International Journal of Scientific Research in Biological Sciences
Vol.8, Issue.4, pp.42-47, August (2021)
Research Paper
E-ISSN: 2347-7520

Effect of oocyte with CLCG on embryo development and implantation rates after ICSI in Freeze-Thaw Embryo Transfer Cycles

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Available online at: www.isroset.org

Received: 05/Jun/2021, Accepted: 20/Jul/2021, Online: 31/Aug/2021

Abstract—Introduction: Oocyte quality plays a major role in understanding the fertility potential of the female, which is primarily reflected by oocytes and their morphological features. A type of cytoplasmic abnormality exhibited by oocytes that can affect the oocyte quality is Centrally located cytoplasmic granulation (CLCG). Morphological variations of the oocyte may result due to different factors.
Aim: To evaluate the consequences of CLCG on embryo development and implantation rates after ICSI in Freeze-Thaw Cycles.
Methods: It was a retrospective study. A total of 418 day3 and 157 day5 patients who had undergone frozen-thawed cycles were recruited. Patients who had embryo transfer with CLCG were grouped as Test group (n = 100, n=90 day3 and n=38, n=42 day5. As per age <30 and age >31) and patients who had embryo transfer with normal oocytes morphology were grouped as the control group (n = 125, n=103 day3 and n=33, n=44 day5. As per age <30 and age >31) respectively.
Results: Among the CLCG and the control group, there was no significant difference observed in the patient's age, FSH, LH, and AMH. However, the maturation and fertilization rate among the patients age >31(P <0.05) years had a significant difference, and we also inferred that the CLCG did not affect the embryo development and implantation rate (P >0.05) in both groups respectively (age <30 and age >31).
Conclusion: These results suggested CLCG might be a normal morphology of oocytes. There was no significant difference in embryo development patients with or without CLCG. The patients who freeze-thawed transferred CLCG embryos had a successful outcome.

Keywords—Cytoplasmic granularity, Embryo Quality, Embryo Transfer. ICSI outcomes.

I. INTRODUCTION

Assisted Reproductive Technology (ART) is the mainspring used to treat infertility patients, the main goal of ART is to provide safe, efficient, and affordable care to optimize the chance of having pregnancy. In-vitro fertilization (IVF), Intracytoplasmic sperm injection (ICSI), and Embryo Transfer (ET) are primary techniques in ART which has to give the most valuable results in the field of infertility. It is important to choose high-quality oocytes for IVF, ICSI in assisted reproduction. Oocyte morphology like cumulus-oocyte complex (COCs), Zona thickness, Polar body (PB), Cytoplasm, and also some intrinsic markers and extrinsic markers have been used as good indicators of oocyte quality[1].

In general, gamete quality plays a major role to treat infertility patients, the cytoplasm of normal oocytes should exhibit uniform granules due to the presence of various organelles. However, granulation at the center of the cytoplasm is regarded as abnormal. Centrally located cytoplasmic granulation (CLCG) is within the center with a clear border that is easily distinguished by a darker appearance which is denser than the other region forming a clear separation, and consider may have an adverse effect on embryonic development. The mechanism of granulation occurrence is still unclear. However, depends upon the patient's age the abnormal cytoplasmic granules may manifest the cytoplasmic immature, and also be associated with chromosomal abnormalities, leading to CLCG.

Serhal et al. (1997) reported normal fertilization and embryonic development in oocytes with abnormal cytoplasmic morphology either homogeneously or centrally localized, but the resulting embryos failed to demonstrate the same implantation potential as those derived from oocytes with normal cytoplasma[10].

Our study is to assess the oocyte quality that may directly influence the cytoplasmic morphology on the success rate of ICSI outcomes of patient's exhibiting CLCG in Freeze-Thaw Embryo Transfer Cycles, and also the data in the

literature studied the factors that affect the oocyte morphology on embryonic development and implantation rate in different age groups.

II. RELATED WORK

The purpose of this study is to understand the implication of CLCG on embryo development and implantation rate after ICSI In Freeze-Thaw Embryo Transfer Cycles.

III. METHODOLOGY

Study Design
This is a retrospective controlled study conducted in a private IVF setup.

Inclusion Criteria
Patients like poor ovarian reserve (POR), tubal factor, polycystic ovarian syndrome (PCOS), endometriosis, unexplained infertility, normozoospermia, and teratozoospermia were included during this study. Consent has been taken from the recruited patients.

Exclusion Criteria
Patients with Fresh embryo transfer, severe oligospermia, azoospermia, or undergone any surgical sperm retrieval (TESA, PESA, TESE, Micro TESA) were excluded.

Ovarian stimulation
The stimulation protocol was followed by downregulation with Gonadotropin-releasing hormone (GnRH) antagonist depending on the patient's age, and BMI. A recombinant menopur was given to the patients for 10-11 days until the size of the follicles reaches 12-14mm. The follicular development was continuously monitored by ultrasound sonography (USG) along with estradiol (E2) levels in the blood. Once the follicles reach the size of 18-20mm, the human chorionic gonadotropin (HCG) was administered. Oocytes retrieval was performed 34-36hrs of HCG trigger under general anaesthesia with the help of transvaginal sonography (TVS) by suction.

Semen preparation
Progressive motile sperm for fertilization were isolated either "swim-up" or two-layer density gradient method, according to the quality of the native semen sample. Sperm motility and concentration were assessed according to World Health Organization criteria before and after preparation. The timing between the sperm preparation and the start of ICSI was comprised of between 30 - 60 minutes.

Oocyte preparation
Follicular fluid was screened and the COCs is pre-washed in equilibrated (37°C) buffer media (G-MOPS, Vitrolife, Sweden), and oocytes were incubated in pre-equilibrated (37°C, 6% CO_2, and 5% O_2) dish containing supplemented fertilization media (G-IVF,Vitrolife,Sweden). The retrieved oocytes were incubated for 2-3 hours.
The cumulus cells were removed using Hyaluronidase (Hyalase,SAGE) at the concentration of 50% (500 µl) of fertilization media and 50% (500 µl) of Hyaluronidase. Denuded oocytes were examined under an inverted microscope at a magnification of x200. Only morphologically mature oocytes were microinjected. Before sperm injection, the cytoplasmic granularity of the oocytes were assessed individually.

ICSI procedure
The microtools required for ICSI and the ICSI procedure itself have been described in detail elsewhere (Van Steirteghem et al., 1995)[8]. Oocytes were clutch by a holding pipette with the polar body at the 6 or 12o'clock position and the injection pipette was inserted at the 3o'clock position. Only motile spermatozoa that appeared morphologically normal were injected. post injection, oocytes were cultured in cleavage media (G-1, Vitrolife, Sweden).

Assessment of Fertilization and Embryo Development.
The fertilization assessment was performed 16-18hrs of post ICSI. by assessing the number of polar bodies and pronuclei. The fertilization rate was outlined as the ratio between the number of diploid zygotes and the number of mature oocytes and embryo cleavage was evaluated after a 46±2hrs culture. Embryo development assessment was performed on day3 including blastomeres number, size, and the percentage of fragmentation. Grade A embryos consist of symmetrical blastomeres of equal size with zero fragmentation. Grade AB embryos had blastomeres of similar size with less than 10% fragmentation covering the embryo surface. Grade B embryos had blastomeres of distinctly unequal sizes and variable fragmentation. Grade C embryos had blastomeres of non-identical size, and severe fragmentation covering greater than 50% of the volume of embryos, and day5 embryo development assessment include the grade of inner cell mass (ICM) and the trophectoderm (TE).

On day3 and day5, the embryo assessment was performed, and embryos were divided as per the grading system (Istanbul consensus), and embryos were cryopreserved with the vitrification method (Kitazato BioPharm, Tokyo, Japan) in cryolocks.

Embryo Transfer
For the FET cycle, the endometrium was prepared by giving supplements and once the endometrium reaches the adequate trilaminar stage (6mm-10mm) monitored by Doppler imaging, the FET was planned.

On the day of transfer, the embryos were thawed by using a kitazato thawing kit (Kitazato BioPharm, Tokyo, Japan). Embryos were incubated for 2hrs post-thaw to check the survival. The embryos were cultured and transferred in G2 plus (G-2,Vitrolife,Sweden) medium, and a Minimum of two or three grades A/B day3 embryos

and a minimum of two day5 embryos were transferred to the patient uterus with the help of cook embryo transfer catheter (Sydney IVF embryo transfer set, cook, Bloomingtom, IN, USA). The whole procedure was monitored by transabdominal ultrasound guidance.

Outcome Measures

The pregnancy was confirmed based on serum Beta hCG levels after Fourteen days of embryo transfer.

The pregnancy rate was calculated on the basis of serum Beta hCG levels (>30 mIU/mL). The implantation of embryos was confirmed after 2weeks of beta hCG positive results by observing gestational sacs in the uterine cavity with the help of transabdominal ultrasonography.

Statistical Analysis

The statistical analysis was performed by using SPSS Statistics Software (SPSS Inc, Chicago, IL, USA). Variables are presented in percentages, mean (M)±standard deviation (SD). The chi-square test was performed to analyze the outcome, the differences were considered significant at P <0.05.

IV. RESULTS AND DISCUSSION

Comparison of Baseline clinical data between two groups of patients: It was found that age might affect the results during the analysis of the clinical data, so the periodic patients were divided by age of <30 and age >31 or Above (Table1). However, there was no significant difference in patient's age, follicle stimulating hormone (FSH) levels, luteinzing hormone (LH) levels, Anti Mullerian Hormone (AMH), and average stimulation ovulation days between the control and experimental group.

Effect of ICSI outcomes on embryonic development potential: There was no significant difference between the control and experimental group in Maturation rate (71.8% vs. 80.4%, *p*=0.127), fertilization rate (80.8% vs. 85.8%, *p*=0.938), and cleavage rate (91.0% vs. 86.6%, p=0.312) with patient's under age of <30 and There was a significant difference seen in patients with age >31 or Above between the control and experimental group in Maturation rate (75.8% vs. 86.5%, *p*=0.003), and fertilization rate (88.0% vs. 90.8%, *p*=0.039). However, there was no significant difference in cleavage rate (84.2% vs. 84.9%, p=0.085) (Table2).

Effect of normal oocytes and CLCG on Pregnancy outcomes in day3 and day5 FET cycles: The total number of embryos transferred in 225 cycles was 675 in day3 FET cycle patient's with CLCG compared with those without CLCG **(a**ge of <30**).** The Clinical pregnancy rate (55.0% vs.40.8%, *p*=0.698), Implantation Rate (28.0% vs.18.0%, *p*=0.226), live birth rate (87.2% vs.76.4%, *p*=0.334), biochemical pregnancy rate (9.0% vs. 9.8%, *p*=1), and abortion rate (12.7% vs.23.5%, *p*=0.254). However, there was no significant difference (P>0.05) in any of the parameters between two group, and also there was no significant difference between the control and experimental group (age >31 or above). The total number of embryos transferred in 193 cycles was 567 in patient's with CLCG compared with those without CLCG, **t**he Clinical pregnancy rate (51.1% vs.38.8%, *p*=0.517), Implantation Rate (23.6% vs.17.7%, *p*=0.577), live birth rate (86.9% vs.87.5%, *p*=0.564), biochemical pregnancy rate (10.8% vs.2.5%, *p*=0.102), and abortion rate (13.0% vs.12.5%, *p*=0.764) respectively (Table3).

The total number of embryos transferred in 71 cycles was 142 in day5 FET cycle patient's with CLCG compared with those without CLCG **(**age of <30**).** There was no significant difference in Clinical pregnancy rate (66.6% vs.68.4%, *p*= 0.564), Implantation Rate (39.3% vs.48.6%, *p*=0.165), live birth rate (81.8% vs. 96.1%, *p*=0.286), biochemical pregnancy rate (0.0% vs.11.5%, *p*=0.083), and abortion rate (18.1% vs.3.8%, *p*=0.179). And also, there was no significant difference seen in control and experimental group (age >31 or above), The total number of embryos transferred in 86 cycles was 172 in patient's without CLCG compared with CLCG. The Clinical pregnancy rate (59.0% vs.56.8%, *p*=0.893), Implantation Rate (38.6% vs.46.4%, *p*=0.558), live birth rate (84.6% vs.84.0%, *p*=0.882), biochemical pregnancy rate (3.8% vs.4.0%, *p*=1), and abortion rate (15.3% vs.16.0%, *p*=1) respectively(Table4).

Discussion

Cytoplasmic granularity can be homogeneous affecting the entire cytoplasm or concentrated in the center with a peripheral ring giving a darkened appearance to the cytoplasm. In conventional IVF and ICSI, where the choice of embryos was restricted to those obtained solely from granular oocytes, term pregnancies have been reported (Veeck,1988,1991). However, oocytes with the darkened and granular center often fail to fertilize and have reduced developmental potential (Veeck,1988,1991; Bedford and Kim,1993). We have demonstrated a normal ICSI fertilization rate with oocytes exhibiting cytoplasmic granularity or a granular center, but there was no significant difference in pregnancy rate was recorded in the study [7].

The cause of these cytoplasmic morphological abnormalities is probably multi-factorial. Ovarian stimulation is known to be detrimental on oocytes quality. Ovulation induction and patient's factors like age, FSH level, AMH, and LH may result in the maturation of abnormal oocytes that would otherwise become atretic in the absence of stimulation. And particularly estrogen and progesterone are intimately involved in the inception of cytoplasmic maturation and the final stage of nuclear maturation of the oocytes (Thibault,1977). Human oocytes retrieved from stimulated IVF cycles have been shown to have a more than 40% incidence of numerical chromosomal abnormalities (Wramsby,1988; Van Blerkomand Henry,1992) [12].

Studies [1,4] have found that central granulation of oocytes had a negative effect on development potential, this study indicates that there was no difference in clinical outcomes between patient's with age of <30 and age >31 or Above

(Table3 and Table4). In the comparative analysis of the study subjects, age was found to be the interfering factor. Therefore, the stratified study was conducted on age with central granulation of oocytes, and This study focused on the pregnancy outcome of the frozen embryo transfer cycle and found no difference in pregnancy, Implantation, and live birth rate in different age groups. These manifest that, the pregnancy outcome of patient's with CLCG oocytes was no affect.

The study of Xiao-fang Yi [1]. makes an appearance that there is no significant effect of CLCG on embryonic development in women with BMI <24. however, this study used cell index extraction software to quantify the CLCG oocytes.

Another study by P.F. Serhal.[7] described that the outcome of ICSI is dependent on the quality of the oocytes retrieved. Normal fertilization and embryo development were achieved after microinjection of oocytes with cytoplasmic abnormalities. However, patient's selection is now more rigorous than at the commencement of the ICSI program. This approach is anticipated to improve the quality of the embryos obtained, and hence the success rates with ICSI.

V. Conclusion and Future Scope

In conclusion, this study suggests that there is no significant effect of CLCG on embryonic development potential, from the aspect of CLCG-to-normal oocytes. There is no significant effect of CLCG on clinical outcomes such as Clinical pregnancy, Implantation, live birth, biochemical pregnancy, and abortion rate respectively. However, CLCG may be a normal form of the oocyte and more samples are needed to further refine the grading standards.

Figures and Tables

Table1. Baseline characteristics of infertile couples undergoing ICSI and selected for CLCG oocyte evaluation

	Under Age of 30 years			Age 31 or above		
Groups	Normal	CLCG	*P*	Normal	CLCG	*P*
cycles(n)	158	138		147	132	
age (years)	27.0±3.0	26.7±3.3		35±5.0	35±5.0	
FSH level (IU/ML)	5.6±4.4	5.8±5.2	ns	6.9±3.1	6.3±3.7	ns
Anti-Mullerian Hormone(ng/mL)	3.8±3.2	4.0±1.9	ns	2.8±1.0	2.6±1.0	ns
LH (IU/ML)	4.6±0.4	4.2±0.2	ns	3.3±0.7	3.8±0.5	ns
GN days (day)	11±2	11±2	ns	11±2	11±2	ns
Results are expressed as n(%) or mean (M) ± standard deviation (SD). A difference was considered significant (s) when P < 0.05; (ns), not significant						

Table2. ICSI outcomes in couples compared to CLCG prevalence

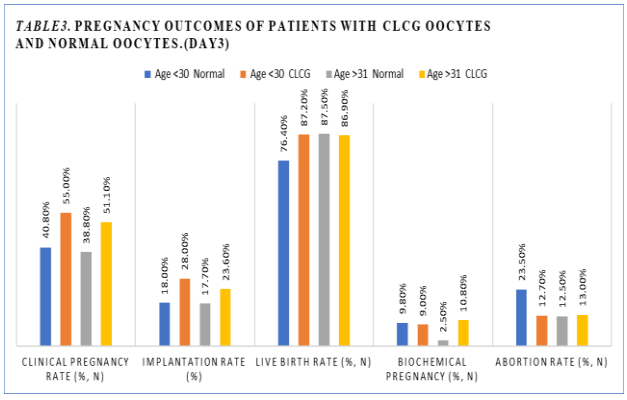
	Under Age of 30 years			Age 31 or above		
Groups	Normal(n=158)	CLCG(n=138)	*P*	Normal(n=147)	CLCG(n=132)	*P*
Oocytes retrieved (n)	2066	1742		1617	1254	
No of MII oocytes(n)	1484	1402		1227	1085	
Maturation rate (%, n)	71.8% (1484/2066)	80.4% (1402/1742)	ns	75.8% (1227/1617)	86.5% (1085/1254)	S (P < 0.05)
Fertilization rate (%, n)	80.8% (1200/1484)	85.8% (1204/1402)	ns	88.0% (1080/1227)	90.8% (986/1085)	S (P < 0.05)
Cleavage rate (%, n)	91.0% (1093/1200)	86.8% (1046/1204)	ns	84.2% (910/1080)	84.9% (838/986)	ns
Results are expressed as n(%) or mean (M) ± standard deviation (SD). A difference was considered significant (s) when P < 0.05; (ns), not significant						

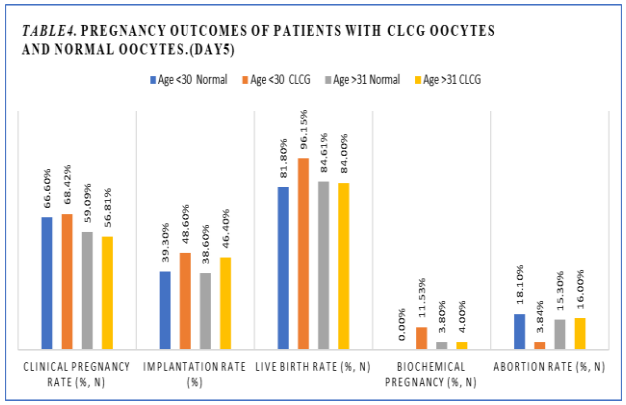
Table3. Pregnancy outcomes of patients with CLCG oocytes and normal oocytes.

	Under Age of 30 years			Age 31 or above		
Groups	Normal	CLCG	*P*	Normal	CLCG	*P*
Day 3 FET cycles(n)	125	100		103	90	
Clinical pregnancy rate (%, n)	40.8% (51/125)	55.0% (55/100)	ns	38.8% (40/103)	51.1% (46/90)	ns
Implantation Rate (%)	18.0% (69/375)	28.0% (84/300)	ns	17.7% (55/309)	23.6% (61/258)	ns
Live birth rate (%, n)	76.4% (39/51)	87.2% (48/55)	ns	87.5% (35/40)	86.9% (40/46)	ns
Biochemical pregnancy (%, n)	9.8% (5/51)	9.0% (5/55)	ns	2.5% (1/40)	10.8% (5/46)	ns
Abortion rate (%, n)	23.5% (12/51)	12.7% (7/55)	ns	12.5% (5/40)	13.0% (6/46)	ns
Results are expressed as n(%) or mean (M) ± standard deviation (SD). A difference was considered significant (s) when P < 0.05; (ns), not significant						

Table4. Pregnancy outcomes of patients with CLCG oocytes and normal oocytes.

	Under Age of 30 years			Age 31 or above		
Groups	Normal	CLCG	*P*	Normal	CLCG	*P*
Day 5 FET cycles(n)	33	38		44	42	
Clinical pregnancy rate (%, n)	66.6% (22/33)	68.42% (26/38)	ns	59.09% (26/44)	56.81% (25/44)	ns
Implantation Rate (%)	39.3% (26/66)	48.6% (37/76)	ns	38.6% (34/88)	46.4% (39/84)	ns
Live birth rate (%, n)	81.8% (18/22)	96.15% (25/26)	ns	84.61% (22/26)	84.0% (21/25)	ns
Biochemical pregnancy (%, n)	0.0% (0/22)	11.53% (3/26)	ns	3.8% (1/26)	4.0% (1/25)	ns
Abortion rate (%, n)	18.1% (4/22)	3.84% (1/26)	ns	15.3% (4/26)	16.0% (4/25)	ns
Results are expressed as n(%) or mean (M) ± standard deviation (SD). A difference was considered significant (s) when P < 0.05; (ns), not significant						





ACKNOWLEDGMENT

We acknowledge the doctors, colleagues who have supported us while conducting this study. We also acknowledge the patients who have participated.

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Awards And Certifications

Awarded Atmanirbhar Bharat Award by Indian Achievers Forum for contribution in Embryology 2021, Awarded as BEST EMBRYOLOGISTS in Bangalore by Xel Research 2021, Awarded as top 100 healthcare leaders in Embryology by IIFA Dubai 2019, Certified Embryologist by Anderson Genetic Lab for Embryo Biopsy. 2019., and Certified Embryology Practioner by American College of Embryology 2017.

Research and Publications

Comparative Study of Clinical Pregnancy Rates Between HCG -Administered Patients Before Embryo Transfer V/S Control Groups, paper presentation at YUVA ISAR 2016 Cochin.

Impact on Fertilization Rate Using Diluted Hyalase - Chandan N1*, Reeta Janet Jessy I2 and Saleem M3, received: April 23, 2020 Published: May 29, 2020 © All rights are reserved by Chandan N., et al. Acta Scientific Women's Health (ISSN: 2582-3205) Volume 2 Issue 6 June 2020.
WEB: https://actascientific.com/ASWH/ASWH-02-0117.php

Impact of Oocytes Insemination Timing on Fertilization and Embryo Development Rate - Chandan N*, Raghunandan K, Reeta Janet Jessy I, Saleem M and

Shwetha C, received: July 20, 2020 Published: August 25, 2020 © All rights are reserved by Chandan N., et al. ACTA SCIENTIFIC Women's Health (ISSN: 2582-3205) Volume 2 Issue 9 September 2020.
WEB: https://actascientific.com/ASWH/ASWH-02-0136.php.,

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