

Follicular Response by using Letrozole alone or combination of Letrozole and Gonadotrophins in patients stimulated for IUI

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Abstract:

IUI with controlled ovarian hyperstimulation is a well known means to achieve pregnancy in unexplained and mild to moderate male factor infertility, which is also inexpensive, easy to perform as office procedure. The success rate is high.

Objective: *To assess the follicular response by Letrozole alone or by combination of Letrozole and Gonadotrophins in patients stimulated for IUI.*

Method: *Prospective observational type of study.*

Place: *CARE BIRDEM*

Time: *10th May 2010 – 31st December 2010*

Population: *All the OPD patients of CARE who were selected for IUI during this period. Patients were divided into two age groups (<30 yrs and >30 yrs)*

Procedure: *Ovulation induction was done by letrozole in one group, and combined letrozole and gonadotrophins in another group. Monitoring was done by TVS for 3 cycles.*

Result: *Mean follicular number was 1.28±0.94 in letrozole and 3.37±0.87 in combined letrozole and gonadotrophins. No. of follicles >18mm was 12 pts (3.54±1.63) in letrozole, and 50 pts (3.44±1.93) in combined letrozole and gonadotrophins. Pregnancy/cycle was 12/77 (5.19%) in letrozole and 28/65 (14.3%) in combined letrozole and gonadotrophins.*

Conclusion: *Number of mature follicle and pregnancy rate is higher in combined letrozole and gonadotrophins group than letrozole alone. There is no significant difference regarding follicular response and pregnancy rate in two age group*

Introduction:

Throughout history, human societies were preoccupied by the process of procreation which is the major aspects of maintaining survival and perpetuation of the human race. Infertility is a well known cause of disharmony and disgrace among couples (mark of displeasure, multiple marriages among Muslims, grounds for divorce, and in the extremes, suicide), since time immemorial. The first written document regarding infertility was the Kahoun Papyrus (2200-1950 B.C.).¹
² Bangladesh is a developing country with an exploding population of 158 million, a total fertility rate of 2.74 per

thousand live births and a growth rate of 1.292%.³ In spite of that, there are a large number of couples who remain childless for years.

Prevalence of infertility throughout the world range from 10%-15% of couple.^{4,5} In a study of rural population of Bangladesh 3.2% have primary infertility and 3% have secondary infertility. Majority of infertile women (40%) believed this to be their fate and 33% accused themselves for infertility.⁶

Prior to the 1960s, infertility was almost exclusively a diagnostic statement Infertility treatment started 50

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years ago with the use of exogenous gonadotrophins.⁷ The introduction of *in vitro fertilization & embryo transfer (IVF-ET)* in 1980s has made it possible for the couple to take advantage of this technology.⁷

Controlled ovarian hyper stimulation combined with IUI of capacitated sperm (COH-IUI) has recently been used to treat a subset of couples infertile in the absence of mechanical compromise of the pelvic viscera, in whom no other efficacious treatment options exist.^{8, 9, 10} The major difference of COH-IUI with IVF is that the normal fallopian tubes can be anticipated to function appropriately and no surgical procedure is required to collect oocytes.^{8, 9, 10} The benefit of IUI in male factor infertility has been accepted because IUI appears to result in higher pregnancy rates than natural intercourse or Intra Cervical Insemination (OR 2.20, 95% CI 1.43-3.39).¹¹ In a review, Hammburg and Insler¹² concluded that, taking into account efficacy, complication rates and cost of infertility treatment women with hypogonadotropic hypogonadism or polycystic ovary syndrome (PCOS) should be offered acceptable methods of ovulation induction and that couples with 'unexplained' or 'multifactorial subfertility' should be exposed to controlled ovarian hyperstimulation with IUI (COH IUI) and only after the failure of these therapies should be offered *in vitro fertilization (IVF)*.¹²

For COH in IUI cycles, ovulation induction protocols are Clomiphene citrate, Aromatase inhibitor (Letrozole) alone or in combination with Gonadotrophins (hMG or rFSH), human chorionic Gonadotrophins (hCG); and the use of GnRH agonist or antagonist combined with Gonadotrophins. hCG is usually used at the end of the stimulation phase to achieve final maturation of the oocytes.¹³ The addition of IUI to Letrozole increase fecundity in couples with unexplained infertility or surgically treated endometriosis.^{14, 15} Ovarian stimulation with letrozole is associated with acceptable pregnancy rates compared with gonadotrophin (cumulative pregnancy rate per couple: 24% vs. 36%),¹⁶ with significant less cost, risk and patient inconvenience.

The combination of IUI with gonadotrophin and letrozole stimulation provides even better results than the combination of IUI with clomiphene.¹⁷

Materials and Methods:

This study was conducted at Centre for Assisted Reproduction (CARE), BIRDEM, Dhaka, during the

period of 10th May to 31st December, 2010. All the infertility patients who were selected for IUI from CARE OPD BIRDEM were included in this study. Informed consent were taken from the couples and interviewed by a structured questionnaire

Inclusion criteria: The female patients with age <40 yrs, history of infertility for at least 2 yrs and with either of the following problems (unexplained infertility, polycystic ovarian syndrome, mild endometriosis) were included. Males with mild to moderate male factor sub fertility with total count (>10 million/ml), motility grade A (> 10%), morphology (>30% N) and erectile dysfunction or hypospadias were included.

Exclusion criteria: Female with bilateral tubal block, severe endometriosis and no ovarian reserve, and male with sperm count <10 million / ml were excluded.

Variables of the study:

Input variable: Type and dose of ovulation inducing agent

Output variable: Follicular size, endometrial thickness, pregnancy outcome

Study Procedure: The patients were divided in to two groups. Group A: Female with young age < 30 years, and Group B: Female with age >30 years.

Superovulation Protocol:

Both groups of patients were stimulated by Letrozole 7.5 mg daily, started on D2 of the cycle and continued for 5 days. Patients were followed by this treatment for 3 cycles of IUI. Follicular response was assessed by TVS on D12 – 13, when at least two follicles attained >18 mm size or single follicle >21 mm size then it was considered satisfactory. This treatment was followed for 3 cycles. The patients who did not respond satisfactorily by 3 cycles of Letrozole were selected for combination of Letrozole and Gonadotrophins. These patients were stimulated by Inj. Gonadotrophins (rFSH 50 iu/ inj. hMG 75 iu) intra muscularly daily starting from D3 for 5-7 days + Letrozole 7.5 mg per day on D₂ – D₆. Patients were followed up by TVS on D12/D13 (follicular size, number and endometrial thickness). In both regims when follicles attain satisfactory size, Inj. HCG (pregnyl/professy) 5000 IUI was given intra muscularly. A single IUI insemination was performed 36 hours after the Inj. of hCG .

Main outcome measure: Number of the follicle 2-3, Size of the follicle >18 mm and Endometrial thickness > 8 mm was considered as satisfactory.

Definition of Pregnancy:

Patients were asked to attend the OPD of CARE BIRDEM 15 days after the missed period. Positive pregnancy test result was defined as pregnancy. Pregnant patients were advised to come for regular antenatal checkup.

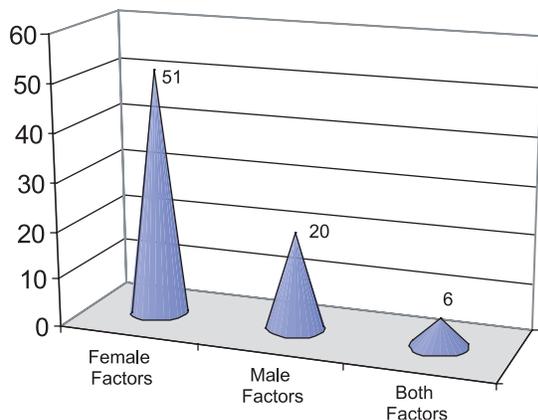
Data processing & analysis: Result of the study was analyzed by standard statistical methods by using SPSS (Statistical Package for Social Science) software, version 16 (SPSS, Inc. Chicago, IL, USA) and Excel windows programme. The data was expressed as mean±SD and median (range) as appropriate. Student’s unpaired t-test was used for calculating the difference between the groups. ÷2 (chi square) test was performed to determine the clustering of different variable of interest. P value of less than 0.05 was considered statistically significant.

Observation and result:

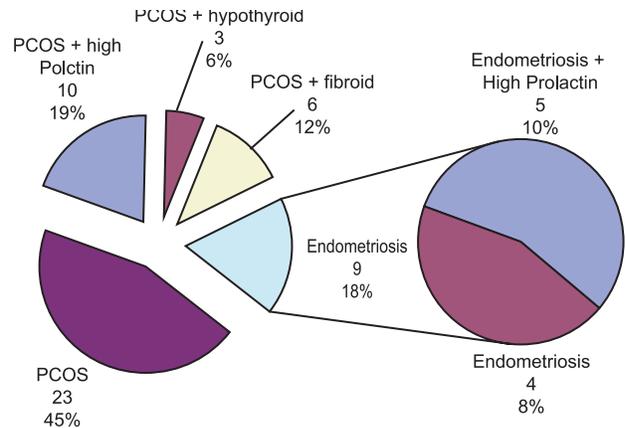
Patients Characteristics

Variables	Mean	SD	Percentage (%)
Age	31.08	4.86	
Duration of infertility	9.45	4.59	
Parity			
Nulliparous (Primary Subfertility)			77.92% (60 pts.)
Multiparous(Secondary Subfertility)			22.8% (17 pts.)
Clinical Presentation			
Oligo/anovulation			74.02% (57 pts.)
Hyperandrogenism			14.28 % (11 pts.)
PCOS (TVS)			55.05% (42 pts.)
BMI (weight in kg/m2)	22.7	3.24	

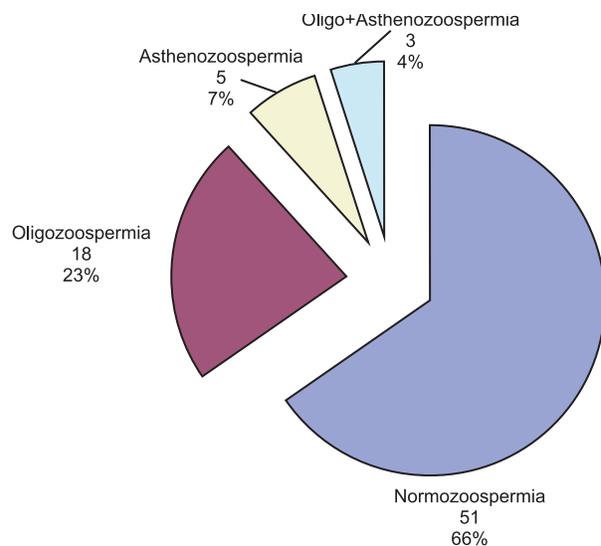
PCOS was detected by TVS and FSH: LH ratio altered in 48.05% of patients. Mean BMI was 22.7±3.24.



Factors responsible for infertility (n=7)



Female factors for infertility



Semen analysis of husband on first visit

Among 77 pts, 35 (45%) had normal FSH: LH ratio and 42 (55%) had an altered ratio. Hyperprolactinaemia was present in 15 (20%) patients. 3 (4%) pts were hypothyroid. 77 patients were evaluated for tubal patency. Laparoscopy was used as a tool in 66 (86%) patients and HSG in 11 (14%) patients. Both tubes were patent in 59 (79%) patients, and one tube was blocked in 18 (23%) patients. During laparoscopy, ovarian drilling was done in PCOS patients.

Table 2: Effect of letrozole and combined letrozole and gonadotrophins on follicular size

Variables	Group A Letrozole (n=77) (cycle=231)	Group B Combined Letrozole and Gonadotrophin (n=65) (cycle=195)
No follicles	38 pts (49.36%)	Nil
pts with follicle size 10-14mm	14 pts (18.9 %)	Nil
pts with follicle size 14-18mm	13 pts (16.88 %)	15 pts (23.07%)
pts with follicle size >18mm	12 pts (15.58 %)	50 pts (76.93%)

Table-III*Outcome in letrozole and combined letrozole and gonadotrophins*

Variables	Group A Letrozole (n=77) (cycle=231)	Group B Combined Letrozole and Gonadotrophin (n=65) (cycle=195)	t	Ç2	P
Total no. of follicles	1.28±0.94	3.37±0.87	7.54		0.0001
No follicles	38 pts (49.36%)	nil			
No. of follicles 10-14mm	2.08±1.68	nil			
No. of follicles 14-18mm	2.18±1.63	3.14±1.93	2.48		0.021
No. of follicles >18mm	(12 pts)3.54±1.63	(50 pts)3.44±1.93	0.374		0.71
Pre-treatment endometrial thickness	5.93±1.06	5.93±1.07	5.37		0.0001
Post-treatment endometrial thickness	7.89±0.93	8.61±1.4	5.83		0.0001
Duration of stimulation (days)	12.2±1.3	10.3±2.8			
Ovulation/cycle	43/231 (18.6%)	174/195 (89.2%)		208.17	0.0001

Mean of total no. of follicles (letrozole vs. combined letrozole and gonadotrophins group 1.28±0.94 vs. 3.37±0.87, p value 0.0001). Endometrial thickness, (letrozole vs. combined letrozole and gonadotrophins 7.14±1.70 mm vs. 8.61±1.4 mm, p value 0.0001)

Comparison of follicular response between two age groups (<30 & >30)

Table-IV*Induction used – Letrozole (7.5 mg/ d for 5 days in a cycle)*

Variables	<30 yrs	>30 yrs	t	Ç2	P
No. of pt	37	40			
No. of induction	111	120			
No. of follicles >18mm in a cycle	3.5	3.6	0.126		0.9
Follicular size >18mm	8 pts (22%)	5 pts (13%)	0.335		0.74
Endometrial thickness(mm)	8.52	8.7	0.556		0.58
Ovulatory cycles (%)	28/111 (25%)	17/120 (14.2%)		3.82	0.051
Pregnancy rate/cycle (%)	9/111 (08%)	3/120 (03%)		2.63	0.11
Pregnancy patient rate	9/37 (24%)	3/40 (07%)		2.96	0.09

In >30 yrs group, only 5 (13%) patients had mature follicles, whereas in <30 yrs 8 (22%) patients had mature follicles. P value is 0.74, which is not significant.

Table-V
Induction used – Inj. FSH (50 IU or hMG, 75 IU for 5-7 days in a cycle)

Variables	<30 yrs	>30 yrs	t test	Ç2	P
No. of pt	30	35			
No. of induction	90	105			
No. of follicles >18mm in a cycle	3.6±1.95	3.3±1.94	0.475		0.64
Follicular size >18mm	20 pts (66%)	30 pts (88%)	1.31		0.2
Endometrial thickness(mm)	8.52	8.7	0.693		0.49
Ovulatory cycles (%)	20/90 (23%)	31/105 (30%)		0.99	0.32
Pregnancy rate/cycle (%)	16/90 (17%)	12/105 (12%)		1.11	0.29
Pregnancy patient rate	16/30 (54%)	12/35 (34%)		1.68	0.19

In >30 yrs age group, 30 (88%) patients had mature follicles, whereas in <30 yrs age group, 20 (66%) patients had mature follicles. P is 0.2.

Table-VI
Comparison of pregnancy outcome between letrozole and combined groups

Variables	Group A Letrozole (n=77) cycle=231)	Group B Comb Letr. & Gonadotrophins (n=65) (cycle=195)	t	§ ²	P value
Duration of stimulation (days)	12.2±1.3	10.3±2.8			
Ovulation cycle	43/231 (18.6%)	174/195 (89.2%)		208.17	0.0001
Pregnancy cycle	12/231 (5.19%)	28/195 (14.3%)		9.39	0.0022
Pregnancy patient rate	12/77 (16%)	28/65 (43%)		11.84	0.0006

Discussion:

COH and IUI are common treatments for couples with unexplained infertility and those with infertility associated with specific diagnoses in which no pregnancy has occurred despite standard directed treatments. Total 77 patients were selected for IUI in our centre CARE BIRDEM. All patients had some sort of past infertility treatment, at various levels from various parts of the country. In the present study, mean age of female patient was 31±4.8 yrs. The mean duration of infertility was 9.5±4.6 yrs. By letrozole there was no follicular development in 38 (49.36%) patients. Mature follicles developed in only 12 (15.58%) patients. On the contrary by combined gonadotrophins and letrozole mature follicles appeared in 50 (76.93%) patients. Average duration of cycle (letrozole vs. combined letrozole and gonadotrophins) was 12.2±1.3 vs. 10.3±2.8 days. Ovulation per cycle (letrozole vs. combined letrozole and gonadotrophins) was 18.6% vs. 89.2%. The p value is 0.0001, which is significant. Endometrial thickness >7 mm (letrozole vs. combined letrozole and gonadotrophins) was 37 (48%) pts vs. 58 (90%) pts. P value is 0.00013, which is significant.

12/231 cycles showed pregnancy in letrozole group, whereas 28/195 cycles showed pregnancy in combined group. Pregnancy per cycle is 5.1% in letrozole group and 14.3% in combined group. Pregnancy patient rate is {12/77 (16%) vs. 28/65 (43%)}. P value is 0.0006 which is significant.

In this study, letrozole was associated with fewer developing and mature follicle, which is not comparable with the study by Mitwally & Casper¹⁴, Al-Omari et al.¹⁸ who showed ovulatory rate by letrozole was 75% and 87.5%, respectively. Another study by Mitwally¹⁵ showed that treatment with letrozole+ FSH required less amount of FSH (616 IU vs. 1590 IU) than FSH alone, and the number of follicle >18mm was higher, in combined letrozole and FSH group (3.3 vs. 1.9, respectively) which is consistent with our study (3.37±0.87 vs. 1.28±0.94).

Another study comparing 3 treatment protocols letrozole+ FSH, CC+FSH and FSH only in patients with unexplained infertility undergoing COH and IUI by Mitwally¹⁴ showed that the total FSH dose was lowest in letrozole+ FSH group, but the endometrial thickness was highest in the letrozole+ FSH group

(9mm). The clinical pregnancy rate was highest in letrozole+ FSH group (22.2%) which is comparable with our study, where pregnancy rate is 14.3% in combined letrozole and gonadotrophins group. The results of this study suggest that the addition of letrozole to FSH can reduce the dose of FSH and medication expense without detrimental effects on endometrial thickness and pregnancy.

In another study by Rumste¹⁹ regarding COS and IUI in unexplained infertility showed that 32% of the couples conceived in four treatment cycles of COS-IUI which is within the range of 20-33% reported by others (Guzick et al.²⁰). In other literatures by Crosignani²¹, Guzick et al.²⁰, Dickey et al.²² pregnancy rate per cycle has been reported to vary from 9-20%. It has been shown that increasing maternal age, longer duration of non-conception and a poor semen quality have a negative impact on the pregnancy rate in couples receiving COS-IUI (Dickey et al. 2002²²). Multifollicular growth contains the risk of multiple pregnancies. Another study by Sarah Healey et al²³ about the effect of letrozole and gonadotrophins in IUI patients showed that they required less gonadotrophin administration (median difference, 300 IU at 95% CI, 225-375 IU) and had a thinner endometrium, (m df 1 mm), developed more follicles >14 mm (m df, 1 follicle 95% CI, 1-2 follicle). But the pregnancy rate did not differ between patients using gonadotrophins alone and those using gonadotrophins+ letrozole (20.9% vs. 21.6%). Between two age groups, in >30 yrs group, only 5 (13%) patients had mature follicles, whereas in <30 yrs 8 (22%) patients had mature follicles. P value is 0.74, which is not significant. Ovulatory cycle was 28/111 cycles, in <30 yrs age group, and 17/120 cycles, in >30 yrs age group. P is 0.051 which is not significant. Pregnancy rate/cycle was 9/111 cycles in <30 yrs age group, and 3/120 cycles in >30 yrs age group. P value is 0.11, which is not significant. Thus there is no statistical significance in follicular response and pregnancy rate by giving letrozole alone in these two groups.

65 patients were poor responders to letrozole were treated with combined letrozole and gonadotrophins. In >30 yrs age group, 31 (86%) out of 35 patients had mature follicles, whereas in <30 yrs age group, 20 (66%) out of 30 patients had mature follicles. P value is 0.2, which is not significant. Therefore there is no significant difference in follicular response between

two age groups by gonadotrophins. But >30 yrs age group required more doses of gonadotrophin (avg of 5-7 doses) than <30 yrs age group.

There is no known national or international study regarding the follicular response of different age groups. There is no national study regarding the controlled ovarian stimulation and IUI, with which to compare.

Conclusion:

Infertility definitely has some tragic dimensions in the family life of many couples. The cause of infertility varies from country to country and in different social groups. Endometriosis, PCOS and male factor are common cause in developed countries, whereas STDs are the main cause in African countries. Incidence of tubal occlusion in our country can be prevented by safe delivery, safe abortion and post abortal care and increasing the awareness about infertility. For PCOS resistant to clomiphene or letrozole, mild endometriosis, unexplained infertility and also mild to moderate male factor infertility, the combination of superovulation and intrauterine insemination is an effective measure for achieving pregnancy. Moreover the effects of superovulation and IUI appear to be independent and additive. IUI and COH is relatively simple, non-invasive, inexpensive and effective measure. It can be provided more easily to more infertile couples in office practices and general hospitals than can the more specialized techniques such as IVF. However, careful selection of patients is required for successful IUI. Studies by Goverde et al.²⁴ on cost effectiveness of IUI and COH showed that it was as effective as IVF in achieving pregnancy (31% vs. 33%) and was more cost effective than IVF. So these two groups of patients should be counselled that IUI and COH is their preferable method, and there is least chance of complications like hyperstimulation syndrome, multiple pregnancy. Many infertile couples of today may have new hope for future days that science will continue to unravel the mysteries of procreation and with the discovery of each mystery more infertile couples will enjoy the fulfilment of bearing a child.

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