ORIGINAL ARTICLE

Clinical Outcome of Intracytoplasmic Sperm Injection using Long Protocol

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ABSTRACT

Primary aim: To study the outcome of intracytoplasmic sperm injection (ICSI) using long protocol. Analysis of the average number of days of stimulation, the incidence of ovarian hyperstimulation syndrome (OHSS), and the number and quality of oocyte retrieved.

Secondary aim: Correlation of the outcome against the stimulation phase length

Method: Retrospective study of 50 patients who underwent *in vitro* fertilisation (IVF) using the long protocol in our ART unit during December 2014 to December 2015. All clinical observations including the patient details, number of days of stimulation, the number of oocytes retrieved, the occurrence of OHSS were collected and tabulated. All clinical data were statistically analyzed.

Result: The clinical outcome of ICSI using long protocol was satisfactory. In the study group, we achieved 72% grade 1 embryo (36) and 2 weeks after embryo transfer the biochemical testing for pregnancy showed positive pregnancy in 52% (26). The average stimulation phase length was 13.5 days, (SD 5.5). The incidence of severe OHSS needing hospitalization and treatment was 2% (1/50). 8 patients observed to have evidence of mild OHSS. OHSS was observed in patients with a shorter period of stimulation phase, mean SPL among OHSS patient was 11.38 (SD -1.6). However, it was not statistically significant (p–0.507). The stimulation phase length did not correlate with the quality of oocyte nor the pregnancy rate as hypothesized theoretically.

Conclusion: The present study shows satisfactory clinical outcome using long protocol,the stimulation phase length did not show significant ill effects in the clinical outcome (72% grade 1 embryo, 52% pregnancy), nor increase complications like severe OHSS.

Keywords: Infertility, *In vitro* fertilisation (IVF)/Intracytoplasmic sperm injection, Long protocol.

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INTRODUCTION

ART using long protocol has been used with success since 1980 (Edwards rg, Steptoe pc, Purdy jm. br j Obstet Gynaecol. 1980;87:737–56. Porter, Smith, Craft et al. lancet. 1984;2:1284–5). However, the regimen, length of stimulation phase, and the pregnancy rate has been varied, resulting in difficulty in counselling regarding the length of the treatment period needed and planning the cycle. GnRH agonist *vs.* antagonist have been studied widely, and the outcome results remain controversial.[1-6] Long Protocol using GnRH agonist has been claimed to be prolonged due to down regulation and stimulation phase, expensive, reduce oocyte quality resulting in poor outcome.[7,8] The influence of ovarian stimulation phase length (spl) is less explored, in a retrospective study it is concluded that stimulation phase length can be used to predict the number of oocyte but not the pregnancy rate.[9] The present study is an attempt to analyse the clinical outcome of icsi using long protocol and the effect of the stimulation phase length (spl) on the outcome of the treatment cycle.

AIM

Primary Aim

To study the outcome of ICSI using long protocol.

Secondary Outcome

- Analysis of the average number of days of stimulation,
- The incidence of OHSS,
- The number quality of oocyte retrieved
- Quality of the embryo
- Number of pregnancy achieved
- Correlation of the outcome against the SPL

METHOD

Retrospective study of 50 cases who underwent ICSI using long protocol all relevant data collected and analyzed statistically

Table 1: Analysis of descriptive data

(Distribution of age, average stimulation phase, ET and number of oocyte retrived in the stimulted cycle)

	N	Minimum	Maximum	Mean	Std. deviation
AGE	50	23	42	31.82	5.086
SPL	50	6	26	13.56	5.429
ET	50	8	14	10.26	1.411
No oocytes	349	3	24	6.54	3.383

Average spl–13.5 days

Long Protocol

- OCP for 3 months
- Injection GnRH agonist (leupride) 3.75 mg IM on 16th day
- D2 USG screening and serum estradiol
- Stimulation with RFSH (Gonal–F) 225 mg SC for 5 days
- Asses USG and serum estradiol
- Depending on response increase or decrease dose/add hMG
- Trigger ovulation with inj. ovitrille (rhCG) 225 IU, SC
- Oocyte retrieval planned 36 hours after the ovulation trigger.
- After preparation of oocyte, ICSI proceeded.
- Quality of embryos assessed.
- ET (max 2) done on D3/D5 depending on the quality of the available embryo.
- Biochemical pregnancy test offered after 2 weeks.

STATISTICAL ANALYSIS

The collected data were analyzed with IBM.SPSS statistics software 23.0 version.
- *Descriptive data:* Frequency analysis (Table 1),
- *Categorical variables:* Percentage Analysis (Table 2)
  Continuous variable: Mean and SD (Table 3)
- *Difference between groups (Pregnancy +ve and -ve with no days of stimulation):* Mann–Whitney U test (Table 4)
- *Multivariate analysis (Grades 1, 2 and 3 with no of days of stimulation):* Kruskal Walli's (Table 5)
- *Relation between variables (no of days of stimulation with no of oocytes):* Spearman's rank test (Table 6)

Treatment Outcome in the Study Cycle

- *Total number of oocyte:* 349 (mean 6.4)
- *Total number of Gr 1 embryo:* 36 (72%)
- *Total number of pregnancy:* 26 (52%)

Table 3: Correlation of stimulation phase length and OHSS

Group Statistics					
OHSS		N	Mean	Std. deviation	Std. error mean
SPL	No	42	13.98	5.799	.895
	Yes	8	11.38	1.685	.596

Mean spl in OHSS cases-11.38 (Sd- 1.6). However not statistically significant *p* value – 0-.507

Table 2: Incidence of OHSS (mild, moderate and severe) and its correlation with polycystic ovary syndrome (PCOS)

			OHSS		
			No	Yes	Total
PCOS	Absent	count	29	6	35
		% of total	58.0%	12.0%	70.0%
	Present	count	13	2	15
		% of total	26.0%	4.0%	30.0%
Total		count	42	8	50
		% of total	84.0%	16.0%	100.0%

Incidence of sever OHSS was 2% (1/50)

DISCUSSION

COH can be achieved using any one among the many protocols available. Despite the disadvantage of the long duration of the stimulation phase in the long protocol it has consistently shown satisfactory results.[10] The long protocol includes pituitary suppression with OCP and GnRH agonist followed by ovarian hyperstimulation with rFSH. Pretreatment with OCP has been observed to lengthen the SPL but prevented early endogenous FSH rise resulting in more mature oocyte.[11] Systemic meta-analysis revealed that GnRH analog used for suppression does not influence the outcome of the cycle.[12] OHSS, a dreaded complication of ART is subjectively increased with the long protocol compared to the short protocol.[13] Prolonged ART cycle is believed to result in poor oocyte quality but in reality, there is no difference in pregnancy rate in prolonged cycles.[14]

The choice of protocol is made by the physician depending on various clinical parameters, and most importantly on the patient's response. The gonadotropin stimulation patients fall under three categories based on their response: (I) high responders; (II) intermediate responders and (III) poor responders.[15,16] Long protocol results in more number of mature embryos resulting in higher pregnancy rate.[10,17] Controversially, poor responders in long protocol produced more oocyte when subjected to antagonist protocol, better embryo quality encountered in agonist flare up protocol.

The present study was conducted in an attempt to assess the clinical outcome of long protocol in the given population. The SPL which is attributed as the disadvantage of

Table 4: Number of oocytes and age *vs.* stimulation phase length Spearman's Rho test

		No days of stimulation	No oocytes	Age
No oocytes	correlation coefficientw	-0.0059	1.000	-.0335[*]
	sig. (2-tailed)	0.684		0.017
	N	50	50	50
Age	correlation coefficient	-0.050	-0.335[*]	1.000
	sig. (2-tailed)	0.733	0.017	
	N	50	50	50

Corelation coefficient is inversely propotional

Table 5: Stimulation shase length (SPL) *vs.* quality of embryo kruskal walli's test

	N	Mean	Std. deviation	Std. ewrror
G1	36	13.44	5.390	.898
G2	8	15.50	7.171	2.535
G3	6	11.67	1.751	.715
Total	50	13.56	5.429	.768

Mean spl in G1 Embryo was 13.44 (Sd-5.3)
No statistical significance when compared with *Spl*
p-value 0.93

Table 6: Stimulation phase length(SPL) *vs.* pregnancy rate

	N	Mean	Std. Deviation	Std. Error
G1	36	13.44	5.390	.898
G2	8	15.50	7.171	2.535
G3	6	11.67	1.751	.715
Total	50	13.56	5.429	.768

+Ve pregnancy seen with mean spl of 14.08 days (5.5)
No correlation between +ve pregnancy and *spl*
p-value 0.3

the long protocol has been analyzed against the various outcome variables to asses if it worsens clinical outcome. In the present study, we have included only patients who underwent a uniform regiment of long protocol.

RESULTS

The average stimulation phase length (SPL) IS 13.5 days (SD-5.5). SPL was observed to be influenced by the age of the patient. Both are inversely proportional (p–0.017). The main complication assed was OHSS. There was evidence of OHSS in 8/50, among which 4% were known cases of PCOS (2/8). Only one patient suffered severe OHSS (1/8). Rest was diagnosed as mild to moderate OHSS who did not need admission or any medical intervention.The influence of SPL on the various clinical outcome measures like OHSS, number of the oocyte, quality of oocyte and pregnancy rate were statistically analyzed

OHSS was observed in patients with a shorter period of stimulation phase, mean SPL 11.38 (SD –1.6). However, it was not statistically significant (*p*–0.507). A definitive negative correlation was seen between the SPL the number of oocytes retrieved, shorter length of cycle resulted in a higher yield of the oocyte. The correlation coefficient being –0.057 (*p*–0.6)

The mean number of oocyte retrieved using the study protocol was 6.98 (SD–4.083). Following ICSI we achieved 72% Gr 1 embryo (36/50). Two weeks after embryo transfer the biochemical testing for pregnancy showed positive pregnancy in 52% (26/50).

CONCLUSION

Long protocol when judiciously used gives satisfactory results, less complications and the length of the stimulation phase does not influence any parameter of clinical outcome. Further studies to ascertain the live birth rate and comparative studies with antagonist protocols are required to acknowledge if the protocol is cost beneficial.

REFERENCES

1. Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception: a Cochrane review. Reproductive BioMedicine Online. 2007 Jan 1;14(5):640-649.
2. Griesinger G, Felberbaum R, Diedrich K. GnRH antagonists in ovarian stimulation: a treatment regimen of clinicians' second choice? Data from the German national IVF registry. Human Reproduction. 2005 Jun 2;20(9):2373-2375.
3. Fauser BC, Devroey P. Precycle Estradiol in Synchronization and Scheduling of Antagonist Cycles Fertil Steril . 2005;83:1607-1611.
4. Ludwig M, Katalinic A, Diedrich K. Arch. The Effect of GnRH Agonist versus GnRH Antagonist on Pregnancy Rate. Gynecol Obstet 2001;265:175-182.
5. Orvieto R, Rabinson J, Meltzer S, Homburg R, Anteby E. GnRH antagonist versus agonist in normoresponders undergoing ICSI: a randomized clinical trial in Iran Clin Exp Obstet Gynecol. 2006;33:197-199.
6. Engel JB, Griesinger G, Schultze-Mosgau A, Felberbaum R, Diedrich K. GnRH agonists and antagonists in assisted reproduction: pregnancy rate. Reproductive biomedicine online. 2006 Jan 1;13(1):84-87.
7. Tarlatzis BC, Kolibianakis EM. Agonist Vs antagonist ,Best Pract Res Clin Obstet Gynaecol. 2007;21:57–65.
8. Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. Human Reproduction. 2002 Apr 1;17(4):874-885.
9. Brie Alport, Allison Case, Hyun Lim, Angela Baerwald. Does the Ovarian Stimulation Phase Length Predict in Vitro Fertilization Outcomes. Int J Fertil Steril. 2011 Oct-Dec;5(3): 134-141.
10. Franco Jr JG, Baruffi RL, Mauri AL, Petersen CG, Felipe V, Cornicelli J, et al. GnRH agonist versus GnRH antagonist in poor ovarian responders: a meta-analysis. Reproductive biomedicine online. 2006 Jan 1;13(5):618-627.
11. Huirne JA, van Loenen AC, Donnez J, Pirard C, Homburg R, Schats R, et al. Effect of an oral contraceptive pill on follicular development in IVF/ICSI patients receiving a GnRH antagonist: a randomized study. Reproductive biomedicine online. 2006 Jan 1;13(2):235-245.
12. Kolibianakis EM, Collins J, Tarlatzis BC, Devroey P, Died rich K, Griesinger G . A systematic review and metaanal¬ysis. Hum Reprod Update. 2006;12: 651-671.
13. HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Bar-Hava%20I1%5BAuthor% 5D andcauthor=true&cauthor_uid=16316846" Bar-Hava I1, HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Yoeli%20R%5BAuthor%5D and cauthor=true and cauthor_uid=16316846" Yoeli R, HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Yulzari-Roll%2 0V% 5BAuthor% 5D and cauthor=true and cauthor_uid =16316846"

Yulzari-Roll V, HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Ashkenazi%20J%5BAuthor%5D&cauthor=true&cauthor_uid=16316846" Ashkenazi J, HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Shalev%20J%5BAuthor%5D&cauthor=true&cauthor_uid=16316846" Shalev J, HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Orvieto%20R%5BAuthor%5D&cauthor=true&cauthor_uid=16316846" Orvieto R. Controlled ovarian hyper-stimulation: does prolonged stimulation justify cancellation of in vitro fertilization cycles? HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/16316846"Gynecol Endocrinol. 2005 Oct;21(4):232-234.

14. Oehninger S. Poor responders in in vitro fertilization (IVF) therapy: the -challenge continues. Facts Views Vis Obgyn 2011;3:101-108.

15. Davis OK. IVF stimulation:protocols for poor responders. Methods Mol Biol 2014;115:329-341.

16. Xiuhua Liao. Optimal usage of the GnRH antagonist protocol Peter Humaidan The Fertility Clinic, Skive Hospital and Aarhus University,Denmark.Int J Clin Exp Pathol 2016;9(11):11965-11971.

17. Roberto M, Donatella C, Vincenza D, Carla T, Antonio P, Massimo M. GnRH antagonist in IVF poor-responder patients: results of a randomized trial. Reproductive biomedicine online. 2005 Jan 1;11(2):189-193.