Original Research Article

Effect of follicular size on the day of trigger on OOCYTE and EMBRYO quality in clomiphene citrate protocol- A comparitive retrospective study

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Abstract

Objective: To compare the effect of follicular size on the day of trigger on the oocyte and embryo quality in clomiphene citrate protocol in patients undergoing IVF/ICSI. **Materials and Methods:** A Retrospective comparative analysis was done in patients who underwent IVF/ICSI with clomiphene citrate protocol between 2016-2018 in craft hospital and research centre kodungallur Kerala. 242 patients who met the inclusion criteria of primary/secondary infertility, <40yrs old, normal male factor. Excluding > 4oys old, endometriosis, PCOS, male factor infertility. **Group A:** included 83 patients with lead follicle measuring 21-23 mm on the day of trigger. **Group B:** Included 159 patients with lead follicle measuring 17-19mm on the day of trigger. Inj ovitrille 250 mcg was given as a trigger. Oocytes retrieved after 36hrs by transvaginal approach. Both groups were compared for number of oocytes retrieved, number of matureoocytes, number of Day 3 good embryos. Statistical analysis was done by independent sample T-test for all variables. **Results:** No statistical difference between age and AFC in both groups. Statistical difference in number of oocytes retrieved (p value -0.055). No statistical difference in number of matureoocytes and number of day 3 embryos. **Conclusion:** Women who underwent IVF/ICSI with clomiphene citrate protocol with a lead > 20mm hadless number of oocytes retrieved when compared to <20mm lead follicle on the trigger dayand had similar number of mature oocyte and day 3 embryos. No beneficial effect by waiting for the lead follicle to reach beyond 20mm in size.

Keywords: Oocyte, Embryo, Clomiphene citrate, PCOS, Endometriosis, Infertility.

Introduction

Controlled ovarian stimulation is critical to assisted reproduction because it increases the number of oocytes undergoing development. Of these, only a portion will be competent for fertilization and development into viable embryos. Understanding the processes of selection, follicular growth, and ovulation has guided the development of this important component of treatment.

The medications, designed to over ride the selection of a single dominant follicle, drive multiple antral follicles into the growth phase. These follicles grow at different rates, andmanagement is guided by their size rather than their competence.

The administration of HCG, mimicking the endogenous luteinizing hormone (LH) surge, is the final event that determines follicular maturity and developmental competence. The timing of its administration is typically guided by the size of the lead follicle or lead follicularcohort.

This treatment is therefore based on an assumption that follicular size predicts the developmental competence of the oocyte. The assumption is based on limited studies using different models from unstimulated cycles to in vitro maturation models in animals[1–7].

Although there is evidence to suggest that follicle size can influence the outcomes, the actual relationship has not been determined. Because the decision to administer human chorionic gonadotropin (hCG) is based largely on the lead follicle.

More specifically, we evaluated the number of oocytes retrieved, number of mature oocytes, the resultant embryo quality, Implantation Rate, Clinical Pregnancy Rate and Live Birth Rate from oocytes originating from a range of lead follicle sizes less than 20mm with comparisons with the lead follicle size more than 20mm in a clomiphene citrate protocol patients undergoing ICSI.

Comparisons within these women allowed us to carefully know any beneficial effect by waiting for the lead follicle to reach beyond 20mm in size to improve the oocyte maturation,number of oocytes retrieved, embryo quality, Implantation Rate, Clinical Pregnancy Rate and Live Birth Rate. This information will be crucial to any quantitative decision-making model.

Objective

To compare the effect of follicular size on the day of trigger on the oocyte and embryo quality in clomiphene citrate protocol in patients undergoing ICSI.

Study design

Retrospective Comparative Study

Primary outcome

LBR

Secondary outcome

CPR, Implantation Rate, No of oocytes retrieved, No of Mature oocytes, No of day 3 good embryos

Materials and methods

A Retrospective comparative analysis was done in patients who underwent ICSI with clomiphene citrate protocol between 2016-2018 in craft hospital and research centrekodungallur, Trissur, Kerala.

Study population

242 patients

Inclusion criteria

Primary/Secondary infertility

<40yrs old, mild endometriosis, Normal male factor, Tubal factors

Exclusion criteria

> 40yrs old, severe endometriosis, PCOS, Uterine anomalies, Male factor infertility. Patients were categorized into two groups depending on their lead follicle size

Group A: included 83 patients with lead follicle measuring 21-23 mm on the day of trigger. **Group B:** Included 159 patients with lead follicle measuring 17-19mm on the day of trigger. 1st or 2nd attempt

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ICSI Patients

Base line scan, E2 levels were measured to confirm the suppressed state on day 2 ofmenstrual cycle.

Clomiphene citrate 100mg daily till trigger day and gonadotropins were started according to BMI, Age, AFC, and previous cycle response if 2nd attempt and doses were adjusted according toresponse.

Ultrasound and estradiol monitoring were performed periodically (frequency based on patient response to medications) throughout the ICSI cycle.

(USG done by one of the 2 consultant in charge in our centre with a similar technique of measuring the mean maximum diameter in two dimensions of each follicle.)

When the lead follicle size between 21-23mm in group A, and lead follicle size 17-19mm in group B Ovitrelle trigger of 250 mcg was given and oocytes retrieved after 36hrs by transvaginal approach.

Once oocytes are retrieved they were stored in Vitromed culture media in tri gas incubator for 1-2hours, followed by chemical denudation and then mechanical denudation.

Oocyte morphology was assessed. Partner's sperm was collected on the day of oocyte retrieval and washed. ICSI was the insemination technique followed in our centre which was done 3-4hrs after oocyte retrieval. Oocytes inseminated by ICSI were transferred to Vitromed culture media. Embryo quality was assessed by Isthanbul consensus and embryos were frozen. Endometrium was prepared frozen transfer by HRT protocol, 2 day 3 embryos were transferred when ET was > 9mm.

Luteal support was given in all the patients in the form of micronized vaginal suppositories 400mg twice a day and oral dydrogesterone 10mg thrice a day till NT scan and oral estrogen was continued till viability scan then tapered and stopped.

Serum beta HCG test was done 2weeks from the progesterone start day and viability scan was done 4 weeks from the embryo transfer date.

Statistical analysis

Data analysis was performed using the statistics package for social sciences. (SPSS version 2.0). For normally distributed data, mean and standard deviation were used to describe data dispersion. Comparison between proportions were calculated using Chi Square test, with a 95% confidence interval. P value of < 0.05 was taken as significant.

Results

Table 1: Demographic and laboratory variables

Variable	Group A (N=83) Lead >20	GROUP B (N=159) Lead<20	P value
AGE	32.37±4.21	32.84±4.16	0.406
AFC	9.91±3.89	10.78±4.43	0.132
AMH	1.8±O.99	2.03±1.35	0.185
TRIGGER DAY E2	2693±141	2313±1140	0.214
ET THICKNESS	10.25±1.18	10.02±0.87	0.38
NO OF DAYS FOR ET PREPARATION ET CYCLES	14.91±6.09	13.38±3.2	0.21

Table 2: Outcome of treatment in group 1 and group 2

Variable	Group A (N=83) Lead >20	GROUP B (N=159) Lead<20	P value
Retrived	8.13±4.66	9.39±4.92	0.055
MII	6.32±4.34	6.91±3.89	0.283
Embryo	4.37±2.82	4.71±2.80	0.371

Table 3: Outcome of retrieved oocytes in group 1 and group 2

Variable	Group A (N=83) Lead >20	GROUP B (N=159) Lead<20	P value
% of MII from RETRIVED OOCYTES	73.09%±25.79	72.1%±22.25	0.753
% DAY 3 Embryo from MII	61.44%±34.67	60.73%±33.76	0.877

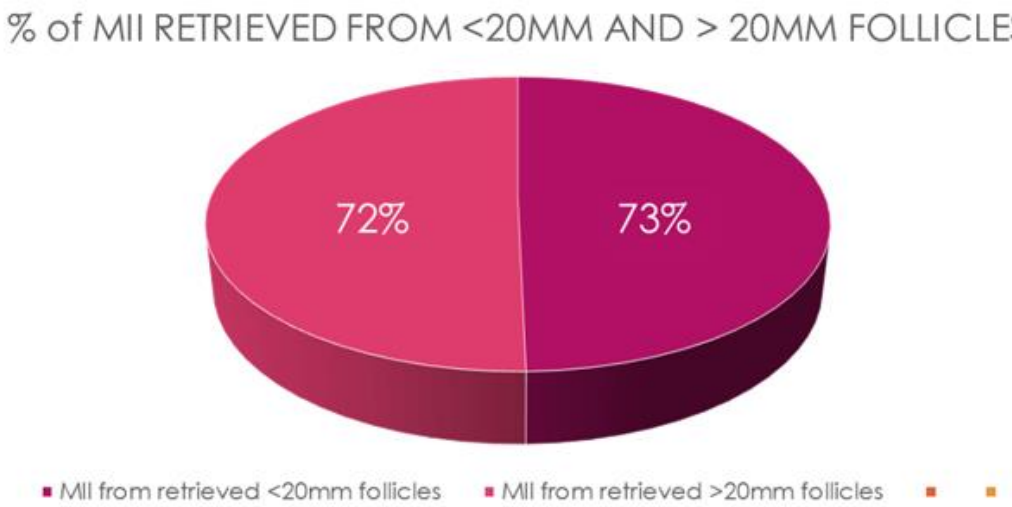

Fig 1

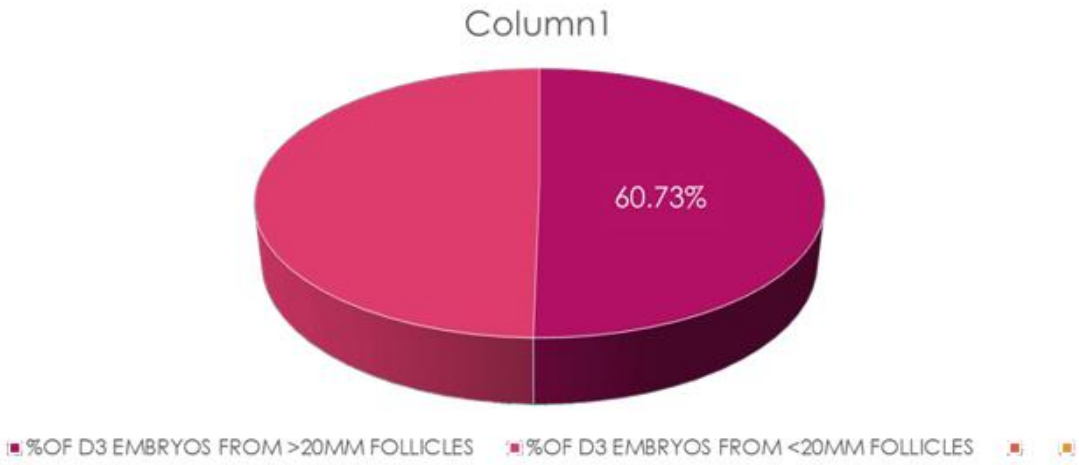

Fig 2: % Day 3 Embryo From MII Retrived

Table 4: Pregnancy outcome in Group 1 & Group 2

Variable	Group A (N=83) Lead >20	GROUP B (N=159) Lead<20	P value
BETA POSITIVE	44(61.1%)	82(61.2%)	0.989
IMPLANTATION RATE	51/144 (35.4%)	99/268 (36.9%)	0.831
CPR	40 (55%)	71 (52.9%)	0.773
LBR	40 (55%)	71 (52.9%)	0.773
Transfer was not done Group A 11 PTS 13.3% Group B 25 PTS 15.7% P-0.631			

Discussion

Jaime M. Knopman et al 2012 [9] had done a retrospective study To analyze the correlation between lead follicle size at HCG trigger and IVF outcome. Four groups were evaluated (<18 mm, 18 - 18.9 mm, 19 - 19.9 mm and ≥20 mm).Conclusions: Delaying the administration of Oocyte trigger to enhance follicular growth does not appear to improve IVF outcome. Larger lead follicles do not yield a higher percentage of mature oocytes, embryos available for transfer or LBR. In this study they had less no of oocytes retrieved from bigger size lead follicle than the smaller size lead follicle on the oocyte trigger day.

Why larger lead follicles not only failed to yield a higher percentage of mature oocytes but also resulted in a lower, albeit not significantly so, live birth rate is not clear. One plausible explanation can be offered by recent data; oocyte maturity is an intricate process that is not simply defined by the presence of the 2nd polar body. Rather, it requires "nuclear maturity" (defined by the presence of the 2nd polar body) as well as cytoplasmic maturity (defined by the appearance of the cumulus- corona complex and the cytoplasm) [10-13].

The completion of both events is necessary to the formation of an oocyte that is capable of developing into a competent embryo. Prolonged follicular stimulation may tamper with these processes damaging a cohort of oocytes and decreasing the likelihood of IVF success.

our study has compared the effect of follicular size on the no of oocyte retrieved , embryo quality, IR, CPR and LBR, in clomiphene citrate protocol who underwent 1 or 2nd attempt icsi with 2 Day 3 good quality embryos transferred after preparing the endometrium by HRT.

In our study also the no of oocytes retrieved from bigger lead follicle were significantly lesscompared to the smaller size lead follicle.

There was no significant difference in No of Mature Oocytes, Day 3 Good Embryos, Implantation Rate, Clinical Pregnancy Rate or Live Birth Rate.

So Ovulation trigger should not be postponed to achieve increased follicular size or improve outcomes in patients undergoing their IVF/ ICSI cycle. This practice increases patient cost and time without increasing the number of oocytes retrieved, the percentage of mature oocytes, the number of embryos available for transfer or the live birth rate.

Limitations

- Our Study was retrospective, in nature thereby limiting our ability to control forconfounding factors.
- Small sample size
- All were frozen embryo transfers
- No data regarding oocyte quality of lead follicle/each follicle.

Conclusion

Women who underwent ICSI with clomiphene citrate protocol with a lead > 20mm had less number of oocytes retrieved when compared to <20mm lead follicle on the trigger day and had similar number of mature oocyte , day 3 embryos, Implantation rate, Clinical Pregnancy Rate or Live Birth Rate. No beneficial effect by waiting for the lead follicle to reach beyond 20mm in size.

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