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Original Research Article

A retrospective study on analyzing the role of oocyte maturation arrest as a causative factor for infertility in unexplained infertility

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ABSTRACT

Background: Birth is often referred to as "the miracle of life" and very rightly so since successful pregnancy represents a complex, a highly coordinated succession of biological processes. In this regard, it is remarkable that pregnancy ever occurs at all. Infertility is a reproductive system disorder affecting 10.7% to 15.5% of couples. Highly heterogeneous in etiology. Investigating the cause is necessary for guiding treatment options. Aim of the study was to determine the percentage of immature oocytes retrieved at the metaphase 1 stage and germinal vesicle stage and its outcome.
Methods: Total 30 cases were included for this retrospective analysis of data done at ARC fertility center, Saveetha Medical College. This study analyzed the data of patients with unexplained infertility who underwent ICSI centers (affiliated to Saveetha University) meeting inclusion criteria and willing to participate in the study.
Results: The mean number of fertilized oocytes among those with more than 25% immature oocyte proportion was 1.18 while among those with less than 25% immature oocyte proportion was 4.13. The chance of fertilization of the oocyte increases when the percentage of immature oocyte was less than or equal to 25%. The association was found to be statistically significant with p-value <0.05.
Conclusions: Thus, some intrinsic oocyte factor could be involved in causing maturation arrest in such couples and resulting in multiple failed IVF cycles.so this oocyte factor could be a major cause in many cases of unexplained infertility.

Keywords: Estrodial level, Immature oocytes, Invitro fertilization, Unexplained fertility

INTRODUCTION

Infertility is a reproductive system disorder affecting 10.7% to 15.5% of couples. Highly heterogeneous in etiology. Investigating the cause is necessary for guiding treatment options. Additionally, the molecular understanding of infertility has the potential to reveal fundamental insight into human reproduction. In human ovary, each fully grown oocyte resumes maturation in response to gonadotropins. This process is completed after the oocyte reaches the M2 stage.[1] Normally in conventional protocols, by the time of retrieval, most of the oocytes will complete their maturation and are collected at the metaphase 2 stage. It is common for few oocytes to remain immature, despite ovarian stimulation and HCG administration. In stimulated IVF cycles, up to 25% to 30% recovered oocyte are immature.[2] In the fetal ovary, oocytes in the primordial follicle initiate meiosis, and pause at prophase I. Until puberty, the oocytes begin the maturation process and resume meiosis in response to

a surge of luteinizing hormone (LH), with germinal vesicle breakdown followed by spindle assembly, chromosome migration, asymmetric division, and extrusion of the first polar body. At this point, the oocytes are mature and arrest at metaphase II until fertilization.[3] During ovulation, the basement membrane of the follicle ruptures releasing a mature oocyte equipped for fertilization. The remaining granulosa and theca cells differentiate into the corpus luteum, which produces progesterone for pregnancy maintenance, or undergoes luteolysis. Meanwhile, in the preovulatory follicle, the oocyte undergoes germinal vesicle breakdown (GVBD) and entry into the first meiotic division (MI) during which chromosomes attach to the meiotic spindle and line up on the metaphase plate. The reduced yield of the immature oocyte is another important aspect in stimulated IVF cycles.[4] Because they have low maturation capacity and seldom yield transferable embryos. When the percentage of the incompetent oocyte is more than 25%, most of the IVF outcome will markedly reduce. In patients who have failed to conceive following many IVF attempts, oocyte maturation arrest appears to arise from intrinsic oocyte abnormality rather than due to sporadic events of abnormal response to ovarian stimulation or poor culture conditions in a certain cycle.[5] High incidents of immature oocytes absorbed in patients with normozoospermia partners and low fertilization rate in previous cycles suggest that fertilization failure and infertility can be due to oocyte factor rather than other factors.[6]

METHODS

Totally 30 cases were included for this retrospective analysis of data done at ARC fertility center, Saveetha medical college in the year 2019. The duration was 4 months from January to April This study analyzed the data of patients with unexplained infertility who underwent ICSI centers (affiliated to Saveetha University) meeting inclusion criteria and willing to participate in the study.

Inclusion criteria

- Patients with 21 to 40 years. Patients with unexplained infertility with more than 10 years of marriage.

Exclusion criteria

- Patients aged more than 40 years. Low ovarian reserve. Patients with uncorrected uterine and adnexal pathology (ex: hydrosalpinx, sub mucous polyps, myomas, and previous difficult ET) smoking and PCOS.

Patient data, including maternal age, height, body mass index (BMI), weight, a period of infertility, and previous embryo transfer attempts were collected from the patient's database file. Variables like age, BMI, previous embryo transfer failures, the total number of oocytes retrieved, number of metaphase 1 oocyte, and number of metaphases 2 also several oocytes at the germinal vesicle stage were all taken into consideration and the data was analysed. All the patients underwent a thorough infertility investigation without any abnormal findings identified. All female patients (25-40) had regular ovulatory cycles and failed to conceive after at least six cycles of ovulation induction. All the patients were in good general health, were non-smokers and none of them reported a family history of infertility. None of them were exposed to any medications for other medical conditions during or just before IVF and embryo transfer, and a thorough evaluation of possible past exposure to environmental and occupational toxicants excessive X-ray irradiation yielded negative results. All the patients exhibited a normal female karyotype (46xx). All of them had normal day 3 FSH levels (3.5-11 iu/l) and a normal response to both ovulation induction of ovulation and ovarian stimulation. All the patients underwent controlled ovarian hyper stimulation with recombinant FSH was started from day 2 or day 3 of the cycle after confirming the pituitary down-regulation, i.e. serum oestradiol level less than 50 pg/mL, serum progesterone level less than 0.9 ng/mL, serum luteinizing hormone (LH) level less than 5 IU/L, endometrial thickness less than 5 mm and no antral follicle larger than 10 mm in each ovary. The starting dose of recombinant FSH was based on the patient's age, body mass index (BMI), antral follicular count (AFC), and serum anti-mullerian hormone (AMH) level. Inj. cetrorelix 0.25 mg was started subcutaneously from day 6 of the cycle. Follicular growth was assessed by serial transvaginal and serum E2, LH, and P4 levels, and recombinant FSH doses were adjusted accordingly. Ultrasound monitoring of follicular growth was done on day 6, day 8, day 10, and then daily till trigger. Serum oestradiol was measured on day 6, day 8, and day10 and before the trigger. Serum LH was measured on day 6, day 8, and day10. And Inj. cetrorelix was continued till the day of the trigger. Trigger was planned when 2 or more follicles reach 18-22 mm size. For ovulation trigger, the patients with serum oestradiol level between 3001-5000 pg/dL were received recombinant HCG 250 mg subcutaneously and the when the levels are more than 5000 pg/dl received triptorelin acetate 0.2 mg, a GnRH agonist subcutaneously as ovulation trigger. And the serum progesterone level was measured 24 hours after the trigger.

Statistical analysis

The study participants were randomized into either GnRH analog or recombinant HCG group by SPSS generated random number. Data entry was made in the Microsoft excel software in codes and analysis was done with an SPSS-20 computer package. Categorical variables are expressed as percentages whereas continuous variables are expressed as mean ± standard deviation. Association between the categorical variable was found by the chi-square test and the relationship

between the continuous variable was assessed by student's t-test. p-value <0.05 was considered as statistically significant.

RESULTS

Table 1, shows about 46.7% of the study participants were in the age group between 35 and 40 years. The mean age was 34.03±3.86 years.

Table 1: distribution of study participants according to age.

Age (in years)	Frequency	Percentage
≤ 30	5	16.7%
30-35	11	36.7%
35-40	14	46.7%
Total	30	100.0%

Table 2, about half of the study participants had infertility for 6 to 9 years. The mean duration of infertility was 9.37±2.87 years. Table 3, 53.3% of the study participants had serum oestradiol level of more than 4500 on the trigger day. Table 4, in about sixteen (53.3%) of the study participants had several oocytes retrieved between 6 to 10 oocytes.

Table 2: Distribution according to duration of infertility.

Duration of infertility (in years)	Frequency	Percentage
6-9	15	50.0%
9-12	9	30.0%
12-15	4	13.3%
15-18	2	6.7%
Total	30	100.0%

Table 3: Distribution according to serum oestradiol level on trigger day.

Serum oestradiol level on trigger day	Frequency	Percentage
≤3000	10	33.3%
3001-4500	4	13.3%
4501-6000	10	33.3%
>6000	6	20.0%
Total	30	100.0%

Table 4: Distribution according to the total number of oocytes retrieved.

A total number of oocytes retrieved	Frequency	Percentage
≤5	3	10%
6-10	16	53.3%
>10	11	36.7%
Total	30	100.0%

Table 5: Distribution according to the number of retrieved oocytes in M1 stage.

The number of oocytes in the M1 stage	Frequency	Percentage
0	1	3.3%
1	3	10.0%
2	11	36.7%
3	7	23.3%
4	4	13.3%
5	3	10.0%
7	1	3.3%
Total	30	100.0%

Table 5, shows in about 60% of the study participants around 2 to 3 M1 stage oocytes were present.

Table 6: Distribution according to the number of oocytes in M2 stage.

Number of oocytes in M2 stage	Frequency	Percentage
Nil	3	10%
1-5	16	53.3%
6-10	11	36.7%
Total	30	100.0%

Table 6, 53.3% of the study participants had several retrieved oocytes in the M2 stage between 1 to 5.

Table 7: Distribution according to the number of embryos fertilised.

Number of embryos fertilized	Frequency	Percentage
1	9	30.0%
2	4	13.3%
3	2	6.7%
5	6	20.0%
6	1	3.3%
Nil	8	26.6%
Total	30	100.0%

Table 7, 73.4% of the study participants had at least one embryo fertilized and about 23.3% had at least 5 embryos successfully fertilized. Table 8, the serum oestradiol level among those were more than 25% retrieved oocytes were immature was 4585 and among those with less than 25% was 4106. Both groups were found to be similar concerning serum oestradiol levels.

Table 9, among the study participants with more than 25% immature retrieved oocyte, 71.4% had poor quality of embryos and among those with less than or equal to 25% immature retrieved oocytes, 62.5% had average quality embryo and 37.5% had good quality embryo. The quality of embryo was improved when the percentage of immature oocytes retrieved was more than 25%.

Table 8: Association between percentage of immature oocytes and serum oestradiol level.

Percentage of immature oocytes retrieved	N	Serum oestradiol level		t	df	p-value
		Mean	Standard deviation			
>25%	22	4585.091	1863.2814	0.840	20.420	0.411
≤25%	8	4106.625	1155.1239			

Table 9: Association between percentage of immature oocytes and quality of embryos.

Quality of embryos	>25%		≤25%		Total	
Poor	10	71.4%	0	0%	10	33.3%
Poor to average	1	7.1%	0	0%	1	3.3%
Average	1	7.1%	5	62.5%	6	20.0%
Average to good	2	14.3%	0	0%	2	6.7%
Good	0	0%	3	37.5%	3	10.0%
Total	14	63.6%	8	36.4%	30	100.0%

X^2 - 18.39 df - 4 p-value - 0.001.

DISCUSSION

The IVF process is complex and often requires numerous treatment cycles. Each IVF treatment cycle begins with the female partner receiving hormonal stimulation to produce multiple mature oocytes, which upon maturation are retrieved by transvaginal oocyte retrieval.[7] Also, the development of micromanipulation, which permits the scientist to inject a single spermatozoon into an oocyte, improved ART outcomes for couples with male factor infertility. Furthermore, the development of sequential media for embryo culture in vitro now allows embryos to be cultured to the later blastocyst stage, enabling the assessment of the developmental potential of the embryos before embryo transfer, thus improving the chances of successful outcomes.[8] Autologous IVF treatment cycles commenced (fresh and frozen embryo transfers), 91.5% of the fresh cycles resulted in oocyte retrieval, 84.5% in fertilization in vitro and embryo transfer, resulting in a clinical pregnancy rate of 22.7% and a live birth rate of 17.2% per treatment cycle commenced.[9] These outcomes highlight the fact that not all IVF treatment cycles result in successful oocyte retrieval, fertilization or embryo development and therefore, the return of embryos to the female partner does not occur for all couples.[10]

In this study 53.3% of the study participants had a serum estradiol level of more than 4500 on the trigger day. In 50% of the study, participants were given agonist as a trigger and another 50% was given HCG as a trigger.

About sixteen (53.3%) of the study participants had several oocytes retrieved between 6 to 10 oocytes. 36.7% of the study participants had two oocytes in the GV stage and 23.3% had three oocytes in the GV stage. In both, the groups with <25% immature oocytes and more than 25% immature oocytes were 9.36 and 9.37, respectively. No difference was found between the groups concerning the duration of infertility.[11]

In these findings the serum estradiol level among those was more than 25% retrieved oocytes were immature was 4585 and among those with less than 25% was 4106. Both groups were found to be similar concerning serum estradiol levels.[12] The mean number of fertilized oocytes among those with more than 25% immature oocyte proportion was 1.18 while among those with less than 25% immature oocyte proportion was 4.13. The chance of fertilization of the oocyte increases when the percentage of immature oocyte was less than or equal to 25%. The association was found to be statistically significant with p-value <0.05.[13] Among the study participants with more than 25% immature retrieved oocyte, 71.4% had poor quality of embryos and among those with less than or equal to 25% immature retrieved oocytes, 62.5% had average quality embryo and 37.5% had good quality embryo.[14] The quality of embryo was improved when the percentage of immature oocytes retrieved was more than 25%. In most couples with unexplained infertility undergoing IVF cycles, there is an increased incidence of immature oocytes (M1 and GV stage) being retrieved resulting in failed IVF cycles because of very low fertilization and poor quality of the oocytes.[15] The mean number of fertilized oocytes among those with more than 25% immature oocyte proportion was 1.18 while among those with less than 25% immature oocyte proportion was 4.13. The chance of fertilization of the oocyte increases when the percentage of immature oocyte was less than or equal to 25%. The association was found to be statistically significant with p-value <0.05.[16]

CONCLUSION

The arrest may be detected not only when oocytes mature but it may occur even after they are successfully fertilized (Balczon et al, Wu et al). Authors intend that this paper should open a further discussion on this topic and will demonstrate to clinicians that this problem is well known

and complex. Neither was it authors intention to indicate in detail the possible approaches, which may solve at least some of the complications described (i.e. cytoplasmic transfer, germinal vesicle replace- ment, metaphase spindle transfer). When these approaches are eventually used, new issues like the `heteroplasmy' and `epigenetic modications' must be considered (De Rycke et al; Hawes et al; St John). Authors hope that future discoveries of the molecular underpinning of hu- man infertility will inform and advance new therapeutic strategies. It was estimated that approximately 0.1% to 1% of women may have defective oocyte maturation, whether absolute or qualitative. The descriptive framework provided here serves to highlight the serious nature of the syndrome of oocyte maturation failure. Levran (60) reported that five of the eight patients with oocyte maturation failure underwent a donor-oocyte cycle with husband's sperm, with four patients achieving pregnancy. At present, use of donor oocytes is the only option available for women with oocyte maturation failure if the defect is profound.

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