests is approximately 10% overall, increases with age and is disproportionately high in women with unexplained infertility; abnormal tests are associated with a poor prognosis, regardless of age, other identified causes of infertility, or type of treatment (Leon speroff *et al.,* 2005; Zorn, 1995). Should all infertile women have an OR test? Certainly, the yield of abnormal tests in young women is very low, except perhaps in those whose infertility is unexplained after an otherwise thorough evaluation. Although an argument for universal screening of all women can be made, OR testing can more strongly be justified for women with any of the following characteristics (Leon speroff *et al.,* 2005; Smotrich *et al.,* 1995). Age older than 35, unexplained infertility, regardless of age, family history of early menopause, previous ovarian surgery (ovarian cystectomy or drilling, unilateral oophorectomy), chemotherapy or radiotherapy, smoking, demonstrated poor response to exogenous gonadotropin stimulation, Over the past several years, ovarian reserve test have emerged as a new, important, and highly useful tool in the evaluation of infertile women. However, they must always be interpreted and applied with caution OR tests are generally reliable, but certainly not infallible. Rigid interpretation or application of test results risks inappropriate recommendations for treatment or for no treatment, and both must be avoided. An abnormal test result does not preclude the possibility of pregnancy. Except perhaps when grossly abnormal test results should, therefore, not be used to deny treatment but only to obtain prognostic information that can help to guide the choice of treatment and best use of available resources. Otherwise, although the probability of pregnancy may be low, are cannot accurately predict who will be among those few with abnormal test results that succeed. Ultimately, regardless what the statistical prognosis may be, the success rate for any individual women will be zero or 100% (Leon speroff *et al.,* 2005; Smotrich *et al.,* 1995; Navot *et al.,* 1987).

Clomiphene citrate: is an orally active synthetic non-steroidal compound with oestrogenic as well as anti-oestrogenic properties, which has traditionally been the treatment of choice in women with an ovulatory PCOS. It displaces oestrogen from its receptors in the hypothalamic – pituitary axis, reduces the negative feedback effect of oestrogen and encourages GnRH secretion. it is administered in an initial daily dose of 50mg on day 2-6 of a spontaneous or induced menstrual period. The dose can be increased by 50mg /day till ovulation is achieved, up to a maximum of 150mg/day. A course of 6 to 12 cycles can be used in women who respond to the drug. It is necessary to monitor follicular response, at least in the first cycle of treatment, with TV scan to minimize the risk of multiple pregnancies. Mid luteal progesterone levels are checked in each cycle. Ovulation is expected to occur in 80% and pregnancy in 35-40% women on Clomiphene (Imani, et al. 2002) approximately 20-25% of women show no response to Clomiphene citrate and are considered to be resistant (Keith Edmonds, 2007).

Adverse reactions: Anti – oestrogenic effects include thickening of cervical mucus and hot flushes in 10% of women. Other side effects include abdominal distension (2%), abdominal pain, nausea, vomiting, headache, breast tenderness, and reversible hair loss. Significant ovarian enlargement occurs in 5%, but OHSS is rare (<1%). The multiple pregnancy rate associated with Clomiphene is 7-10%. A putative link with ovarian cancer has been described in women receiving more than 12 cycles of clomiphene (Keith Edmonds, 2007).

Aromatase inhibitors: have been used as alternatives to clomiphene in view of their lack of anti-oestrogenic effects. They suppress oestrogen production and mimic the central reduction of negative feedback by ovarian oestrogen (Keith Edmonds, 2007). Femara (Letrozole) is a prescription medication Licensed to treat breast cancer in postmenapausal women. When a women uses femara for infertility, the medicine is taken at the beginning of the menstrual cycle. Temporally decrease the amount of oestrogen in the body sends a message to the brain to increase the production of substances that stimulate the ovaries. This often causes ovulation in women who do not normally ovulate or who not ovulate regularly. In many ways, femara works similarly to

clomid however, femara be less likely to cause certain problems with cervical mucus. Clomid causes cervical mucus problems, leading to vaginal dryness or cervical mucus changes that interfere with motility of sperm. Femara is also likely to cause the lining of the uterus to thin, as clomid sometimes does. A thin uterine lining can make pregnancy less likely. Nobody has yet identified the optimal dose for letrozole 2.5, 5 and 7.5 mg. Different studies comparing these doses regimen have occasionally prefer one does or another, but there is no conclusive data that one dose is better than another and the length of treatment is for five days.

Who is a candidate for femara?

Doctors prescribe femara under specific fertility circumstances:

After clomid has been tried and failed to bring on ovulation.
After ovulation occurred with clomid, but the endometrium was very thin.
After ovulation with clomid, but failure to conceive after 6 cycles.
After experiencing sever side effects with clomid.

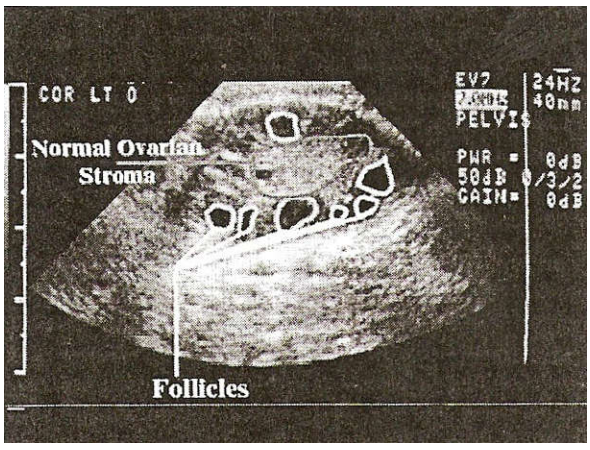
What are the side effects and risks when using femara?

There is some controversy concerning the use of femara for infertility. Femara can cause miscarriages or birth deformities. It is unclear that these cases the women were taking femara while pregnant or just took it priorly seems that the drug is risky when taken during pregnancy. Side effects include headache, nausea, hot flashes, night sweats, joint pain, bloating, and fatigue (http://www.getting pregnant – fast.com).

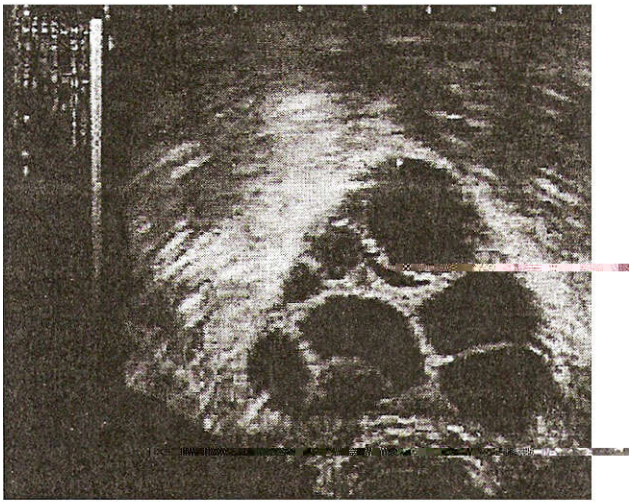
Location of ovaries: The ovaries are usually found at the level of the uterine fundus where the uterus becomes triangular.

Size: In a menstruating women, ovaries normally measure approximately 3×1.5×1 cm. The size and appearance of the ovaries depend on both age and the stage of the menstrual cycle. In the younger adult they are almond shaped, solid, a greyish pink. Previous investigators have documented that the ovary reduces in size with increasing age, regardless of whether the women has given birth. Ultrasound ovarian volume has also been used to predict the risk for ovarian hyperstimulation syndrome. Ovarian volume During a women's life, ovarian volume changes from 0.7cm2 at the age of 10 years, to 5.8cm2 at the age of 18 years. However, at the age of 40 years, the ovaries tend to decrease in size and they decrease even further after menapouse.

Antral follicle counts:



U/S picture shows normal ovarian stroma and multiple small growing follicles during follicular phase



U/S picture shows mature follicle

The number of antral follicles are counted early in the follicular phase is found to be a predictor of the number of oocytes collected in an IVF program. This test might be representing of ovarian reserve, and indeed an age-related decrease in antral follicle count has been observed. passive OR testing transvaginal ultrasound (TVUS)

Diminished OR means that fewer follicles are available for stimulation and recruitment by fertility drugs. The use TVUS was first suggested in 1966 but was not seriously considered at that time. It should be performed with an empty bladder to bring the uterine fundus and ovaries closer to the vaginal fornices, i.e., closer to the endovaginal transducer probe (Peter *et al.,* 1997). In Gynecology transabdominal probe tend to range from 3 to 5 MHZ and transvaginal probe from 5-8.0MHZ.

Transvaginal ultrasound:

Advantages:

1. High probe frequency giving increased resolution of pelvic structures.
2. Diagnosis of early pregnancy viability approximately one week before transabdominal scan.
3. Emptybladder.
4. Not dependant on habitus.

Disadvantages:

1. Internal examination.
2. Possible discomfort.
3. Not suitable for all patents.
4. Chaperone required.

A small amount of gel is applied to the transducer tip and the tip and shaft of probe covered with a non spermicidal condom apply small amount of lubricant gel to allow easy insertion to probe, when a transvaginal examination is being performed, palpation can be used to modify the position of pelvic structures to optimize the image quality. Three-dimensional ultrasound (3D): To avoid the difficulties in outlining or measuring ovarian size, 3D ultrasound has been proposed using a dedicated volumetric probe or a manual survey of the

ovary. It has been used to measure ovarian and stromal volumes, providing information that is not available from two dimensional (2D) ultrasound. Data are transferred to a computer and can be analyzed later.

Normal ultrasound of ovaries: The ovaries are oval structures, usually closely related to the iliac vessels on the pelvic side-wall. They are less echogenic than the uterus and may contain variable number of small cysts/follicles. Ovarian size varies according to the age and hormonal status of the patient. Less than 7.5ml is considered normal in menstruating women, and in post menopausal women, the volume should be less than 3ml. The ovarian volume is calculated using the formula for appellate ellipse, i.e 0.5(length× depth ×width). The position of the ovarian varies. They may be deep in the pouch of Douglas or high up on the pelvic side-wall. Ovaries can be seen in over 90% of normal menstruating women and approximately 60% of post menopausal women, depending on the age, visualization of intra ovarian vessels depend on ovarian activity the most commonly used indices to assess perfusion are resistive index (RI) and pulsatility index(PI) (Roger *et al.,* 1998).

Normal value for FSH during follicular phase

First half 3.9 – 12.0 mIu/ml.
Second half 2.9-9.0 mIu/ml.
Ovulation peak 6.3 – 24.0 mIu/ml.
Normal value for LH during follicular phase 1.68 – 15 mIu/ml.
Ovulation peak 21.9 – 56.6 mIu/ml.

MATERIALS AND METHODS

This study was carried out at fertility center of Al-Saddar teaching hospital in Al-Najaf city, include 75 infertile women attending the outpatient clinic of fertility center in the early follicular phase of menstrual cycle were offered participation in a prospective observational study from January 2009 to August 2009. consent was obtained from all participants. 75 infertile women divided into 3 groups (25 patients) : 25 patient regarded as control group i.e without treatment for ovulation induction. 25 patient regarded as CCCT group i.e women treated with clomiphene citrate drug and the last 25 patient regarded as femara group treated with letrozole drug. All of the 3 groups their ages ranges from 20-40 years. At first visit, in the second day of MC history taken from the patient included name, age, type of infertility either primary or secondary and duration of it, cause of infertility either male or female factor, also ask about result of HSG, if any trial of I.V.F or I.U.I and the result of it. After history taken, patients underwent evaluation of ovarian morphometry by TVUS (Medscan long wave with frequency 20- MHZ, vaginal probe 7-10 MHZ). The exclusion criteria after history, complete examination and investigations included those P.C.O.S, tubal causes of infertility and sever male causes. Ovarian images were procured in the saggital and coronal planes and the frozen image reflecting the largest ovarian dimensions was utilized for the measurement of ovarian length, width and depth (thickness) by cm as per standard clinical practice. number of small antral follicle (2-9 mm), size of large follicle and endometrial thickness (cm). All of these measured for each ovary and endometrium in Day 2 and Day 10 of MC. Mean ovarian diameter calculated by this formula (mean ovarian length+ mean ovarian width /2) per cm2, while mean ovarian volume calculated by the formula (0.5 × length × width × depth) per cm3. the endometrium was measured by longitudinal scans including the whole endometrium at the point of its maximum thickness. Both endometrial outer limits were included in the measurement, assuming that the amount of intracavity fluid was not significant. After these ultrasonic measurements, serum samples were collected in Day 2 and Day 10 of MC and stored at -20 °c until the assessment of markers of ovarian reserve (FSH and LH) by ELISA (EL × 800). Absorbance microplate reader is a single- channel reader- assay system, designed to automatically perform end point analysis for avariety of ELISA- based applications.The data they produce are managed by an on board processor, via a 2- line × 24- character LCD screen and membrane switch. Comparison of these data done between all three groups. The first group (control group), measure FSH, LH, and TVUS taken in Day2 of M.C and repeated these measurement in Day 10 of M.C without any treatment while second group (CCCT group) measure FSH, LH and TVUS done at Day2 and in the same day the patient given clomiphone citrate tab 100mg/dialy for 5 days and then in Day 10 repeated the same measurement while in 3rd group (femara group) given letrozole tab 2.5mg× 2/daily from Day2 for 5 days and same measurement taken. After that each group of our patients according to FSH level day 10 divided into normal or abnormal, normal if FSH < 12 mIu/ml, abnormal if FSH > 12 mIu/ml and see differences between other parameters accordingly.

Statistical analysis: The SPSS (statistical package for social sciences) 15 for Windows was used for all analysis. Descriptive statistics (mean, standard deviations), and t-student test were used to demonstrate results. Differences between means of each paired treatment of the two groups: control and CCCT, and the second group control and Femara were compared by using t-student test. One Way ANOVA and L.S.D were assessed for comparison of three studied groups. P≤ 0.05 was considered statistically significant Seventy – five patients were enrolled over the study period.

RESULTS

The continuous data are reported as mean ± SD. The mean age for control group (25 patients) was 29.60 ±6.50, while mean age for CCCT group (25 patients) was 30.44±6.72 and mean age for femara group (25 patients) was 31.84±6.523. The age parameter of all groups divided into 3 groups: less than 30 years, 30-40 years, more than 40 years. Mean and SD calculated accordingly.

- The age parameter of control group: (14/25) at age of less than 30years with 56%, mean age was 25.14±3.50. (8/25) at age between 30-40 years with 32%, mean age 32.75±2.12. (3/25) at age more than 40 years with 12%, mean age was 42.00±1.73.
- The age parameter of CCCT group: (11/25) at age less than 30 years with 44%, mean age was 25.45±2.77, (11/25) at age between 30-40 years, with 44%, mean age was 33.36 ±3.50. (3/25) at age more than 40 years, with 12%. mean age 41.66±3.21.
- The age parameter of femara group: (8/25) at age less than 30 years, with 32%, Mean age was 24.37±3.37. (12/25) at age between 30-40 years, with 48% mean age was 33.08±2.57. (5/25) at age more than 40 years, with 20% Mean age 40.80±0.83. Most infertile patient in our research have had primary infertility. (21/25), with 84% of control group with duration of 2-15 years, (21/25), with 84% of CCCT group with duration of 2-18 years. (22/25), with 88% of femara group with duration of

Table 1. Demonstrates General Demographics and Descriptive statistics of Control, Femara and CCCT Groups

Parameters	Control (25)	CCCT Group(25)	FEMARA (25)
Age	29.60±6.506	30.4400±6.727	31.84±6.523
Less than 30Yrs	25.143±3.505 (14)56%	24.454±2.770 (11)44%	24.375±3.378 (8)32%
30-40Yrs	32.750±2.121 (8)32%	33.364±3.500 (11)44%	33.083±2.575 (12)48%
More than 40Yrs	42±1.732 (3)12%	41.667± 3.214 (3)12%	40.800±0.837 (5)20%
Type of Infertility			
1º Infertility			
No.	21(84%)	21(84%)	22(88%)
Duration	2-15	2-18	1.5 - 19
2º Infertility			
No.	4(16%)	4(16%)	3(12%)
Duration	3-5	5-12	4 - 9

Table 2. Comparisons of Day 2 mean ovarian volume, mean no. of small antral follicle, mean ovarian diameter, FSH, LH, and endometrial thickness for all studied group according to age groups

Parameters	Age groups(years)			P
	< 30yrs (33/75)44%	30-40yrs (31/75)41.33%	> 40yrs (11/75)14.67%	
Mean ovarian volume	8.65±1.407	6.577±1.321	5.601±1.528	0.002*
Mean no of antral follicle	5.88±1.1.198	4.50±1.282	3.72±1.0 49	0.012*
Mean ovarian diameter	2.84±0.120	2.544±0.083	2.375±0.194	0.000*
FSH	6.35±2.65	5.80±2.69	6.40±1.72	0.488NS
LH	5.34±1.66	5.48±1.62	4.19±1.01	0.032*
Endometrium thickness	0.36±0.02	0.37±0.032	0.33±0.03	0.073NS

*Significant at 5%, NS not significant (F-test, One Way ANOVA)

Table 3. Hormonal Assay comparisons between Control, CCCT and Femara groups for day 2 and day 10 of MC

Groups	LH DAY2 (mIU/ml)	LH DAY10 (mIU/ml)	FSH DAY2 (mIU/ml)	FSH DAY10 (mIU/ml) Normal	FSH DAY10 (mIU/ml) Abnormal
Control group Mean ±SD	5.47±3.753	7.81±1.619	5.99±2.780	6.811±1.542 22/25(88%)	12.543±0.299 3/25(12%)
CCCT group Mean ±SD	4.37±3.191	9.37±1.176*(a)	6.07±2.641	6.894±1.886 18/25(72%)	20.368±11.447 7/25(28%)
Femara group Mean ±SD	6.83±6.422(b)	9.419±4.341*(a)	6.31±3.547	5.395±1.897*(a,b) 20/25(80%)	23.634±11.669*(a) 5/25(20%)

D10 FSH (Normal<12mIU/ml) (abnormal>12mIU/ml) (*L.S.D. Significant at P≤0.05) a =significance differences with Control group, b= significance differences with CCCT group.

1.5-19 years, while secondary infertility accounts for the remaining patients 4/25, with 16% of control group with duration 3-5 years, 4/25, with 16% of CCCT group with duration 5-12 years While 3/25, with 12% of femara group with duration 4-9 years.

For ovarian volume measurement, there is decrease in mean ovarian volume with increasing age, by a significant P. value of (0.002) between all 3 age groups (less than 30 years, 30-40 years, more than 40 years), while measurement of mean number of small antral follicle also there is decrease in a number of small antral follicle with increasing age by significant P. value of (0.012) between all age groups. Also for mean ovarian diameter there is decrease in mean ovarian diameter with increasing age by significant P. value of (0.000) between all age groups. For FSH measurement day 2, there is decrease in FSH level day 2 in age group of 30-40 years and then return to increase in age more than 40 years by non significant P. value (0.488). For LH measurement, there is increase in LH level day2 for age group 30-40 years than day 2 for age group less than 30 years, and return to decrease for age group more than 40 years by significant P. value (0.032). For endometrial thickness, there is significant decrease with age by P. value of 0.073. As shown in figure 1,2,3,4,5. for mean value ±SD of LH level day 2 there is non significant differences between control and CCCT, while, there is significant increment of LH level day 2 of femara group 6.83±6.42 than CCCT which is 4.37±3.19. While LH level day 10 show there is significant increment in CCCT by 9.37±1.17 than control group of 7.81±1.61 and significant increment of LH level day 10 of femara group 9.419±4.34 than control group of 7.81±1.61. For FSH day 2, there is non significant differences between three groups, while for FSH day 10 according to our classification normal when FSH < 12 mIu/ml and abnormal when FSH > 12 mIu/ml. for control group day 2 FSH was 5.99± 2.780, with normal FSH there is increment in FSH level by 6.811± 1.542 (22 patients out of 25 with 88%), while for abnormal group there is a high increment in FSH level by 12.543 ± 0.299 (3 patients out of 25 with 12%). For CCCT group, day 2 FSH was 6.07 ± 2.641 while for day 10 FSH for normal group there is increment by 6.894 ± 1.886 (18/25 patients with 72%) while for abnormal group, there is high increment by 20.368 ± 11.447 (7/25 patients 28%). For femara group, day 2 FSH was 6.31 ± 3.547 while day 10 FSH for normal group was 5.395 ± 1.897 (20/25 patient with 80%) while for abnormal group, there is high increment by 23.634 ± 11.669 (5/25 patients with 20%). In this table comparison done for age, mean ovarian diameter, mean ovarian volume, AFC and endometrial thickness for all our groups day 10 according to normal FSH (< 12 mIu/ml) and abnormal FSH (> 12 mIu/ml). For age parameter, there is non significant changes for all studied groups in normal and abnormal FSH levels. While for mean ovarian diameter, in control group, there is significant decrease in abnormal group by 2.903± 0.278 by P. value of 0.041 than normal FSH group of 3.405±0.715. for CCCT group also there is significant decrease in mean ovarian diameter in abnormal FSH group of 2.019±0.631 than normal FSH group of 3.225± 0.937 by P. value of 0.003.

Table 4. Comparison of studied Parameters within groups according to D10 FSH

Parameters	Group	Normal FSH<12mIu/ml	Abnormal FSH>12mIu/ml	P value (t-test)
Age	Control(Mean± SD)	30.046±6.615	33.333±5.507	0.365N.S
	CCCT(Mean± SD)	30.294±5.676	30.75±9.019	0.878N.S
	Femara(Mean± SD)	31.40±6.286	33.60±7.925	0.512 N.S
mean ovarian diameter	Control(Mean± SD)	3.405±0.718	2.903±0.278	0.041 *
	CCCT((Mean± SD)	3.225±0.937	2.019±0.631	0.003 **
	Femara(Mean± SD)	3.177±0.347	1.650±0.440 **(a,b)	0.001 **
mean ovarian volume	Control(Mean± SD)	10.405±1.718	8.903±1.278	0.036 *
	CCCT(Mean± SD)	9.225±1.937	8.018±1.631	0.048 *
	Femara(Mean± SD)	12.150±0.720 *(b)	10.160±0.616 *(a,b)	0.032 *
antral follicle count	Control(Mean± SD)	7.012±0.121	5.462±0.241	0.042 *
	CCCT(Mean± SD)	6.123±0.220	4.240±0.123	0.033 *
	Femara(Mean± SD)	5.462±0.310 *(a,b)	3.012±0.146 *(a,b)	0.037 *
Endometrial thickness	Control(Mean± SD)	1.077±0.057	0.833±0.031	0.022 *
	CCCT(Mean± SD)	0.982±0.055	0.830±0.075	0.023 *
	Femara(Mean± SD)	1.190±0.058 *(a,b)	0.860±0.052	0.001*
ANOVA-test	a =differences with control, b = differences with CCCT groups			

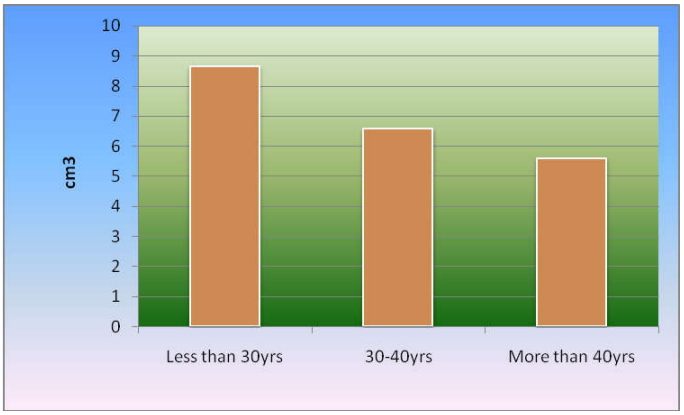


Fig. 1. D2 Mean ovarian volume of studied groups according to age groups

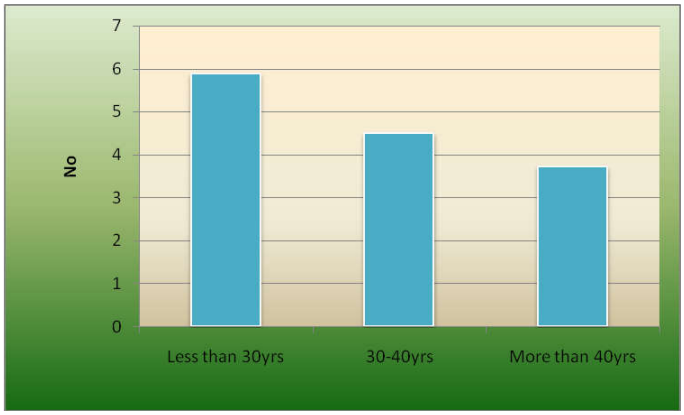


Fig. 2. D2 Mean No. of antral follicle of studied groups according to age groups

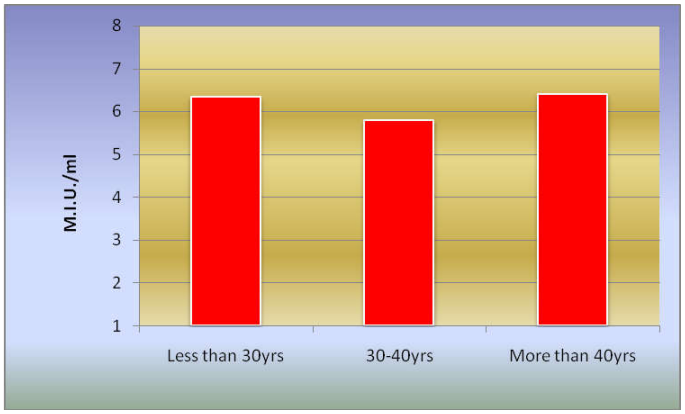


Fig. 3. D2 FSH of studied groups according to age groups

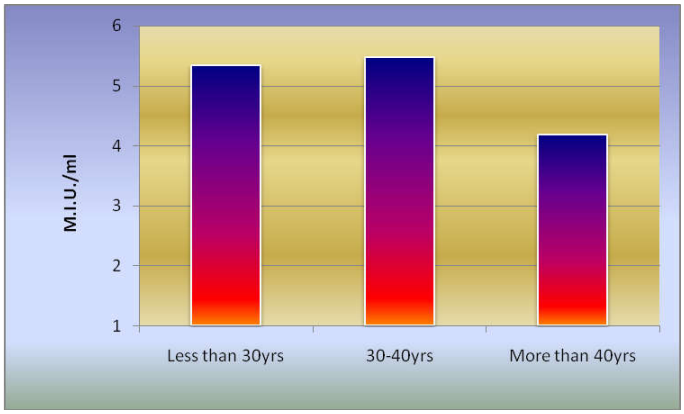
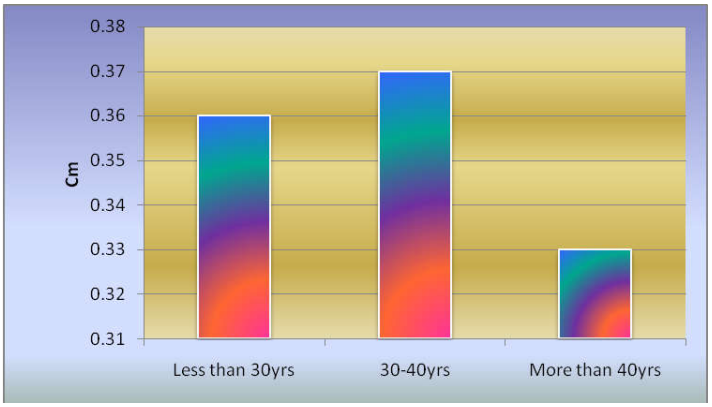


Fig. 4. D2 LH of studied groups according to age groups



Fig(5): D2 Endometrial Thickness of studied groups according to age groups

For femara group, also there is significant decrease in abnormal FSH group of 1.650 ± 0.440 than normal FSH group of 3.177±0.347 by P. value of 0.001. In general there is non significant changes in mean ovarian diameter for all studied groups in normal FSH group while there is a significant decrease in mean ovarian diameter for femara group than control and CCCT in abnormal FSH group. For mean ovarian volume, in control group there is significant decrease in abnormal FSH group of 8.903± 1.278 than normal group by 10.405 ± 1.718 by P. value of 0.036, while for CCCT there is significant decrease in mean ovarian volume in abnormal FSH group of 8.018 ± 1.631 than normal FSH group of 9.225±1.937 by P. value of 0.048, while for Femara group there is significant decrease in abnormal FSH group of 10.160±0.616 than normal FSH group of 12.150±0.720 by P. value of 0.032. In general femara group had significant increase in mean ovarian volume than CCCT group in normal FSH group, also femara group had a significant increase in mean ovarian volume than CCCT and control groups in abnormal FSH group. for AFC, in control group, there is significant decrease in AFC in abnormal FSH group of 5.462±0.241 than normal FSH group of 7.012±0.121 by P. value of 0.042. while for CCCT there is significant decrease in abnormal FSH group of 4.240± 0.123 than normal FSH group of 6.123 0.123 by P. value of 0.033, while for femara group, there is significant decrease in abnormal FSH group of 3.012 ± 0.146 than normal FSH group of 5.462 ± 0.310 by P. value of 0.037. In general in the femara group, there is significant decrease in AFC in normal FSH group than control and CCCT and significant decrease in AFC than CCCT and control group in abnormal FSH group. For endometrial thickness, in control group there is significant decrease in abnormal FSH group of 0.833± 0.031 than normal FSH group of 1.077 ± 0.057 by P. value of 0.022, while for CCCT group, there significant decrease in abnormal FSH group of 0.830±0.075 than normal FSH group of 0.982±0.055 by P. value of 0.023, while for femara group, there is significant decrease in abnormal FSH group of 0.860± 0.052 than normal FSH group of 1.190±0.058 by P. value of 0.001. In general, there is significant increase in endometrial thickness for femara group than CCCT and control groups. So in our study, there is significant effect of high FSH level > 12mIu/ml day 10 of M.C on ovarian volume, ovarian diameter, AFC and endometrial thickness.

Table 5. Number and Percentage of large follicle in the studied groups in day 10 for normal FSH < 12mIu/ml

	Control group		CCCT group		Femaral group	
	No	%	No	%	No	%
Large follicle	3/25	12	7/25	28	12/25	48

Follicle size (15-20mm) in day 10. For control group , number 3/25 , so % is 12, while for CCCT group number 7/25 , so % is 28 , while for femara group number is 12/25 , so % is 48. In this table femara group had better effect on follicular maturation than CCCT and control group , while CCCT had better effect on follicular maturation than control group.

DISCUSSION

The spontaneous pregnancy prognosis and the pregnancy prognosis in ART programs are age-dependant, though age is an epidemiological estimate that is not very precise in predicting individual OR. Many tests have been developed to screen for diminished OR, and these are important because nowadays pregnancy is increasingly being postponed for a variety of reasons. Thus, it is women in their late thirties and couples with un explained subfertility who will most likely benefit from these tests. When discussing OR, a distinction should be made between the two parts of this entity. On one hand, ovarian response can be measured by the reaction in an ART program, and this might reflect the quantity of the pool of oocytes in the ovaries. On the other hand, the chance to achieve a pregnancy can be studied (in both ART cycles and spontaneous cycles), and this might reflect the no. of oocytes left in the ovaries as well as the quantity of the oocytes. The first factor is interesting from a scientific point of view. However, these two factors should be considered separately when interpreting the results of the studies (Bukman and Heineman, 2001). OR is related to the age and can be estimated to OR tests, such as AFC, ovarian volume, Ovarian diameter, endometrial thickness and various endocrine parameters (day 10 FSH and LH). In our study we found these is significant relationship of age with mean ovarian volume, mean ovarian diameters, small AFC and basal LH level, but we found no significant changes with endometrial thickness and basal FSH level. In our study, basal FSH and LH conc. were normal (<10mIu/ml) in all the three groups (control, CCCT and femara). Haadsma *et al.* 2007 found that the no. of small antral follicle (2-6 mm) is significantly related to age and also, independent of age, to all endocrine OR tests tested, suggesting the no. of small antral follicles represent the functional OR. In a retrospective study by Frattarelli *et al.;* lauria-costa 2000 Ovarian measurements were performed by TVUS in the early follicular phase of the MC in 278 patients.

The authors demonstrated an inverse association between the Ovarian diameter (computed as an average of length and width) and age, basal FSH levels and the basal FSH : LH ratio. While Stacea Bowen, John Norian, MD and Lubna Pal, MBBS, MR COG, MS in 2008 have a attempted to explore the association of the individual ovarian measurements (i.e ovarian length and width) as well as the overall mean ovarian diameter (average of the length and width) independently with the OR, as reflected by the highest reported FSH value for each participant, as well as with advancing age. Their data confirm that the individual ovarian parameters (width, length or an average of the two) reliably reflect OR in pre-menopausal infertile women. They have further attempted to adjust for potential cofounders that may influence ovarian size including a history of smoking (Sharara, F.I ; Beatse, SN ; Leonardi, MR; Navot cigarette smoking accelerates the development of diminished OR as evidenced by the CCCT, 1994) and ovulatory status (i.e larger ovarian size associated with annovulatory status, specifically P.C.O.S, as well as BMI. while all the three ovarian measurements demonstrate negative association of statistical significance with increasing FSH levels (and hence declining OR) ,and with advancing age, the magnitude of these associations was most robust for ovarian width. Many medical studies have correlated ovarian size with a woman's ability to respond adequately to medications. Women with small ovaries tend to respond more poorly to fertility medication than those with larger ovaries. Ovarian volume is quick and cost – effective. Amir Lass[1], Jonathan Skull and Robert M.L. Winston, 1997, recommended that ovarian volume should be measured by TVUS before ovulation induction in all patients, regardless of age, and stimulation planned accordingly. Their results suggests that women who have a mean ovarian volume of < $3cm^3$ have a very high chance of failure to respond to exogenously applied stimuli.

About basal endometrial thickness, in our study we found no significant changes, because in all cases, we measured endometrial thickness in day 2, or day 3 where endometrium in its maximum shedding process, this is the first and secondly properly because, we exclude those woman with P.C.O.S. In our study shows there is no significant differences between LH levels day 2 and day 10 for all studied groups, that its mean LH level within normal limits (<15m IU/ml in follicular phase of MC). This is consistent with Mukerjee T, Coper man AB who were found that LH measurements may also have predictor value for OR, but FSH is considered a better marker since as menopause approaches, FSH rises sooner, and more dramatically than LH. There may be place for combined FSH and LH testing to estimate OR. In our study, the level of basal FSH was below 10mIu/ml (5.99±2.780 – 6.31±3.547) as shown in table no. 3. Basal FSH conc. was considered abnormal if it exceed 10mIu/ml. Winslow *et al.,* 1991 was found that cycle day 3 serum FSH conc. is an indirect estimate of OR, it being a measure of the amount of inhibin B/oestradial that a cohort of follicles is producing and the feedback effects at the level of the pituitary. Many studies have been carried out to determine the importance of the day 3 FSH conc. (Muasher *et al.,* 1988; Scott *et al.,* 1989 ; Toner *et al.,* 1991; pearl stone *et al.,* 1992 ; Ebrahim *et al.,* 1993; Ahmed Ebbiary *et al.,* 1994). All of these studies concerned data from ART cycles. Patients with low basal FSH conc. (threshold values vary between 10.8 and 25 Iu/ l) responded better to ovulation induction, as demonstrated by the no. of mature oocytes. Some studies show FSH to be a better predictor than age, although an age- related decline in fecundity remained (Scott and Hofmann, 1995). In one study (Martin *et al.,* 1996) no pregnancies were found in cycles with cycle day 3 FSH conc. ≥ 20 mIu/ml, whilst in other studies age was seen to be a better predictor for I.V.F outcome than basal FSH (Hall *et al.,* 1999). One study (Ahmed Ebbiary *et al.,* 1994) showed that sub fertile women with high FSH conc. had poorer follicular growth in a natural cycle when compared with sub fertile women with normal FSH concentration.

No data were available to assess the predictive value of basal FSH screening in woman of the general infertile population. Martin Js, Nisker JA, 1996 were found that a single measurement of day 3 FSH may not represent actual OR. When testing reveals elevated FSH, this result should be confirmed in a later cycle. However, interpretation of fluctuations across multiple cycles is controversial. Among patients with a series of day 3 FSH values that include at least one unfavorable (elevated) FSH test, a low response to ovulation induction has been observed. In our study we used CCCT as a predictor for OR, and we found that 18 patients out of 25 patients (72%) with a normal FSH levels (< 12 mIu/ml) and 7 out of 25 patients (28%) with abnormal FSH (>12mIu/ml), we found no significant differences regarding age groups, but we found that there is significant relationship of the level of FSH day 10 with the ovarian diameter, ovarian volume, no. of small antral follicle and endometrial thickness. All of these results found to be statistically significant. CCCT has been validated by several authors for predicting OR. The test involves the administration of 100mg clomiphene citrate on day 5-9, and the determination of FSH conc. on day 3 and 10 (Navot *et al.,* 1987 ; Tanbo *et al.,* 1989, 1992 ; Lonmaye *et al.,* 1990 ; Scott *et al.,* 1993, 1995). With normally responding ovaries, the clomiphene citrate- dependent rise in FSH will be suppressed by Oestradiol and inhibin B produced by the follicles. An abnormal test is defined as an abnormal high FSH on day 3 and/or day 10. Age related differences in the CCCT outcome have been noted (Nader and Berkowitz, 1991). several publications have described the value of CCCT result in relation to the reaction to ovarian stimulation and pregnancy rates. An abnormal test is seen more often in older women beginning in the early thirties, and predicting a lower pregnancy rate in spontaneous cycles (Scott *et al.,* 1993) as well as in stimulated cycles (Loumaye et al./ 1990) Women with a normal test result respond better to control ovarian stimulation, and in this way the CCCT provides a reliable individual pregnancy for the ovarian response (tando *et al.,* 1989). The CCCT appears to be more sensitive than day 3 FSH alone (Scott and Hofmann, 1989), the likelihood ratio of a positive test in relation to the chance to become pregnant varying between 6.2 and 14.5. In one study (Tanbo *et al.,* 1992), the cancellation rate was found to be much higher in woman with an abnormal test result. Recently, a relationship was found between the no. of follicles counted by ovarian biopsy and the outcome of this test (aulekli *et al.,* 1999). One study performed to determined the cycle to cycle variability of cycle day 10 FSH in the CCCT (Hannoun *et al.,* 1998) showed significant inter-cycle variability, but no correlation with the potential to achieve pregnancy was evaluated.

The results of the different studies indicate that the CCCT should be able to differentiate between women with either normal or decreased OR. Navot *et al.* 1987 found that when undergoing CCCT, the first step is to measure day 3 FSH and E2. then 100mg of clomiphene is administrated on cycle days 5 through 9, and FSH and E2 measurements are repeated and cycle day 10. in general, a high day 10 FSH suggests poor OR. Lonmanye E, Billion JM, mine JM et al 1990 describe the CCCT, 18 patients out of 51 had abnormal responses. Of those with abnormal responses, only one pregnancy resulted (1 of 18, or 6%). The pregnancy rate among those with normal CCCT response was substantially higher (14 of 33, or 42%). Scott *et al.* 1993 found that the evolution of a large no. of infertility patients found a 10% prevalence of abnormal CCCT responders. Kahraman *et al.,* 1997, found the CCCT to be a better predictor of OR than day 3 FSH measurements alone. Femara drug is used to induce ovulation, it acts to suppress Ostrogen in the body and increase FSH and LH which result in development of ovarian. Follicle, thus helping to bring ovulation. In our study, we try to found the efficasy of the femara drug as a predictive test for OR, we found that, 20 patients out of 25 patients (80%) with a normal FSH levels and 5 patients out of 25 patients (20%) with abnormal FSH. We found no significant differences regarding age groups, but we found that there is significant relationship of the level of FSH day 10 with the ovarian diameter, ovarian volume, no. of small antral follicle and endometrial thickness. Also we found that femara group is probably better than CCCT group in predicting OR as it appear statistically significant. So we can use femara similar to CCCT in predicting OR. Data from early trials confirm the effectiveness of letrozole, the most commonly used agent (Homburg 2003). In the only randomized controlled trial comparing letrozole (2.5mg daily cycle day 3-7) and clomiphene treatment (50mg daily cycle day 3-7), conducted in normal ovulatory women. Peak endometrial thickness was similar despite markedly lower estrogen levels in women receiving letrozole. Some early studies suggested that the pregnancy result with letrozole far exceeded than clomiphene citrate and were possible even higher than gonadotrophins. Pregnancy rates with letrozole are similar to those seen with clomiphene and are similar lower than the pregnancy rates

seen with gonadotrophins. Older patients have a lower success than younger patients.

Conclusion

1. 1-CCCT is more predictive of OR compared with age, basal serum FSH and LH.
2. 2-We can use femara as predictive test for OR and probably this needs further study.

Abbreviations

FSH - Follicular, Stimulating, hormone
LH - Luteinizing hormone
GnRH - Gonadotrophin – releasing hormone.
PCOS - Polycystic ovarian syndrome.
COCP - Combined oral contraceptive pills.
PID - Pelvic inflammatory disease
OR - Ovarian reserve
CCCT - Clomiphene citrate challenge tests.
MC - Menstrual cycle
TVUS - Trans vaginal ultra sonography.
HSG - Hystero salpingography
IVF - Invitro fertilization
IUI - Intrauterine insemination.
SD - Standard deviation
SPSS - Statistical package for social sciences
AFC - Antral follicle count.
No. - Number.
ART - Assisted reproductive technique
CONC. - Concentration

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