EVALUATING THREE ALTERNATIVE PROTOCOLS FOR IMPROVING OVARIAN RESPOSE OF THE POOR RESPONDERS UNDERGOING ASSISSTED REPRODUCTIVE TECHNIQUES

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By

Yasser Ibrahim Orief

MBBCh Alex
MS Ob/Gyn Alex

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SUPERVISORS

Prof. Dr. Ibrahim Ahmed Medhat

Professor of Obstetrics and Gynecology,
Faculty of Medicine,
University of Alexandria.

Prof. Dr. Mahmoud Ali Abo Ollo

Professor of Obstetrics and Gynecology,
Faculty of Medicine,
University of Alexandria.

Ass. Prof. Dr. Hisham Ali Saleh

Assistant Professor of Obstetrics and Gynecology,
Faculty of Medicine,
University of Alexandria.

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TABLE OF CONTENT

	ACKNOWLEDGEMENT	i
	TABLE OF CONTENT	ii
	ABBREVIATIONS	iii
	LIST OF TABLES	v
	LIST OF FIGURES	vi
I.	INTRODUCTION	1
II.	AIM OF THE WORK	32
III.	MATERIALS AND METHODS	33
IV.	RESULTS	37
V.	DISCUSSION	54
VI.	SUMMARY AND CONCLUSIONS	60
VII.	RECOMMENDATIONS	63
VIII.	LITERATURE CITED	64
IX.	PROTOCOL	
X.	ARABIC SUMMARY	

ABBREVIATIONS

ANOVA	Analysis of variance
ART	Assisted reproductive techniques
CC challenge test	Clomiphene Citrate Challenge Test
COH	Controlled ovarian hyperstimulation
DOR	Diminished ovarian reserve
E2	Estradiol
EFORT	Exogenous FSH ovarian reserve test
EGF	Epidermal growth factor
F test	Fisher exact test
FSH	Follicle stimulating hormone
GAST	Gonadotrophin-releasing Hormone Agonist Stimulation Test
GnRH-a	Gonadotrophin releasing hormone analogue
GnSAF	Gonadotrophin surge-attenuating factor
GV	German vesicle
hCG	Human chorionic gonadotrophin
hMG	Human menopausal gonadotrophin
i.m.	Intramuscular
ICSI	Intracytoplasmic sperm injection
IGF	Insulin like growth factor
IU	International unit
IUI	Intrauterine insemination
IVF	In vitro fertilization
LH	Luteinizing hormone
MI	Metaphase I
MII	Metaphase II
OHSS	Ovarian hyperstimulation syndrome
P	Progesterone

P value	Probability value
PCOS	Polycystic ovarian syndrome
Pgm	Picogram
rFSH	Recombinant FSH
s.c.	Subcutaneous
SPSS	Statistical package for social sciences
TVS	Transvaginal ultrasonography
χ^2	Chi-square test

LIST OF TABLES

Table		Page
1	Clinical characteristics of the patients under study.	38
2	Clinical characteristics of the treatment cycles.	39
3	Attempted oocyte retrieval and oocyte recovery rates per started cycle.	41
4	Cycle cancellation rate and causes of cycle cancellation.	42
5	Number and quality of the retrieved oocytes.	44
6	Successful and failed fertilization per oocyte recovery.	46
7	Embryo scoring.	48
8	Embryo transfer.	49
9	Pregnancy and clinical pregnancy rates per started cycle.	50
10	Pregnancy outcome.	51
11	Live birth rate.	52

LIST OF FIGURES

Figure		Page
1	Serum Progesterone and endometrial thickness on day of hCG administration.	40
2	Serum estradiol on day of hCG administration.	40
3	Number of ampoules and Duration of induction.	40
4	Attempted oocyte retrieval and Oocyte recovery rates per started cycle.	41
5	Cycle cancellation rate.	43
6	Causes of cycle cancellation.	43
7	Number of oocytes retrieved.	45
8	Quality of oocytes retrieved.	45
9	Successful and failed fertilization per oocyte recovery.	47
10	Embryo scoring.	48
11	Pregnancy and clinical pregnancy rates per started cycle.	50
12	Pregnancy outcome.	51
13	Live birth rate.	53

Chapter I

INTRODUCTION

INTRODUCTION

One of the greatest challenges in clinical reproductive endocrinology is evaluation and management of patients who respond poorly to exogenous gonadotrophins and who are categorized as "low responders". Identifying potential low responders is of critical clinical importance. These patients require specialized management to optimize the number and quality of oocytes that may be available for assisted reproductive technologies (ART) procedures. Even with optimal management, their clinical pregnancy and delivery rates are diminished compared with age matched controls, and patients should be managed accordingly. [1]

Physiologic changes in ovarian function with age

Virtually all clinicians are aware of the age related diminution in reproductive potential. As women become older, their chances for becoming pregnant decline. A detectable decrease in the reproductive efficiency is present by the time women are in their late twenties and very few women are able to conceive beyond their mid-forties.
This age related decline in the reproductive function has several important characteristics and is related principally to changes at the oocyte level. [2]

One of the most clinical aspects to understand the impact of the age related diminution on ovarian reproductive function is the difference between a decline in quantitative ovarian responsiveness (a low responder) and a decline in ovarian reserve (low quality oocytes with very poor potential to produce a viable pregnancy). These two factors are closely related but not identical. [3] Although not fully accepted, the most dominant criteria for poor ovarian response are small numbers of follicles developed or oocytes retrieved and low estradiol (E2) levels after the use of a standard stimulation protocol. [4]

Ovarian reserve is formed in utero as a result of oogonial mitosis counterbalanced by oogonial atresia and meiosis. Abnormalities in genes responsible in germ cell migration, mitosis or meiosis could compromise ovarian reserve. At birth 1-2 million follicles are present, to decrease to 300,000 at puberty, of those only 400-500 ovulate, the rest undergo atresia. Follicular growth is divided into 3 phases; pre-antral, tonic antral growth phase, and exponential growth phase. During the FSH dependent phases, tonic and exponential phases, follicles pass through 8 classes with dramatic increase in granulosa cell number, and follicle diameter. Class V follicles are present at the end of the luteal phase to be recruited, by the initial FSH rise. As one follicle is selected, it inhibits FSH by its marked E2 production, leading to atresia in the rest of the cohort, this results in dominance, and mono-ovulation. Raising FSH above a threshold, before dominance, would rescue the rest of the follicles in the cohort, with multifollicular response to hMG. In 9-24% of patients follicular response to ovarian stimulation is less than average “low responders”. [5] This is a very heterogonous group that could present with old age, high basal FSH, occasionally high basal E2, low antral follicle count by ultrasound, and aberrant responses to dynamic testing such as GnRH-a test, clomiphene challenge test. At a cellular level, their granulosa cells are inherently abnormal, and their embryo implantation potential is compromised. In general low responders could either have low ovarian reserve, or normal ovarian reserve, the latter represent different etiologies and several treatment options are available.

Ovulation induction

Optimal ovarian response to stimulation is crucial for the efficiency of assisted reproduction procedures. When starting ovarian stimulation, however, it is sometimes very difficult to predict the ovarian response to gonadotrophin stimulation. Both ovarian hyperstimulation syndrome (OHSS) and poor response are undesired conditions. The aim of ovarian stimulation is to strike a balance between giving a dose high enough to allow the growth of 8–12 follicles while at the same time minimizing the risk of ovarian hyperstimulation syndrome.

The different ovarian responses to gonadotrophins may be due not only to different follicle sensitivities to FSH and FSH pharmacodynamics, but also to other factors that can be predicted and evaluated before choosing the right kind of protocol for ovulation induction and gonadotrophin dosage. It has been recognized that clinical factors such as age, body weight and FSH basal concentrations may influence ovarian response. [(6)] Furthermore, women with polycystic ovarian syndrome (PCOS) have proven to be more sensitive to gonadotrophin stimulation with a tendency to respond excessively to stimulation and increased OHSS risk; [(7)] such women therefore benefit from a reduced starting dose.

Patients considered ‘standard’ because they are aged below 40 years, with a regular menstrual cycle and normal basal FSH concentrations, may also present marked variability in ovarian response to treatment when a standardized dose is administered.

Many clinical trials have been performed with a view to discover the optimal gonadotrophin starting dose for assisted reproduction procedure, particularly following the introduction of recombinant FSH preparations (r-FSH). The starting dose in patients undergoing their first treatment and younger than 40 years typically ranges from 100 to 250 IU/day, but there is no universal agreement on the most advantageous initial FSH dose.

FSH threshold and FSH window:

It is accepted that assisted reproduction is most successful when a reasonable number of healthy, mature-sized follicles is induced to grow in a cohort by exogenous FSH administration. Follicular recruitment generally refers to the induction of growth and maturation of gonadotrophin sensitive antral follicles by FSH (and LH). This is based upon the concept that in the normal menstrual cycle, follicles attain FSH sensitivity on a daily basis, but only those selected by the peri-menstrual rise in FSH are induced to grow. [(8)]

The two gonadotrophins demonstrate essential trophic and permissive roles that can be defined for follicles at different stages of advanced development. However, other elements, such as growth factors and insulin, are responsible for the much longer earlier stages of follicular development in the complex sequence of events comprising the life history of human ovarian follicles. In reality, the gonadotrophin sensitive phase is restricted only to the final couple of weeks of existence prior to ovulation. It is interesting that the FSH receptor message is present even in primordial follicles, but growth (initial recruitment)

takes place under the control of other factors, even in the absence of FSH stimulation. This can be seen in the clinical circumstance of hypogonadotrophic hypogonadism, where antral follicles are present, and only a few days of FSH injections can achieve ovulation. Correspondingly, the preceding 3–4 months development, from 'initial recruitment' to the antral stages, occurs with negligible contribution from the gonadotrophins and their receptors. [9]

The concepts of clinical control of follicular recruitment may address, on the one hand, short-term antral follicle maturation with gonadotrophins and other factors, and on the other, long term primordial follicle cohort development, influencing the size and development of the available antral follicle cohort.

In relation to assisted conception, human menopausal gonadotrophin (hMG) was used alone and with clomiphene by Edwards and Steptoe [10] (1975) from the earliest days of IVF. The history of the elucidation of FSH has been reviewed in the past. [11] The high FSH levels, which occur during the luteofollicular phase 4 days before menses, give rise to continued growth of a limited number or cohort of follicles. [12, 13] These follicles become dependent on continued stimulation by gonadotrophins during the follicular phase, and each growing follicle possesses a threshold requirement for stimulation by circulating FSH.

The concept that FSH concentrations should be above a certain level 'FSH threshold' was first introduced by Brown [14] in 1978 and substantiated more recently by Schoemaker [15, 16] and others. [17] The 'FSH threshold' means in this case the magnitude of FSH that is required for stimulation of ongoing follicle growth and ovulation. The duration of the 'FSH window' during which FSH levels are above the threshold required to stimulate ongoing development, determines the number of follicles that can develop to the pre-ovulatory stage, and finally this 'window' is opened to therapeutic intervention with exogenous FSH. [18, 19]

The aim of stimulation for IVF is to surpass the FSH threshold of all follicles within the cohort, producing a group of mature, synchronous follicles after 10–15 days of treatment. The biological activity of FSH is the sum of a complex combination of processes, and these effects may be sub-served by a number of intermediaries. The important processes are the release from the pituitary, survival in the circulation, transport and receptor binding, activation of signal transduction pathways, clearance from the circulation and modifications during circulation, and finally also receptor-binding desensitization. [20]

Gonadotrophin preparations have been used for ovarian stimulation since the early days of IVF. [21, 22] There is a narrow dose range for use of FSH between a threshold level required to stimulate growth of a follicle and the maximal dose above which over-stimulation can occur. [23]

Definition of poor responder

Unfortunately, there is no universally accepted definition for the "low", "poor", "bad" or "non" responder, although these patients have definitely lower pregnancy rates compared with `normal' responders. Numerous criteria have been used to characterize poor response. The number of developed follicles and/or number of oocytes retrieved after a standard-dose ovarian stimulation protocol are the most important two criteria for defining poor ovarian response. The proposed number varies among different authors, and ranges from less than three to less than five dominant follicles on the day of hCG administration, [24, 25, 26] and/ or less than three to less than five retrieved oocytes. [27, 28, 29]

Peak E2 level is another criterion used, as it correlates with the number of developing follicles. A peak E2 level of <300 to <500 pg/ml has been proposed as being crucial for defining poor response, [30, 31] although a level <100 pg/ml on day 5 of stimulation has also been suggested. [32] An elevated day 3 FSH level ranging from >7 to >15 mIU/ml has been proposed as an additional criterion to define poor ovarian response. [33, 34, 35, 36]

Likewise, an advanced patient age of >40 years, disappointing or no response to the clomiphene challenge test [37] and a failed "Lupron screening test" [38] may be predictive of poor response. More logically, but not necessarily more accurately, criteria such as at least one cancelled IVF cycle, [39] increased number of hMG or FSH ampoules used (>44) [40], increased (>300 IU/day) gonadotrophin dose used and prolonged duration of gonadotrophin stimulation [41] have been suggested. It should be noted that, in a variety of studies, these criteria have been used either alone or in combination, thereby highlighting the complexity, the lack of uniformity in definitions and also the major difficulties encountered when comparing the different strategies proposed.

Some authors have suggested a classification of poor responders. Thus, according to Gorgy and Taramissi, [42] there are two subgroups of poor responders. The first subgroup includes the young (aged <37 years) and slim (body weight <70 kg), who developed less than five follicles following 9 days of ovarian stimulation with 225 IU/day and did not reach oocyte retrieval, or required >600 IU of gonadotrophin per retrieved oocyte if they did reach that stage. The second subgroup included patients aged >37 years and weighing >70 kg who had their cycles cancelled due to less than five developed follicles following 9 days of ovarian stimulation with 300 IU/day of gonadotrophins. Similarly, others, [43] proposed that three subgroups of low responders should be identified:

(i) Patients with a low response to previous IVF attempts but normal basal FSH levels;
(ii) young patients with persistently high FSH levels; and
(iii) older patients with an abnormal endocrinological profile.

Clinical experience has not shown any real advantage in subcategorizing patients however, as no significant differences regarding response to ovarian hyper-stimulation protocols have been observed. In fact, low ovarian response is confirmed only after the patient has failed ovarian stimulation following an accepted as `normal' or `standard' ovarian stimulation regimen.

Causes of low ovarian response

Although several possible etiologies have been suggested, it seems that a diminished ovarian reserve (DOR) is the principal factor of poor ovarian response. [44, 45] Diminished ovarian reserve means diminished number of residual recruitable follicles within the ovary. [46] It is the most important cause, and perhaps the only predictable one.

Causes of DOR include advanced age, ovarian pathology, previous surgery, chemotherapy, or radiotherapy, and smoking. Unexplained DOR is occasionally seen in younger patients and is probably related to low original follicular endowment, or accelerated depletion, and these patients are probably at increased risk of pre-mature ovarian failure. [47]

Alternatively, a decreased number of FSH receptors available in granulosa cells, [48] defective signal transduction after FSH receptor binding, [49] the presence of a special FSH receptor-binding inhibitor in the follicular fluid, [50] disturbance in local intra-ovarian autocrine/paracrine regulation with predominance of inhibitory growth factors like EGF, over facilitatory ones like IGF-II. [51] These patients might benefit from administration of growth hormone [52] or substances that promote endogenous growth hormone release like arginine, [53] Clonidine, [54] and pyridostigmine. [55]

Other causes could be also postulated like an inappropriate local vascular network for the distribution of gonadotrophins, [56] exaggerated suppression and perhaps direct effect of Gn-RH agonist on the ovary. [57, 58]

Interception of the cycle at a point when the number of ready follicles is few is a theoretical cause, but might explain the occasional, non-consistent poor response seen in some patients. Although it contradicts the well established notion that follicular recruitment occurs under all circumstances in a number that is proportionate to the residual pool, [59] it gains some credibility from the significant variations seen in basal FSH levels [60] and from the claimed effectiveness of oral contraceptives pre-treatment in improving ovarian response. [61] The presence of auto-antibodies against granulosa cells and lowered circulating gonadotrophin surge-attenuating factor (GnSAF) bioactivity have also been proposed. [62]

Prediction of ovarian reserve

As currently there is no accurate and 100% predictive test available to assess ovarian response, there is likewise no screening test to detect poor ovarian response, and only ovarian reserve can be assessed. Several tests have been suggested for this purpose, but at present there is no definitive evidence for their predictive value. Clearly, the ideal test is the response of the ovaries to ovarian stimulation.

The results of ovarian reserve testing are often viewed by patients as either good news or bad. The bad news is that, for patients with low ovarian reserve, implantation rates are generally poor and the possibility of successful pregnancy is very limited. When faced with such information, couples are often devastated. They may have a variety of reactions - including, anger, denial or depression, all of which are normal and natural expressions of grief.

When couples receive a diagnosis of poor ovarian reserve, it is often helpful for them to spend time talking to each other, examining their parenting needs. They may want to ask themselves, "What is the most important thing to us about having a family? Is it the opportunity to provide love and guidance to a child? Is the genetic link essential? Is it important to experience pregnancy and childbirth? Is childfree living a realistic alternative?" By answering such questions, the couple will be in a better position to assess other options. Many patients have enlisted the aid of support groups, therapists, or compassionate physicians to help them through this crucial time. Whatever the outcome, patients receiving such shocking news will need time to mourn their loss and find a way toward acceptance. [63]

What is ovarian reserve?

As a woman ages, her supply of oocytes gradually declines over time until the oocytes are depleted at menopause. Although we expect the ovary to age in a certain way, there are times when it doesn't behave as predicted. That's why screening for ovarian reserve is a fundamental part of the initial evaluation for infertility patients of any age.

The term "ovarian reserve" refers to a woman's current supply of oocytes, and is closely associated with reproductive potential. In general, the greater the number of remaining oocytes, the better the chance for conception. Conversely, low ovarian reserve greatly diminishes a patient's chances for conception.

Methods of assessing ovarian reserve

Since a woman's chronological age is the single most important factor in predicting a couple's reproductive potential, age has often guided infertility treatment choices. However, age alone doesn't tell the whole story. Consequently, researchers have developed (and are continuing to develop) more refined methods of predicting a couple's response to infertility treatment. Some of the more sophisticated tools for assessing fertility potential include the measurement of FSH, LH, estradiol, and inhibin-B. Additionally, because patients should not be subjected to all tests, decisions regarding which method(s) to use are guided by practitioner experience.

Even though several sophisticated tools exist for measuring ovarian reserve, most fall short of what we consider ideal sensitivity and specificity. Also, how best to interpret ovarian reserve tests is controversial, since clinical experience is still evolving with these tests. Even so, most infertility patients should be periodically evaluated for the possibility of impaired ovarian reserve before pursuing any advanced fertility treatment. [64]

Finite supply of oocytes

Even before birth, a woman's oocytes begin to diminish in number. During the 20th gestation week, a female embryo contains about seven million oocytes. At birth, the number of oocytes has already dropped to about 200,000. The number of oocytes continues to decline as the woman ages, until no oocytes remain when the woman reaches menopause.

Fortunately, women are naturally equipped with an ample supply of extra oocytes. The number of oocytes a woman has at birth far exceeds the average number of menstrual cycles she will have during her lifetime. Therefore, when women undergo fertility treatment to boost oocyte production, the risk for premature menopause is not different than it would be for other women. [65]

The effects of female age on ovarian reserve

Menstrual cycles that occur near the end of the ovaries' lifetime are associated with older oocytes of poorer quality. [66] In general, ovarian age parallels chronological age. But since that is not always the case, it is vitally important for clinicians to assess an infertility patient's ovarian reserve. This is particularly true for women over the age of 35.

Conception and childbirth in women of advanced age has always been uncommon. This fact was recognized from the beginning of recorded human history .Later, studies looking at communal societies found solid evidence of reduced fertility associated with older women. [67] More recent work has validated these findings. [68, 69] The father's age, however, appears to have only a marginal influence on fertility. [70]

The effect of maternal age on fertility has been the subject of considerable research. In one such study, pregnancy success rates as a result of timed therapeutic insemination (intrauterine insemination; IUI) were evaluated in terms of maternal age. The poorest outcomes were consistently seen among women 36 years of age or older. [71] This observation was confirmed by research in an in vitro fertilization (IVF) setting, where women 37 years of age or older had a 9% ongoing pregnancy rate compared to a 26% ongoing pregnancy rate in patients younger than 30 years. [72]

In the clinical practice, IVF treatments involving women aged 40 or older have been associated with fewer oocytes obtained per cycle, low estradiol (E2) on the day of hCG administration, and considerably lower embryo implantation rates when compared to women 32 years of age or younger. Even if pregnancy does occur in women who are 40 years or older, there is a high risk for unfavorable outcomes. As maternal age increases, miscarriage occurs more frequently, as does the chance of fetal chromosomal abnormalities. [73]

The experience of the western countries with donor-oocyte cycles (although we do not agree with these techniques) offers further evidence that infertility and live birth rates are strongly influenced by the age of oocytes. Younger oocytes from donors under the age of 35 are typically used in donor-oocyte cycles. This greatly improves the reproductive outcome. [74, 75] While the recipient's age does have some negative impact on implantation rates, the effects appear limited. In fact, even if the recipient is over 40, clinical pregnancy rates approaching 59% have been achieved when donor (younger) oocytes are used. [76]

Testing of ovarian reserve

The methods for assessing ovarian reserve are classified into two groups: passive testing and dynamic testing. The goal of both approaches is to provide information regarding oocyte quality and quantity [64]

A. Passive ovarian reserve testing

- Measuring FSH and LH
- Measuring Estradiol
- Measuring Progesterone
- Measuring Inhibin-B
- Transvaginal Ultrasound
- Measuring circulating gonadotrophin surge-attenuating factor (GnSAF) bioactivity
- Measuring follicular fluid concentration of insulin-like growth factor-I (IGF-I)
- Measuring serum concentrations of antimüllerian hormone

B. Dynamic Ovarian Reserve Testing

- Clomiphene Citrate Challenge Test (CC challenge test)
- Gonadotropin-releasing Hormone Agonist Stimulation Test (GAST)

Measuring FSH and LH

It became apparent that early follicular-phase FSH levels played an important role in pregnancy outcomes. Researchers soon discovered that day 3 FSH could be very useful in predicting response to ovulation induction and IVF. [77]

Age related changes in FSH levels

A series of studies in the 1970s and 1980s characterized the endocrinological aspects of the transition through the climacteric. Sherman and Korenman documented that women with normal ovulatory cycles commonly begin having subtle elevation in their FSH levels beginning in their thirties. [78, 79] Other authors confirmed these findings, consistently showing that the first elevation occurs in the early follicular phase. [80, 81] Although these studies did not evaluate the relation between FSH levels and ovarian reserve (pregnancy potential), they documented that FSH concentrations increase around the same general time that the incidence of diminished ovarian reserve increases. These data provided the initial background data when evaluating various forms of FSH screening.

From the previous data, it is concluded that as the woman 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. A rise in early follicular-phase FSH is also accompanied by a decline in oocyte quality, and some investigators have linked such FSH elevations to fetal abnormalities. [82]

The elevation in FSH concentrations are not accompanied by changes in circulating estradiol (E2) or progesterone (P) levels throughout the menstrual cycle [83, 84] Thus 19 and 49 year old women with regular menstrual cycles have comparable E2 and P levels through the various phases of their cycles. Thus there is no lack of hormonal support of the endometrium to explain the age related decline in fertility.

Batesta et al evaluated LH, FSH, E2, P, inhibin and endometrial biopsies in younger (age 20-30) and older (age 40-50) volunteers. [85] FSH concentrations were increased and inhibin levels were decreased in the older group. None of the other variables were different. The same investigators also measured the 24 hour mean FSH and LH levels in the early follicular and mid-luteal phases of the cycle in both the younger and the older groups. The 24 hour mean FSH level concentrations were significantly higher in the older group in both phases of the cycle compared with the younger group, whereas no differences were noted for LH.

Basal day 3 FSH concentrations and pregnancy rates

Since FSH has such high predictive value, should FSH always be measured? And if so, what values are important?

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. Adding to this uncertainty is the fact that no data exists which describes FSH patterns in a "control" population of fertile women. [86] Arbitrarily applying day 3 FSH tests to all women (even those with no infertility history) is controversial, and can offer confusing results. Some clinicians have therefore questioned the usefulness of widespread FSH screening. [87]

While it is unwise to rely on a single test to fully assess ovarian reserve, considerable data exists that provides some general guidelines about which FSH values are most significant. In one center, women undergoing IVF with a day 3 FSH of less than 15 mIU/ml were twice as likely to conceive as women with FSH values between 15 and 24.9 mIU/ml. [88] Other investigators confirmed these results, and FSH values emerged as superior to maternal age as a method for determining reproductive outcome in IVF. [89] Indeed, one series reported that when day 3 FSH levels exceed 20 mIU/L, conception rates fell sharply. [90]

Intercycle variability in basal FSH levels

Traditionally, clinicians have relied on cycle day 3 FSH test results to help assess ovarian function. However, 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. [91] 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 reproductive physiology. [92]

Patients with low FSH values (suggesting satisfactory ovarian reserve) 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 ovarian reserve.

A single measurement of day 3 FSH may not represent actual ovarian reserve. 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. [93] Another analysis revealed that patients with both high and low FSH values across multiple cycles performed as low responders during IVF. [94] However, other investigators regard variable FSH results differently. Some consider relatively low day 3 FSH values permissive for IVF or other fertility treatments during that cycle. Preliminary data suggest that if FSH returns to a "normal" level after an abnormal (high) test in a previous month, conception rates for IVF may be approximately 35% for patients under the age of 40. [95]

LH measurement may also have predictive value for ovarian reserve, but FSH is considered a better marker since as menopause approaches, FSH rises sooner and more dramatically than LH. [96, 97] There may be a place for combined FSH+LH testing to estimate ovarian reserve, as some investigators have suggested an increased FSH: LH ratio may predict an elevation in FSH alone. [98]

Current status of basal FSH screening

Elevated basal day 3 FSH concentrations are highly predictive of diminished ovarian reserve as defined by poor gonadotrophin responsiveness and pregnancy rates in patients undergoing complex ovulation induction or one of the assisted reproductive technologies. The test is simple, inexpensive and routinely available. The studies performed to date are limited to clinical circumstances requiring complex ovulation induction.

Measuring Estradiol

Investigators initially thought estradiol (E2) would be a more specific marker for ovarian reserve than either FSH or LH. Unfortunately, subsequent research has shown a weak relationship between E2 and ovarian response to fertility treatments. Evaluation of cycle day 3 E2 in IVF patients revealed no clear association between E2 and treatment outcome. [88]

In a population of IVF patients without pre-treatment GnRH-a suppression, cycle day 3 E2 and FSH were compared to reproductive outcome. The researchers observed that even when FSH values were less than 20mIU/mL, no pregnancy occurred when day 3 E2 was greater than 75pg/mL. [99] This result was supported by others who observed better outcomes for women aged 38-42 when day 3 E2 was less than 80pg/mL and FSH was normal. [100] From these studies it seems that evaluating both E2 and FSH was a better predictor of ovarian reserve than using either measurement alone.

Low day 3 E2 levels, combined with normal FSH, have been associated with improved stimulation response, higher pregnancy rates, [101] and lower cycle cancellation rates. [102] Interestingly, researchers reported that measurement of E2 one day earlier (on cycle day 2) did not enhance the predictive value of ovarian response. [103]

High levels of E2 early in the menstrual cycle suggest an inappropriately advanced stage of follicular development. This may occur as the ovary ages, or when ovarian follicular cysts remain from a prior menstrual cycle. The follicular cysts can interfere with oocyte "recruitment" in the treatment cycle, naturally leading to a poor response to fertility treatment.

Measuring Progesterone

A decline in ovarian reserve has also been associated with a short follicular phase, early LH surge, and premature elevation of progesterone. [104] Initially, it was though progesterone might be a useful tool for ovarian screening. However, daily E2 and progesterone testing performed in volunteers with ovulatory cycles revealed no differences in E2 or progesterone as a function of age. [97] Researchers then turned their attention from "static" progesterone assessment to the study of progesterone patterns in the context of dynamic testing. In that setting, some investigators found high progesterone levels (1.1ng/mL) on day 10 of clomiphene citrate challenge tests (CC challenge test) to be associated with short follicular phases, diminished ovarian reserve, and reduced potential to achieve pregnancy. [105]

Measuring Inhibin-B

Inhibin-B is an ovarian hormone secreted from the granulosa cells of the antral follicles and has a direct suppressive effect on FSH release. [106] Although present in ovulating women, it is not normally found in postmenopausal women. As early as 1932, researchers suspected that a non-steroidal regulator of FSH secretion might exist, [107] but it was not until 1976 that this hypothesis was actually confirmed. [108]

Unfortunately, the initial interest for inhibin-B as an ovarian reserve screening tool was tempered by the lack of a satisfactory way to study it. If it is measured by standard FSH-release methods, more than seven active forms of inhibin, as well as inactive inhibin constituents are found in human serum. [109]

Studies are currently underway to uncover more information about inhibin-B. [110, 111] Although measuring inhibin-B is still considered investigational as a way to screen ovarian reserve, a number of advances have helped make measurement of inhibin-B a clinical reality. Nevertheless, it is critical for clinicians using inhibin-B in ovarian reserve testing to understand exactly which assay is being used, and acknowledge limitations in measurement methods.

Inhibin-B may prove to be a beneficial marker for ovarian reserve assessment because it fluctuates during the menstrual cycle, and is significantly reduced in women over the age of 35. [112] One center observed that when day 3 inhibin-B was less than 45pg/mL, the response to fertility treatment was lower, the cancellation rate was higher, the number of

retrieved oocytes was less, and the pregnancy rate was significantly reduced when compared to subjects with day 3 inhibin-B values greater than or equal to 45pg/mL. [113]

In an effort to broaden the diagnostic capability of inhibin-B, some researchers have proposed including this test as an experimental component of the clomiphene citrate challenge test. [114] Although these early reports confirm that inhibin-B can enhance current tools that measure ovarian reserve, more data are needed before meaningful normal ranges for inhibin-B can be routinely applied in clinical practice. [115, 116]

Transvaginal Ultrasound

A. Ovarian volume

Diminished ovarian reserve means that fewer follicles are available for stimulation and recruitment by fertility drugs. By allowing physicians to view the ovaries and assess the number of follicles, transvaginal ultrasound can aid in the assessment of ovarian volume, thus, decreased ovarian volume was first proposed, [117]

Previous investigators have documented that the ovary reduces in size with increasing age, regardless of whether the woman has given birth or not. [118] Other researchers have found that the lower the ovarian volume, the greater the dose of fertility drugs required to stimulate the ovaries. [119]

Ultrasound ovarian volume has also been used to predict the risk for ovarian hyperstimulation syndrome. [120] However, it has been theorized that ultrasound ovarian volume done at the beginning of the treatment cycle is more closely related to the number of follicles found during the pre-treatment period rather than the number of oocytes developed during treatment. [121]

B. Basal antral follicle counts

Recently, it was proposed that the antral follicle count in the early follicular phase provides better prognostic information on the occurrence of poor response during hormone stimulation for IVF than does the patient's chronological age and available endocrine markers. The magnitude of the gonadotropin response and the number of the oocytes that may be retrieved correlates quite nicely with these studies. [122] So, it could be concluded that decreased basal antral follicle count [123] and a significantly decreased ovarian stromal blood flow [124] are proposed to be associated with low ovarian reserve. However, there is no threshold value that essentially predicts extremely low pregnancy rates. Patients with less than five follicles visible in the basal state (usually examines at the same time that the day 3 FSH concentrations were obtained before starting any medications) needed more medication and had produced fewer oocytes but still had ongoing pregnancy rates up to 35%. Although significantly lower than their age related counterparts, these rates are still high enough to justify an IVF cycle [1].

Multivariate analyses involving basal FSH and inhibin B levels to a logistic model with the antral follicle count, may significantly improve the prediction of poor ovarian response in IVF. [122]

Measuring circulating gonadotrophin surge-attenuating factor (GnSAF) bioactivity, follicular fluid concentration of insulin-like growth factor (IGF)-I and serum concentrations of antimüllerian hormone

Recently, the predictive value of the reduced GnSAF production and bioactivity [62] and low insulin-like growth factor (IGF)-I concentrations in follicular fluid [125] were evaluated in poor responders. Furthermore, it has been proposed that decreased serum concentrations of antimüllerian hormone may be a novel marker for ovarian ageing. This correlates with the number of antral follicles and age, but less strongly with FSH levels, while its decrease occurs earlier than do changes in other markers associated with ovarian ageing. [126]

The clomiphene Citrate Challenge Test

In contrast to the static measurements of ovarian reserve mentioned previously, the clomiphene citrate challenge test (CC challenge test) is a dynamic approach. Its purpose is to stimulate the ovary to initiate oocyte production in response to clomiphene citrate. In theory, the clomiphine citrate was designed to detect low ovarian reserve that would not be discovered by a single FSH and/or E2 measurements.

The CC challenge test is based on the assumption that adequate ovarian reserve is associated with a healthy group of developing follicles. This healthy group of follicles should be capable of producing enough inhibin and E2 to suppress FSH production and resist the effects of clomiphene.

Clomiphene works by shutting down the estrogen receptors on the hypothalamus and tricking the hypothalamus into thinking that the patient doesn't have enough estrogen. In response, the hypothalamus works harder to induce the pituitary gland to produce more FSH and LH. This, in turn, initiates follicular growth.

When undergoing CC challenge test, the first step is to measure day 3 FSH and E2. Then 100mg of clomiphene is administered on cycle days 5 through 9, and FSH and E2 measurements are repeated on cycle day 10. [127] In general, a high day 10 FSH suggests poor ovarian reserve.

The CC challenge test and pregnancy rates

In the original report describing the CC challenge test, 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 CC challenge test response was substantially higher (14 of 33, or 42%). [127]

Several other investigators have confirmed the good predictive value of CC challenge test before treatment. [128, 129, 130] Tanbo et al studied 91 women over age 35 and found abnormal CC challenge test in 37 patients. Twenty of the 37 patients also had an elevated

basal FSH concentration on cycle day 3. Only one patient had an abnormal value on day 3 with a normal value on day 10. The predictive value of an abnormal test was 85% for cycle cancellation due to poor ovarian responsiveness, and 100% for failing to conceive. [129, 130] Loumaye et al also evaluated the CC challenge test, but defined an abnormal test by adding the day 3 and day 10 FSH values together. In their series of 114 patients, the predictive value of an abnormal test for failing to conceive was 100%. [128]

The CC challenge test screening in the general infertility population

The data generated during the initial evaluation of the CC challenge test were similar in nature to that evaluating basal FSH concentrations alone. The CC challenge test evaluates the predictive value of the test in assisted reproductive programs or in patients undergoing complex ovulation induction. There were legitimate concerns that since the CC challenge test reflected the inability of the developing cohort of follicles to suppress FSH concentrations into the normal range, that the test would be predictive only of the quality of the cohort as a whole. If a single follicle in the cohort possesses good reproductive potential (even if the others did not), the nature processes of recruitment and selection could lead to ovulation of the highest quality follicle, and the predictive value of the CC challenge test would be diminished.

Scott et al completed a long term prospective evaluation of CC challenge test screening in women from the general infertility population [131] Approximately 105 of the 236 patients who were evaluated and followed for a minimum of one year had an abnormal CC challenge test. The incidence of the abnormal test rose with age and was 3% when < 30 years, 7% at 30-34 years, 10% at 35-39 years and 26% for women over 40 years. Most importantly, the pregnancy rates in the patients with diminished ovarian reserve were markedly lower (9%) than those with adequate reserve (43%). Evaluation of the relation between the eventual clinical diagnosis and CC challenge test results in these 236 couples showed a very high incidence of abnormal tests in the patients with unexplained infertility. In fact, the incidence of abnormal CC challenge test was highest among patients with unexplained infertility (38%) and was unaffected by age. This supports that diminished ovarian reserve is an etiology of infertility, and that couples with abnormal tests should not be considered to have unexplained infertility.

Day 3 versus day 10 elevations during the CC challenge test

Another report found clomiphine citrate to be a better predictor of ovarian reserve than day 3 FSH measurement alone. It seemed possible that day 10 values may have a different prognosis than those on day 3 since they require a provocative test to be unmasked. In fact, pregnancy rates were extremely poor even if only the day 10 samples were abnormal. No difference in counseling may be justified if only the day 10 level is elevated. [132]

Current status of CC challenge test screening

An abnormal CC challenge test has excellent predictive values for diminished ovarian reserve and poor long term pregnancy rates in natural cycles, during ovulation induction, and in IVF. Although the test is quite specific, it has limited sensitivity with a significant

age related diminution in reproductive potential occurring even among women with normal test results.

The test may be superior to basal FSH screening because it is two to three times more sensitive than basal FSH alone. Although abnormal day 3 FSH values appear to be accompanied by abnormal day 10 values in most cases, the current literature does not contain enough data to recommend omission of the day 3 sample.

Gonadotropin-releasing Hormone Agonist Stimulation Test

A gonadotropin-releasing hormone agonist initially elevates E2, and then profoundly suppresses both FSH and LH. [133] This is sometimes called a "flare-effect". More than a decade ago, it was theorized that low ovarian reserve might be detected by evaluating differences in LH, FSH, and E2 levels following the administration of GnRH-a during IVF. [90] This approach was later formalized as a diagnostic tool known as the GnRH-a stimulation test, or GAST. [134]

The purpose of GAST is to evaluate changes in E2 on cycle day 2 and 3 following administration of GnRH-a. Patients with greater elevations of E2 have correspondingly higher pregnancy rates. Four GAST E2 patterns have emerged:

1) prompt E2 elevation, then decrease by cycle day 4
2) delayed E2 rise with fall by cycle day 6
3) persistent E2 elevation
4) no E2 response after GnRH-a

Clinical pregnancy rates for these groups were strikingly different: 46%, 38%, 16%, and 6% were observed in patterns 1 through 4, respectively. [72] In summary, GAST has been a better predictor of the functional abilities of the ovary than either FSH or age. [134]

The exogenous FSH ovarian reserve test (EFORT) was proposed by Fanchin et al. [135] The EFORT is similar to the GAST which gauges ovarian responsiveness to exogenous FSH. The test is predictive of ovarian response and to a lesser degree pregnancy rates. Specific thresholds that may be used to direct treatment await more rigorous definition in further studies. Because the GAST and EFORT are costly and involves an injection and repeated blood tests, they are not widely used in clinical practice

Management of poor ovarian responce

The problem of maximizing follicular responsiveness has been extensively studied since investigators began describing dramatically lower success rates in IVF patients who produced fewer follicles during stimulation. Strategies have ranged from simply increasing the dose of exogenous gonadotropins, the use of adjunctive agents such as GnRH analogues and growth hormone, and the use of micromanipulation. With virtually every protocol, improvements in overall responsiveness have been shown for some patients. Inspite of this fact, the incremental improvement in pregnancy rates has generally been quite small. These data continue to emphasize the importance of qualitative changes in these patients´ oocytes since many will have sufficient improvements in the quantity of the oocytes recovered to have routine numbers of embryos transferred.

For these reasons, it may be insufficient to simply evaluate various treatment regimens by comparing peak E2 concentrations, the number of the follicles that develop, the number of oocytes recovered or the number of the embryos that are available for transfer. While pilot studies may legitimately compare ovarian responsiveness, any meaningful and definitive evaluation must also include an assessment of implantation and pregnancy rates [(1)].

High doses of gonadotrophins

The initial unresponsiveness to gonadotrophin stimulation unavoidably leads the clinician to increase the dosage of medication for ovarian hyperstimulation, and indeed this action forms part of the definition of the low responder. According to most authors, the common initial dose for poor responders is at least 300 IU/day. Few studies have been conducted on this issue however, as high doses of gonadotrophins have been used in the large majority of regimens employed.

Cedrin-Durnerin et al., [(136)] in a prospective randomized study, compared a high fixed versus a step-down dose of gonadotrophins on a minidose flare-up GnRH agonist regimen. Patients were pretreated for 14 days with 10 mg/day norethisterone, followed by 100 mg triptorelin s.c. from day 1, reduced to 25 mg s.c. from day 3 of the cycle. Purified FSH at a dose of 450 IU/day i.m. was administered from day 3 either in a fixed manner or decreasing to 300 IU/day, and finally to 150 IU/day. These authors showed, by applying this flare-up GnRH agonist protocol, that there were increased cancellation rates and similar pregnancy rates but, as expected, a significantly reduced number of ampoules of GnRH were used.

Doubling of the starting hMG dose, from 225 IU to 450 IU/day from day 5 of ovarian stimulation onwards, has been evaluated in a prospective randomized study conducted by Van Hooff et al on 1993. [(137)] These authors concluded that such an approach was ineffective in enhancing ovarian response in low responders, this being in accordance with the hypothesis that follicular recruitment occurs only during the late luteal and early follicular phases of the menstrual cycle.

Similar doses (450 IU/day) were also used in a retrospective study done by Karande et al. [138] where in 34 poor responders the mean number of retrieved oocytes (2.67) was still low, as was the pregnancy rate (12%). The latter group concluded therefore that there was no benefit from the incremental increase in FSH dose. The same conclusions were reached in another retrospective study performed by Land et al. [139] where 126 previously poor responders were given 450 IU/day hMG. The women showed an increased number of oocytes retrieved, but the pregnancy rate remained low (3.2%). Conversely, in a prospective study by Hofmann et al., the effects of the increased gonadotrophin doses in poor responders were evaluated as improved. [140] The authors showed increased pregnancy rates (33.3 versus 6.7%) and lower cancellation rates (9 versus 35%), by using 450 IU/day of purified FSH (in step-down fashion), rather than 300 IU/day. The benefits of using a very high dose of purified FSH were also reported in a retrospective study done by Crosignani et al. [141] which included 116 poor responders and resulted in satisfactory follicular growth in70% of cases.

In general, high doses of gonadotrophins have been used by the vast majority of authors and form a clear part of all protocols for ovarian stimulation in poor responders. Never the less, the results of studies evaluating the use of gonadotrophins in these patients remains controversial, though the true prospective randomized studies have shown either minimal or no benefit.

Use of recombinant FSH versus purified urinary FSH

The use of recombinant FSH (rFSH) appears to be associated with better assisted reproduction results than do either purified urinary FSH or hMG [142, 143] As patients included in these studies were not poor responders, these results stimulated the concept that rFSH might also improve oocyte and embryo quality in poor-response patients. The use of rFSH versus purified FSH in poor responders was evaluated in a small (15 versus 15 patients), prospective randomized study performed by Raga et al. [144] The authors found an increased mean number of oocytes collected (7.2 versus 5.6), improved pregnancy rates (33 versus 6%) and lower cancellation rates (13 versus 40%). Similarly, another prospective study done by De Placido et al., although with historical controls, [145] assessed the efficacy of 300 IU rFSH versus the same dose of purified FSH in the flare-up protocol involving 28 cycles of poor-responder patients in each group. These authors suggested that rFSH was associated with a significantly larger number of oocytes retrieved (2.4 versus 1.7) and significantly increased pregnancy rates (14.3 versus 0%).

It seems therefore, that there is no evidence that rFSH produces better results in poor responders, though larger prospective randomized trials are needed to elucidate this issue further.

Luteal initiation of FSH

This interesting approach was first suggested in 1998 by Rombauts et al. [146] Healthy, small, antral follicles are present in late follicular phase, and initiation of their further development occurs under action of the premenstrual FSH rise. [147] The hypothesis was that earlier administration of FSH might increase the number of recruited follicles by opening the recruitment window earlier, in the late luteal phase of the preceding cycle. In a

prospective randomized controlled study in two patient groups (n = 20 each), these authors administered leuprolide according to a standard short or long follicular protocol. The control group received 150 IU/day rFSH from cycle day 3, while the study group received the same dose from day 25 of the previous cycle. Unfortunately, the results were not encouraging, with increased cancellation rates (33 versus 25%), decreased number of oocytes collected (4.5 versus 6) and lower pregnancy rates (0 versus 5%); in addition, increased FSH doses (1950 versus 1500 IU) were needed for a longer stimulation period (15 versus 11 days).

Flare-up GnRH agonist regimens: short and ultra-short protocols

The flare-up regimens involve early follicular phase initiation of the GnRH agonist, with minimal delay before the onset of gonadotrophin administration. [148, 149] There are two theoretical advantages with this approach: first, the ovarian suppression is not excessive; and second, the initial stimulation of the GnRH receptors and consequent secretion of endogenous gonadotrophins enhances the effects of the exogenously administered gonadotrophins. It has been proposed that by decreasing the GnRH agonist dose, a lighter ovarian suppression could be obtained and, hence a better response to gonadotrophin stimulation could be achieved. Furthermore, several microdoses of GnRH agonists in the flare up protocols have been tested, the aim being to achieve gonadotrophin release while eliminating the phenomena of increased LH, androgen and progesterone secretion noted in the classic flare-up protocols. [150, 151]

Thus, at least in theory, these regimens would be suited to patients with low ovarian response. Although widely used, there are no prospective randomized controlled trials of flare-up protocols which can be used to assess their efficacy compared with standard protocols.

Standard-dose flare-up regimens

In a prospective study with historical controls and using an ultrashort protocol, [148] according to which seven patients were administered 0.5 mg/day buserelin during only the first three days of the cycle, a 0% cancellation rate and 42.9% pregnancy rate were found. Similarly, in a prospective study with no controls, the same flare-up regime was used in three categories of various responders after a Lupron screening test. [152]
The group of 53 poor-responding patients had a low cancellation rate (11.3%) and a good pregnancy rate (29%) despite the low number of oocytes retrieved. In accordance with these data, others [41] compared retrospectively the flare-up versus the luteal GnRH agonist regimen, and observed higher pregnancy rates (20.4 versus 11.7%) and lower cancellation rates with the flare-up protocol.

By contrast, other authors failed to confirm any substantial benefit of using a classic flare-up protocol. In a prospective study with historical controls performed by Karande et al, [35] 80 poor responders were treated using a classic flare-up GnRH agonist regimen with leuprolide 0.5 mg/day from cycle day 2, and 450-600 IU/day hMG from cycle day 3. The authors found an increased number of retrieved oocytes (10 per cycle), but otherwise no improvement, with a high cancellation rate (23.4%) and a low pregnancy rate (13.4%).

Hence, most studies (that are non-randomized) conducted to assess the standard dose flare-up protocols demonstrated a degree of improvement.

Micro-dose GnRH agonist flare-up regimens

The idea of minimizing the dose of the GnRH agonist created the so-called 'mini' and `micro' dose flare-up GnRH agonist regimens. Improved outcome was observed in a prospective, non-randomized trial done by Surrey et al, [153] who treated 34 patients who had poor response and no pregnancies in a previous IVF attempt with the long luteal regimen, with a microdose flare-up GnRH agonist regimen (leuprolide 80 mg/day s.c. from day 3). The cancellation rate was significantly decreased in the flare-up microdose protocol, and the clinical pregnancy rate was increased. Impressive results using the same microdose leuprolide protocol were also reported in a prospective study demonstrated by Schoolcraft et al, [154] here, 32 poor responders were pretreated for 21 days with a combined oral contraceptive (COC). On day 3 post-COC, each patient received leuprolide 40 mg b.i.d. and GH 4 IU/day (i.m.), followed on day 5 post-COC by a high dose of gonadotrophins (450 IU purified FSH). The cancellation rate was 12.5%, a mean of 10.9 oocytes per patient was retrieved, and a very optimistic pregnancy rate of 50% was obtained. Even lower doses were used in a prospective study by Scott and Navot, [150] where in leuprolide was given to 32 women at a dose of 20 mg b.i.d. from cycle day 3, followed by high doses of FSH from cycle day 5. The authors found higher peak E2 levels and a higher number of retrieved oocytes.

Despite these findings, the initial optimism for the microdose flare-up regimen was not supported by the results of other studies. Hence, in a retrospective analysis one group [155] compared a microdose flare-up regimen with a long luteal with decreasing dose of GnRH agonist, and observed significantly higher cancellation rates (22.5 versus 8.2%, P = 0.032), lower clinical pregnancy rates (47.3 versus 60%, P = NS) and a decreased number of oocytes retrieved per cycle (13.3 versus16.5, P = NS) with the microdose flare-up regimen. The results derived from the use of reduced-dose GnRH agonist flare-up regimens are controversial. There is a trend towards improvement, but larger controlled and randomized studies are needed to support this issue.

Luteal initiation of GnRH agonist regimens

These regimens are characterized by the use of relatively low doses of GnRH agonists commencing in the mid-luteal phase of the cycle and usually ending at the time of menses or shortly thereafter, in combination with high doses of gonadotrophins. The possible mechanism of action is the reduced effect of the GnRH agonists on their ovarian receptors, [156, 157] that results in reduced ovarian suppression and consequently, in increased ovarian response. Despite the early discontinuation of the agonist, the incidence of premature LH surge is low but the results are quite contradictory.

Only two published prospective randomized controlled trials showed no statistically significant increase in pregnancy rates, whereas seven prospective trials with historical controls and one retrospective study demonstrated improved outcome. In a prospective randomized, controlled trial involving 78 cycles, a `stop agonist' regimen was compared

with a standard long luteal protocol. [158] Thus, the use of GnRH agonist (buserelin 1 mg/day intranasally or triptorelin 0.1 mg/day, s.c.) was initiated on day 21 of the pre-stimulation cycle and ceased on the day of confirmed pituitary suppression (E2 level<140 pmol/l). Ovarian stimulation was induced with the use of225 to 375 IU/day hMG or purified FSH (i.m.), commencing on the day of down-regulation. No improvement was found, as the mean number of the retrieved oocytes remained unchanged and no significant increase in the pregnancy rate was noted. Similar results were observed in another well-designed, prospective, randomized, controlled trial, [159] where the `stop' versus `non-stop' protocol of GnRH agonist, plus high doses of gonadotrophins were compared. The authors used leuprolide (1 mg, s.c.) from day 21 of the cycle, ceasing on the day of menses, followed by 375-450 IU hMG and/or purified FSH daily (i.m.). A significantly higher number of aspirated follicles was found (8.7 versus 5.3 per cycle, P = 0.027), but there was no significant difference in either cancellation rate (5.7 versus 2.8%) or pregnancy rate (18.7 versus 14.3%).

These results are not supported by other studies which, although prospective, had historical controls. Thus, in a prospective analysis involving 224 cycles demonstrated by Faber et al, [36] a low-dose midluteal GnRH agonist (leuprolide 0.5 mg, s.c.) was administered but then discontinued with the onset of menses. All of the patients had received the GnRH agonist for at least 7 days, after which 450-600 IU of purified FSH or hMG were administered (i.m.) daily. The dose of gonadotrophins was decreased 2 days prior to hCG administration, and this was referred to as the `stop-Lupron protocol'. The authors reported a low cancellation rate of 12.5%, a high number of oocytes aspirated per cycle (11.1), and an impressive clinical pregnancy rate per transfer (32%). Interestingly, among the poor responders the authors did not find any statistically significant difference when comparing the use of hMG/purified FSH with purified FSH alone. In accordance with this approach, another group, [160] in a 52 cycle, non-randomized prospective study, administered 0.5 mg leuprolide s.c. from day 21 to the next cycle's day 2. After this, 300-450 IU/day hMG and purified FSH were used for controlled ovarian stimulation. The patients showed a good response to stimulation (mean 7.5 oocytes per cycle) and encouraging pregnancy rates (20.5% per embryo transfer). The same protocol was also assessed by another group in an 82-cycle prospective analysis with historical controls; both high pregnancy rates (33.3%) and cancellation rates (31.6%) were observed. [35]

Switching between various GnRH agonists did not seem to make any difference. Thus, in a prospective study involving 36 poor responders, the use of nafarelin (0.6 mg/day, commenced in the midluteal phase and discontinued on day 5 of ovarian stimulation resulted in an enhanced efficacy of the gonadotrophin treatment [39]. Herein, the number of retrieved oocytes was increased by 28%, cancellation rates were decreased (to 8.3%) and pregnancy rates increased (to 19.4%). In another prospective study using the same GnRH agonist at the same dosage but discontinuing it on day 1 of the next cycle, 39 poor responders were treated and showed a positive effect on the number of retrieved oocytes and the pregnancy rates (10.7 versus 2.8%). [161] Subsequently, reducing the GnRH agonist had encouraging results (increased number of oocytes collected, decreased total gonadotrophins used), as shown in another prospective study by Olivennes et al. [162] These authors used 0.1 mg/day leuprolide s.c. from cycle day 21, reducing it to 0.05 mg/day on down-regulation day in 98 cycles. However, the cancellation rate remained high (24%) and the pregnancy rate relatively low (16.3%).

Administration of a single dose depot GnRH agonist preparation (leuprolide 3.75 mg) on day 21 of the pre-stimulated cycle was also assessed in a prospective study including 27 cycles. [163] These authors observed a significantly increased pregnancy rate and number of oocytes retrieved, while the cancellation rate was decreased.

GnRH antagonist regimens

The relatively new GnRH antagonist regimens aim to avoid the premature LH surge and, at the same time, to utilize the maximum of the ovarian oocyte cohort by minimizing the suppressing effects of the GnRH analogues on the ovarian receptors, thus avoiding ovarian suppression at the stage of the follicle recruitment. [164]

In a study conducted by Craft I. et al, [165] they used clomiphene citrate (100 mg/ daily, from cycle days 2 to 5) combined with the appropriate dose of gonadotrophins (mean 375 IU/day), in 24 cycles of poor responding patients. The GnRH antagonist cetrorelix was started on cycle day 6 at a dose of 0.25 mg/day. Compared to previous results with GnRH agonists in the same patients, fewer abandoned cycles (29.2 versus 56.5%), increased number of retrieved oocytes per cycle (6.4 versus 4.7) and increased pregnancy rates per transfer (23.5 versus 10%) were observed. There was also a reduction in the amount of gonadotrophin injections used. Nevertheless, the above mentioned results did not reach statistical significance.

Another prospective randomized study [166] reported that the use of GnRH antagonists, together with high doses of gonadotrophins (300 IU/day hMG+ 300 IU/ day purified FSH from cycle day 2) in previous poor responders, was associated with lower cancellation rates (20 versus 25%) and increased pregnancy rates (20 versus 6.25%), as compared with gonadotrophins alone. However, these differences were not statistically significant and no change in the number of the oocytes retrieved was observed. The same authors [167] in a subsequent prospective randomized controlled trial compared the multidose GnRH antagonist protocol with the flare-up GnRH- agonist regimen in poor responders (24 cycles in each group). These authors observed significantly less oocytes retrieved per cycle (4.5 versus 5.5) in the antagonist group ($P = 0.032$), but no significant difference was seen in either the cancellation or pregnancy rates (25 versus 20.83% and 22.3 versus 26.3%, respectively).

The available limited data, derived from small or preliminary studies, do not show any advantage from the use of GnRH antagonists. Therefore larger, controlled, prospective randomized trials using GnRH antagonists are necessary to investigate this issue.

Adjunctive use of aromatase inhibitors

The aromatase inhibitor, letrozole, approved in 1997 for treating advanced breast cancer [168] falls in the category of new products for which numerous other possible applications exist. Designed for suppressing estradiol (E_2)production, products such as letrozole could become useful either in the short-term for inducing or facilitating ovulation or in the longer-term for treatment of endometriosis and possibly, uterine fibroids. This situation resembles that which prevailed some 15 years ago with GnRH agonists. Approved for

treating prostate cancer, GnRH agonists were soon tested and used in an array of other applications in gynecology, including in controlled ovarian hyperstimulation (COH). [169] We now know the multitude of uses for GnRH agonists, whereas those of aromatase inhibitors are unraveling before our eyes.

Aromatase P450, the estrogen synthase that converts androgen to estrogen, is physiologically expressed in a variety of tissues, including the ovary, placenta, skin, adipose tissue and brain. [1710, 171, 172] In certain pathological conditions, such as breast cancer and pelvic endometriosis, very high expression levels have been reported. [173, 174] In the endometrium, aromatase P450 is also expressed under pathological conditions, and local estrogen biosynthesis is thought to be integral to the pathophysiology of a variety of uterine disorders, including adenomyosis, fibroids and endometriosis. [175, 176]

Aromatase is a cytochrome *P*-450 haemoprotein-contaromatase inhibitory enzyme complex that catalyses the rate-limiting step in the production of estrogens, i.e. the conversion of androstenedione and testosterone into estrogens. [177, 178] The aromatase enzyme is a good target for selective inhibition because estrogen production is a terminal step in the biosynthetic sequence.

Recently, a group of highly selective aromatase inhibitor, including letrozole and anastrozole, has been approved for use in post-menopausal women with breast cancer to suppress estrogen production. These aromatase inhibitors have a relatively short half-life (48 h) compared with clomiphine citrate, and therefore administration of one of these new aromatase inhibitors in the early follicular phase should result in drug levels in the body that are extremely low or absent during the peri-ovulatory and luteal phases of the cycle due to its rapid elimination from the body. [179, 180] In addition, since no estrogen receptor down-regulation occurs, no adverse effects on estrogen target tissues, as observed in clomiphine citrate-treated cycles, would be expected.

It is hypothesized that it may be possible to mimic the action of clomiphine citrate, without depletion of estrogen receptors, by administration of aromatase inhibitors in the early part of the menstrual cycle. This use of the aromatase inhibitors would result in release of the hypothalamic–pituitary axis from estrogenic negative feedback, thereby increasing gonadotrophin secretion and resulting in stimulation of ovarian follicles. [181, 182, 183, 184] Adding clomiphene citrate (CC) to FSH for controlled ovarian stimulation decreases FSH dose required for optimum stimulation. However, because of its anti-estrogenic effects, CC may be associated with lower pregnancy rates offsetting the FSH-dose reduction benefit.

Mitwally and Casper performed a study to test the hypothesis that the use of the aromatase inhibitor, letrozole, in conjunction with FSH for controlled ovarian stimulation, would decrease the dose of gonadotrophins required for controlled ovarian stimulation similar to clomiphine citrate with FSH when compared with FSH only as a control. [185] It was a prospective pilot study that included women with unexplained infertility undergoing controlled ovarian stimulation and intrauterine insemination. Thirty-six women received the aromatase inhibitor letrozole & FSH, 18 women received CC & FSH and 56 women received FSH only. Each woman received one treatment regimen in one treatment cycle. All patients were given recombinant or highly purified FSH (50–150 IU/day) starting on

day 3 to 7 until day of hCG. The FSH dose needed was significantly lower in letrozole & FSH and CC & FSH groups compared with FSH-only without a difference in number of follicles >1.8 cm. Pregnancy rate was 19.1% in the letrozole & FSH group, 10.5% in the CC & FSH group and 18.7% in the FSH-only group. Both pregnancy rate and endometrial thickness were significantly lower in CC & FSH group compared with the other two groups. Estradiol (E_2) levels were significantly lower in the letrozole & FSH group compared with the other two groups. They suggested that the concomitant use of the aromatase inhibitor, letrozole, during controlled ovarian stimulation results in a reduction of the dose of FSH required to achieve a mean of three mature follicles prior to intrauterine insemination without the deleterious peripheral anti-estrogenic effects often observed with CC. In addition, the low physiological levels of estrogen in the letrozole & FSH group may contribute to an improvement in pregnancy rates compared with the CC & FSH study group.

The reduced FSH dose required for controlled ovarian hyperstimulation associated with aromatase inhibition may be due to a central and/or a peripheral mechanism of action. Centrally, inhibition of estrogen synthesis by an aromatase inhibitor may release the estrogenic negative feedback on the hypothalamus and/or pituitary resulting in an increase in endogenous gonadotrophin secretion leading to enhancement of ovarian follicular development. Peripherally, at the ovarian level, inhibition of the conversion of androgens into estrogens by aromatase inhibition may lead to temporary accumulation of the androgens. Androgens were found to increase follicular sensitivity to FSH through amplification of the FSH receptor gene expression either directly or through other mediators such as the insulin-like growth factor system. [186, 187, 188, 189] Other mechanisms yet to be determined may also be working at the peripheral level and need further study to improve our understanding of follicular development in both health and disease.

Thus, three possible advantages are seen in using aromatase inhibitors such as letrozole in COH:

(i) Simplicity of CC cycles without anti-estrogenic effects on endometrium and mucus;

(ii) Increased responsiveness to FSH leading to better responses in poor responders and

(iii) Decreased tendency for premature luteinization.

A fourth hypothetical benefit of using aromatase inhibitors in COH is that, they may improve the endometrial receptivity with subsequent improvement in the implantation rate and increasing the pregnancy outcome. This hypothesis is concluded from the observation that a subgroup of infertile women expresses high levels of aromatase P450 in the endometrium. In order to determine whether endometrial aromatase P450 mRNA expression is prognostic of IVF outcome or not, Jan Brosens et al., [190] quantified transcript levels in biopsy specimens from a cohort of 150 subfertile patients awaiting IVF treatment using real-time quantitative PCR. Aromatase P450 transcripts were detected in all endometria examined, although the levels varied considerably between samples, ranging from 0.22 to 486.6 arbitrary units (a.u.). The clinical pregnancy rate in women with high endometrial aromatase P450 mRNA levels ($\geq$8.3 a.u.; n = 21) was 9.5% compared with 30.1% in those patients with low expression levels (<8.3 a.u.; n = 101) ($P < 0.05$). The

cycle day of the endometrial biopsy, cause of infertility, age, parity, number of oocytes collected and number of embryos transferred did not differ between patients with high versus low endometrial aromatase P450 mRNA levels ($P > 0.1$). The results of the present study indicate that the level of aromatase P450 expression represents one of a multitude of factors that determine IVF treatment outcome. However, this study is limited in its size, and the prognostic value of measuring endometrial aromatase P450 expression levels for predicting IVF treatment outcome requires further validation in a larger cohort of patients

However, many questions are remaining about using aromatase inhibitors in COH:

(i) What are the optimal timing and duration for aromatase inhibitors' use in COH?
(ii) What is the optimal dose of aromatase inhibitors needed to give sufficient response in COH?
(iii) Is there is a need for GnRH antagonists to counteract the risk of premature LH surge?
(iv) Do aromatase inhibitors affect the quality of oocytes?

Timing of aromatase inhibitor treatments

Understandably, the first pilot trials followed a path paved by a long experience with CC: 5 days of treatment, starting on cycle day 3 of spontaneous or induced menses. The favorable results reported in anovulatory [184] and ovulatory patients [191] support the clinical soundness of this simple approach. Yet in certain women, FSH rises earlier or later in relation to menses with consequences on the ovarian response to letrozole that still need to be clarified. Hence, the patho-physiological groundwork remains to be done for possibly optimizing COH regimens by synchronizing letrozole treatments to the timing of the endogenous FSH signal. [192, 193] That letrozole was not continued beyond cycle day 7 in trials published to date may have been well inspired. More prolonged treatments might further hamper the E_2 rise and carry negative consequences on follicular maturation, oocyte quality and the endometrium. Conversely, further extending letrozole treatment, for example until day 9, might yield a larger oocyte crop by further extending the duration of the FSH window.

A distinct objective of aromatase inhibitors is to improve the ovarian response in the so-called 'poor responders' by lowering intra-ovarian E_2, increasing intra-ovarian androgens and enhancing the sensitivity of FSH receptors. [194, 195] Here, the optimal timing of aromatase inhibitor treatment may markedly differ from what has already been tested. If the objective is to enhance the sensitivity of FSH receptors by increasing intra-ovarian androgens, the optimal timing of letrozole treatment may well be 'before' rather than 'when' the ovary is exposed to FSH (endogenous and/or exogenous). Hence, aromatase inhibitor treatments may possibly be more efficient for this objective if given in a 'priming' manner when FSH levels and follicular recruitment are still inhibited by endogenous or exogenous E_2. [196]

Further trials are evidently needed to map out the best treatment paradigms for using letrozole in poor ovarian responders.

Dosing letrozole

The dose of letrozole used in most COH trials, 2.5 mg/day, is efficacious in breast cancer patients, leading to near complete suppression of E_2 production and undetectable E_2 levels. [168] This dose should therefore suffice for maximizing the stimulating signal sent to the hypothalamo-pituitary axis. Yet, higher doses may more profoundly inhibit the ovarian response and thus further extend the FSH window by impairing the ovarian production of E_2. The recent data, [197] support this concept, with more mature follicles seen in women who received a higher dose of letrozole (5 mg/day) despite lower levels of circulating E_2. On the contrary, a lower dose of letrozole (<2.5 mg) or a decreasing one (starting with 2.5 mg/day and decreasing thereafter) could potentially permit a longer therapy (beyond day 7) with possibly, lesser needs for exogenous FSH in combined letrozole/FSH COH protocols. Here again, further trials are urgently needed.

Risk of premature LH surge and need for GnRH antagonists

An observed effect of aromatase inhibitors used in 'CC-like' protocols is a decrease in E_2 production with approximately a 50% diminution in E_2/mature follicle in peripheral blood on the day of hCG administration [184, 185, 191]

As LH surge is induced by a late follicular rise in E_2 levels that feeds back positively on the hypothalamo-pituitary axis, [198] we would anticipate that letrozole delays LH elevation. Yet, actual findings may differ because the onset of LH elevation reflects a balance between the dose-dependent stimulation of E_2 and an antagonizing action of follicular proteins. [199] Thus, the lower E_2 per follicle levels observed in letrozole cycles will delay the LH surge only if it is not accompanied by a parallel decline in follicular protein(s) that antagonize(s) LH elevation.

From the report of Mitwally and Casper [185], we can learn that women having an LH surge in letrozole–FSH cycles have higher E_2/mature follicles (600 pmol/l) than those who did not surge (460 pmol/l), which is consistent with our expectations. Yet these E_2 values were markedly lower than those seen in women who surged in CC–FSH and FSH-only COHs (1193 and 1514 pmol/l, respectively). Hence, the data of Mitwally and Casper suggest that letrozole not only lowers E_2 but also the follicular protein(s) that antagonize(s) the LH surge, because the E_2 levels that triggered an LH surge were lower than in non-letrozole COH.

Quality of oocytes

The worthy pregnancy rates reported in the pilot trials published to date predict well for the oocyte quality in letrozole and letrozole–hMG/FSH cycles. Reports of human IVF data in letrozole cycles are however urgently needed for a definitive answer to this question.

In conclusion, to the best of our knowledge, all the published data relating clinical experience with aromatase inhibitors in COH alone [184, 191, 197] or in combination with exogenous FSH [185, 200] in women suffering from chronic oligo-anovulation [184, 191] or ovulating spontaneously [197, 200] These studies provide positive proof of principle and

should serve as groundwork for future prospective trials assessing the true merit of these new approaches. Therefore larger, controlled, prospective randomized trials using aromatase inhibitors in poor responders are necessary to investigate this issue.

Adjunctive use of GH or GH-releasing factor or pyridostigmine

The hypothesis that GH stimulates ovarian steroidogenesis, follicular development and enhances the ovarian response to FSH was proposed in 1986. [(201)] This action of GH is believed to be mediated via the IGF-1 that acts in synergy with FSH, amplifying its effects on granulosa cells. [(202)] This was the theoretical basis for the introduction of GH or GH-releasing factor (GH-RF) in the IVF treatment of poor responders. Usually, 4 to 12 IU GH are administered s.c., commencing on the day of ovarian stimulation with gonadotrophins. A total of nine prospective trials have been reported; four of these were non- randomized but had historical controls. Seven of the studies revealed essentially no change or no significant improvement in the clinical results.

In one large multi-centre prospective randomized, double-blind, placebo-controlled trial, [(203)] GH-RF was administered and caused an increase in endogenous levels of GH. However, the final cancellation and pregnancy rates were similar to those found for the protocol without GH-releasing hormone (12.5 versus 16% and 8.3 versus 8% respectively). No statistically significant improvement in cancellation and pregnancy rates were reported.

In another double-blind, placebo-controlled trial [(204)] in which 4 IU/day of GH was used as adjuvant therapy. Increasing the GH dose to 12 IU/day in a long luteal GnRH agonist regimen led to similar results in a prospective study with historical controls. [(40)] The above discouraging findings were also reported by others, [(205)] who treated 21 previous poor responders with 12 IU/day GH in a prospective double-blind, placebo controlled study and found no significant differences in serum E2 levels, duration of the follicular phase, total hMG dose and number of oocytes between the placebo or GH cycles. Likewise, in a small prospective randomized double-blind placebo controlled study; another group administered 18 IU GH on alternative days in a classic flare-up triptorelin protocol with 300 IU/day of hMG, in 14 cycles of poor responders [(206)] ,unfortunately, the results were also disappointing. The same conclusion was also reached in two additional small studies. [(207, 208)] Optimistic results were reached in a small prospective study with historical controls, [(209)] where 10 patients had higher numbers of oocytes collected (7.5 versus 3.5, $P < 0.001$) and improved pregnancy rates (60%).

GH secretion can also be increased by acetylcholine, which inhibits somatostatin secretion at the hypothalamic level. [(210)] Pyridostigmine is an acetylcholinesterase inhibitor which, by enhancing the action of acetylcholine, can increase GH secretion. This approach was evaluated in a randomized double-blind placebo-controlled study [(211)] which included 70 poor responders who were given 120 mg/day pyridostigmine orally, from the day of down-regulation until the day of hCG, along with a long luteal GnRH agonist regimen (triptorelin 0.1 mg on day 21, hMG/FSH 300 IU/day, i.m.). Compared with placebo, pyridostigmine was associated with a significantly lower number of ampoules used (38.4 versus 48.3), a higher number of oocytes collected (5.9 versus 3.7) and improved (but not significantly significant) pregnancy rates (25.7 versus 11.4%).

In a recent Cochrane Review, a meta-analysis was conducted of the trials assessing the effectiveness of GH adjuvant therapy in women undergoing ovulation induction. [212] In previous poor responders, the common odds ratio for pregnancy per cycle instituted was 2.55 (95% CI 0.64-10.12). No significant difference was noted in either the number of follicles and oocytes, or gonadotrophin usage.

Therefore, these published data do not support any benefit from the use of GH as adjuvant therapy in poor responders.

Adjunctive use of glucocorticosteroids (dexamethasone)

It has been suggested that dexamethasone may directly influence follicular development and oocyte maturation via its isoform (11- bHSD) in the granulosa cells [213] or indirectly, by increasing serum GH and consequently intrafollicular IGF-1. [214] In addition, it may provoke immuno-suppression within the endometrial microenvironment. [215]

To date, no studies have been reported involving poor responders. In one double-blind, placebo-controlled prospective randomized study in 290 cycles of normal responders (aged <41 years), dexamethasone was administered at 1 mg/day in the long luteal protocol until the day prior to oocyte retrieval, [216] and the authors found a significantly lower cancellation rate (2.8 versus 12.4%, P = 0.001). These findings provided great encouragement, as they reveal a very low incidence of poor response with the use of corticosteroids; however, the data are limited and can only be considered as preliminary. Consequently, further trials are needed to support the role of corticosteroids in patients confirmed as poor responders.

Adjunctive use of nitric oxide (NO)-donors (L-arginine)

Adjunctive use of nitric oxide (NO)-donors (L-arginine) in humans, increased vascularization appears to play a critical role in the selection, growth and maturation of follicles in both natural and IVF cycles. [217]

L-Arginine is a potential vasodilator as a NO-donor; in fact, NO is derived in vivo from L-arginine by a NO-synthase enzyme that is either cytokine inducible or calcium-dependent. [218] It is also thought that NO is involved in follicular maturation and selection, [219] possibly due to its participation in peri-ovulatory vasodilatory modulation, as proven in a rat model. [220]

Promising results were presented by one group [221] in a prospective randomized study in which two groups of 17 poor responders were compared, each of which was treated with the GnRH agonist flare-up regimen, high doses of purified FSH and orally administered L-arginine. Significantly higher numbers of collected oocytes were found (4.1 versus 1.6, P = 0.049), as well as a higher, though not significantly so, pregnancy rate (17 versus 0%, P = NS) and a lower cancellation rate (11 versus76%, P = 0.001) in the L-arginine group. Clearly these preliminary results require further verification, and additional studies are

needed to investigate the role of these agents in ovarian stimulation, particularly in poor responders.

Pretreatment with COC or progestogens

Combined oral contraceptives (COC) administration aims to suppress endogenous gonadotrophins and, at the same time (through its estrogen component), generate and sensitize more estrogen receptors. Unfortunately, the administration of COC acts as a type of pituitary suppression in its own right.

A few prospective and randomized studies have shown that COC pretreatment may be beneficial with regard to ovarian response and clinical pregnancy rates. [222, 223] This suggestion was not confirmed by pretreatment with progestins alone, [224] although the data were obtained from a patient cohort which excluded poor responders.

Although several investigators have used COC pretreatment in other experimental protocols for poor responders, only one retrospective study has been reported on this topic. [225] These authors showed that COC administration prior to the GnRH- agonist protocol was associated with higher pregnancy rates and lower cancellation rates.

In conclusion, although there is a general feeling that COC pretreatment might be of assistance in the ovarian response of poor responders, only a minimal amount of published data exist which document this hypothesis.

Routine use of ICSI

Based on the hypothesis that higher fertilization rates can be achieved with ICSI than with conventional IVF, [226] one group [227] proposed the use of ICSI with the very few and valuable oocytes of their poor responders, in order to improve pregnancy rates. This proposal was tested in a prospective randomized study which however, showed no difference in the clinical results.

Assisted hatching of zona pellucida

The concept of this intervention is to increase the implantation potential in the few embryos that poor responders produce. The routine use of assisted hatching of the zona pellucida remains controversial, though it has been suggested that women with multiple IVF failures or those aged over 38 years might benefit from standard application of this technique. [228, 229] Nevertheless, the published studies evaluate the use of assisted hatching in patients with poor prognosis for IVF, and not in true poor responders.

A highly significant and even more impressive increase in both implantation (33 versus 6.4%) and pregnancy (64 versus 19%) rates was found, in a prospective trial where 33 poor-prognosis patients were evaluated. [230] The inclusion criteria were increased day 3 FSH levels, age >39 years, or multiple prior IVF failures. In a similar study [229] pregnancy rates were found to be improved (23.9 versus 7%) in women aged over 38 years and who had had more than three failed IVF attempts. Using the same patient criteria, another group [231] observed increased pregnancy rates (52 versus 32%) in a 100-cycle prospective

randomized trial, while others [232] obtained similar results in women aged over 35 but less than 43 years. Increased pregnancy rates (30 versus 7%) were found in another prospective randomized study [233] in which among others, 38 patients with poor prognosis were evaluated. In an additional prospective randomized study conducted by Mansour et al., a significantly increased pregnancy rate (23 versus7.3%) was observed in women aged >40 years and/or with at least two failed IVF attempts. [234] It was noteworthy that even those patients with the poorest prognosis had a mean of at least eight retrieved oocytes per cycle, which automatically excludes them from the poor-response category.

By contrast, in a large-scale prospective study in 312 cycles involving women aged >38 years, no difference was found in pregnancy rates after routine use of assisted hatching. [235] Of note was the fact that these women required only 225 IU hMG/day to produce a mean 6.05 oocytes per cycle, compared with the control participants who responded poorly, producing only 3.74 oocytes per cycle. Similar results were shown in another prospective study, [236] where assisted hatching was applied in patients aged >36 years, though these women unquestionably were not poor responders, having a mean of almost 10 retrieved oocytes per cycle.

It is clear that all of these studies involve potential, but not truly poor responders. Hence, well-designed studies involving documented poor responders should be conducted in order to assess the efficacy of this intervention.

Natural cycle

Some authors have proposed that if a woman does not respond to ovarian stimulation, then the use of her own natural cycle oocyte(s) should be considered. This approach is less invasive and less costy for the patient. Although the results of many studies have been published in the area of natural cycle IVF, very few have involved solely poor responders, thus, three historically controlled prospective studies and one uncontrolled study that simply included older women were identified.

In the first study, which was prospective in nature and included historical controls, [237] it was suggested that the outcome was improved, with a mean of 0.9 oocytes per cycle(versus 1.5) being aspirated. In addition, the cancellation rates were significantly lower (18.8 versus 48%) and ongoing pregnancy rates per cycle were higher (18.8 versus 0%).

Encouraging results were obtained from another group in a prospective study. [238] Standard ICSI was performed on retrieved MII oocytes in 25 natural cycles in 17 patients with poor response in at least two previous cycles. Oocyte retrieval was attempted in twenty cycles (80%), oocytes were recovered in 13 of them (65%). All of the recovered oocytes were MII (except one retrieved from a secondary follicle), and all MII oocytes were injected. Fertilization and cleavage occurred in 11 cycles (Fertilization rate 87%) and embryo transfer was cancelled in two patients. Pregnancies occurred in five patients, one of them was chemical and four ended in live births (36% per transfer, 20% per attempted retrieval, and 16% per started cycle). They concluded that natural cycle ICSI is a low cost alternative with reasonable success rate in women with previous low response to stimulation due to low ovarian reserve.

By contrast, in another prospective study with historical controls, [239] comparable results were reported between the natural and stimulated cycles, in which at least one oocyte was aspirated in 82% of the patients while the full-term pregnancy rate was 9%. Similar results were found in a prospective study with no controls (44 cycles), in which patients aged over 44 years (i.e. potential but not proven poor responders) were included. [240] Successful oocyte aspiration was achieved in almost half of the cycles (48.5%), and the ongoing pregnancy rate was 2.08% per cycle.

Therefore, the available data relating to the use of natural cycle in poor responders are not only limited in extent but also contradictory in nature.

Is there a link between an extremely poor response and early ovarian failure?

Traditionally, the peri-menopause was described as the time when ovarian function was so altered by the continuous depletion of oocytes that clinical symptoms, such as menstrual irregularities, appeared. In recent years, assisted reproductive technologies have given us a better understanding of the events that precede the menopause. [241]

It is now realized that an accelerated decline of ovarian function begins much earlier than previously thought, most likely in the mid-thirties. [242] At around this time the total remaining number of follicles in the ovaries has been shown to be near 25,000 [243] and there is an accelerated loss of follicles, as well as qualitative changes in the remaining follicles.

These follicles have fewer granulosa cells, which demonstrate diminished production of steroids and glucoproteins [244, 245, 246] and also decreased mitosis and increased apoptosis. [247, 248] As a result of compromised endocrine, paracrine and autocrine signals, there is altered communication between the granulosa cells and the oocytes, which result in abnormal nuclear and cytoplasmic maturation within the oocyte. [249, 250, 251, 252] The clinical result is an increase in the incidence of aneuploidy, [253] an increase in the incidence of miscarriage and a decrease in fertility rates both spontaneous and with assisted reproduction technologies. [254, 255]

This marked reduction of reproductive potential occurs without obvious clinical symptoms of endocrine deficiency. The term used to describe a woman's reproductive potential, as it relates to the process of follicular depletion and oocyte quality, is ovarian reserve. It is well established that, in general, ovarian reserve declines with age. However, the rate of this decline seems to vary among individuals and depends on the medical history and various environmental and genetic factors. Severe endometriosis, pelvic inflammatory disease, ovarian surgery, various systemic illnesses, chemotherapy and smoking are all known factors affecting the ovarian reserve. [256, 257, 258, 259, 260] Although there is evidence that poor performance in the various dynamic tests for ovarian reserve, including IVF, is associated with low pregnancy rates, there has not been evidence, so far, that this poor performance is also linked to an earlier menopause. One interesting study [261] reported the identification of a group of 12 infertile women, initially diagnosed as having unexplained or anovulatory infertility, who had a normal baseline hormonal profile and did not respond to repeated ovarian stimulation with gonadotrophins. They all developed ovarian failure

within a few months. The mean age of the patients in that group was 39.8 years (range 34–43), the mean FSH at first evaluation was 5.4 IU/l and, following the diagnosis of non-response, 53.5 IU/l. The mean time elapsed between the two tests was 8.8 months. They concluded that non-response to gonadotrophin stimulation might be the first detectable sign of impending menopause.

Other recent studies have also shown that poor ovarian response could be the first sign of ovarian ageing (early ovarian failure or early menopause). [262, 263, 264] The various existing tests for ovarian reserve generally have good specificity but rather poor sensitivity. Although abnormal results predict poor responses, there are women, especially older women, who have good test results but respond poorly to ovarian stimulation.

The mechanisms by which chronological age could diminish pregnancy rates independent of FSH are not fully understood. Similarly with the clomiphene citrate challenge test: although it is quite specific, it has limited sensitivity, with a significant age-related diminution in reproductive potential occurring even among women with normal test results. [265]

We do not know the prevalence and the exact mechanism of premature decline of ovarian reserve, especially in terms of oocyte quality, in the general population. One possible mechanism is a generally higher intrinsic rate of atresia. Another possibility is that some patients have had a destructive process that left behind fewer follicles but the same proportion of `good' oocytes. [266]

Various medical, environmental [267, 268] and genetic factors [269, 270] may be involved. An interesting hypothesis is that this process may have occurred in fetal life. In an epidemiological study, [271] menopause occurred at a younger age in women who had low weight at 1 year. Women who had an early menopause tended to be short at birth. It was suggested that growth retardation in late gestation, leading to shortness at birth and low weight gain in infancy, might be associated with a reduced number of primordial follicles in the ovary, leading to an earlier menopause. In another study, [272] the volume percentages of primordial follicles in the ovaries of severely growth-retarded fetuses of different gestational ages were significantly lower than those observed in age-matched controls.

In a very interesting report, diminished ovarian reserve in normally cycling women was a predictor of unfavorable lipid levels and increased cardiovascular risk. [273] An exciting area of research would be to investigate the possibility of delaying or even reversing the accelerated decline of the ovarian reserve in some women.

Chapter II

AIM OF THE WORK

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The present study was intended to compare three alternative protocols of controlled ovarian hyperstimulation (COH) in patients with poor ovarian response to standard long protocol of GnRH-a administration in assisted reproductive techniques (ART). The three protocols were:

1. The antagonist protocol.
2. The aromatase inhibitor protocol.
3. The agonist stop protocol.

Chapter III

MATERIALS AND METHODS

MATERIALS AND METHODS

This was a non-randomized prospective study in which sixty infertile ladies were recruited from the infertility clinic of El-Shatby Maternity University Hospital and a specialized IVF center. All the patients accepted and consented to the in vitro fertilization and intra-cytoplasmic sperm injection (IVF – ICSI) program.

Criteria for inclusion

* The age of the patients was ranging from 20 to 40 years.
* The period of infertility was at least 2 years.
* All patients were indicated for ICSI
* All the patients had
    - Day 3 serum FSH < 12 mIU/ml.
    - Day 3 serum E2 < 80 pg/ml.
* All the patients had a poor ovarian response to standard long protocol of GnRH-a administration in previous ICSI cycles, i.e.: failure to produce an adequate number of mature follicles (five dominant follicles or less).
* No contra-indication to pregnancy.

Criteria for exclusion

* Previous history of ovarian or pelvic surgery.
* Endometriosis.

All patients were subjected to

(1) Detailed history taking
(2) General examination including breast examination.
(3) Local examination; abdominal and pelvic examination.
(4) Routine investigations (complete blood picture, complete urine analysis, fasting blood sugar)
(5) Infertility investigations including:

* Husband semen analysis.
* Hysterosalpingography.
* Trans- vaginal ultrasound for
    - Detection of the antral follicles number and size in the early follicular phase.
    - Size, direction of the uterus as well as the thickness and pattern of the endometrium.
    - Follicular scanning.
* Hormonal profile
    - Early follicular phase (day 3) serum FSH.
    - Serial assays of serum estradiol level (day 3, day 7 and day of HCG).
    - Serum progesteone level on day of HCG.

The patients were categorized into three groups

Group A

Consisted of 20 patients treated with urinary human menopausal gonadotropins (u-hMG) (Merional®, IBSA) and urinary follicle stimulating hormone (u-FSH) (Fostimon®, IBSA) in a dose of five to six ampoules per day starting from the third day of the cycle till the criteria for the administration of human chorionic gonadotrophin, hCG (Profasi®; Serono; or Pregnyl®; Organon) is reached (i.e.; when two or more graffian follicles are > 18-20 mm and the endometrial thickness is > 8 mm).

GnRh-antagonist, cetrorelix (Cetrotide®; ASTA Medica AG, Frankfurt/Main, Germany and Serono International S.A.) was also given subcutaneously using the multiple dose protocol with a dose of 0.25 mg per day starting when the follicle size reaches 14 mm till the criteria for the administration of human chorionic gonadotrophin (hCG) was reached.

Group B

Consisted of 20 patients treated with the aromatase inhibitor, letrozole (Femara®; Novartis, USA), 2.5 mg/day from the third day of the menstrual cycle together with Human menopausal gonadotrophin (u-hMG) and follicle stimulating hormone (u-FSH) in a dose of five to six ampoules per day till the criteria for the administration of human chorionic gonadotrophin (hCG) was reached.

Group C

Consisted of 20 patients treated with the GnRH agonist ,triptorelin (Decapeptyl®; Ferring). in a dose of 0.1 mg subcutaneously beginning in the mid luteal phase of the previous menstrual cycle till suppression of the pituitary gland occurs around the third day of the next cycle (i.e.; when the basal serum E2 < 50 pgm/ ml), then u-hMG & u-FSH were started in a dose of five to six ampoules per day till the criteria for the administration of human chorionic gonadotrophin (hCG) was reached.

The development of the ovarian follicles was monitored by both transvaginal ultrasound measurement of the mean follicular diameter as well as serial assays of estradiol level during the follicular phase. The dose and duration of u-FSH and u-hMG treatment were adjusted during the monitoring of the follicular development according to the patient's response including the number of the growing follicles and estradiol levels. When the criteria for human chorionic gonadotropin (hCG) administration was reached, 10 000 IU of hCG was given subcutaneously to trigger ovulation. Retrieval was scheduled 34 hours later using trans-vaginal ultrasound guided needle aspiration.

The cycle was cancelled if only one mature graffian follicle was obtained after controlled ovarian hyperstimulation.

Following oocyte retrieval, the patients received luteal phase support in the form of micronized natural progesterone vaginally in a dose of 600 mg/day (i.e.; two vaginal ovules taken three times daily) to continue preparing the endometrium.

After oocyte retrieval, the cumulus and corona radiata were removed mechanically under a stereomicroscope, after exposure to 0.5% hyalouronidase solution (Sigma Company, Deisenhofen, Germany) for 30 seconds. mature oocytes were injected (ICSI). This was followed by transfer of the embryos in the appropriate time. Patient β-hCG was measured for diagnosis of pregnancy 14 days after embryo transfer and then measured serially to monitor the rise in its titre. Implantation was noted later by the appearance of the gestational sac in the uterus using transvaginal ultrasonography (TVS). Clinical pregnancy was confirmed by observing fetal cardiac pulsation 4 weeks after positive pregnancy test by TVS. In these cases, the administration of progesterone was continued up to week 12 of gestation.

Intracytoplasmic sperm injection (ICSI)

For the procedure of ICSI, both holding and injection pipettes were obtained commercially (Humagen Fertility Diagnostics, USA), and the ICSI procedure was performed using Narashige micromanipulators (Narashige, Japan) under Hoffman modulation optics.

Just before the ICSI procedure the sperm suspension was placed in a 10 µl droplet of 10% polyvinyl-pyrrolidone (Medicult) at the 3 o'clock position. Injection of the oocyte was performed in microdroplets of Medicult IVF medium under mineral oil (International Medical, The Netherlands).

A single motile morphologically normal spermatozoon that had migrated to the 9 o'clock position was selected, immobilized by touching its tail with the injection micropipette, and then aspirated tail first into the pipette. The oocyte to be injected was secured with the holding pipette (9 o' clock position) adjacent to the polar body (6 o'clock position). The micropipette containing the sperm was then inserted through the zona pellucida and the oolemma into the ooplasm at the 3 o'clock position of the oocyte. Penetration of the oolemma was confirmed by aspiration of some cytoplasm into the micropipette and the spermatozoon was then slowly injected. The pipette was withdrawn gently and the oocyte released from the holding pipette.

Assessment of fertilization and cleavage

Oocytes were examined for fertilization 16–18 h after ICSI. Cleavage of the oocytes was assessed on day 2 (48 h) and day 3 (72 h) before transfer into the uterus.

The embryos were graded on a scale of 1 to 4. [274] *Grade 1* embryos (class A) were the best embryos, containing even-sized, symmetrical blastomeres with no obvious fragmentation; *grade 2* (class Af) had blastomeres of uneven size or the total cytoplasmic mass contained <10% fragmentation; *grade 3* embryos (class B) had 10–50% of their cytoplasm fragmented; and *grade 4*embryos (class C) showed >50% cytoplasmic fragmentation.

Statistical analysis

The various outcome measures are expressed as mean ± SD. Fertilization; implantation and pregnancy rates were compared between the three groups and tested for significant difference by the χ^2 test, Student's *t*-test, and analysis of variance (ANOVA) test. A *P*-value of <0.05 was considered as statistically significant. The statistical tests were performed with the statistical package for social sciences (SPSS) for windows, version 10 software. [275]

Chapter IV

RESULTS

RESULTS

The study population consisted of sixty infertile couples with a poor ovarian response to standard long protocol of GnRH-a administration in previous ICSI cycles. The couples were then divided into three subgroups each with twenty patients; each patient was enrolled in the study only once. The three groups underwent controlled ovarian hyperstimulation (COH) using three alternative protocols, the agonist stop, the antagonist and the aromatase inhibitor protocols and the treatment outcome of the three groups was compared.

Baseline clinical characteristics

The three groups were comparable with respect to all Baseline clinical characteristics (Table I). There was no clinical significance as regard the age (Fisher exact test, “F test”, 0.6273, P value 0.5376). The mean age was 35.25±3.79 years for the agonist stop group, 36.2±4.39 years for the antagonist group and 36.6 ± 3.50 years for the aromatase inhibitor group. The mean duration of infertility was 9.15± 5.31 years for the agonist stop group, 9±5.911 years for the antagonist group and 11.65± 6.474 years for the aromatase inhibitor group showing no statistically significant differences between the three groups (F test 1.264, P 0.2900).

There was also no statistically significant differences between the three groups as regard the basal serum FSH level (P 0.2248), the basal serum estradiol level (P 0.0563) as well as the number of the antral follicles (P 0.852). The mean basal serum FSH level was 9.899 ± 2.234 mIU/ml for the agonist stop group, 8.68 ± 2.635 mIU/ml for the antagonist group and 8.842±2.286 mIU/ml years for the aromatase inhibitor group. The mean basal serum estradiol level was 50.925±16.89 mIU/ml for the agonist stop group, 39.225±11.57 mIU/ml for the antagonist group and 46.66±16.672 mIU/ml for the aromatase inhibitor group. The mean number of the antral follicles was 6.9± 2.4687 for the agonist stop group, 6.7±1.6575 for the antagonist group and 7.05±1.6375 for the aromatase inhibitor group.

Table I: Clinical characteristics of the patients under study.

	Agonist stop	Antagonist	Aromatase inhibitor	Test	P value	Significance
Number of patients	20	20	20			
Number of cycles	20	20	20			
Age	35.25±3.79	36.2±4.39	36.6 ± 3.50	F 0.6273	P 0.5376	NS
Infertility duration	9.15± 5.31	9±5.911	11.65± 6.474	F 1.264	P 0.2900	NS
Basal FSH	9.899 ± 2.234	8.68 ± 2.635	8.842±2.286	F 1.5322	P 0.2248	NS
Basal estradiol	50.925±16.89	39.225±11.57	46.66±16.672	F3.0272	P 0.0563	NS
Antral follicles number	6.9± 2.4687	6.7±1.6575	7.05±1.6375	F 0.1605	P 0.852	NS

- Values are mean ± SD (Standard deviation).
- NS = Non significant.

Clinical characteristics of the treatment cycles

There was a significant relationship between the number of ampoules needed during controlled ovarian hyper-stimulation (COH) among the three groups (F test 1.2530, P <0.001) where the mean number of the ampoules was significantly reduced in the aromatase inhibitor group (49.45± 9.37) and the antagonist group (58.35±14.28) compared with the agonist stop group (66.45±9.90). There was also a significant relationship between the Maximal serum E2 level on the day of hCG among the three groups (F test 1.2530, P <0.001) where it was high in the agonist stop group (1468.29±883.3 pg/ml) compared with the antagonist group (827.3±365.19 pg/ml) and the aromatase inhibitor group (551.1±285.69 pg/ml).

On the other hand, there was no significant relation between the three groups (P >0.05) as regard the mean serum progesterone level on the day of hCG (0.994±0.519 ng/ml for the agonist stop group, 0.875±0.312 ng/ml for the antagonist group and 0.917±0.379 ng/ml for the aromatase inhibitor group), the mean endometrium thickness on hCG day (8.752± 1.504 mm for the agonist stop group, 8.22±1.795 mm for the antagonist group and 7.983±1.1 mm for the aromatase inhibitor group) and the Duration of induction (11.85±2.058 days for the agonist stop group, 11.4±1.602 days for the antagonist group and 11.25±1.208 days for the aromatase inhibitor group) (Table II, Figure I,II,III).

Table II: Clinical characteristics of the treatment cycles.

	Agonist stop(1)	Antagonist (2)	Aromatase inhibitor (3)	F test	P value	Significance
Maximal serum E2 level	1468.29±883.3	827.3±365.19	551.1±285.69	12.891	<0.001	* 1:2,3
Progesterone on hCG day	0.994±0.519	0.875±0.312	0.917±0.379	0.3864	0.6813	NS
Endometrium thickness on hCG day	8.752± 1.504	8.22±1.795	7.983±1.1	1.2529	0.2939	NS
Number of ampoules	66.45±9.90	58.35±14.28	49.45± 9.37	1.2530	<0.001	* 1:2,3 ; 2:3
Duration of induction	11.85±2.058	11.4±1.602	11.25±1.208	1.2531	0.4971	NS

- Values are mean ± SD (Standard deviation).
- NS = Non significant.
- * = Significant P<0.05.

Figure I: Serum Progesterone and endometrial thickness on day of hCG administration.

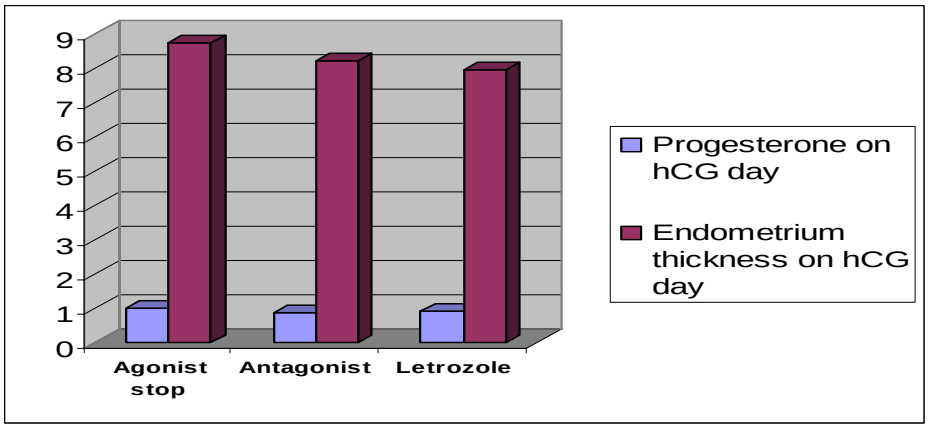


Figure II: Serum estradiol on day of hCG administration.

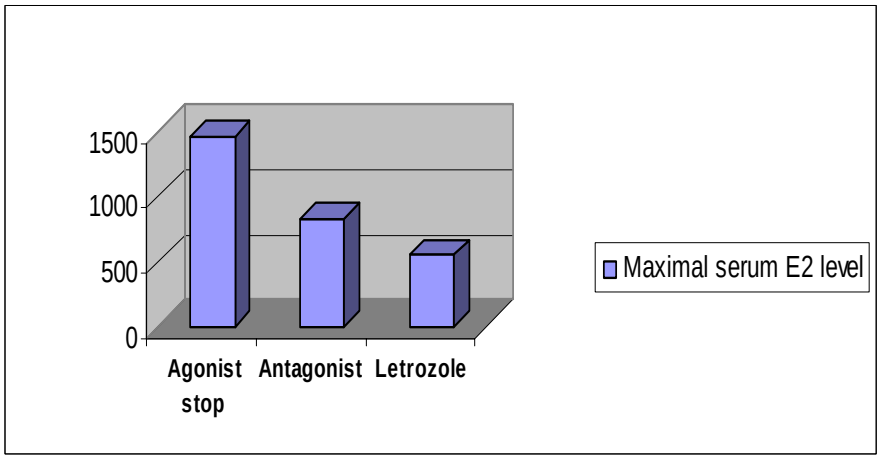
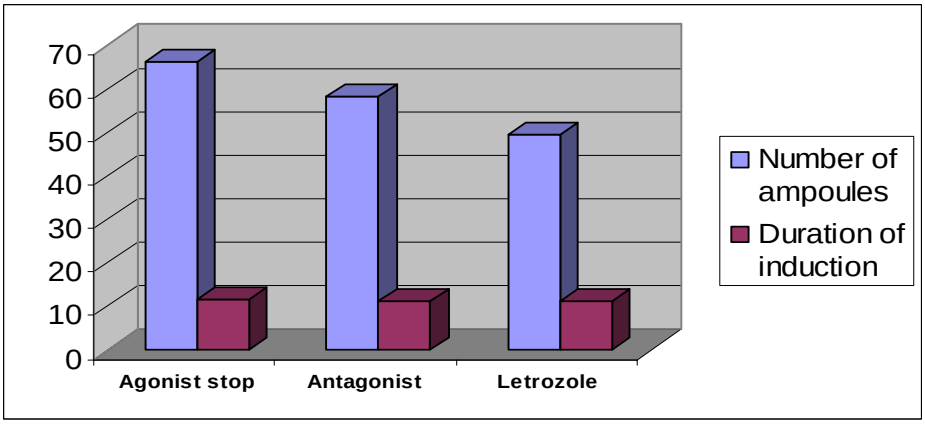


Figure III: Number of ampoules and Duration of induction.



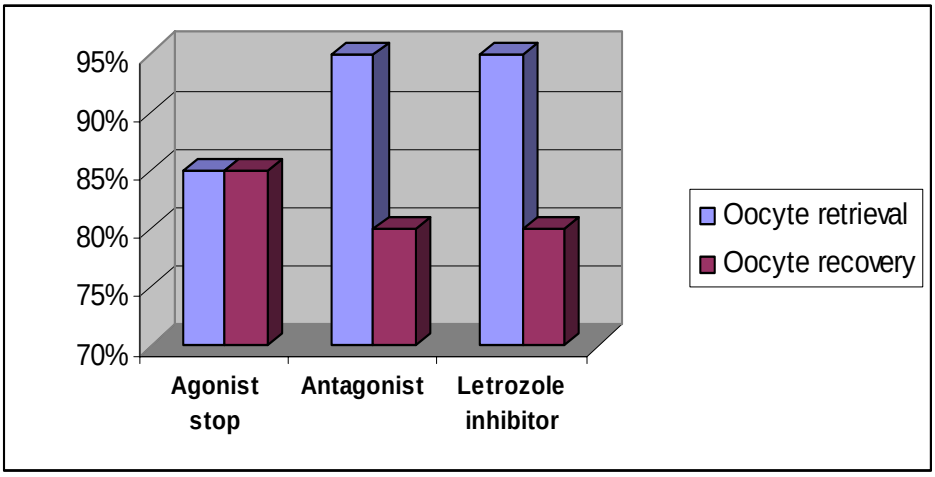
Oocyte retrieval was performed in seventeen cycles out of twenty (85%) in the agonist stop group and in nineteen cycles out of twenty (95%) in both the antagonist and the aromatase inhibitor groups with no statistical significance between the three groups (Chi-square test, χ^2 1.7454, P 0.4178). Oocytes were then recovered in all the attempts in the agonist stop group (85%) and in only sixteen cycles (80%) in both the antagonist and the aromatase inhibitor groups with no statistical significance between the three groups (χ^2 3.0128, P 0.2216) (Table III, Figure IV).

Table III: Attempted oocyte retrieval and oocyte recovery rates per started cycle.

	Agonist stop	Antagonist	Aromatase inhibitor	χ^2 test	P
Attempted oocyte retrieval per started cycle	17/20 (85%)	19/20 (95%)	19/20 (95%)	1.7454	0.4178 (NS)
Oocyte recovery per started cycle	17/20 (85%)	16/20 (80%)	16/20 (80%)	3.0128	0.2216 (NS)

- NS = Non significant.

Figure IV: Attempted oocyte retrieval and Oocyte recovery rates per started cycle.



Cycle cancellation rate and causes of cycle cancellation

Four cycles out of twenty (20%) were cancelled in the agonist stop group, three cycles (75%) were cancelled because of no response to stimulation and the fourth cycle (25%) was cancelled due to no fertilization of the retrieved oocytes after performing ICSI. In the antagonist group, seven cycles out of twenty (35%) were cancelled, the causes of cancellation were no response to stimulation (one cycle, 14.30%), no oocytes on retrieval (three cycles, 42.85%) and no fertilization (three cycles, 42.85%). As regard the aromatase inhibitor group, four cycles out of twenty (20%) were cancelled, the causes of cancellation were no responce to stimulation (one cycle, 25%) and no oocytes on retrieval (three cycles, 75%). There was no statistical significance between the three groups regarding the Cycle cancellation rate and causes of cycle cancellation (χ^2 1.6, P 0.4493) (Table IV, Figure V and VI).

Table IV: Cycle cancellation rate and causes of cycle cancellation.

	Agonist stop	Antagonist	Aromatase inhibitor	χ^2 test	P value	Significance
Cycle cancellation rate	4/20 (20%)	7/20 (35%)	4/20 (20%)	1.6	0.449	NS
No response to stimulation	3/4 (75%)	1/7 (14.30%)	1/4 (25%)			
No oocytes on retrieval	0 (0%)	3/7 (42.85%)	3/4 (75%)			
No fertilization	1/4 (25%)	3/7 (42.85%)	0 (0%)			

- NS = Non significant.

Figure V: Cycle cancellation rate.

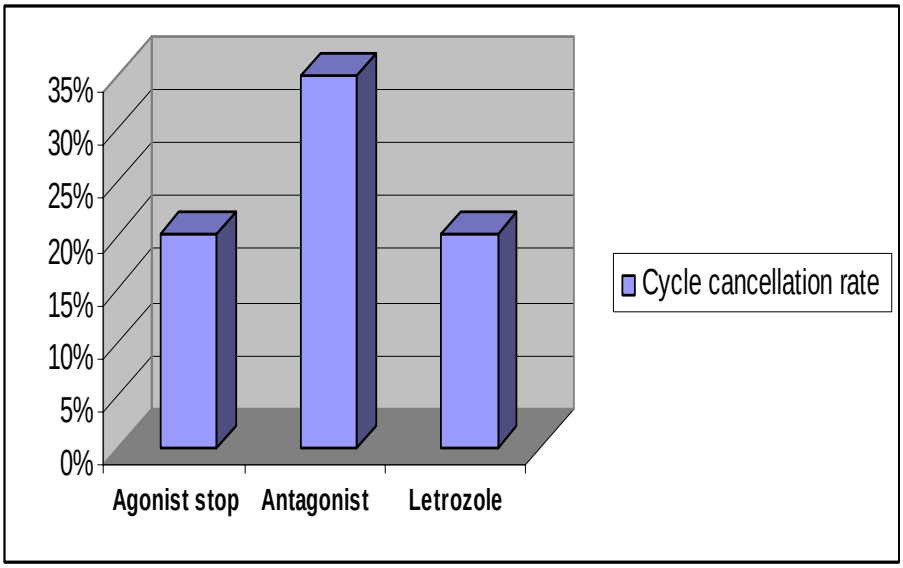
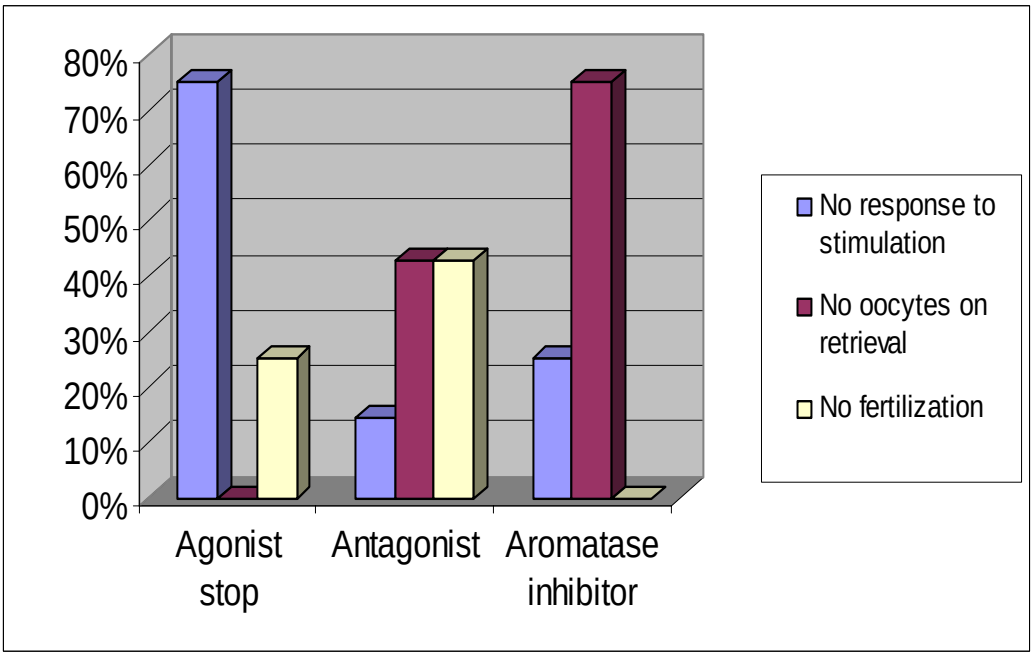


Figure VI: Causes of cycle cancellation.



Number and quality of the retrieved oocytes

There was no statistical difference between the three groups regarding the number of the oocytes retrieved (F test 1.327, P 0.2742) where the mean number of the retrieved oocytes was 5±2.263 for the agonist stop group, 3.83±3.88 for the antagonist group and 3.52±1.98 for the aromatase inhibitor group. However, regarding the oocyte quality, there was a statistical significant difference between the agonist stop group and the aromatase inhibitor group regarding the mean MII oocytes number (4.47±1.91 for the agonist stop group and 2.68±1.52 for the aromatase inhibitor group), such statistical significant difference was not present for the antagonist group (3.16±2.89). Comparable number of MI and GV (Germinal Vesicle) oocytes was retrieved in the three groups with no statistical significance encountered. For the MI oocytes, the mean number was 0.294±0.469 for the agonist stop group, 0.388±0.978 for the antagonist group and 0.631±0.683 for the aromatase inhibitor group. For the GV oocytes, the mean number was 0.235±0.752 for the agonist stop group, 0.277±0.460 for the antagonist group and 0.210±0.418 for the aromatase inhibitor group (Table V, Figure VII and VIII).

Table V: Number and quality of the retrieved oocytes.

	Agonist stop(1)	Antagonist(2)	Aromatase inhibitor (3)	F test	P value	Significance
Number of oocytes retrieved	5±2.263	3.83±3.88	3.52±1.98	1.327	0.2742	NS
M II Oocytes	4.47±1.91	3.16±2.89	2.68±1.52	3.170	0.0503	*1:3
MI Oocytes	0.294±0.469	0.388±0.978	0.631±0.683	0.9982	0.3755	NS
GV Oocytes	0.235±0.752	0.277±0.460	0.210±0.418	0.0685	0.9338	NS

- Values are mean ± SD (Standard deviation).
- NS = Non significant.
- * = Significant P<0.05.

Figure VII: Number of oocytes retrieved.

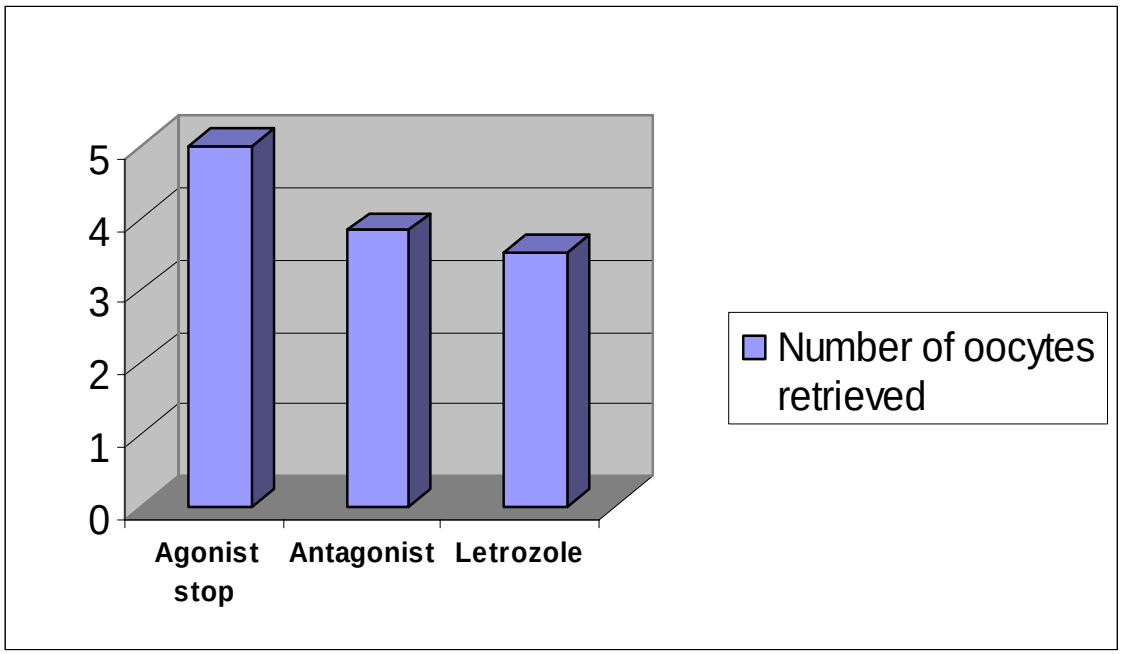
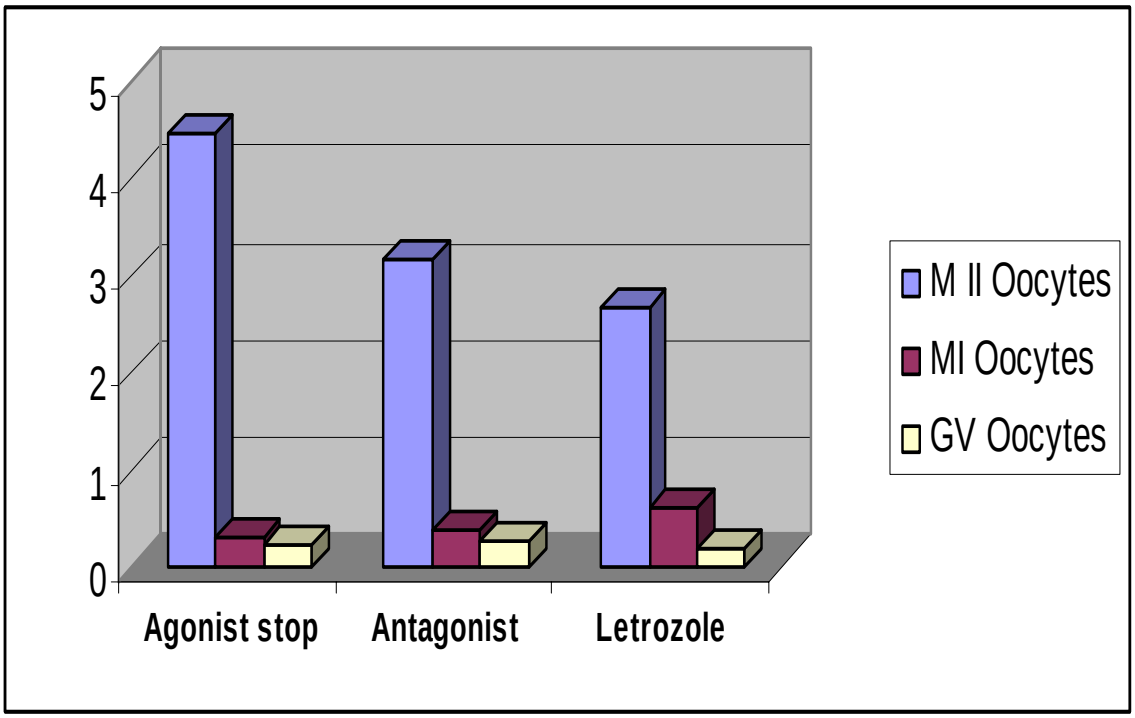


Figure VIII: Quality of oocytes retrieved.



Fertilization rate

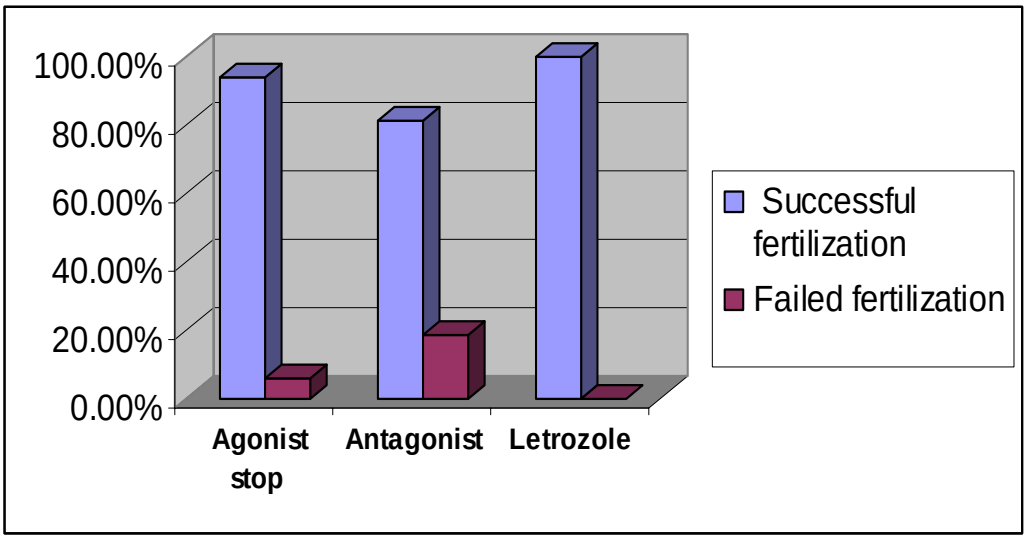
There was no statistical significance between the three groups as regard the successful fertilization rate per oocyte recovery (χ^2 3.932, P 0.1400) where sixteen out of seventeen cycles (94.12%) in the agonist stop group, thirteen out of sixteen cycles (81.25%) in the antagonist group and all the cycles (100%) with successful oocyte recovery in the aromatase inhibitor group showed successful fertilization. However, there was a statistical significant difference between the agonist stop and the other two groups as regard the number of the fertilized oocytes (F 3.887, P 0.027). The mean number of the fertilized oocytes was more in the agonist stop group (4.35±2.26) compared with the antagonist group (2.61±2.40) and the aromatase inhibitor group (2.68± 1.53) (Table VI, Figure IX).

Table VI: Successful and failed fertilization per oocyte recovery.

	Agonist stop (1)	Antagonist (2)	Aromatase inhibitor (3)	Test	P value	Significance
Successful fertilization per oocyte recovery	94.12%	81.25%	100%	χ^2 3.932	0.1400	NS
Failed fertilization	5.88%	18.75%	0%	χ^2 3.932	0.1400	NS
Number of fertilized oocytes	4.35±2.26	2.61±2.40	2.68± 1.53	F 3.887	0.027	*1:2,3

- Values are mean ± SD (Standard deviation).
- NS = Non significant.
- * = Significant P<0.05.

Figure IX: Successful and failed fertilization per oocyte recovery.



Embryo scoring

There was a statistical significant difference between the agonist stop and the other two groups as regard the number of class A embryos (F test 4.1825, P 0.0207) and the number of the four celled class A embryos (F test 5.1519, P 0.009) which was higher in the agonist stop group than the other two groups. The mean number of the class A embryos was (3.176±1.380) in the agonist stop group, (1.833±1.757) in the antagonist group and (1.947± 1.393) in the aromatase inhibitor group. The mean number of the four celled class A embryos was (2.11±1.576) in the agonist stop group, (1.055±1.211) in the antagonist group and (0.8421± 0.958) in the aromatase inhibitor group.

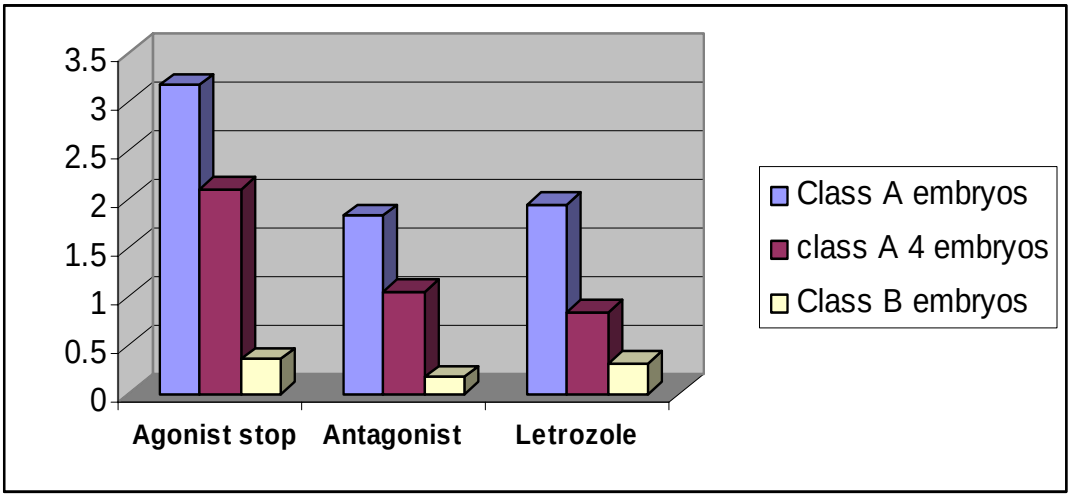
As regard the class B embryos, there was no statistically significant difference between the three groups. The mean number of the class B embryos was (0.3529±0.7018) in the agonist stop group, (0.1666±0.514) in the antagonist group and (0.3157±0.4775) in the aromatase inhibitor group (Table VII, Figure X).

Table VII: Embryo scoring.

	Agonist stop (1)	Antagonist (2)	Aromatase inhibitor (3)	F test	P value	Significance
Class A embryos	3.176±1.380	1.833±1.757	1.947± 1.393	4.1825	0.0207	*1:2,3
Number of 4 celled class A embryos	2.11±1.576	1.055±1.211	0.8421± 0.958	5.1519	0.009	*1:2,3
Class B embryos	0.3529±0.7018	0.1666±0.514	0.3157±0.4775	0.534	0.5890	NS

- Values are mean ± SD (Standard deviation).
- NS = Non significant.
- * = Significant P<0.05.

Figure X: Embryo scoring.



Embryo transfer

Embryo transfer was performed in sixteen out of twenty cycles (80%) in both the agonist stop and the aromatase inhibitor groups and in only thirteen cycles (65%) in the antagonist group. The mean number of embryos transferred per cycle was (2.75±1.650) in the agonist stop group, (1.65±1.755) in the antagonist group and (2.15±1.531) in the aromatase inhibitor group, there was no statistical difference between the three groups (P>0.05) (Table VIII).

Table VIII: Embryo transfer.

	Agonist stop	Antagonist	Aromatase inhibitor	Test	P value	Significance
Embryo transfer per started cycle	80%	65%	80%	χ^2 1.6	P 0.449	NS
Number of embryos transferred per cycle	2.75±1.650	1.65±1.755	2.15±1.531	F 2.233	P 0.1164	NS

- Values are mean ± SD (Standard deviation).
- NS = Non significant.

Pregnancy outcome

Positive pregnancy test was found in seven cycles (35%) in the agonist stop group, five cycles (25%) in the antagonist group and in only three cycles (15%) in the aromatase inhibitor group. In the agonist stop group, positive cardiac pulsation (i.e., clinical pregnancy) was found in the seven cases, three of them (15%) continued till full term, three (15%) ended as abortion and one (5%) as tubal ectopic pregnancy. In the antagonist group, one of the cycles (5%) was chemical pregnancy, three (15%) ended as abortion and only one (5%) was ongoing pregnancy which ended with preterm delivery at 34 weeks. In the aromatase inhibitor group, the first patient (5%) was chemical pregnancy, the second (5%) was blighted ovum and the third (5%) was ongoing pregnancy which ended with preterm delivery at 35 weeks. There was a statistically significant difference in the clinical pregnancy rate between the agonist stop and the aromatase inhibitor groups (χ^2 5.625, P 0.018) where it was higher in the agonist stop group (35%) than in the aromatase inhibitor group (5%) (Table IX, X - Figure XI, XII).

Table IX: Pregnancy and clinical pregnancy rates per started cycle.

	Agonist stop (1)	Antagonist (2)	Aromatase inhibitor (3)	χ^2 test	P value	Significance
Pregnancy rate	7/20 (35%)	5/20 (25%)	3/20 (15%)	2.133	0.3441	NS
Clinical pregnancy rate	7/20 (35%)	4/20 (20%)	1/20 (5%)	5.625	0.018	*1:3

- NS = Non significant.
- * = Significant P<0.05.

Figure XI: Pregnancy and clinical pregnancy rates per started cycle.

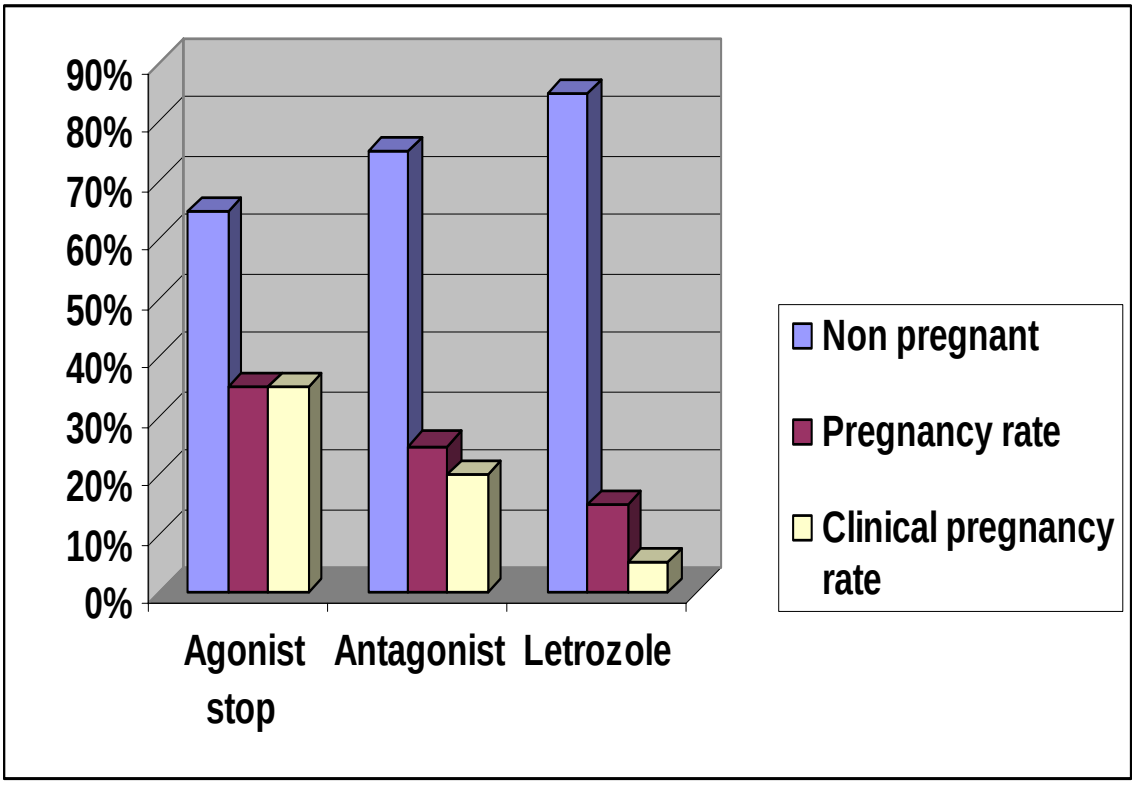
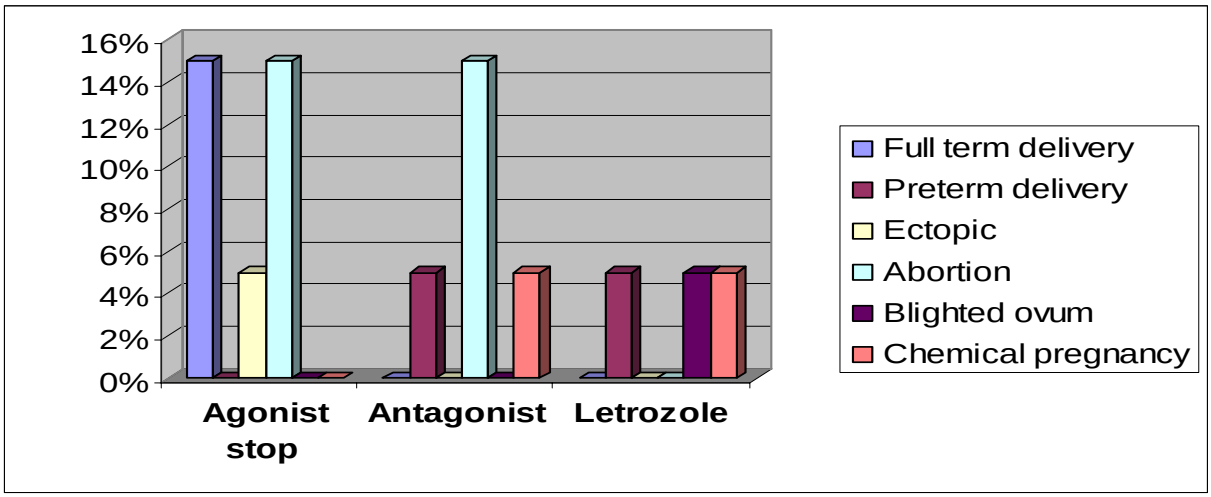


Table X: Pregnancy outcome.

	Agonist stop	Antagonist	Aromatase inhibitor
Full term delivery	3/20 (15%)	0 (0%)	0 (0%)
Preterm delivery	0 (0%)	1/20 (5%)	1/20 (5%)
Ectopic	1/20 (5%)	0 (0%)	0 (0%)
Abortion	3/20 (15%)	3/20 (15%)	0 (0%)
Blighted ovum	0 (0%)	0 (0%)	1/20 (5%)
Chemical pregnancy	0 (0%)	1/20 (5%)	1/20 (5%)

Figure XII: Pregnancy outcome.



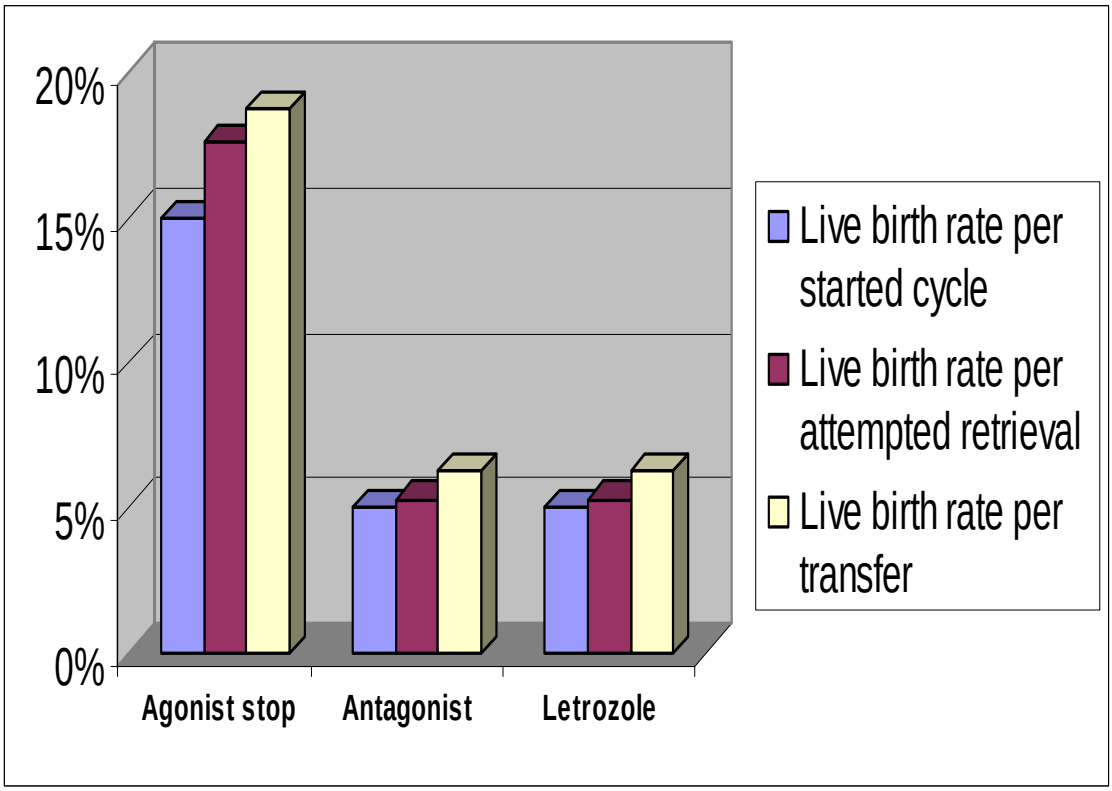
The live birth rate per started cycle was 15% (three out of twenty cycles) for the agonist stop group, 5 % (one out of twenty cycles) for the antagonist group and 5 % (one out of twenty cycles) for the aromatase inhibitor group. The live birth rate per attempted retrieval was 17.6% (three out of seventeen cycles) for the agonist stop group, 5.3% (one out of nineteen cycles) for the antagonist group and 5.3% (one out of nineteen cycles) for the aromatase inhibitor group. The live birth rate per transfer was 18.75% (three out of sixteen cycles) for the agonist stop group, 6.25% (one out of sixteen cycles) for the antagonist group and 6.25% (one out of sixteen cycles) for the aromatase inhibitor group. Again, there was no statistical difference between the three groups regarding the live birth rate (P>0.05) (Table XI, Figure XIII).

Table XI: Live birth rate.

	Agonist stop	Antagonist	Aromatase inhibitor	χ^2 test	P value	Significance
Live birth rate per started cycle	3/20 (15%)	1/20 (5%)	1/20 (5%)	1.745	0.4178	NS
Live birth rate per attempted retrieval	3/17 (17.6%)	1/19 (5.3%)	1/19 (5.3%)	2.179	0.3362	NS
Live birth rate per transfer	3/16 (18.75%)	1/16 (6.25%)	1/16 (6.25%)	1.786	0.4094	NS

- NS = Non significant.

Figure XIII: Live birth rate.



Chapter V

DISCUSSION

DISCUSSION

Low ovarian response to stimulation still represents one of the most intractable problems of infertility treatment. Clinicians have historically approached low responders in two ways. A first option is to refuse patients entry into in-vitro fertilization (IVF) cycles. Such an approach is, however, obviously discriminatory and serves no practical purpose except for protecting a programmer's pregnancy rate (a rather self-serving and medically inappropriate choice). This leaves, as the only ethical choice, attempts to maximize outcomes in IVF cycles for low responders by attempting to improve their historically poor pregnancy and delivery rates.

In poor responder patients, the induction of a controlled multifollicular growth is a big challenge. Several stimulation protocols with different doses of gonadotrophins have been suggested but unfortunately no protocol is really effective and the ideal approach to this group of patients has not been well established. Moreover, whatever protocol is used, the clinical outcome is poorer than that observed in normoresponder patients and it seems to be related to female age, to the number of oocytes retrieved and to the number of embryos transferred.

Gonadotrophins

When the standard daily dose of gonadotrophins (225-300IU) fails to induce a proper multifollicular growth, the obvious clinical approach is to increase the dose of gonadotrophins, and high doses of gonadotrophins have been used by the vast majority of the authors in poor responder patients. Therefore, it was decided to treat the patients with an increased dose of urinary human menopausal gonadotropins (hMG) and follicle stimulating hormone (FSH) in a dose of five to six ampoules per day (375-450 IU/ day) starting from the third day of the cycle till the criteria for the administration of human chorionic gonadotrophin (hCG) is reached (i.e.; when two or more graffian follicles are > 18-20 mm and the endometrial thickness is > 8 mm),in order to evaluate if a better ovarian response could be achieved. The gonadotrophins dose was tailored through the treatment cycle according to the patient response on the basis of ultrasound follicular scanning and serial serum estradiol levels. There was a significant relationship concerning the number of ampoules needed during controlled ovarian hyper-stimulation (COH) among the three groups (F test 1.2530, P <0.001) where the mean number of the ampoules was significantly reduced in the aromatase inhibitor group (49.45± 9.37) and the antagonist group (58.35±14.28) compared with the agonist stop group (66.45±9.90). Unfortunately, minimal benefit was obtained with an increased dose regarding the number of the retrieved oocytes and the fertilization rate.

The results of several studies evaluating the use of a high dose of gonadotrophin in patients who fail to respond to the standard protocol are still controversial. Our results are in agreement with those of Akman et al and Nikolettos et al which showed that increased doses of gonadotrophins were not able to influence ovarian response and to increase pregnancy rates in poor responders [166, 276] conversely; other reports give favorable effects that include increased pregnancy rates and reduced cancellation rates. [140, 141] It is speculated that these patients require a specific stimulation protocol to retrieve the

maximum number of oocytes. These patients are likely to have a reduced ovarian reserve with an outcome that will be poor independently of the gonadotrophin dosage administered. Another fact which should be put in mind is that, not all low responders are similar, as they represent a very heterogeneous group. According to their basal FSH concentrations, young low responders with high basal FSH concentrations have a poor outcome based on the low quality of the oocytes retrieved. However, young low responders with normal day 3 FSH concentrations, although their ovarian reserve may be compromised, might benefit from increasing the doses of gonadotrophins in order to proceed to oocyte retrieval and avoid a new cancelled cycle, their chances of achieving a pregnancy seem to be similar to those of normal responders.

GnRH analogues

In normo-responder patients the combination of gonadotrophins and gonadotrophin releasing hormone (GnRH) agonists lowers cancellation rate, raises the number of pre-ovulatory follicles, oocytes retrieved and good quality embryos for transfer, thus leading to better pregnancy rates. Conversely, in poor responder patients it is not clear whether the use of GnRH agonists is advantageous or detrimental. The GnRH agonists may have a direct ovarian effect, acting to modulate ovarian steroidogenesis and oocyte maturation, and sometimes may induce excessive over-suppression with insufficient concentration of serum estradiol and a reduced or absent follicular responses. For this reason, in all those patients who fail to obtain adequate multifollicular growth with the long GnRH agonist protocols, the options are either to decrease the length of suppression by decreasing the duration of GnRH agonist use (short and ultra-short regimens) or to lower or to stop (after pituitary suppression) the dose of GnRH agonists initiated during the luteal phase to allow for down-regulation without complete inhibition of ovarian response.

In our study, we decided to compare the agonist stop protocol in cases of poor responders with the antagonist as well as the aromatase inhibitor protocols. For the agonist stop protocol, 20 patients started the GnRH agonist (triptorelin) in a dose of 0.1 mg subcutaneously beginning in the mid luteal phase of the previous menstrual cycle till suppression of the pituitary gland occurs around the third day of the next cycle (i.e.; when the basal serum E2 < 50 pgm) then hMG&FSH started in a dose of five to six ampoules per day till the criteria for the administration of human chorionic gonadotrophin (HCG) was reached. We got improved results in terms of oocyte quality, fertilization rate, embryo quality and clinical outcome. Regarding the oocyte quality, there was a statistically significant difference between the agonist stop group and the aromatase inhibitor group regarding the mean MII oocytes number (4.47±1.91 for the agonist stop group and 2.68±1.52 for the aromatase inhibitor group), such statistically significant difference was not present for the antagonist group (3.16±2.89).
There was a also a statistically significant difference between the agonist stop and the other two groups as regard the number of the fertilized oocytes (F 3.887, P 0.027). The mean number of the fertilized oocytes was more in the agonist stop group (4.35±2.26) compared with the antagonist group (2.61±2.40) and the aromatase inhibitor group (2.68± 1.53). In addition, the embryos were of better quality in the agonist stop than in the antagonist or the aromatase inhibitor groups. As stated before, **t**here was a statistical significant difference between the agonist stop and the other two groups as regard the number of class A embryos (F test 4.1825, P 0.0207) and the number of the four celled class A embryos (F

test 5.1519, P 0.009) which was higher in the agonist stop group than the other two groups. The mean number of the class A embryos was (3.176±1.380) in the agonist stop group, (1.833±1.757) in the antagonist group and (1.947± 1.393) in the aromatase inhibitor group. The mean number of the four celled class A embryos was (2.11±1.576) in the agonist stop group, (1.055±1.211) in the antagonist group and (0.8421± 0.958) in the aromatase inhibitor group. Finally, There was a statistical significant difference in the clinical pregnancy rate between the agonist stop group and the aromatase inhibitor group (χ^2 5.625, P 0.018) where it was higher in the agonist stop group (7 cases, 35%) than in the aromatase inhibitor group (1 case, 5%).

The mechanism by which the agonist stop protocol apparently improves ovarian responsiveness is unknown. It is possible that GnRH agonists have a direct inhibitory effect on the ovaries and that, by reducing the dose or stopping it altogether, it removes this suppression and increases ovarian response. [157, 277] The mechanism of continued suppression (despite 11.8±2.05days of stimulation) of premature LH surges under this protocol is also still unknown and needs to be further investigated. Continuous suppression of LH after stopping GnRH agonist has been reported by Sungurtekin and Jansen who stated that: [278] a 5 day course of GnRHa appeared to suppress endogenous GnRH activity within 48 h and remained so for at least 1 week afterwards.

Our results are in agreement with the work of the Norfolk group [36] Who used the work of Feldberg et al [34] to develop a protocol under which they terminated GnRHa (Lupron) with the onset of menses and followed up with high-dose gonadotrophin therapy (the `stop-Lupron' protocol). They argued that such a protocol maximizes ovarian response without losing the benefits of GnRHa down-regulation and presented their experience with 182 low responders undergoing 224 IVF–embryo transfer cycles. Only one patient amongst 80 cycles triggered a spontaneous, premature LH surge prior to HCG administration. They also reported a clinical pregnancy rate, ongoing pregnancy rate per transfer and implantation rate of 32%, 24% and 9% respectively. Their cycle cancellation rate was only 12.5% (28 cycles) and they noted no difference in outcome between stimulation with FSH alone versus FSH with HMG. Although most of the published trials (prospective studies with historical controls) report a reduction in the amount of gonadotrophin administered and improved results in terms of number of oocytes retrieved and clinical outcome [34, 36] with the use of GnRH agonist stop protocol, in two prospective randomized control trials by Dirnfeld et al [279] and Garcia-Velasco et al [280], improvements in reproductive outcome were not reported.

GnRH antagonists

The use of GnRh antagonists in the mid-late follicular phase during ovarian stimulation prevents the premature LH surge while not causing suppression in the early follicular phase. With this stimulation regimen, it is possible to obtain a more natural follicular recruitment without any inhibitory effect possibly induced by the GnRH agonist and therefore it has been suggested by several authors as a suitable protocol for poor responders. However, with this approach also there is conflicting results in the literature.

In our study, a group of twenty poor responder patients were stimulated with a dose of five to six ampoules per day starting from the third day of the cycle, GnRh-antagonist,

cetrorelix (Cetrotide®; ASTA Medica AG, Frankfurt/Main, Germany and Serono International S.A.) was given subcutaneously using the multiple dose protocol with a dose of 0.25 mg per day starting when the follicle size reaches 14 mm till the criteria for the administration of human chorionic gonadotrophin (hCG) was reached. The cycle characteristics and the reproductive outcomes were compared with the agonist stop protocol and the aromatase inhibitor group. The results showed that a significantly lower number of ampoules (P <0.001) was used in the cetrorelix group (58.35±14.28) versus the agonist stop protocol group (66.45±9.90). These results indicate the possibility of reducing the amount of gonadotrophins, the length of stimulation and the overall cost normally associated with the agonist protocol. The present findings are in accordance with data presented by Craft et al. in 1999 as well as Nikolettos et al. at 2001. [165, 276] who reported a significantly lower number of ampoules used in the cetrorelix group (49.3 ± 4.3) versus the long agonist group (72.6 ± 6.8) (P 0.0001).

In our study, there was no significant relation between the three groups regarding the duration of induction, the oocyte recovery rate as well as the cycle cancellation rate. However, there was a statistical significance difference between the antagonist group and the agonist stop group regarding the mean number of the fertilized oocytes where it was lower in the antagonist group (2.61±2.40) than in the agonist stop group (4.35± 2.26). In addition, the mean number of class A embryos in the antagonist group (1.833±1.757) was significantly lower than that of the agonist stop group (3.176±1.380). The same statistical significant difference goes with the mean number of the four celled class A embryos which was high in the agonist stop group (2.11±1.576) than in the antagonist group (1.055±1.211). We did not achieve a significant improvement in the reproductive outcome regarding the clinical pregnancy as well as live birth rate when we compared the antagonist group with the agonist stop or the aromatase inhibitor groups. Our results are in agreement with the two prospective studies done with Craft et al in 1999 [165] and Akman et al in 2000 [166] who achieved no significant difference in the reproductive outcome using the antagonist protocol in poor responder patients. However, better results were achieved by Akman et al in 2001[167] in another prospective randomized study where the antagonist regimen was compared with the flare up regimen. Similarly, in a prospective non randomized study, D' Amato et al in 2004 [281] retrieved a significantly higher number of oocytes and a significantly lower cancellation rate by using GnRH antagonists in combination with clomiphene citrate and gonadotrophins. Similar results were reported in one retrospective study by Fasouliotis et al in 2003. [282]

The limited data available show conflicting results on the use of the GnRH antagonists in poor responder patients. The value of GnRH antagonists in poor responders is not clear at present, as the majority of studies performed are either retrospective [276, 282, 283] or prospective but not randomized trials.[281] There is only one RCT performed by Akman et al in 2001in which GnRH antagonists were compared with GnRH agonists in poor responders. [167] That study, however, although showing promising results for GnRH antagonists, was underpowered to allow solid conclusions to be drawn. There is a need for further well-designed larger prospective randomized controlled studies in this category of patients with poor prognosis in order to evaluate the use of GnRH antagonists.

Aromatase inhibitors

The success of aromatase inhibition by letrozole in inducing ovulation in anovulatory women with PCOS [182] and augmenting ovulation in ovulatory women [184] has been reported previously by Mitwally and Casper. It has also been shown that when letrozole is used with FSH, a significant reduction occurs in the FSH dose needed for COH. [184] The successful use of letrozole in increasing ovarian sensitivity to gonadotrophins and inducing superovulation in poor responders [284] prompted us to evaluate the potential role of letrozole–FSH combination in achieving successful IVF outcome in women with poor ovarian response.

In a non randomized prospective trial, a group of twenty patients were treated with the aromatase inhibitor, letrozole (Femara®; Novartis, USA) in a dose of 2.5 mg/day from the third day of the menstrual cycle together with Human menopausal gonadotrophin (hMG) and follicle stimulating hormone (FSH) in a dose of five to six ampoules per day till the criteria for the administration of human chorionic gonadotrophin (hCG) was reached. The results were then compared with two other stimulation protocols, the agonist stop and the antagonist protocol, where twenty patients were also enrolled under each of these protocols. Compared with the agonist stop group (66.45±9.90) and the antagonist group (58.35±14.28) the Let-FSH group (49.45± 9.37) received a significantly (P <0.001) lower number of gonadotrophin ampoules per day. The maximal serum E2 level was significantly (P <0.001) lower in the aromatase inhibitor group (551.1± 285.69 pg/mL) than in the agonist stop group (1468.29±883.3 pg/mL). Our results are in agreement with those obtained by Goswami et al [285] who evaluated whether incorporation of letrozole could be an effective low-cost IVF protocol for poor responders in their randomized, controlled, single-blind trial. In their study, thirty-eight women with a history of poor ovarian response to gonadotrophins were recruited. Thirteen women (Let-FSH group) received letrozole 2.5 mg daily from day 3–7, and recombinant FSH (rFSH) 75 IU/day on days 3 and 8; and 25 women (GnRH-ag-FSH group) underwent long GnRH agonist protocol and stimulated with rFSH (300–450 IU/day). Compared with the GnRH-ag-FSH group (2865±228 IU), the Let-FSH group (150±0 IU) received a significantly (P < 0.001) lower total dose of FSH. Except for terminal E_2, which was significantly higher (P < 0.001) in the GnRH-ag-FSH group (380±46 pg/ml) than the Let-FSH group (227±45 pg/ml), the treatment outcomes in all other respects, including pregnancy rate, were statistically comparable. It is of particular importance to note that despite the use of around 20-fold lower dose of FSH in the Let-FSH group, which entailed significant reduction of the cost of treatment, the outcomes in all major respects including pregnancy rate were comparable between the groups. They therefore concluded that adjunctive use of letrozole may form an effective means of low-cost IVF protocol in poorly responding women.

However, in our study, the agonist stop protocol gave better statistical significant results (p < 0.05) regarding the mean number of MII oocytes (4.47±1.91), the mean number of fertilized oocytes (4.35± 2.26), the mean number of class A embryos (3.176±1.380), the mean number of four celled class A embryos (2.11±1.576) when compared with the aromatase inhibitor group (MII oocytes 2.68±1.52, number of fertilized oocytes 2.68± 1.53, Class A embryos 1.947± 1.393 and 4celled class A embryos 0.8421± 0.958). Consequently, the clinical pregnancy rate was significantly better (P 0.018) in the agonist stop protocol (7 cases, 35%) when compared with that of the aromatase inhibitor

group (1 case, 5%). It is not yet known whether the use of aromatase inhibitor has its detrimental effect on the oocyte quality and hence on the fertilization and pregnancy rate or not. Further studies are needed to evaluate that context.

The precise mechanism of the ovarian effects of letrozole is as yet unexplored; however, some of the earlier observations and propositions on the effects of letrozole can be extrapolated to formulate a hypothesis. During the reproductive years, estrogens are chiefly produced in the ovary under the stimulation of aromatase. As the menopausal stage approaches, there occurs a decline in ovarian estrogen production; however, the extragonadal sites, notably adipocytes, continue to contribute peripheral production of estrogens that may act locally as paracrine or even intracrine factors. [286, 287]

Because of selective inhibition of aromatase, letrozole significantly inhibited the overall production of estrogens, which was reflected in the decreased levels of terminal E_2 in the aromatase inhibitor group. Consequent withdrawal of the negative feedback effects of estrogens may allow the pituitary to produce more endogenous FSH. Moreover, attenuated aromatization may secondarily lead to accumulation of follicular androgens, which may increase the follicular sensitivity through amplification of FSH receptor gene expression [288] or stimulate insulin-like growth factor-I, which may act in synergy with FSH. [189, 289] All these effects may have phenomenal importance in the letrozole-mediated promotion of follicular maturation. It may be significant in this context to emphasize that the absence of GnRH down-regulation in this proposed low-cost protocol may entail premature LH surge and luteinization However, possibly due to the small sample size; this problem was not encountered in the present study.

The cost of treatment, chiefly owing to the high cost of gonadotrophins, is frequently prohibitive. Suggestions have therefore been made that natural cycle IVF, which may produce high-quality embryo(s) without a high cost involvement, may be considered for so-called elderly poor responders; however, likelihood of pregnancy has been reported to be low. [240] The present study bears the promise that as an alternative to natural cycle IVF, letrozole may have future prospects as a cost-saving stimulation protocol for IVF in women with poor ovarian response. Nevertheless, larger randomized studies are needed to confirm these data. It must also be taken into consideration that the use of letrozole as a low-cost IVF protocol, though exciting, has to be evaluated further carefully, as letrozole and other aromatase inhibitors have not been extensively used in women of high reproductive age. This has been the main reason for employing selectively elderly women for this study.

Chapter VI

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Following the introduction of IVF (in vitro fertilization) and embryo transfer in 1978, this procedure has resulted in thousands of pregnancies and opened a vast new frontier of research and treatment for the infertile couple. Pregnancy rates with IVF improve as the number of high quality embryos available for transfer increases; therefore, using ovarian stimulating agents to produce multiple oocytes for IVF are advantageous. Clomiphene citrate, human menopausal gonadotrophin, and subsequent generations of these products are commonly used as stimulating agents. In conjunction with the stimulating agents, gonadotrophin-releasing hormone (GnRH) agonists, antagonists and other preparations serve as adjuvants for successful control of all events in the induction process.

There is a general consensus on the clinical fact that transfer of optimum number of high quality embryos selected from the cohort of available embryos is necessary to maintain a high pregnancy rate. For this reason those IVF cycles with a low response and a reduced number of oocytes and embryos will have few chances of producing a pregnancy.

Poor ovarian response to super-ovulation treatment is observed in a certain group of patients, the so-called 'low responders'. A 'poor response' in the context of in-vitro fertilization (IVF) can be defined as failure to produce an adequate number of mature follicles(less than four dominant follicles), and/or a peak estradiol concentration less than a defined minimum (less than 300 pg/ml). The cut-off points implied in this definition vary between different centers. Many authors decide to cancel the IVF cycle when their defined minimum concentrations are not reached despite the lack of evidence of improved outcome in subsequent cycles.

It is obvious that various management strategies have been proposed to improve ovarian response to gonadotrophins, but these have been met with limited success, and the ideal stimulation protocol for the low responder has yet to be formulated.

In our non randomized prospective study, we decided to compare the agonist stop protocol in cases of poor responders with the antagonist as well as the aromatase inhibitor protocols. The study group consisted of sixty poor responder women subdivided into three subgroups; each group consisted of twenty patients.

In our results, we found that the mean number of the ampoules was significantly reduced in the aromatase inhibitor group (49.45± 9.37) and the antagonist group (58.35±14.28) compared with the agonist stop group (66.45±9.90). There was also a significant relationship between the maximal serum E2 level on the day of hCG among the three groups (F test 1.2530, P <0.001) where it was high in the agonist stop group (1468.29±883.3 pg/ml) compared with the antagonist group (827.3±365.19 pg/ml) and the aromatase inhibitor group (551.1±285.69 pg/ml).

We got improved results in the agonist stop group in terms of oocyte quality, fertilization rate, embryo quality and reproductive outcome. Regarding the oocyte

quality, there was a statistical significant difference between the agonist stop group and the aromatase inhibitor group regarding the mean MII oocytes number (4.47±1.91 for the agonist stop group and 2.68±1.52 for the aromatase inhibitor group), such statistical significant difference was not present for the antagonist group (3.16±2.89). There was a also a statistical significant difference between the agonist stop and the other two groups as regard the number of the fertilized oocytes (F 3.887, P 0.027). The mean number of the fertilized oocytes was more in the agonist stop group (4.35±2.26) compared with the antagonist group (2.61±2.40) and the aromatase inhibitor group (2.68± 1.53). In addition, the embryos were of better quality in the agonist stop than in the antagonist or the aromatase inhibitor groups. There was a statistical significant difference between the agonist stop and the other two groups as regard the number of class A embryos (F test 4.1825, P 0.0207) and the number of the four celled class A embryos (F test 5.1519, P 0.009) which was higher in the agonist stop group than the other two groups. The mean number of the class A embryos was (3.176±1.380) in the agonist stop group, (1.833±1.757) in the antagonist group and (1.947± 1.393) in the aromatase inhibitor group. The mean number of the four celled class A embryos was (2.11±1.576) in the agonist stop group, (1.055±1.211) in the antagonist group and (0.8421± 0.958) in the aromatase inhibitor group. Finally, There was a statistical significant difference in the clinical pregnancy rate between the agonist stop group and the aromatase inhibitor group (X^2 5.625, P 0.018) where it was higher in the agonist stop group (7 cases, 35%) than in the aromatase inhibitor group (1 case, 5%).

Despite the plethora of predictive tests for low ovarian response, the poor responder is revealed definitively only during ovarian stimulation. Furthermore, there is no uniformity in the definition of the `poor' response, thereby rendering many of the clinical trials incomparable. On the other hand, an accurate prediction of low ovarian response would help the clinician to choose the most suitable alternative to classic IVF treatment.

A second limitation of the published studies is the selection of the control group, with most of the prospective studies using historical controls and comparing patients with their prior `failed' IVF cycles.

A third limitation is that, at present, there have been no reports of any large-scale, prospective, randomized, controlled trials of the different management strategies. At present, the results from only a few prospective–and even fewer prospective, randomized, double-blind, placebo-controlled trials have been reported, and all have been small in terms of patient numbers.

The use of very high doses of gonadotrophins to stimulate the ovaries is clearly unavoidable due to the lack of any initial ovarian responsiveness. Nevertheless, the results have been controversial with the prospective randomized trials showing either minimal or no benefit. Additionally, the few available relevant studies have suggested that the use of recombinant FSH might improve outcome.

A significant improvement was demonstrated with the use of the low-dose, mid-luteal onset, GnRH agonist regimens, that discontinue with the initiation of ovarian stimulation,

followed by high doses of gonadotrophins (GnRH agonist `stop' protocols), according to the prospective studies with historical controls. However, the well-designed prospective trials failed to confirm this, and showed no significant improvement. In addition, further studies are required to determine whether early cessation of GnRH-a leads to an increase in LH concentrations in the late induction phase, and subsequently to decrease implantation.

The few data available from the use of the GnRH antagonists do not show any benefits at present, though it is possibly too early to comment at this time. Certainly, further evaluation in this area is necessary.

To the best of our knowledge, the published data about the clinical experience with aromatase inhibitors provide positive results regarding decreasing the dose of gonadotrophins needed for controlled ovarian hyperstimulation with conflicting results regarding the oocyte quality, the fertilization and the clinical pregnancy rate. However, many questions about the use of aromatase inhibitors in COH still need to be answered regarding the optimal dose, the optimal timing and duration for aromatase inhibitors' use in COH as well as is there is a need for GnRH antagonists to counteract the risk of premature LH surge. Therefore larger, controlled, prospective randomized trials using aromatase inhibitors in poor responders are necessary to investigate this issue.

In conclusion, there is a clear need to standardize the definitions of low ovarian response. Well-designed, large-scale, randomized, controlled trials are needed to assess the efficacy of the different management strategies. The current results which are available are perhaps somewhat disappointing, but this should not be too surprising as most poor ovarian response women appear to have occult ovarian failure. Thus, the exhausted ovarian apparatus is unable to react to any stimulation, no matter how powerful this might be. The ideal stimulation for poor responders still remains a challenge, as the hypothesized diminished oocyte cohort and poor oocyte quality cannot be reversed within the limits of our present capabilities.

Chapter VII

RECOMMENDATIONS

RECOMMENDATIONS

It is very important to diagnose, which patients are most likely to present a poor response to conventional ovarian stimulation protocols. Low ovarian responders with good ovarian reserve will have some chances that by applying new protocols and combining new drugs, improve their response and have higher pregnancy rates. Data from the literature as well as our own data suggest that the “agonist stop” protocol can improve the reproductive outcome in such patients. However, these protocols should be tested against each other in well-designed, large-scale, randomized, controlled trials.

Chapter VIII

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LITERATURE CITED

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Chapter IX

PROTOCOL

بسم الله الرحمن الرحيم

Evaluating three alternative protocols for improving ovarian response of the poor responders undergoing assisted reproductive techniques.

تقييم ثلاثة طرق علاجية مختلفة لتحسين استجابة المبيض في حالات ضعف الاستجابة أثناء عمليات الاخصاب المساعد

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By

Yasser Ibrahim Orief

MBBch. Alex.
Ob/Gyn Alex.

Assistant lecturer
Of obstetric and gynaecology
Elshatby maternity university hospital

Faculty of medicine ,

Alexandria University.

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جامعة الاسكندرية

إيفاء جزئيا لشروط الحصول علي درجة

دكتور أمراض النساء و التوليد

من الطبيب

ياسر إ براهيم عريف

بكالوريوس الطب و الجراحة (الاسكندرية)
ماجستير أمراض النساء و التوليد (الاسكندرية)

مدرس مساعد بقسم أمراض النساء و التوليد

مستشفي الشاطبي الجامعي لأمراض النساء والتوليد

كلية الطب

جامعة الإسكندرية

2003

SUPERVISORS

المشرفون

Prof. Dr. ***Ibrahim Ahmed Medhat***
Professor of obstetrics
and gynaecology ,
Faculty of medicine ,
Alexandria university

أ. د/ *ابراهيم أحمد مدحت*
أستاذ أمراض النساء و التوليد
كلية الطب
جامعة الاسكندرية

Prof. Dr. ***Mahmoud A. Abo Ollo***
Professor of obstetrics
and gynaecology ,
Faculty of medicine ,
Alexandria university.

أ.د/ *محمودعلي أبو عللو*
أستاذ أمراض النساء و التوليد
كلية الطب
جامعة الاسكندرية

Ass. Prof. Dr. ***Hisham Ali Saleh***
Assistant Professor of obstetrics
and gynaecology ,
Faculty of medicine ,
Alexandria university.

د/ *هشام علي صالح*
أستاذ مساعد أمراض النساء و التوليد
كلية الطب
جامعة الاسكندرية

INTRODUCTION

Following the introduction of IVF and embryo transfer in 1978, this procedure has resulted in thousands of pregnancies and opened a vast new frontier of research and treatment for the infertile couple. Pregnancy rates with IVF improve as the number of high quality embryos available for transfer increases; therefore, using ovarian stimulating agents to produce multiple oocytes for IVF are advantageous. Clomiphene citrate, human menopausal gonadotrophin, and subsequent generations of these products are commonly used as stimulating agents. In conjunction with the stimulating agents, gonadotrophin-releasing hormone (GnRH) agonists, antagonists and other preparations serve as adjuvants for successful control of all events in the induction process.[1]

There is a general consensus on the clinical fact that transfer of optimum number of high quality embryos selected from the cohort of available embryos is necessary to maintain a high pregnancy rate. For this reason those IVF cycles with a low response and a reduced number of oocytes and embryos will have few chances of producing a pregnancy.[2]

Poor ovarian response to superovulation treatment is observed in a certain group of patients, the so-called 'low responders' [3] A 'poor response' in the context of in-vitro fertilization (IVF) can be defined as failure to produce an adequate number of mature follicles(less than

four dominant follicles), and/or a peak oestradiol concentration less than a defined minimum (less than 300 pg/ml). The cut-off points implied in this definition vary between different centers.[4] Many authors decide to cancel the IVF cycle when their defined minimum concentrations are not reached despite the lack of evidence of improved outcome in subsequent cycles.[4]

In general, low responders could either have low ovarian reserve, or normal ovarian reserve, the latter represent different etiologies. Disturbance in local intra-ovarian autocrine/ paracrine regulation with predominance of inhibitory growth factors like EGF (epidermal growth factor) over facilitatory ones like IGF-II (insulin like growth factor II) was suggested as a cause of low ovarian response[5]. Other authers postulated that there is an exaggerated suppression and perhaps direct effect of Gn-RH agonist on the ovary.[6,7] Impaired gonadotrophin delivery to ovarian tissues due to impaired pharmacokinetics,[8]or severe peri-ovarian adhesions[9] was also suggested. Inappropriate induction protocols and low doses of gonadotropins used for controlled ovarian hyperstimulation could also be responsible for low ovarian response.[11]

It is very important to diagnose, which patients are most likely to Present a poor response to conventional ovarian stimulation protocols. It is mandatory to know the patient's plasma basal levels of FSH and estradiol together with personal data such as the age and the previous history of the patient. Low ovarian responders with good ovarian reserve will have some chances that by applying new protocols and combining new drugs, improve their response and have higher pregnancy rates. Poor oocyte yield in such patients may result

in cycle cancellation, fewer embryos available for transfer and decreased pregnancy rates.[10]

Numerous strategies to improve ovarian stimulation in poor responders have been proposed. These include variations in the type, dose and timing of gonadotropins, gonadotropin-releasing hormone agonists and gonadotropin-releasing hormone antagonists.[11] Other workers give adjuvants to the induction protocols such as growth hormone supplementations , aromatase inhibitors , L- Arginine, low dose dexamethasone and others. [12]

The addition of GnRH antagonists to ovarian stimulation protocols might be a new hope for poor responder IVF patients. GnRH antagonists induce a rapid decrease in LH and FSH, preventing and interrupting LH surges. Due to their rapid onset of action, they do not require a desensitization period, and this allows their use in the late follicular phase. Antagonists could be particularly useful for patients in whom the ovaries are over sensitive to the direct suppressive effect of agonists. GnRH antagonists could thus replace GnRH agonists in ART cycles with poor response. Two protocols for assisted reproduction technology (ART) were designed: the single-dose protocol allies simplicity and efficacy, while the multiple-dose protocol is efficient and could reduce monitoring of the cycle. Compared with GnRH agonists, the use of the GnRH antagonists significantly reduces the dose of gonadotrophin and duration of treatment required. The GnRH antagonists are useful in both good and poor responders in preventing premature LH surge.[13,14]

Owing to the fact that GnRH-a may have a direct negative effect on folliculogenesis and oocytes through their receptors on the ovary, which is apparent especially in poor responders, discontinuation of GnRH-a during ovarian stimulation for IVF may have a beneficial effect on both E2 and oocyte production. Embryo cleavage rates and morphology may be significantly improved, this may be due to improved oocyte quality. The efficacy of gonadotropin treatment was enhanced when GnRH-a was discontinued.[15]

The use of the aromatase inhibitor letrozole with FSH for ovarian stimulation in poor responders undergoing ovarian superovulation and intrauterine insemination has been studied by some authors who demonstrate a potential benefit of aromatase inhibition for improving ovarian response to FSH in poor responders.[16] Administration of letrozole in the early menstrual cycle would release the pituitary-hypothalamic axis from the estrogenic negative feedback, similar to the effect of clomiphine citrate but without estrogenic receptors down-regulation, and the resulting increase in gonadotropin secretion could stimulate follicular development. Also, letrozole may act locally in the ovary to increase follicular sensitivity to FSH by increasing the local androgen production in the ovary because conversion of androgen substrate to estrogen is blocked. The accumulating androgens increase follicular sensitivity to FSH through androgenic amplification of FSH receptor gene expression.[16]

It is obvious that various management strategies have been proposed to improve ovarian response to gonadotrophins, but these have been met with limited success, and the ideal stimulation protocol for the low responder has yet to be formulated.[17]

In this study three new protocols for the management of ICSI patients with a low response to controlled ovarian hyperstimulation will be investigated.

AIM OF THE WORK

The present study is intended to compare three alternative protocols of controlled ovarian hyperstimulation (COH) in patients with poor ovarian responce to standard long protocol of GnRH-a administration in assisted reproductive technologies (ART). The three protocols are:

1. The antagonist protocol
2. The aromatase inhibitor protocol
3. The agonist stop protocol

MATERIAL

The study will include 60 infertile ladies recruited from the infertility clinic of El-Shatby Maternity University Hospital and a specialized IVF center. The patients will be accepting and consenting to the in vitro fertilization and intra-cytoplasmic sperm injection (IVF – ICSI) program.

Criteria for inclusion :

* The age of the patients is ranging from 20 to 40 years.

* The period of infertility is at least 2 year.

* All patients are indicated for ICSI

* All patients will have

- Day 3 serum FSH < 12 mIU/ml.[18]
- Day 3 serum E2 < 80 pg/ml.[19]

* All patients will have a poor ovarian response to standard long protocol of GnRH-a administration in previous in vitro fertilization (IVF) cycles, i.e.: failure to produce an adequate number of mature follicles (five dominant follicles or less).[4]

* No contra-indication to pregnancy.

Criteria for exclusion:

- Previous history of ovarian or pelvic surgery.
- Endometriosis

METHODS

All patients will be subjected to:

(1) Detailed history taking

(2) General examination including breast examination.

(3) Local examination; abdominal and pelvic examination.

(4) Routine investigations (complete blood picture, complete urine analysis, fasting blood sugar)

(5) Infertility investigations including:

* Husband semen analysis.
* Hysterosalpingography.
* Trans- vaginal ultrasound for
  - Detection of the antral follicles number and size in the early follicular phase
  - Size, direction of the uterus as well as the thickness and pattern of the endometrium
  - Follicular scanning
* Hormonal profile
  - Early follicular phase (day 3) serum FSH, E2

These patients will be randomly subdivided into three groups:

Group A :

Consists of 20 patients will be treated with human menopausal gonadotropins (hMG) and follicle stimulating hormone (FSH) in a dose of five to six ampoules per day starting from the third day of the cycle till the criteria for the administration of human chorionic gonadotrophin (HCG) is

reached (i.e.; when two or more graffian follicles are > 18-20 mm and the endometrial thickness is > 8 mm).

GnRh – antagonist (cetrorelix)will be also given subcutaneously using the multiple dose protocol with a dose of 0.25 mg per day starting when the follicle size reaches 14 mm till the criteria for the administration of human chorionic gonadotrophin (HCG) is reached.

Group B :

Consists of 20 patients will be treated with the aromatase inhibitor (letrozole) 2.5 mg/day from the third day of the menstrual cycle together with Human menopausal gonadotrophin (hMG) and follicle stimulating hormone (FSH) in a dose of five to six ampoules per day till the criteria for the administration of human chorionic gonadotrophin(HCG) is reached.

Group C :

Consists of 20 patients will be treated with the GnRH agonist (triptorelin) in a dose of 0.1 mg subcutaneously beginning in the mid luteal phase of the previous menstrual cycle till suppression of the pituitary gland occurs around the third day of the next cycle (i.e.; when the basal serum E2 < 50 pgm) then hMG&FSH are started in a dose of five to six ampoules per day till the criteria for the administration of human chorionic gonadotrophin (HCG) is reached.

In all patients, when the criteria for human chorionic gonadotropin (HCG) administration are reached, 10 000 IU of HCG will be given to trigger ovulation. retrieval will be scheduled 34 hours later using vaginal ultrasound guided needle aspiration.

The cycle will be cancelled if only one mature graffian follicle is obtained after controlled ovarian hyperstimulation is carried out.

Following oocyte retrieval, patients will receive luteal phase support in the form of micronized natural progesterone vaginally in a dose of 600 mg/day (i.e.; two vaginal ovules taken three times daily) to continue preparing the endometrium.

After oocyte retrieval, mature oocytes will be injected (ICSI). This will be followed by transfer of the embryos in the appropriate time.
The main outcome measures will include:

- Implantation rate (the number of sacs per number of embryo transferred)
- Clinical pregnancy rate (positive serum pregnancy test 14 days from the time of oocyte collection)

The secondary outcome measures will include:

- Maximal serum E(2) level
- Follicular development
- Dose and duration of gonadotropin therapy
- Cycle cancellation rate
- Number of oocytes retrieved
- Number of embryos transferred.

RESULTS

Statistical analysis of the data will be carried out by using statistical computer programs. Suitable statistical tests will be utilized for categorical and quantitative variables. [20]

DISCUSSION

The data obtained from the results will be discussed regarding the modern national and international literatures and journals.

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Chapter X

ARABIC SUMMARY

الملخص العربي

اسفر التقدم السريع في وسائل الاخصاب المعملي و محاولة فهم فسيولوجيا الخلية منذ عام ١٩٨٧ عندما تمت ولادة أول طفلة عن طريق تقنية أطفال الأنابيب و حتي الأن عن فتح باب الأمل علي مصراعيه أمام الكثير من المرضي الذين يعانون من عدم القدرة علي الانجاب للحصول علي الطفل الذي طالما انتظروه. و حيث أنه من المعروف أن معدلات الحمل عقب تقنية الاخصاب المعملي و أطفال الأنابيب تعتمد اعتمادا كبيرا علي عدد الاجنة عالية الجودة المتاحة حيث تزداد بزيادتها. أصبح استخدام المستحضرات الطبية المحفزة للتبويض للحصول علي عدد كبير من البويضات لاستخدامها في عمليات الحقن المجهري مهما للغاية ويوجد حاليا الكثير من هذه المستحضرات في السوق العلاجي.

يوجد حاليا اتفاق عام بين العلماء علي أن نقل عدد مثالي من الأجنة عالية الجودة من مجموعة الأجنة الناتجة من عملية الحقن المجهري ضروري لحدوث نسبة حمل عالية. لهذا السبب فانه في حالات ضعف الاستجابة المبيضية أثناء عمليات الاخصاب المساعد للمستحضرات الطبية المحفزة للتبويض و الذي ينتج عنه عدد قليل من البويضات و بالتالي عدد قليل من الأجنة المرتجعة فان فرص حدوث الحمل ستكون قليلة.

ان استجابة المبيض الضعيفة لتحفيز التبويض و التي يتم رصدها في حالات معينة يطلق عليها " حالات ضعيفة الاستجابة" يمكن تعريفها بالفشل في انتاج عدد جيد من الحويصلات الناضجة " أقل من أربع حويصلات سائدة" و أن تركيز هرمون الأستراديول في الدم أقل من٣٠٠ بيكوجرام بالملليلتر المكعب. و حتي الأن لا يوجد أتفاق عام بين المراكز البحثية المختلفة علي هذا التعريف حيث يختلف الحد الفاصل للتعريف من مركز لأخر.

و قد استحدث العلماء العديد من الطرق لمحاولة تحسين ضعف الاستجابة المبيضية فمنهم من يستخدم مستحضرات طبية جديدة للتحفيز و منهم من يلجأ الي زيادة جرعات الأدية المحفزة التقليديةو أخرون يقومون باستخدام أدوية مساعدة كهرمون النمو و الكورتيزون و غيرها. ومن الواضح أنه قد تم استحداث الكثير من الطرق لتحسين الاستجابة المبيضية ولكن لم يحقق معظمها النجاح المنشود و حتي الأن لا يوجد بروتوكول مثالي متفق عليه لاستخدامه في مثل هذه الحالات.

في هذه الدراسة تمت مناقشة ثلاثة طرق علاجية جديدة لتحسين استجابة المبيض في حالات ضعف الاستجابة أثناء عمليات الاخصاب المساعد. تمت الدراسة علي عدد ستين مريضة تعانين

من ضعف الاستجابة المبيضية أثناء عمليات سابقة للاخصاب المساعد و قد تم تقسيم المرضي الي ثلاث مجموعات كل منها يتكون من عشرين مريضة

- المجموعة اللأولى: بروتوكول مضاد الهرمون المطلق للجونادوتروفين.
- المجموعة الثانية: بروتوكول مثبط انزيم الأروماتيز.
- المجموعةالثالثة: بروتوكول ايقاف مماثل الهرمون المطلق للجونادوتروفين.

و قد أسفرت الدراسة عن وجود علاقة ذات دلالة احصائية من حيث انخفاض متوسط عدد أمبولات الجونادوتروفين المستخدمة في المجموعة اللأولى (٥٨.٣٥±١٤.٢٨) و المجموعة الثانية (٤٩.٤٥±٩.٣٧) بالمقارنة بالمجموعةالثالثة (٦٦.٤٥±٩.٩٠) و قد تبين أيضا وجود علاقة ذات دلالة احصائية من حيث انخفاض المستوي الأقصي لهرمون الأستروجين بالدم في المجموعة اللأولى (٨٢٧.٣±٣٦٥.١٩ بيكوجرام/مل) و المجموعة الثانية (٥٥١.١±٢٨٥.٦٩ بيكوجرام/مل) بالمقارنة بالمجموعةالثالثة (١٤٦٨.٢٩±٨٨٣.٣ بيكوجرام/مل).

كما أسفرت الدراسة عن وجود علاقات ذات دلالة احصائية فى نتائج المجموعةالثالثة (بروتوكول ايقاف مماثل الهرمون المطلق للجونادوتروفين) بالمقارنة بنتائج المجموعتين الأولى و الثانية .

- من ناحية جودة البويضات أسفرت الدراسة عن وجود علاقة ذات دلالة احصائية في متوسط عدد بويضات الميتافيز من المرحلة الثانية بين حالات المجموعةالثالثة (٤.٤٧±١.٩١) و المجموعة الثانية (٢.٦٨±١.٥٢) و لم تتحقق هذه العلاقة مع حالات المجموعة اللأولى.
- و قد تبين أيضا و جود علاقة ذات دلالة احصائية في متوسط عدد البويضات المخصبة بين بين حالات المجموعةالثالثة (٦٤.٣٥±٢.٢) بالمقارنة بنتائج المجموعتين الأولى و الثانية (٢.٦١±٢.٤٠ للمجموعة اللأولى - ٢.٦٨±١.٥٣ للمجموعة الثانية).
- بالاضافة الي ذلك أسفرت الدراسة عن وجود علاقة ذات دلالة احصائية فى نتائج المجموعةالثالثة(بروتوكول ايقاف مماثل الهرمون المطلق للجونادوتروفين) بالمقارنة بنتائج المجموعتين الأولى و الثانية من حيث جودة الاجنة حيث كان متوسط عدد الاجنة من المستوى الاول "أ" في المجموعةالثالثة (٣.١٧٦±١.٣٨٠) بالمقارنة بالمجموعة اللأولى (١.٨٣٣±١.٧٥٧) و المجموعة الثانية (١.٩٤٧±١.٣٩٣) و كان متوسط عدد الاجنة من المستوى الاول "أ" ذات الخلايا الاربعة في المجموعةالثالثة (٢.١١±١.٥٧٦) بالمقارنة بالمجموعة اللأولى (١.٠٥٥±١.٢١١) و المجموعة الثانية (٠.٨٤٢١±٠.٩٥٨).

- تبين وجود علاقة ذات دلالة احصائية من حيث متوسط عدد الحمل الاكلينيكي بين حالات المجموعةالثالثة (بروتوكول ايقاف مماثل الهرمون المطلق للجونادوتروفين) (٧ حالات بنسبة ٣٥%)و حالات المجموعة الثانية (بروتوكول مثبط انزيم الأروماتيز) (حالة واحدة بنسبة ٥%).

انـه لمـن الـضروري تـشخيص الحـالات الأكثـر عرضـة لـضعف الاسـتجابة لبروتوكـولات التحفيز التقليدية خصوصا عندما يكون لديها احتياطي مبيضي جيد حيث أن فرص الحمل قد تزداد لديها عند أستخدام بروتوكولات جديدة للتحفيز و بادخال أدوية جديدة لتحسين الأستجابة المبيضية. و قد أسفرت هذه الدراسة أن بروتوكول ايقاف مماثل الهرمون المطلق للجونادوتروفين من الممكن أن يحسن النتائج الانجابية لهذه المجموعة من المرضي. في النهاية نوصي بعمل دراسات أخري لمقارنة الطرق المختلفة لمحاولة تحسين ضعف الاستجابة المبيضية مع التوصية بزيادة عدد عينات الدراسة.

الملخص العربي

المشرفون

أ. د/ ابراهيم أحمد مدحت

أستاذ أمراض النساء و التوليد
كلية الطب- جامعة الاسكندرية

أ.د/ محمودعلي أبو علو

أستاذ أمراض النساء و التوليد
كلية الطب- جامعة الاسكندرية

د/ هشام علي صالح

أستاذ مساعد أمراض النساء و التوليد
كلية الطب- جامعة الاسكندرية

تقييم ثلاثة طرق علاجية مختلفة لتحسين استجابة المبيض في حالات ضعف الاستجابة أثناء عمليات الاخصاب المساعد

رسالة علمية

مقدمة الى كلية الطب- جامعة الاسكندرية

إيفاء جزئيا لشروط الحصول علي درجة

دكتوراه أمراض النساء و التوليد

مقدمة من

ياسر إ براهيم عريف

بكالوريوس الطب و الجراحة (الاسكندرية)

ماجستير أمراض النساء و التوليد (الاسكندرية)

كلية الطب

جامعة الإسكندرية

٢٠٠٦