# Original Article

## Number of Days of Controlled Ovarian Stimulation (COS) and Outcome of Assisted Reproductive Technology

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## Abstract

Assisted reproductive technology (ART) has become most widely used procedure worldwide for the couples with subfertility. Also, predictability for the success rate in ART is challenging and is of paramount importance in counseling and treating the couples. Whether the prolonged period of COS would have an impact on pregnancy outcome is of concern. Hence this study has been designed to compare the number of days of stimulation and ART outcome. Aim: To assess the predictive value of number of days of stimulation in ART success rate and to use it as a counseling tool. Materials and methods: It is a retrospective study conducted in the Department of Andrology & Reproductive Medicine, Chettinad Superspecialty Hospital, Kelambakkam, Chennai. Study included 200patients from January 2009 to December 2014. Results: The proportion of women who had clinical pregnancy was 32.35%, 25.24% and 23.07% in Group A, B & C respectively. The differences in clinical pregnancy among the study groups were statistically not significant (Chi square value 1.28, p value 0.452). Number of women who had live births were 26.47%, 23.30% and 15.38% among the stimulation days 6 to 9, 10-13 and more than 14 days respectively. There is no statistical difference in Live birth rate (p-value 0.343). Conclusion: To conclude, it is clearly demonstrated that as the number of days of ovarian stimulation increases, there is a decline in the Clinical pregnancy rate and Live birth rate though it is not statistically significant.

Key Words: Ovarian stimulation, assisted reproduction, pregnancy outcome, gonadotropins, agonist.

## Introduction

Assisted reproductive technology (ART) has become most widely used procedure worldwide for the couples with subfertility and it has been estimated that more than 5million babies are born out of ART worldwide[1]. Predictability of the success rate in ART is challenging and is of paramount importance for counseling and treating the couples. Ovarian stimulation is an integral part of ART programme for multifollicular growth and supernumerary embryos available for transfer and freezing. But, ovarian response to stimulation is highly unpredictable in most women and is subject to many variables like age of the couple, duration of infertility, body mass index (BMI), cause of infertility, baseline FSH level, antimullerian hormone levels (AMH), antral follicle count (AFC) etc[2]. Out of these, female age is recognized as indicator for predicting the oocyte quality[3]. However, it is not a modifiable factor in improving the success rate of ART.

The average duration of ovarian stimulation has been found to be between 8-10days. It has been noted that women with decreased ovarian reserve and advanced age group take longer time to achieve desirable follicular maturity. For predicting the success rate of ART with respect to number of days of stimulation, only few studies are available[4-6]. With variable results, whether this prolonged period of COS would have an impact on pregnancy outcome is of concern. Hence this study has been designed to compare the number of days of stimulation and ART outcome in our department.

## Objective

To assess the predictive value of number of days of stimulation in ART success rate and to use it as counseling tool.

## Materials and methods

It is a retrospective study conducted in the Department of Andrology & Reproductive Medicine, Chettinad Super Specialty Hospital, Kelambakkam, Chennai. Study included 200 patients from January 2009 to December 2014.

Inclusion criteria: Female age <=35yrs,Unexplained infertility, Normozoospermia, 1st cycle ART, IntraCytoplasmic Sperm Injection (ICSI) cycles, Short flare GnRH agonist protocol, Dose of Gonadotropins 225IU/300IU/375IU per day, All fresh embryo transfer cycles. Exclusion criteria: Endometriosis, Polycystic ovaries, Adenomyosis, Fibroid uterus, Hydrosalpinx , Elective freezing of all embryos, Frozen embryo transfers.

## Methodology

Patients were recruited on Day 2/3 of menstrual cycle either after OCP withdrawal or natural cycle. In the

antral follicle count, which tells us about the ovarian reserve. Follicle Stimulating Hormone (FSH) test was also done. FSH is found to be the simplest, cost effective and still widely used measure for ovarian reserve test[7]. Accuracy in determining the ovarian reserve was similar with AMH and AFC as a single test and combining the two tests did not improve the prediction of ovarian response[7]. Hence, AFC has been considered as the best test for predicting the ovarian reserve[8]. Gonadotrophin dosage were decided, according to the age of the women, Body mass index, previous treatment cycle response, Number of antral follicles, Basal FSH level. All the patients included in the had short flare GnRH agonist protocol. It is an unique protocol followed in our department with the results comparable with the other standard protocols. In this protocol, GnRH agonist analogue Injection Leuprolide acetate 1mg was started subcutaneously from Day 2 of menstrual cycle and it was continued till the day of hCG trigger. It produces initial flare effect for recruitment of more follicles from the cohort and after continues administration it produces down regulation and prevents premature LH surge. Along with GnRH agonist, urinary Gonadotropins (Human Menopausal Gonadotropins) were started from Day 3 of the cycle and it was also continued till the day of hCG trigger. Patients were reviewed with first ultrasound after 5days of stimulation, according to the follicular response dosage were adjusted. Once, three or more follicles reached 18mm or more, urinary hCG trigger was given 35-36hrs prior to oocyte pick up. Timing of trigger was not influenced by weekend scheduling since our department has in house clinicians and embryologists.

Sperm preparation was done by swim up method. Intracytoplasmic Sperm Injection (ICSI) was done for all Metaphase 2 oocytes. There is a better fertilization rate obtained after ICSI as compared to IVF (68% versus 46%) and total fertilization failure following ICSI and IVF treatment was seen in 4.4% and 25% of the cycles respectively[9]. Hence, our policy is to do ICSI for all in view of referral for tertiary care. Luteal support was started with vaginal micronized progesterone 200mg thrice daily after oocyte pick up. Embryo transfer was done on Day 2,3,4 or 5 depending on the quality and the number of available embryos.

Urine pregnancy test and Serum Beta hCG were measured after 14days of embryo transfer. If the test was found to be positive, after 2weeks transvaginal ultrasound was planned to confirm clinical pregnancy. Clinical pregnancy is defined as appearance of Gestational sac detected by transvaginal ultrasound. Patients were followed up through pregnancy and delivery details either by personal or phone contact.

## Measured outcomes

1. Clinical Pregnancy Rate (CPR) is calculated as number of clinical pregnancy per embryo transfer.
2. Live birth rate (LBR) is calculated as number of live births per Embryo transfer.

## Statistical analysis

Number of days of ovarian stimulation was the primary explanatory variable. Occurrence of clinical pregnancy and Live birth were the primary outcome variable. The descriptive analysis of the explanatory and outcome variables was done by frequencies and percentages. Other relevant parameters like age of the female partner, BMI, Day 2 FSH level, Antral Follicle count, total dose of gonadotropin, number of Dominant follicle, Number of oocyte retrieved, total number of M2 oocytes, number of embryos transferred were compared between the study groups, using ANOVA test. Calculating the odds ratio and t's 95% Confidence Interval and p-value using binary logistic regression analysis assessed the association between number of days of stimulation and occurrence of clinical pregnancy and live birth. IBM SPSS version 21 was used for statistical analysis.

| Parameter | Frequency | Percentage (%) |
|---|---|---|
| Group A | 74 | 37.0 |
| Group B | 110 | 55.0 |
| Group C | 16 | 8.0 |

**Table 1** - Number of study participants in each study group

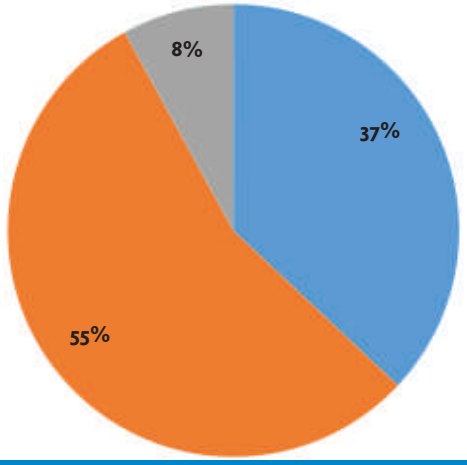


**Fig 1** - Pie chart showing distribution study population among three study groups

| Baseline characteristics and outcome. | Group A (N=74) | Group B (N=110) | Group C (N=16) | P value |
|---|---|---|---|---|
| Female age (years) | 30.71 | 30.28 | 30.71 | 0.761 |
| BMI (kg/m²) | 25.12 | 25.52 | 26.07 | 0.231 |
| Total AFC | 11.18 | 10.24 | 7.07 | 0.006 |
| Day 2 FSH (mIU/ml) | 7.10 | 7.42 | 7.83 | 0.598 |
| Total dose of gonadotropin (IU) | 2445.8 | 3555.2 | 4558.9 | <0.001 |
| Dominant Follicle | 8.15 | 8.89 | 6.14 | 0.167 |
| No. of Oocytes retrieved | 8.35 | 9.70 | 6.64 | 0.164 |
| No. of Metaphase 2 oocytes | 6.20 | 7.53 | 6.0 | 0.272 |
| No. of embryos transferred | 2.30 | 2.46 | 2.23 | 0.282 |

**Table 2** - Comparison of baseline characters among the three groups.

## Results

Total of 200 women were included in the study. The number of women who received stimulation for 6 to 9 days (Group A) was 74(37%), for 10-13days(Group B) was 110(55%) women and the remaining 16(8%) had received stimulation for more than 14 days (Group C). (Table 1).

The mean age and BMI of the women were about 30 years and 25 kg per m2 respectively, which was comparable among the three study groups with no statistical difference. (Table 2,2a)

| Parameter | Mean ± STD | F statistic | P value |
|---|---|---|---|
| I. **Female Age** | | | |
| Group A | 30.71 ± 3.05 | 0.273 | 0.761 |
| Group B | 30.28 ± 3.03 | | |
| Group C | 30.71 ± 2.26 | | |
| II. **BMI** | | | |
| Group A | 25.12 ± 3.70 | 1.476 | 0.231 |
| Group B | 25.52 ± 3.62 | | |
| Group C | 26.07 ± 2.99 | | |

**Table 2a** - ANOVA test comparing the baseline parameters among the three groups.

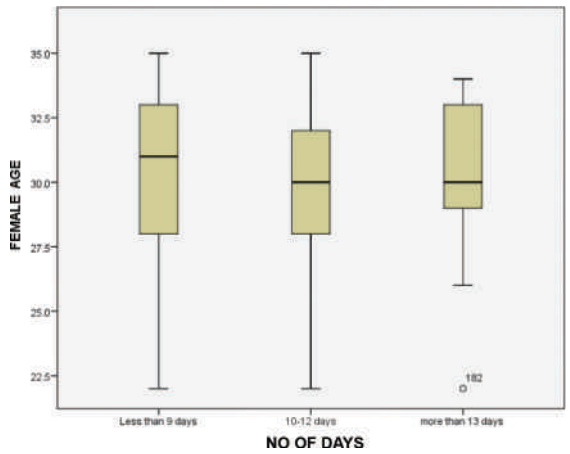


**Fig 2** - BOX and WHISKER PLOT comparing the age of the women among the there study groups (N=200)

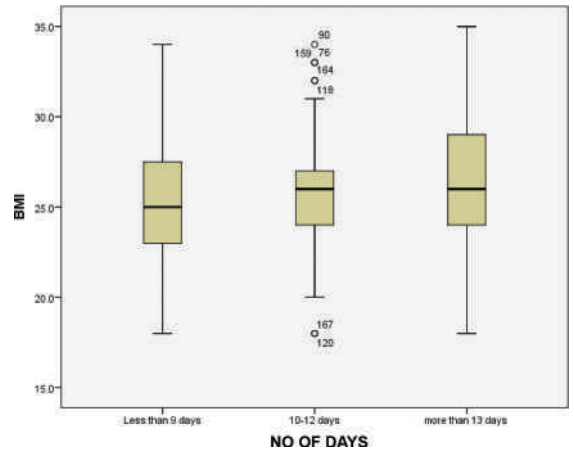


**Fig 3** - BOX and WHISKER PLOT comparing the BMI of the women among the three study groups (N=200)

Statistically significant difference was observed in the mean values of total antral follicle count and total dosage of gonadotropin. The differences in the mean values of other parameters were statistically not significant. (Table 3)

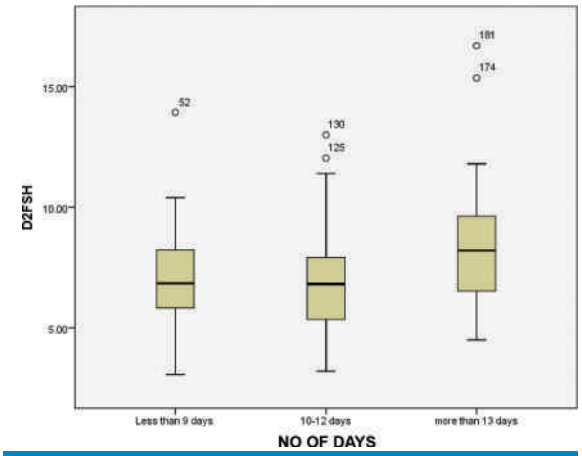


**Fig 4** - BOX and WHISKER PLOT comparing D2 FSH of the women among the three study groups (N=200)

| Parameter | Mean ± STD | F statistic | p-value |
|---|---|---|---|
| I. **D2 FSH** | | | |
| Group A | 7.10 ± 1.82 | 0.515 | 0.598 |
| Group B | 7.42 ± 2.27 | | |
| Group C | 7.83 ± 2.08 | | |
| II. **Total AFC** | | | |
| Group A | 11.18 ± 4.57 | 5.317 | 0.006 |
| Group B | 10.24 ± 4.42 | | |
| Group C | 7.07 ± 3.19 | | |
| III. **Total dose** | | | |
| Group A | 2445.8 ± 548.6 | 55.81 | <0.001 |
| Group B | 3555.2 ± 836.4 | | |
| Group C | 4558.9 ± 1138.6 | | |
| VI. **Dominant Follicle** | | | |
| Group A | 8.15 ± 4.31 | 1.806 | 0.167 |
| Group B | 8.89 ± 4.09 | | |
| Group C | 6.14 ± 3.11 | | |
| VII. **Retrieved** | | | |
| Group A | 8.35 ± 5.52 | 1.893 | 0.164 |
| Group B | 9.70 ± 5.71 | | |
| Group C | 6.64 ± 3.60 | | |
| VIII. **M2** | | | |
| Group A | 6.20 ± 4.50 | 1.310 | 0.272 |
| Group B | 7.53 ± 4.10 | | |
| Group C | 6.00 ± 3.80 | | |
| IX. **Number of embryo transferred** | | | |
| Group A | 2.30 ± 0.944 | 1.632 | 2.282 |
| Group B | 2.46 ± 0.661 | | |
| Group C | 2.23 ± 0.842 | | |

**Table 3** - ANOVA test comparing the treatment related factors among the three groups

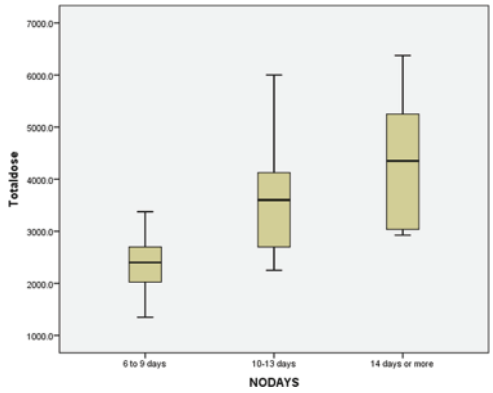


**Fig 5** - BOX and WHISKER PLOT comparing total dose of FSH of the women among the three study groups (N=200)

## Primary Outcomes

The proportion of women who had clinical pregnancy was 32.35%, 25.24% and 23.07% in Group A, B & C respectively. The differences in clinical pregnancy among the study groups were statistically not signifi cant (Chi square value 1.28, p value 0.452).

| Parameter | Pregnancy2groups | | Chi Square Value | p Value |
|---|---|---|---|---|
| | Yes | No | | |
| Group A (74) | 22 (32.35%) | 52 (67.65%) | 1.28 | 0.452 |
| Group B (110) | 26 (25.24%) | 84 (74.75%) | | |
| Group C (16) | 3 (23.07%) | 13 (76.93%) | | |

**Table 4** - Association of number of days with clinical pregnancy (N=200)

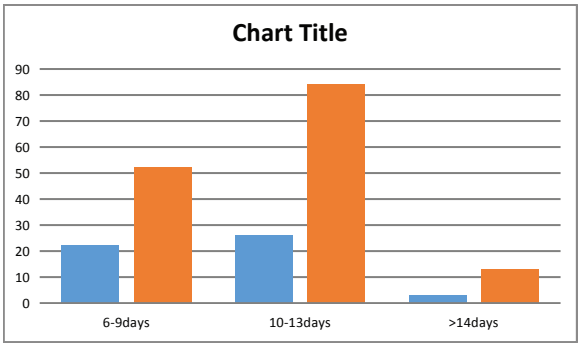


**Fig 6** - Bar chart comparing clinical pregnancy among 3 study groups

Among the women who had embryo transfer, Number of women who had live births were 26.47%, 23.30% and 15.38% among the stimulation days 6 to 9, 10-13 and more than 14 days respectively. There is no statistical difference in Live birth rate (p-value 0.343)

| Parameter | End Result | | Chi square value (Fisher's exact test) | P-value |
|---|---|---|---|---|
| | Live birth | Miscarriage | | |
| Group A | 18 (26.47%) | 4 (5.90%) | 2.14 | 0.343 |
| Group B | 24 (23.30%) | 2 (1.94%) | | |
| Group C | 2 (15.38%) | 1 (7.62%) | | |

**Table 5** - Comparison of end result between the study groups

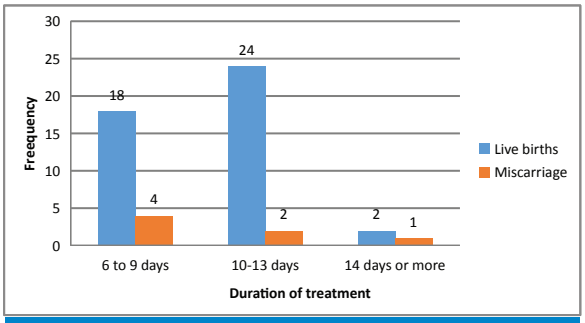


**Fig 7** - Bar chart comparing clinical pregnancy among 3 study groups

## Discussion

In ART cycles, duration of ovarian stimulation culminates with achieving optimum number of dominant follicle. Clinically, Ovarian stimulation is continued until two or three follicles have reached a size of 17–18 mm mean diameter[10]. Kerin et al clearly says that ideal stimulation protocols should result in development of at least three follicles beyond 17 mm so that it will yield at least two embryos for transfer[10]. As believed in practice 10-14 days cycle length is not constant in every patient and it is not an independent factor for predicting pregnancy outcome. On the contrary, the association between the daily dose of gonadotropins and outcome is well established[11].

In our study, we had 200 patients who met our inclusion criteria. In literature search, we found very few studies comparing the duration of ovarian stimulation and the outcome. The duration of ovarian stimulation ranged between 6 and 17 days. We grouped the patients as Group A, B, & C. Group A patients had stimulation between 6 and 9days, Group B patients had stimulation between 10 and 13days and Group C between 14 and 17 days of stimulation. According to Martin et al, there is no significant difference in the pregnancy rate when the duration of ovarian stimulation are less than 9days, between 10 and 11days and more than 12days[4]. Whereas Meleen et al, differ that when the number of days of stimulation exceed beyond 13days it affects the pregnancy outcome[5]. Based on the above observations, we decided to group our length of ovarian stimulation more than 14 days as Group C.

Age is considered as the best predictor of the oocyte quality and it is known to be the most important factor in determining the pregnancy potential in regularly cycling women[12]. The mean age of patients in the other two studies ranged between 34 and 36years. In our study, mean age of the patients in all groups was similar with the mean age of 30.71 +/- 3.05years. As the woman's age advances, there is an increased requirement of gonadotropins and length of stimulation, still resulting in decline in the pregnancy rate[13]. Body Mass Index is an independent factor for the number of days of stimulation. Obesity had been reported to influence both stimulation length and cycle outcome[14]. Meleen et al had mean BMI of the three groups as 26kg/m2, which was not statistically significant. In our study, we had mean BMI of 25kg/m2, which was comparable among all three groups.

There are various tests available to assess the ovarian reserve. Basal follicle stimulating hormone (FSH) levels measured on day 3 of the menstrual cycle is the most widely used ovarian reserve test to assess the ovarian response to stimulation, for over two decades now[15]. We prefer to consider day 2 serum FSH and AFC as best predictor for quantitative assessment of the ovarian reserve in our population. There was a significant difference in the total antral follicle count and the total gonadotropin dosage required among the three groups. Meleen et al and Martin et al reported similar observation in their studies. Group C women required more doses of gonadotropin in our study. From the above observation, it is clearly demonstrated that women with low antral follicle count required more doses of gonadotropin and longer days of stimu lation.

Different stimulation protocols may show wide variation in the number of days of stimulation[16]. We eliminated the confounding factor by using similar stimulation protocol for all our patients. Martin et al used long agonist protocol for all their patients and Meleen et al, included long agonist, antagonist and antagonist flare protocol.

Clinical pregnancy rate in group A, B and C were 32.35%, 25.24% and 23.07% respectively. It was comparable with the study by Meleen et al, 36%, 37.8% and 24.4% respectively among the three groups. The success of any ART programme is defined by take home baby rate[17]. The live birth rate in Group C was only 15.38% whereas Live birth rate in the other two groups were 26.47% and 23.07% were higher than group C. Though there is no statistical difference between the live birth rates among the three groups, still it is clinically higher in the Groups A and B compared to group C. Duration of ovarian stimulation may not be helpful in predicting the success rate prior to stimulation, it may still be useful in counseling the patients regarding the success rate during the cycle. Our results were comparable with the study by Meleen et al, that is 30%, 30.0% and 24.4%respectively among the three groups.

Though the dosage of gonadotropin required was more in group C who had low antral follicle count, live birth rate also remained low. Increasing the starting dose of gonadotropin stimulation in potential low responders is not an effective approach. No significant improvement in oocyte or embryo yield, or pregnancy rates were observed following such an upward dose adjustment.

Major strength of the study is that all the patients in my study population had only unexplained infertility, similar stimulation protocol and type of Gonadotropins. Limitations are small population size and it is a retrospective type of analysis.

## Conclusion

From our observation, it is clearly demon strated that as the number of days of ovarian stimulation increases, there is a decrease in the Clinical pregnancy rate and Live birth rate though it is not statistically significant.

When the antral follicle count is low, the total dose of gonadotropin required is more, which was statistically significant and found to be associated with prolonged number of days of stimulation.

It is the inherent ovarian response, which decides the degree of response rather than the actual drug, dose or duration.

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## Any form of physical will do

Benefits of exercise are well established. Regular exercise helps to reduce the incidence of chronic ailments and untimely death. Recommended weekly requirement is 150 minutes of moderate activity or 75 minutes of vigorous activity. While some manage to achieve the goal with a fairly evenly distributed activity throughout the week (regularly active), others may try to fulfil all their exercise needs during the weekends in one or two bursts of physical activity (weekend warriors). Yet others may be "insufficiently active". Do all these approaches lead to similar benefits? In a pooled analysis of nearly 64000 subjects aged 40 or older, researchers from Loughborough university of UK tried to find link between mortality and exercise habits over a period of 14 years (1994 to 2008). They discovered that regardless of the type or frequency, for all those who engaged in physical activity (Regularly active, weekend warriors, insufficiently active), the all-cause mortality risk was at least 30 percent lower than those who were totally inactive, although it was lowest in the regularly active. The important thing is to be physically active even if does not fulfil recommended minimum.

(Gary O'Donovan et al., JAMA Internal Medicine, doi:10.1001/jamainternmed.2016.8014, published online 9 January, 2017)

- Dr. K. Ramesh Rao