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Female Fertility Clock Closes to Tick around the Age of 43

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Abstract

Objective: To investigate if attempting *In Vitro* Fertilization (IVF) in women over 40 is of worth. **Materials & Methods:** Two hundred and thirty nine women over 40 years of age undergoing IVF were enrolled. Long protocol was used for down-regulation followed by stimulation and IVF-ET. The primary outcome measure was live-birth rate. Secondary outcomes included rates of miscarriage, cycle cancellation, obstetrical complications, and maternal and fetal adverse events. **Results:** Clinical pregnancy rates per cycle were 42.85 %, 39.34 %, 34.09 %, 27.27 % and 16.66 % for 40, 41, 42, 43 and 44 years old, respectively. Absolute difference in live birth rate in age 40 - 44 *vs* control, -15 percentage points; 95 % Confidence Interval (CI) -45 to 15.1, -18.77 percentage points; 95 % CI,(-56.31 to 18.79), -23.77 percentage points; 95 % CI,(-71.31 to 23.81), -25.82 percentage points; 95 % CI, (-77.48 to 25.84) and, - 27.72 percentage points; 95 % CI, (-83.16 to 27.76), respectively. Intra-uterine growth restriction and preterm delivery occurred significantly more frequently in the age group of 42 and above. Only one IVF cycle in patients aged 44 resulted in delivery. **Conclusion:** It appears that IVF treatment should be limited to patients not older than 43 years, with adequate ovarian response.

Keywords: Age, Live Birth Rate, Ovarian Function

1. Introduction

During the past three decades, the modern world has witnessed an increase in the age at first birth and the number of women delaying childbearing[1]. Commensurate with this has been a 150 % increase in the number of women giving birth between ages 35 and 39 years and a steady increase in those aged between 40-44 years[2]. This late-motherhood trend has been attributed to late marriages, and educational, professional, financial or personal restraints forcing a reproductive difficulty for the women.

Decline in female fecundity as a function of age is very well demonstrated[3]. Menken *et al*. provided compelling evidence for this trend in their report stating effects of age at marriage on fertility in which as high as 64 % women over the age of 40 failed to conceive[4]. Psychosocial bonding and crave for baby from own oocyte often drives to choose for autologous oocyte despite knowing the fact of age-related drop in fertility. With the rising number of women who choose to delay pregnancy until an advanced age, practice of Assisted Reproductive Technologies (ART) has increased accordingly[5]. The growing popularity and success of ART has given the women the impression that female fertility may be manipulated at any stage of life[6], an erroneous assumption. While ART may overcome this age-related decline to some extent, there appears to be an upper limit beyond which no pregnancies will occur using a woman's own oocytes. The biological basis of decreased fertility with age appears to

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involve several factors: attrition and over-utilization of follicles leading to menopause with the quality of existing oocytes and average intercourse frequency declining with age. Other factors include ageing of the reproductive tract, particularly the uterus, chromosomal abnormalities and decrease of endometrial receptivity[7,8].

Medical literature on ART outcomes in women over the age 40 is composed largely of reports involving mainly multiparous patients[9]. Since multiparous patients have many unique problems that often are unrelated to problems of first-time mothers, results from these studies may not be of help to physicians counseling first-time patients about pregnancy outcomes[10].

Furthermore, pregnancy in older women is associated with many confounding factors e.g., parity, pre-existing diabetes mellitus and/or hypertension, which should be taken into account if the risks associated with advanced maternal age are to be quantified[11]. In general, the current fertility treatment of normal older woman offers little advantage over expectant management in terms of cumulative pregnancy rates except when oocyte donation is utilized. The aim of this study was to assess the extent of ART success in women 40 years and above regarding pregnancy, and live birth rate using autologous oocyte, and to investigate the rate of adverse outcomes for the mother and baby.

2. Materials and Methods

This retrospective study was conducted at Institute of Reproductive Medicine, Kolkata, a referral centre for the treatment and management of infertility and O&G complications, from March 2008 to December 2013. Women at the age of 40 years and above were eligible in the study group if were attempting to conceive for the first time. Women between the ages of 30 and 39 years formed the control.

Patients were asked to report any symptoms of Ovarian Hyper-Stimulation Syndrome (OHSS) like abdominal distension, nausea, vomiting, and respiratory distress following the day of stimulation. Patients in the study group were analyzed on the basis of the incidence of mild and moderate OHSS. The investigation was performed with approval from the Research Ethics Board of the Institute (Ref. No. IRM/HEC/BNC-34/5-11-2007) and written informed consent was obtained from all study participants.

The subjects for the present investigation were selected from a total of 639 women desiring to conceive for the first time. Donor or surrogate cycles, ZIFT or GIFT and frozen embryo transfer were excluded from the study. Among the rest 502 subjects, 46 women had irregular menstruation, and a high follicle stimulating hormone level on day 3 who were put on an oral estrogen/progesterone therapy for 3 cycles. 13 women in control and 14 in study group after medication had normal FSH and were included in the study. Eventually, 483 women formed the study population. After excluding patients from the control arm on the basis of communication problem, declined consent or discontinuation of the treatment, 207 subjects and 239 women formed the control and patient populations, respectively. None of the women had any major medical problem. Sub-stratification of the patient arm based on age, comprised 32.21 % women of 40 years, 25.52 % of 41 years, 18.41 % of 42 years, 13.81 % of 43 years and 10.04 % of 44 years (Figure 1).

Women in control group were treated with Gonadotropin-Releasing Hormone (GnRH) agonist (Leupride acetate, Sun Pharmaceutical Ind Ltd., Mumbai; 0.5 mg daily *s.c.*) starting 1 week before the expected menses (~day 21). After down-regulation was achieved, the agonist dosage was reduced to 0.2 mg daily and ovarian stimulation was commenced from day 3 with 150–300 IU of recombinant-FSH (rFSH) (inj. Gonal-F, Merck Serono Specialities Pvt. Ltd., Italy). Serial ultrasound monitoring was scheduled from 7th day of gonadotropin stimulation, followed by subsequent ultrasound monitoring according to the patient's response. The study group underwent controlled ovarian stimulation in ART cycles using long and antagonist protocols in the form of GnRH agonist or antagonist (Ganirelix, Organon, Netherlands). Stimulation was followed by human menopausal gonadotropin (hMG) (75 or 150 IU) (Menopur, Ferring Pharmaceuticals, Kiel, Germany) as required or rFSH (75 or 150 or 225 IU) (Newmon R, LG Life Sciences, Seoul, Korea) or a combination of both depending on ovarian response. The dose of gonadotropin received was adjusted according to the response. GnRH agonist was continued (except in 10 patients where stop protocol was used) up to and including the day of human chorionic gonadotropin (hCG) administration. Ovulation was triggered with a single bolus dose of 10,000 IU of urinary hCG (Pregnyl, The Netherlands) followed by transvaginal ultrasound-guided oocyte retrieval 35 hours later. All follicles 12 mm or larger were aspirated. Conventional IVF or Intra-

Cytoplasmic Sperm Injection (ICSI) was performed in the study group (n = 239) or control (n = 207) as indicated. Culture media used was vitrolife (Vitrolife, Goteborg, AB, Sweden). Embryos were scored according to morphological criteria (cell number, regularity of blastomeres, and fragmentation). Equal sized blastomeres and 3 embryos of the highest quality were transferred in all patients on day 2.

Serum FSH, LH and E_2 levels were measured using fully automated electro-chemiluminscence technology using an Immulite platform (Ortho Clinical Diagnostics, Mumbai, India). Intra- and inter-assay coefficients of variation were less than 10 %. The β-hCG >25 IU/L or gestational sac on trans-vaginal sonography 2 weeks after embryo transfer was considered positive for pregnancy. Cycle cancellation was identified when no embryo was transferred in the absence of sufficient oocyte retrieved, or embryo or unsuitable endometrium.

The primary outcome measure was the rate of live births. Secondary outcomes included cycle cancellation rate, rates of miscarriage, intrauterine fetal death and obstetrical complications. Such complications included pregnancy-induced hypertension, gestational diabetes mellitus, intra-uterine growth restriction, and preterm delivery. The rates of maternal thrombocytopenia (defined as a platelet count of <150,000 per cubic millimeter), bleeding episodes (i.e., the amount of vaginal blood loss at delivery), and skin reactions were assessed by telephone at 3-month intervals by the research team and verified on the basis of obstetrical medical reports. In cases in which a congenital or neonatal abnormality was suspected, a neonatologist made the final diagnosis.

The primary outcome was assessed in all women. The incidences of preterm delivery, Gestational Diabetes Mellitus (GDM), Intra-Uterine Growth Restriction (IUGR) and congenital or neonatal abnormalities were calculated for women who had an ongoing pregnancy beyond 12 weeks of gestation. Adverse events were evaluated for all women.

Differences in dichotomous outcomes among the two groups were analyzed with the use of chi-square test or Fisher's exact test when the expected cell frequencies fell below five. Differences in live-birth rates were expressed as absolute differences and relative risks, with associated 95 % confidence intervals, with the control group as the reference. One-way analysis-of-variance was used to compare continuous outcome measures. *P* value less than 0.05 was considered to indicate statistical significance. The Statistical Package for Social Sciences version 17.0 (SPSS Inc, Chicago, Illinois, USA) was used for the statistical analysis. Data are presented as mean ± SD.

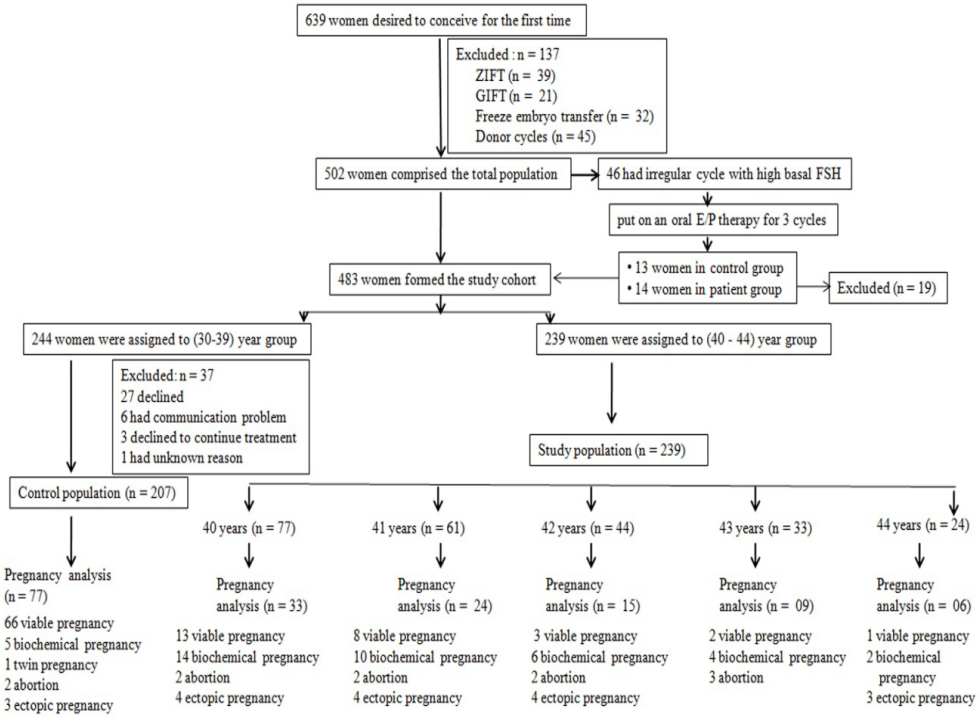

Figure 1. Enrollment and outcomes.

3. Results

A total of four hundred and forty six women were enrolled, with 239 (53.58 %) in the study cohort and 207 (46.41 %) patients in the control population, respectively. Women who were excluded from the study were advised oocyte donation programme. Table 1 summarizes the baseline characteristics of the study population. Mean age of enrollment into patient cohort was 41.47 ± 5.41. The older patients had a significantly longer period of infertility, more previous

IVF cycles, and a higher mean basal FSH and lower Antral Follicle Count (AFC) and AMH (p <0.001) than the younger population. Difference of body mass index between the two groups is near to significance (p < 0.054) with a mean value of 22.87 ± 3.45 kg/m^2 in the younger group and 23.61 ± 4.41 kg/m^2 in the older one.

Out of 239 patients studied, 36.4 % became pregnant and ~31 % of those who became pregnant gave a live birth. Table 2 describes the characteristics of the treatment cycle among patients ≥ 40 years old according to women's age. No differences were found for any of the parameters between the 41, 42, 43 age groups. However, the amount of gonadotropins required and endometrial thickness differed significantly (p < 0.001) in the age group above 40 with increasing gradually from the age 41 till 44. Proportions of women who gave birth to a live infant were 16.88 %, 13.11 %, 6.81 %, 6.06 % and 4.16 % in different subgroups, respectively compared to 31.88 % in control population. No differences in live birth rate were found between the two sub-divisions of the control population. Therefore, the data concerning these patients are grouped together. Absolute difference in live birth rate: age 40 *vs.* control, -15 percentage points; 95 % confidence interval [CI] -45 to 15.1; age 41 *vs.* control, -18.77 percentage points; 95 % CI, (-56.31 to 18.79); age 42 *vs.* control, -23.77 percentage points; 95 % CI, (-71.31 to 23.81); age 43 *vs.* control, -25.82 percentage points; 95 % CI, (-77.48 to 25.84); age 44 *vs.* control,- 27.72 percentage points; 95 % CI, (-83.16 to 27.76), respectively (Table 2).

Table 1. Baseline characteristics of the patients*

Baseline characteristic	Study population (n = 239)					Control
	40	41	42	43	44	30 - 39
Age (yrs)	---	---	----	---	----	34.67 ± 3.28
No. of patients (%)	77 (32.21)	61 (25.52)	44 (18.41)	37.22 (13.38)	25 (10.46)	207
Body Mass Index†	23.61 ± 4.41	24.27 ± 2.97	24.89 ± 4.17‡	25.06 ± 4.66‡	25.27 ± 4.49‡	22.87 ± 3.45
Duration Of Marriage (yrs)‡	10.12 ± 2.62	12.31 ± 1.8	11.55 ± 3.06	13.34 ± 1.06	15.03 ± 2.15	3.14 ± 2.62
FSH (IU/L)	7.2 ± 1.55	7.16 ± 2.37	7.64 ± 1.47	7.83 ± 0.57‡	8.06 ± 0.37‡	6.79 ± 2.13
LH (IU/L)‡	7.1 ± 1.35	7.0 ± 2.4	6.51 ± 1.21	6.2 ± 0.81	5.44 ±1.19	4.45 ± 1.56
E_2 (pg/ml)‡	1250 ± 120	1040 ± 160	960 ± 110	843 ± 102	560 ± 91	1730 ± 110‡
AFC‡	9.46 ± 4.72	7.0 ± 0.51	7.73 ± 4.19	7.03 ± 1.26	5.59 ± 2.3	13.91 ± 8.75
AMH (ng/ml)‡	0.9 ± 1.12	0.6 ± 1.38	0.5 ± 0.88	0.4 ± 0.45	0.3 ± 0. 41	1.42 ± 2.0
Resistance Index‡	0.79 ± 0.04	0.81± 0.07	0.82 ± 0.06	0.84 ± 0.09	0.95 ± 0.11	0.65 ± 0.03
Pulsality Index	1.29 ± 0.03	1.87 ± 0.08‡	1.94 ± 0.07‡	1.98 ± 0.11‡	1.99 ± 0.15‡	1.15 ± 0.04

*All values are expressed in Mean ± S.D.
†The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ p < 0.05 for comparison against control.
FSH: Follicle stimulating hormone; AFC: Antral follicle count; AMH: anti-Mullerian hormone.

Table 2. Live - birth rate (Primary outcome)[#]

Variable	Age group (years)					
	40	**41**	**42**	**43**	**44**	**Control (30 – 39)**
Intention to treat population						
No. of patients	77	61	44	33	24	207
Live birth – no. (%)	13 (16.88 %)	8 (13.11 %)	3 (6.11 %)	2 (6.06 %)	1 (4.16 %)	66* (31.88 %)
Relative risk (95 % CI)	0.49 (0.27 to 0.91)	0.35 (0.16 to 0.72)	0.25 (0.08 to 0.76)	0.22 (0.05 to 0.86)	0.14 (0.02 to 1.02)	1.00
Absolute difference in live birth rate (95 % CI)- %	-15 (-45 to 15.1)	-18.77 (-56.31 to 18.79)	-23.77 (-71.31 to 23.81)	-25.82 (-77.48 to 25.84)	-27.72 (-83.16 to 27.76)	------
Women who became pregnant						
No. of patients	30	23	15	09	06	77
Live birth – no. (%)	13 (43.33 %)	8 (34.78 %)	3 (20 %)	2 (22.22 %)	1 (16.66 %)	66* (85.71 %)
Relative risk (95 % CI)	0.38 (0.23 – 0.65)	0.35 (0.19 -0.66)	0.54 (0.31 -0.94)	0.16 (0.04 -0.56)	0.19 (0.03 -1.16)	1.00
Absolute difference in live birth rate (95 % CI)- %	-42.38 (-127.39 to 42.41)	-50.93 (-101.88 to 50.97)	-65.71 (-197.16 to 65.74)	-63.49 (-190.47 to 63.54)	-69.05 (-207.18 to 69.11)	-----

[#]Absolute differences and relative risks were calculated for the comparison between patients of different age groups (40, 41, 42, 43, and 44) and the control group. *$p > 0.01$ values are for all comparisons. CI denotes confidence interval.

Table 3. Secondary outcomes

Outcome	**Study population**					**Control**	***p*-Value†**
Age (years)	**40**	**41**	**42**	**43**	**44**	**30 -39**	
Complications of early pregnancy							
No of patients	11	8	7	4	3	77	------
Abortion	2	2	3	2	2	2	1.00
Ectopic pregnancy	2	0	1	1	0	3	0.91
Complications of late pregnancy							
No of patients	7	6	3	1	1	61	-----
GDM	2	2	1	0	1	14	0.66
PIH	1	1	1	1	1	8	0.84
IUFD	1	1	0	0	0	5	0.59
IUGR	2	1	2	1	1	10	0.88
Preterm delivery	2	1	2	1	1	14	0.79
Maternal adverse events							
No of patients	11	8	7	4	3	80	-------
Thrombocytopenia[‡]	1	0	0	0	0	10	0.48
Nose bleed	2	1	0	1	0	4	0.55
GIT problem	8	6	6	2	3	62	0.89
Neonatal events							
Congenital and neonatal anomalies	1	0	0	0	1	6	1.00

† *p* values are for all comparisons.
Complications of early pregnancy and maternal adverse events were calculated for all 446 women who underwent randomization.
Ongoing pregnancy outcomes and neonatal events were evaluated for 164 women with ongoing pregnancy beyond 12 weeks of gestation.
‡ Platelets were measured only in women with ongoing pregnancy beyond 12 weeks of gestation. Thrombocytopenia was defined as a platelet count below 150,000 per cubic millimeter.
§ Among the congenital abnormalities, the two events were central adrenal insufficiency; in the control age group six events were one trisomy 21 [Down's syndrome]), (trisomy 18), resulting in death 6 days after birth and prenatal supraventricular tachycardia, preauricular tags and one trisomy 9 mosaicism, resulting in small size for gestational age and a heart-valve abnormality.

A steep decline in live birth rate was observed in women over 40. No significant differences in secondary outcomes were observed among the six groups, except that women in the age group of 42 and above where intra-uterine growth restriction and preterm delivery occurred significantly and more frequently (Table 3). No serious maternal adverse events were reported.

There were no significant interactions between the study-group assignment and the experience of having previous IVF cycles or ectopic pregnancy or abortion.

4. Discussion

The decline in fertility remains a complex issue for women who, for a variety of reasons, are attempting to have children at an advanced age. In this study, we report that gradual increase of age in women over 40 decreases chances of a healthy clinical pregnancy and it practically comes to an end around the age of 43. Live birth rates were highest (37.66 %) in 40 year old age group among the patient cohort while control group documented 40.58 % live birth rate. Among secondary outcomes, most notable are an increased tendency of intra-uterine growth restriction and preterm delivery occurring in almost half the women at an age over 42.

The most critical statistic that couples contemplating fertility treatment need to understand is the odds of them taking home a healthy baby. A recent study by Spandorfer concluded that IVF was a reasonable option for women aged 45 years with normal ovarian reserve and a production of at least five oocytes[12]. However, eying at the difficulty of the situation, a group from Israel concluded that IVF treatment should be limited to patients not older than 43 year old with adequate ovarian response[13]. In line with this finding, we also report age around 43 is the "living on edge" for women for conceiving a pregnancy by IVF.

It appears that maternal age is the most significant prognostic factor for IVF success or failure[14]. Impact of chronological aging (telomere deletion and chromosomal shortening) is more or less constant affecting oocyte quality in majority of women[15]. On the other hand, uterine vascular defect (diabetes, hypertension) and immune response vary from women to women. However, unlike egg deficiency, all 40-plus women do not suffer from deficient uterine receptivity because endometrial receptivity[16] depends on uterine vasculature and immunomodulatory changes in different endometrial compartments[17] although not reflected precisely in the Table 1. Therefore, identification of women over 40 and above is warranted for the maximum benefit from IVF. Here comes the importance of ovarian reserve or ovarian response to stimulation. Since one marker is not sufficient to predict[18], commonly used markers like AFC, and AMH are used which both show gradual decline with the increase of age with a concomitant increase of basal FSH (Table 1).

The impressive improvements in assisted reproduction technologies over the last two decades have had little impact on the prognosis of women with advanced age[19]. Treatment of such patients still yields low success rates and, on the top of that, even when a pregnancy is achieved, the toll of pregnancy loss with secondary complications is very high. In a recent study, Serour *et al.*[20] reported the cancellation rate of 16 % per initiated cycle, which is similar to the 16.6 % in Tsafrir *et al.* study[21]. Our data reports approximately 35.71 % fetal loss in different patient strata with a high incidence of gastrointestinal problems often found in women above 40 (Table 3).

Reports indicate increased risks in women aged over 40, with a step-change in risk above the age group[7,15,18]. In older women, one would expect to find higher incidence of disorders such as diabetes and hypertension[22]. However, we did not find a higher incidence of pregestational diabetes although we found significantly high incidence of gestational diabetes. It is possible that in the study group some of the women diagnosed with diabetes during pregnancy had previously undiagnosed pregestational diabetes. In our study, the incidence of maternal ICU transfer is about 1.1 % which is much higher than the incidence in our general population (0.04 %). It is also to be remembered that babies of older women are at an increased risk of serious adverse outcomes, including intrapartum-related perinatal death, and early neonatal death[23]; however, no such cases were observed as all deliveries were conducted by caesarian section at about 36 weeks of gestation. Hence, counselling of the couple is important in women of advanced age, and oocyte donation or adoption programme would be a more reasonable alternative, if applicable.

A limitation of the current study warrants consideration. Long term down regulation followed by gonadotropins has been applied to the participants as per the conventional protocol of the institute. Short flare up protocol might be useful for poor responders as mentioned in the Bologna criteria; however, cuing to the success rate of GnRH-a in our institute and elsewhere we preferred the former. However, prevalence of failed prior IVF cycles among the women in our study population was 10 %, which makes us confident that there was no selective referral of women without failed IVF.

In conclusion, the incidence of pregnancy late in life was associated with a high prevalence of adverse birth outcomes, particularly in events during ongoing pregnancy. Women ≥40 with singleton pregnancies were at a significantly higher risk for both maternal and neonatal complications compared to group of pregnancies achieved by conventional IVF.

5. Conflict of Interests

None.

6. References

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