Original Article

Effect of stimulation phase length (SPL) on IVF/ICSI outcomes: a prospective study

Deepmala Deepmala, Kuldeep Jain, Bharti Jain

KJIVF & Laparoscopy Centre, Gagan Vihar, New Delhi, India

Abstract

Aim/Objectives: To determine whether SPL influences number of follicles on trigger day, oocyte retrieved at ovum pick up, grade A/B oocyte cumulus complex (OCC), M2 oocytes, fertilisation-rate, cleavage-rate, endometrial thickness, and clinical-pregnancy-rate (CPR) in IVF/ICSI cycles. **Settings/Design:** A prospective observational study conducted over a period of six months at an IVF/ICSI centre. **Methods/Material:** 152 patients enrolled for IVF/ICSI cycles except those undergoing frozen embryo transfer, donor oocyte/sperm, and surrogacy program. 16 patients were further excluded due to cycle cancellation and data of 136 patients were analysed. Individualised patient treatment with individualised controlled ovarian stimulation planned for each patient according to their clinical, ultrasound, and hormonal profile. The study parameter (stimulation phase length (SPL)) was further subdivided into short (<10 days), medium (10-12 days), and long (>12 days) and its associations with outcome parameters studied. **Statistics:** SPSS program for windows, version 17.0 used. Continuous variables compared using ANOVA. Spearman's correlation was used to find the association among various variables. Categorical variables were compared using Chi square test. $P < 0.05$ indicates significant difference. **Results:** The number of follicles and oocytes retrieved were significantly higher in medium SPL ($P < 0.05$). But there were no significant differences in number of grade A/B OCC or M2 oocytes among all three SPL groups ($P > 0.05$). There was no association between SPL and fertilisation rate, cleavage rate, or endometrial thickness. The CPR was 41.91% (57/136). The CPR was 31.6%, 46.7%, and 37.5% in short, medium, and long SPL, respectively. Though CPR appears higher in medium SPL but it is not statistically significant ($P > 0.05$). **Conclusions:** Optimising SPL between 10 and 12 days may serve as non-invasive marker for follicle/oocyte quantity but not quality. SPL cannot predict IVF/ICSI outcomes.

Keywords: CPR, fertilisation rate, follicles, oocytes, SPL

Address for correspondence: Dr Deepmala, DGO, DNB OBGY, DCR fellow, KJIVF & Laparoscopy Centre, 24 Gagan Vihar, New Delhi-51, India.
E-mail: deepmala23122008@gmail.com
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INTRODUCTION

The prevalence of infertility is approximately 15% worldwide. The Indian Society of Assisted Reproduction 2018 states that infertility affects 10–14% of population. This is approximately 27.5 million couples in India. With this burden of infertile couples, the focus of treatment has shifted, from systematic correction of each factor to providing the most efficient and cost-effective treatment, which is Assisted Reproductive Technology (ART).[1] Efforts have been taken to evaluate the factors affecting IVF/ICSI outcomes.

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Ovarian stimulation is an integral part of ART, the goal of which is to induce development of multiple dominant follicles and to recruit many mature oocytes to improve chances for conception.[2,3] Along with the number of oocytes collected, the ability of retrieved oocyte to get fertilised and form healthy embryo is important for successful pregnancy.[4,5]

Follicle diameter and serum oestradiol levels are the commonly used primary markers for follicle maturity.[6,7] Age, follicular stimulating hormone (FSH), antral follicular count (AFC), and anti-Mullerian hormone (AMH) have been extensively studied as quantitative markers of ovarian reserve.[8,9] The mean follicular growth rate during spontaneous menstrual cycle is 1.42 mm/day whereas it is 1.36 in oral contraceptive cycles and 1.69 mm/day in stimulated cycles. But whether this increased growth rate in stimulated cycle affects ART outcomes is largely unstudied.[10] The accelerated growth of follicles will also depend upon length and dosage of gonadotropins utilised. Therefore the total duration of gonadotropin or the stimulation phase length (SPL) may serve as an indirect and non-invasive method of measuring growth profiles of the follicles.

Nayadu[11] in 1991 reported that slow early follicular growth during ovarian stimulation is associated with poor IVF/ICSI outcomes. Another study in 2003 stated that the clinical pregnancy rate was 84% in normal ovulators whereas it was 21% in early ovulators.[12] In 2006, Dr Ryan Martin[13] retrospectively studied the impact of dose and duration of gonadotropins on IVF outcomes of 140 women. The women with longer duration of stimulation had fewer oocyte yields. But they did not find any significant difference in clinical pregnancy rates. Whereas Meleen Chauang[14] in 2010 stated that 13 days or more of stimulation had decreased live birth rate.[15]

These studies indicate a potential role of SPL in determining oocyte number and quality but differ in their opinion about its role on embryo and pregnancy outcomes. The studies conducted till now were largely retrospective and the effects of SPL on oocyte, embryo, and IVF/ICSI outcomes are largely unevaluated and need further studies.

Therefore we designed a prospective study of women undergoing IVF/ICSI to find the effect of SPL on IVF/ICSI outcomes. The main objectives of the study were to determine whether SPL influences number of follicles on trigger day, oocyte retrieved at ovum pick up (OPU), number of grade A/B oocyte cumulus complex (OCC), number of M2 oocytes, fertilisation rate, cleavage rate, endometrial thickness (ET), and clinical pregnancy rate (CPR). SPL was further divided into three groups of short (<10 days), medium (10–12 days), and long (>12 days) with an objective to determine an optimal SPL that results in competent oocytes and successful embryo and pregnancy outcomes. This would allow clinicians to tailor patient stimulation protocols to maximise the chance of a successful treatment.

SUBJECTS AND METHODS

A prospective observational cohort study over a period of 6 months (August 2018 to January 2019) was conducted at an IVF/ICSI centre. Ethical clearance was obtained from Independent Ethical Committee of Indian Fertility Society. 152 patients were enrolled for IVF/ICSI cycles after detailed counselling and written informed consent except those with frozen embryo transfer, donor oocyte/sperm, and surrogacy program.

Out of these 16 patients were further excluded due to cycle cancellation (0-poor ovarian response, 5-ovarian hyperstimulation syndrome, and 1-poor endometrium) as their outcomes could not be compared. Data of remaining 136 patients were analysed.

Individualised patient treatment was planned according to patient's profile and protocol (agonist/antagonist) decided. In agonist protocol GnRH agonist was given from 21st day of previous cycle and serum LH and E2 levels were measured on Day 2. Ovarian stimulation started once adequate downregulation was achieved. In antagonist protocol ovarian stimulation, was started on Day 2/Day 3 of cycle and GnRH antagonist added after 5 days of stimulation to prevent premature LH surge. Individualised controlled ovarian stimulation was started as per patients anti-Mullerian hormone (AMH) levels, Day 2 hormone profile (serum FSH, LH, E2), and Antral Follicle Count (AFC). Initial dose of gonadotropin (Gn) was noted. Serial follicular monitoring was done and gonadotropin dose adjusted till at least three follicles of 18 mm were seen and trigger in the form of recombinant human chorionic gonadotrophin (rHCG) 250 microgram was given subcutaneously. OPU was done after 36 hours of trigger. Total dose of gonadotropin and number of follicles >12mm were noted on the day of trigger. SPL was defined as the number of days from starting gonadotropin to the day of trigger and this was noted. On the day of OPU the number of grade A/B OCC were

noted. Further in cases designated for ICSI the number of M2 oocytes after denudation were also noted. Grading of OCC done as Grade A (mature), Grade B (immature), Grade C (postmature), Grade D (atretic). Grading of oocytes after denudation for ICSI done as GV (germinal vesicle), M1 (metaphase 1), M2 (metaphase 2).[16] IVF/ICSI (D0) was performed according to the patient's treatment plan, number and grade of OCC/oocytes obtained. Check for fertilisation, cleavage, morula, and blastocyst was done according to fixed standard laboratory protocol. Fertilisation rate was defined as the percentage of fertilised embryos (embryos with two pronuclei and two polar body) out of total number of OCC inseminated or oocytes injected. Cleavage rate was defined as percentage of cleaved embryos out of total number of fertilised embryos.

According to treatment plan either D3 transfer of three embryos or D5 transfer of two blastocysts were done and surplus embryos were either vitrified or observed and discarded according to the patients consent. ET was measured for all patients on the day of embryo transfer. Serum Beta HCG was done 15 days after embryo transfer and if positive, ultrasonography was done after 7 days or later to detect intrauterine gestational sac and document clinical pregnancy.

The study parameter was SPL and it was further subdivided into short (<10 days), medium (10–12 days), and long (>12 days). The outcome parameters were number of follicles on the trigger day, total number of OCC/oocytes retrieved at OPU, number of Grade A/B OCC, number of M2 oocytes, fertilisation rate, cleavage rate, ET, and CPR.

STATISTICAL ANALYSIS

SPSS program for windows version 17.0 was used. Continuous variables were presented as mean ± SD (standard deviation) and categorical variables were presented as absolute number and percentage. Data were checked for normality before analysis using Shapiro Wilk test. Normally distributed continuous variables were compared using Analysis of Variance test (ANOVA). The Kruskal Wallis test was used for those variables that were not normally distributed and further comparisons were done using Mann-Whitney U test. Spearman's correlation was also used to find the association among various variables. Categorical variables were compared using Chi square test. For all statistical tests, $P < 0.05$ was taken to indicate a significant difference.

RESULTS

The percentage of patients with short (<10 days), medium (10–12 days), and long (>12 days) SPL were 27.9% (38/136), 66.1% (90/136), and 5.9% (8/136), respectively. The range of SPL was between 8 and 14 days. There were no significant differences in mean age, mean BMI, distribution of parity and type of infertility and mean duration of infertility among all the three groups of SPL ($P > 0.05$) [Table 1].

The mean number of follicles >12mm on the trigger day and the number of oocytes retrieved at OPU were significantly higher in SPL of 10–12 days ($P = 0.002$ and 0.011 respectively). But there were no significant differences in the mean number of grade A/B OCC or M2 oocytes among all three groups of SPL ($P = 0.056$ and 0.100). On univariate analysis with spearman correlation graph, there is a parabolic association or trend seen between SPL and follicles, oocytes retrieved, Grade A/B OCC, and M2 oocytes. This means that these variables increased as the number of days of gonadotropin stimulation (SPL) increased, peaked at 11 days and then again fall as the SPL further increased. But due to rising and falling trend there were no significant linear correlation found [Table 2, Figure 1].

There were no significant differences in mean fertilisation rate, mean cleavage rate, and mean ET among the three SPL groups ($P = 0.258$, 0.821, 0.211 respectively). No associations were observed between SPL and fertilisation rate, cleavage rate, or ET. The CPR was 41.91 % (57/136) among the study population. No significant differences were seen in CPR among the three SPL groups (*P* of short/medium SPL is 0.114, medium/long SPL is 0.745 and short/long SPL is 0.618) [Table 2].

Starting and total dose of gonadotropins and the protocol used were the independent factors affecting SPL on

Table 1: Comparison of demographic parameters among the three SPL groups

	SPL			*P* value
	<10 days	10–12 days	>12 days	
Age (yrs)*	31.03 ± 3.94	32.14 ± 3.34	33.00 ± 1.77	0.162
BMI (kg/m^2)[a]	27.44 ± 2.76	27.76 ± 2.56	28.48 ± 1.38	0.564
Parity[b]				0.616
0	31 (81.6%)	79 (87.8%)	7 (87.5%)	
1	7 (18.4%)	9 (10%)	1 (12.5%)	
2	0 (0%)	2 (2.2%)	0 (0%)	
Type of infertility[b]				0.914
1 Primary	22 (57.9%)	50 (55.6%)	5 (62.5%)	
2 Secondary	16 (42.1%)	40 (44.4%)	3 (37.5%)	
Duration of infertility[a]	4.55 ± 2.67	5.20 ± 2.43	5.25 ± 1.04	0.115

[a]Mean ± SD. [b]Percentage

Table 2: Comparison of outcome parameters among the three SPL groups

Outcome parameters Mean ± SD	SPL			*P* value
	<10 days	10–12 days	>12 days	
Follicles > 12 mm on the trigger day	**9.89 ± 3.11**	**13.24 ± 5.51**	**9.75 ± 4.33**	**0.002**
Oocytes collected at OPU	8.39 ± 2.94	11.08 ± 5	8.38 ± 2.67	0.011
Grade A/B OCC	**7.61 ± 2.51**	**9.53 ± 4.52**	**7.13 ± 2.36**	**0.056**
M2 oocytes	7.00 ± 2.36	8.86 ± 4.45	7 ± 2.07	0.100
Fertilisation rate	**86.52 ± 11.5**	**82.02 ± 14.68**	**82.91 ± 8.03**	**0.258**
Cleavage rate	97.02 ± 14.46	93.93 ± 12.59	95.42 ± 8.53	0.821
Endometrial thickness	**9.24 ± 1.85**	**9.46 ± 1.49**	**9.60 ± 2.01**	**0.211**
Clinical pregnancy rate	12/38 (31.6%)	42/90 (46.7%)	3/8 (37.5%)	0.277

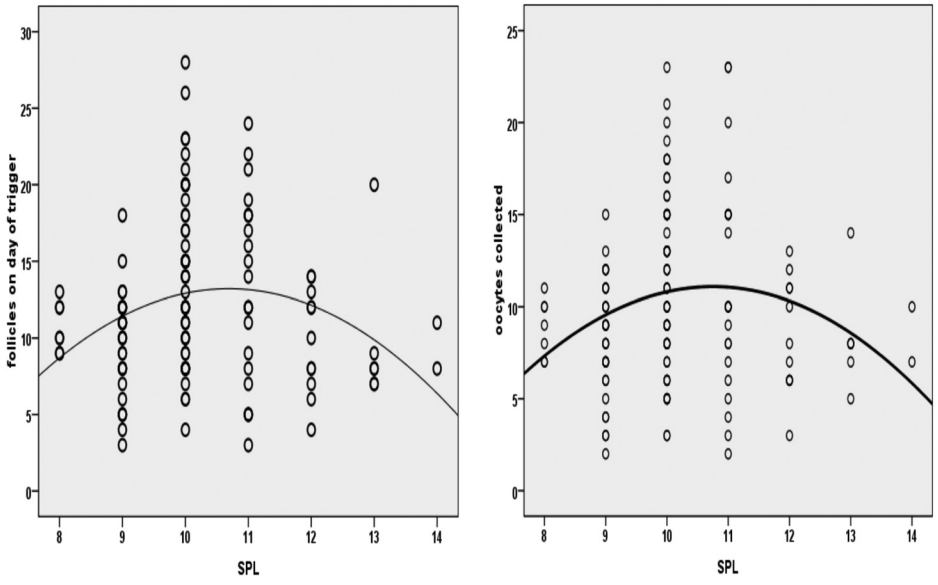

Figure 1: Correlation graph showing parabolic trend between SPL and number of follicles >12 mm on the trigger day and number of oocytes collected at ovum pickup

multivariate logistic regression analysis [Table 3]. There was a significant difference in mean starting dose (217.11, 246.67, 300.00 international units) and mean total dose (1898.68, 2780.00, 6050.00 international units) of gonadotropin among the three SPL groups of <10 days, 10–12 days, and >12 days, respectively ($P = 0.001$ and <0.001). The increase in starting and total dose was in direct proportion to the increase in number of days of stimulation. The percentage of patients landing up in SPL <10 was significantly higher when antagonist protocol is used ($P = 0.004$). Whereas the percentage of patients landing up in SPL 10–12 days was significantly more when agonist protocol is used ($P = 0.033$). In SPL group of >12 days, patients with agonist protocol was more than that with antagonist protocol but the difference was not significant ($P = 0.231$).

DISCUSSION

IVF/ICSI outcomes are affected by multiple factors which include maternal age, patient's history and hormonal profile, ovarian response, the quality and quantity of gametes, the embryo-endometrium crosstalk, and also the laboratory quality and the experience of the clinician. But once a woman enters an IVF/ICSI program, none of these factors are readily modifiable except the stimulation of ovarian functions. The studies done till now largely indicate a potential role of SPL in determining oocyte number and quality but differ in the opinion about its role on embryo and pregnancy outcomes. And most of these studies were retrospective. Therefore we conducted this prospective study to evaluate SPL or the duration of gonadotropin stimulation as an indirect, non-invasive and modifiable predictor for IVF/ICSI outcomes.

The data of 152 patients were collected and after exclusion of 16 patients due to cycle cancellation, the data of 136 patients were analysed.

Among the outcomes which measured ovarian functions, the analysis of difference in mean numbers is significant for the number of follicles and oocytes retrieved among

Table 3: Independent factors affecting SPL

	Unstandardised coefficients		Standardised coefficients	*t*	*P* value	95.0% confidence interval for B	
	B	Std. error	Beta			Lower bound	Upper bound
(Constant)	8.363	0.360		23.240	0.000	7.651	9.075
Starting dose of gonadotropin (IU)	−0.009	0.001	−0.488	−6.532	<0.001	−0.012	−0.006
Total dose of gonadotropin (IU)	0.001	0.000	1.166	16.090	<0.001	0.001	0.001
Protocol	0.314	0.125	0.118	2.516	0.013	0.067	0.560

To identify potential factors associated with SPL univariate analyses were performed. Multivariate logistic regression model was used to identify independent factors for SPL. An enter approach was used to enter new terms into the model, with a limit of $P < 0.05$ to enter the terms.

the three SPL groups. Also the parabolic association seen on correlation graph is paralleling this observation [Table 2, Figure 1]. This means all these variable increase with SPL, maximise at 10–12 days, and then decrease. The analysis of difference in mean numbers is not significant for the number of grade A/B OCC and M2 oocytes [Table 2]. Still some parabolic association is seen on correlation graph.

The above observations mean that an increase in the dose of gonadotropin in slow responders and a decrease in the dose of gonadotrophin in fast responders to achieve a SPL of 10–12 days may optimise the number or increase the number of follicles and the total number of oocytes retrieved. But this does not necessarily indicate a higher output in terms of grade A/B OCC or M2 oocytes. Therefore optimising SPL between 10 and 12 days may serve as a marker for quantitative ovarian outcome but not the qualitative as it depends more on intrinsic ovarian function and synchronisation of cytoplasmic and nuclear maturation of the oocyte.

Our study supports the findings of the following studies done earlier. In 2004 MP Portmann[17] concluded that though ultrasound and serum hormonal parameters can guide for administration of trigger but they may not necessarily reflect an appropriate synchrony between nuclear and cytoplasmic maturation. In 2011 Brie Alport[18] stated that the number of follicles formed on trigger day and the number of oocytes retrieved decreased with SPL greater or less than 11. Kovacs[19] in 2016 found that despite similar embryo outcome, SPL of 8 days or less was associated with lower oocyte retrieval and clinical pregnancy rate when compared with SPL of 9–12 days. In a recent study in 2018 by Amir Javed[20]; no significant difference in the number of M2 oocytes retrieved in the two groups of SPL (8–10 days and 11–13 days).

In addition, we found that starting dose and total dose of gonadotropins and the protocol used were the independent factors affecting SPL. The antagonist protocol is associated with short SPL whereas the agonist protocol is associated with medium and long SPL. This is also explained by the fact that in agonist protocol there is prolonged downregulation and it takes longer duration and sometimes larger doses of gonadotropins to achieve an optimal follicular development. Whereas in antagonist protocol there is no downregulation, therefore the dose of gonadotropins needed is much less but the chances of premature LH surge are higher. Also the increase in total dose is in direct proportion to the increase in number of days of stimulation and as the starting dose is increased, the SPL also increases. But this does not imply that the number of follicles will increase with starting or total dose as the relation between SPL and follicles/oocytes is not linear.

Maternal age-associated ovarian ageing and shortened follicular phase are associated with adverse pregnancy outcomes. [21] Advanced maternal age may need more dose of gonadotropins. But our study did not find any correlation between age and SPL.

Further in our study SPL did not find any association between SPL and fertilisation rate or cleavage rate. This means SPL cannot predict the fertilisation potential of an oocyte which again depends upon intrinsic functions of gametes. Also there was no association between SPL and endometrial thickness. The CPR was 41.91 % among the study population. The CPR was 31.6%, 46.7%, and 37.5% among the patients with SPL of <10 days, 10–12 days, and >12 days, respectively. Though the percentage appears higher in the SPL group of 10–12 days but on statistical analysis there were no significant differences in CPR among the three SPL groups. Our study supports the findings of following studies. In 2006 Dr Ryan Martin[13] stated that SPL may not be an independent predictive factor for embryo or pregnancy outcomes. Alport[18] in 2011 found no association between SPL and endometrial thickness and pregnancy outcomes. In their study SPL was not affected by maternal age.

These above observations indicate that SPL cannot be used as a predictor of embryo development, endometrium, or clinical pregnancy outcome. The

results from this study suggests that the IVF/ICSI outcomes, specially the pregnancy potential depends more on the ovary's ability to form mature follicles and competent oocytes and not on the speed at which they develop follicles due to stimulation.

In our study an individualised approach was used for controlled ovarian stimulation to maximise the IVF/ICSI success. The wide range of SPL between 8 and 14 days was observed depending on patient's profile and requirement. We found three clinical pregnancy positives in SPL of >12 days and 12 clinical positives in SPL <10 days [Table 4]. Though the outcomes in terms of fertilisation or clinical pregnancy do not differ significantly among the three groups of SPL (short, medium, long), but still pregnancy is possible at any SPL. These observations are clinically relevant because they suggest that cycles should not be cancelled based on short or prolonged stimulation alone.

Similar inference was given by Bar-hava[22] in 2005. They concluded that the length of stimulation phase does not affect the clinical pregnancy rate and the cycles should not be cancelled only on the basis of prolonged stimulation. But there are studies stating that prolonged stimulation of 13 days or more was significantly associated with poor clinical pregnancy rate.[15] On the contrary another study in 2016 found that the stimulation length of 8 days or less was associated with lower oocyte retrieval and clinical pregnancy rate when compared with stimulation phase length of 9–12 days.[19] These reports do necessitate further and continued research.The positive effect of optimising SPL between 10 and 12 days on follicular growth and oocyte retrieval but not on grades of OCC or M2 oocytes, embryo, or pregnancy outcomes suggests that the qualitative development of follicles and oocytes is more important than quantitative development following ovarian stimulation. This is also supported by a study done in 2006 by Merce et al.[23] stating that pregnancy potential is not associated with the number of dominant follicles developed. Therefore further studies are needed to find the markers for qualitative aspect of follicle and oocyte functions. These may include genomics, proteomics, or metabolomics.

In a prospective study it would be unethical to subject any patients to a fixed SPL and then study the outcomes in IVF/ICSI as this would compromise the patient's results. The results of our study might be restricted due to relatively small sample size over small duration of 6 months insufficient to collect live birth data. Therefore a substantially large sample size of individualised controlled ovarian stimulation over longer duration which could evaluate live birth data would be required to completely evaluate the effects of SPL on different aspects of IVF/ICSI outcomes.

Table 4: Clinical pregnancy positives distribution among whole range of SPL

SPL (days)	8	9	10	11	12	13	14
Clinical pregnancy positive	5	7	24	13	5	1	2

CONCLUSIONS

In the prospective study done above we concluded that optimising SPL between 10 and 12 days may serve as a non-invasive marker for follicle/oocyte quantity but not quality which plays a vital role in determining pregnancy success. SPL is not associated with fertilisation rate, cleavage rate, endometrial thickness, or CPR. Therefore length of stimulation phase cannot predict IVF/ICSI outcomes. It is also suggested that cycles should not be cancelled based on short or prolonged stimulation alone. Continued prospective research is warranted in this field with substantially larger sample size to evaluate the potential of SPL as a non-invasive marker of IVF/ICSI success.

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Conflicts of interest
There are no conflicts of interest.

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