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Original Research Article

Oocyte central granularity and its relation to pregnancy outcome following intracytoplasmic sperm injection-freeze-all cycles

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ABSTRACT

Background: Oocyte quality is instrumental in the development of early embryos and may account for the difference in the rates of fertilization, cleavage, implantation and pregnancy outcome. Centrally located cytoplasmic granularity is a rare morphological feature that can be observed in certain cases. We conducted this study with the sole aim to compare the pregnancy outcomes from embryo derived exclusively from oocytes with dense central granularity with embryos derived from oocytes with normal morphology in intracytoplasmic sperm injection (ICSI)-freeze- all cycles.
Methods: A retrospective case control study performed during a period of 1 year and 10 months from Jan 2018-Oct 2019 at craft hospital and research centre India; a tertiary heath care centre. The 62 infertile women undergoing ICSI FET cycle with oocyte cohort having all central granularity oocytes were included as study participants after fulfilling the inclusion and exclusion criteria. The 200 women undergoing ICSI- frozen embryo transfer (FET with normal morphology oocyte were taken. Implantation rate and clinical pregnancy rate were evaluated as primary outcomes. Ongoing pregnancy rate, live birth rate, fertilization rate and top-quality embryo were taken as secondary outcomes.
Results: The implantation rate and clinical pregnancy rate was 21.48% and 40.33% in study group compared to 35.012% and 58.5% respectively in control group which was statistically significant (p=0.002; 0.012 respectively).
Conclusions: Presence of dense central granularity in the oocyte can be used to predict poor ART outcome in terms of low implantation and clinical pregnancy rates.

Keywords: ICSI, FET, Oocyte dense central granularity, Pregnancy outcomes

INTRODUCTION

Selecting the most viable embryos for transfer is a challenge in assisted reproduction. It has been reported that embryo viability also correlates with oocyte morphology. It is important to choose the high-quality oocytes for *in vitro* fertilization (IVF) or ICSI in assisted reproduction. Introduction of ICSI technique offers a good opportunity to evaluate denuded oocytes before fertilization and analyses the correlation between oocyte morphology and embryo viability. Oocyte quality is a important prognostic marker as the nuclear and cytoplasmic maturity of the oocyte may be directly related to success rate of ICSI.

Studies analyzing morphological characteristics of oocytes report that good quality oocytes should have an intact first polar body, a clear, moderately granular cytoplasm, a small perivitelline space and a colourless zona pellucida.[1-4] It is seen that more than 50% of collected oocytes after controlled ovarian stimulation (COS) present one or more anomalies.[5,6] Cytoplasmic abnormalities include dark cytoplasm, dark incorporations, spots, granules, refractile bodies and vacuoles. In general, the cytoplasm of normal oocytes should exhibit uniform fine granules due to the presence of various organelles in the cytoplasm.[3,4] However, granularity or granulation at the center of the cytoplasm is considered abnormal and is diagnosed as a large dark, spongy granular area.[7] Cytoplasmic granularity

of oocyte can be homogenous or centrally located, and slight or severe. The severity of granularity is based on the diameter of the granular area and the depth of the lesion. Moreover, there is dearth of data in literature between oocyte morphology and pregnancy rates. Hence, we conducted a study to compare the pregnancy outcomes from embryo derived exclusively from oocytes with dense central granularity with the embryo derived from oocytes with normal morphology.

METHODS

It was a retrospective study design carried out at craft hospital and research centre, Kerala over a period of 22 months from January 2018 to October 2019. The study protocol was reviewed and authorized by the institutional ethics committee. After fulfilling the inclusion and exclusion criteria study participants and controls were selected. Study participants included women with oocytes showing dense central granularity in all of the oocytes in a cohort (n=62). Control group included women with all normal morphology oocytes (n=200).

The primary outcome was implantation rate (defined as the percentage of embryos which successfully undergo implantation compared to the number of embryos transferred in a given period) and clinical pregnancy rate (defined as the evidence of intrauterine gestational sac with cardiac activity of the foetus at around 6-8 weeks' gestation). The secondary outcomes were fertilization rate, top quality embryo, live birth rate (defined as the delivery of a live-born >24 weeks of gestation), ongoing pregnancy rate (defined as the number of pregnancies that have completed ≥20 weeks of gestation) and miscarriage rate (defined as the proportion of women with pregnancy loss before 20 weeks of gestation) per embryo transfer cycle.

Inclusion criteria

For the study included women with age ≤40 years, primary or secondary infertility, mild male factor infertility and ICSI-FET cycles.

Exclusion criteria

Consisted of women with age more than 40 year age, patients with coexisting oocyte morphological defects like PVS debris and SER , uterine cavity anomalies, fibroid, endometriosis or hydrosalpinx , infertile women with poor ovarian response having less than/ equal to 3 oocytes retrieved, severe male factor infertility required surgical sperm retrieval, patients with karyotype abnormalities requiring PGD, women undergoing FET with thin endometrium <8 mm, recurrent implantation failure cases.

Patient selection

Based on clinical profile, controlled ovarian hyperstimulation and gonadotropin dose were decided and adjusted according to follicular growth and estradiol levels. Ovulation trigger given when more than 2 follicles reached 18 mm with either recombinant hCG or agonist and oocyte pick up was done 36-38 hours post trigger. Cumulus oocyte complex (COC) was kept in culture media in trigas incubator for next 1-2 hours, followed by denudation. Oocyte morphology was assessed under 200x magnification in an inverted microscope. Oocytes with dense granularity were identified according to diameter of granular area and depth of lesion. An oocyte with >50% cytoplasm affected by granulation, exhibiting a crater-like appearance was classified as dense central granularity.

ICSI was the insemination technique. Day 3 embryo quality was assessed by the Lucinda Veeck classification. For FET endometrium was prepared by a suitable protocol. Day 3 frozen embryo transfer was done with 2-3 good quality embryo with an optimal endometrium thickness of ≥8 mm Luteal phase support with micronized progesterone 400 mg vaginally twice a day and dydrogesterone 10mg orally thrice a day along with the estradiol valerate oral or vaginal tablets was given till Beta hCG results. Luteal phase support was continued till 10 weeks if beta hCG positive. Transvaginal ultrasound was performed 4-5 weeks after the embryo transfer to localize intrauterine gestational sac. The clinical pregnancy was confirmed after the intrauterine pregnancy sac was seen.

Statistical analysis

Data analysis was performed using the statistics package for social sciences (SPSS 20.0). For normally distributed data, mean and standard deviation were used to describe data location and dispersion. Comparison between case and controls were evaluated by Man-Whitney U test and Chi-square test with 95% confidence interval.

RESULTS

The basal parameters are given Table 1 (below) without any statistical difference between cases and controls.

Table 1: Baseline parameters amongst cases and controls.

Variables	Case, (n=62)	Control, (n=200)	P value
Age (Years)	31.41± 6.47	31.77± 4.66	0.637
AMH	4.10± 3.58	5.14± 3.78	0.519
Stimulation days	8.14± 0.94	8.04± 1.45	0.574
Estradiol on trigger day	2439.18± 1202.97	2542.650± 1261.80	0.566
Gonadotrpin total dose	1915.14± 544.23	1896.9± 284.82	0.918
No. of embryo transferred	2.21	2.14± 0.41	0.338
Endometrial thickness	9.57± 5.74	10.23± 7.66	0.419

In the above comparison of baseline characteristics, we find no significant difference in the terms of age, AMH. number of stimulation days or number of embryos transferred amongst the case and the control group.

Table 2: Comparison of primary pregnancy outcome in cases and controls.

Primary outcomes	Case, (n=62) (%)	Control, (n=200) (%)	P value
Implantation rate per cycle	21.48	35.012	0.002
Clinical pregnancy rate per cycle	40.33	58.5	0.012

The implantation rate per cycle in dense central granularity is 21.48% which is significantly lower than in women with normal morphology oocytes. The clinical pregnancy rate is also significantly lower in the case cohort as compared to the control oocyte cohort.

Table 3: Comparison of secondary pregnancy outcomes between cases and controls.

Variables	Case, (n=62) (%)	Control, (n=200) (%)	P value
Ongoing pregnancy rate	25.8	26	0.975
Miscarriage rate	3.2	3.5	0.388
Live birth rate per cycle	11.29	29	0.004
Fertilization rate	76±21	81±22	0.27
Top quality embryo	61±26	62±20	0.74

The live birth rate with embryos derived from dense granularity oocytes is 11.29% which is significantly lower than in the controls (29%). However, the fertilization rate and top embryo quality doesn't show any significant difference in both the groups.

DISCUSSION

Serhal et al firstly defined centrally located cytoplasmic granulation (CLCG) as the central region of the oocyte cytoplasm appeared to be denser than other regions, forming a clear separation, and considered to have an adverse effect on embryonic development.[7] Fancsovits et al and Sun et al considered the occurrence of CLCG might be related to patient's age and gonadotropin stimulation.[8,9] The use of gonadotropin during ovarian hyper stimulation (COH) in ART destroys the developmental regulation and synchronization of oocytes, mainly the nuclear and cytoplasm maturity are not synchronized. However, the most plausible hypothesis that could explain CLCG dysmorphism is cytoplasmic immaturity which could be responsible for embryonic aneuploidy in 52-57%.[10]

Previous studies done by Fancsovits et al, Kahraman et al, Merviel et al and Serhel et al have shown significant lower implantation rate, clinical pregnancy rate and LBR in patients with embryo derived from dense central granularity oocytes compared to women with the normal morphology oocytes.[7,8,10,11] Thus proving that the CLCG oocytes had a negative impact on embryonic development. Esfandiari et al and Xiao et al found similar fertilization rate, embryonic development potential and pregnancy outcome in patients with CLCG oocytes and those with normal cytoplasm.[12,13]

Cytoplasmic granularity can be homogenous affecting whole cytoplasm, or concentrated in the center with a clear peripheral ring giving a darkened appearance to the cytoplasm. There are conflicting reports on the relationship between the oocyte morphology and fertilization rates or implantation rates.[2] According to De Sutter et al oocyte morphology is believed to be insignificant in terms of fertilization, embryo quality or clinical pregnancy rates.[2] Cytoplasmic granulation may be a poor prognostic factor because it may be a sign of cytoplasmic immaturity. It is believed that the cause of the cytoplasmic morphological abnormalities it is actually multifactorial. According to Fancsovits et al and Sun et al the occurrence of CLCG might be related to patient's age and gonadotropin stimulation.[8,9] Ovulation induction may result in maturation of abnormal oocytes that would otherwise become atretic in the absence of stimulation.[13] The role of stimulation protocols could also be another reason postulated for cytolpasmic granularity.[8] As per Fancsovits et al study, the type of gonadotrophin used for ovarian stimulation had no effect on the occurrence of small or large cytoplasmic granules. Within FSH stimulation cycles, r-FSH resulted in a higher number of oocytes with more than 3 small granules, and less oocytes with large granules, as compared with u-FSH cycles.[8] The most accepted hypothesis till date that could explain CLCG dysmorphism is cytoplasmic immaturity which could be responsible for embryonic aneuploidy in 52-57%.[10] This was also supported by Sun et al study, Van Blerkom and Henry et al.[9,13] Moreover, the negative impact on embryo viability and low clinical pregnancy rates may be explained by the higher rate of aneuploidy in dysmorphic oocytes. The resulting embryos thus fail to demonstrate the same implantation potential as those derived from oocytes with normal morphology. A few studies also suggest that some oocyte anomalies may also related to epigenetic defects in the offspring.[14,15]

In our study we established the role of dense central granularity on implantation and clinical pregnancy. In our study significant lower implantation rate, clinical pregnancy rate and LBR is noted in patients with embryo derived from dense central granularity oocytes compared to women with the normal morphology oocytes. Our finding is in accordance with Fancsovits et al, Kahraman et al, Merviel et al, Serhel et al and Ebner et al.[6,8,10,11] However, the fertilization rate and the number of top-quality embryo didn't show significant difference between

cases and controls. Xiao et al found similar fertilization rate, high quality embryo rate and clinical pregnancy outcome in patients with CLCG oocytes and those with normal cytoplasm.[16] We can explain this by considering the possibility that the embryo transplanted by CLCG oocytes were not necessarily the ones from CLCG oocytes as these studies evaluated a mixed oocyte cohort and hence the results were not conclusive.

Our study has a few strengths when compared to previous studies on central granularity oocytes. This study is focused specifically on embryos derived from oocyte cohort having all oocytes with dense central granularity unlike the previous studies where only a particular percentage of the oocyte cohort was affected with dense central granularity. However, there are certain limitations too. It is a retrospective study design and the sample size is small. It doesn't assess the cumulative pregnancy rate. Lastly, it is not evident whether the cause effect relation between all oocytes with oocyte dense central granularity can be extrapolated to the population where only few oocytes may have dense central granularity. But larger studies need to be conducted for further evidence.

CONCLUSION

Presence of dense central granularity in the oocyte can be used to predict poor ART outcome in terms of low implantation rates, clinical pregnancy rates and live birth rates. Thus, it can be used as a marker of low success rates in IVF/ICSI. As far as we know it is specifically on the embryo derived exclusively from dense granularity oocyte cohort unlike previous studies that evaluated pregnancy outcomes mixed oocyte cohort.

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