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Impact of Oocytes with Morphological Abnormalities on Blastocyst Formation Rate

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Abstract— **Objective:** Aim of the study is to evaluate the intracytoplasmic and extracytoplasmic abnormalities of the human oocytes and their influence on potential embryo development and blastocyst formation rate outcome.
Exclusive criteria: Cycles from cryopreserved oocytes and the patient underwent agonist stimulation protocol were excluded, Severe male factor infertility (oligozoospermia<10 million/ml)Oocyte morphology was evaluated at the time of ICSI and scored according to the scoring system described by Martin-Wilding et al. The inseminated oocytes were cultured according to standard protocol. Fertilization was assessed at 17-18 hours and cleavage was assessed at 26 hours after insemination respectively. Embryo grading was done on day 5 using Gardner's embryo grading system.
Material and Methods: A Single center, prospective observational study with patients undergoing intracytoplasmic sperm injection (ICSI) treatment between September 2020 to January 2021 was included. Oocytes are graded according to their maturity for all oocytes and subsequently assessed for fertilization, cleavage, and blastocyst grading. Correlation between oocyte morphology and fertilization rate, cleavage rate, and blastocyst rate assessed using appropriate statistical method using (SPSS) version 16.0.
Result: A total of 50 patients and oocytes were studied, in that 371 oocytes underwent intracytoplasmic sperm injection (ICSI) with fertilization rate 78.4 %(291/371), cleavage rate 81%(236/291), and blastocyst rate 66.5%(157/236). Embryo grades were A-37.5%, B-50.9 %, and C-11.% oocyte morphology was not predictive of fertilization, cleavage, and blastocyst rates.
Conclusion: There is no statically difference between blastocyst formation rate between morphologically normal oocytes and oocytes with morphological abnormalities like CLGC, SER, and Refractile body.

Keywords—oocyte abnormalities, ICSI, Blastocyst rate, intracytoplasmic sperm injection,

I. INTRODUCTION

Infertility is inability of a couple to attain conception or bring a pregnancy to term after 12 or more months of normal unprotected sexual activity. Initially, test-tube baby Louis Brown was born on 25, July 1978. ART technique IVF-in-vitro fertilization, ICSI- Intracytoplasmic sperm cell injection IVF and ICSI had been widely used to treat couples with the non-male factor infertility and severe male factor, severely impaired sperm cell characteristics and former IVF unsuccessful cycle.(1) Minor attention has been focused on oocyte morphology in standard IVF techniques because it is often difficult to assess the cytoplasmic morphology of the oocyte and exact stage of oocyte maturation, gametocyte square measure invariably enclosed by cumulus complicated or corona cells at the time of assortment. Cumulus cells act as nutrition supplements for gametocyte maturation. (2)

Following the removal of the cumulus–corona cells in preparation for ICSI, gametocyte analysis is additionally correct. Gametocyte maturation is predicated on the nuclear maturation standing, the morphology of the living substance, and also the look of the extracytoplasmic structures. (3) The presence of the primary cell (IPB) is generally thought-about to be a marker of gametocyte nuclear maturity. However, recent studies exploiting polarized light microscope have shown that oocytes displaying a cell should still be immature. Solely those displaying a cellular division spindle (MS) will be thought about as true, mature, Metaphase II (MII) stage oocytes. Nuclear maturity alone is, in fact, not enough to see the standard of associate degree gametocytes. Nuclear and protoplasm maturation ought to be completed in a coordinated manner to make sure optimum conditions for consequent fertilization.(4) a perfect mature human gametocyte, supported by morphological characteristics, ought to have a 'normal-looking living substance, one cell, associate degree applicable zona pellucid (ZP) thickness.

II. STUDY DESIGN

The study was a prospective observational comparative study.

III. EXCLUSIVE CRITERIA

Cycles from cryopreserved oocytes and the patient underwent agonist stimulation protocol were excluded, Severe male factor infertility (oligozoospermia<10 million/ml) oocyte morphology was evaluated at the time of ICSI and scored according to the scoring system described by Martin-Wilding et al. The inseminated oocytes were cultured according to standard protocol. Fertilization was assessed at 17-18 hours and cleavage was assessed at 26 hours after insemination respectively. Embryo grading was done on day 5 using Gardner's embryo grading system.(5)

IV. MATERIAL AND MATHODS

The study was conducted at Wing's Women's hospital, Ahmadabad, India. After obtaining ethical clearance from (The Independent Ethics Committee, Indian Fertility Society Flat No.302, 3rd Floor, Kailas Building, Kasturba Gandhi Marg, C.P,New Delhi-110001 is registered (Registration ` ECR/222/indt/DL/2015/RR-18) with Drug Controller General of India, Directorate General of Health Services, New Delhi as per the Rule 122D of the Drugs and Cosmetics Rules 1945..).It was a prospective observational study conducted between September 2020 to January 2021. All infertile couples undergoing ICSI, irrespective of their cause of infertility. Oocyte quality was assessed and scores were calculated. Correlation between Oocyte morphology and oocyte score with embryo grade, fertilization rate, and cleavage rate were assessed. Women undergoing controlled ovarian stimulation(COS), antagonist protocol, between the age group 25 to 35 years were included. Those with severe male factor infertility or where TESA sample was used. Oocyte retrieval was done 35 hours after the ovulation trigger. The oocytes were incubated for 3 hours after retrieval to facilitate their final maturation and then denuded for ICSI. Oocyte morphology was evaluated at the time of ICSI and scored according to the scoring system described by Martin-Wilding *et al.* The inseminated oocytes were cultured according to standard protocol. Fertilization was assessed at 17-18 hours and cleavage was assessed at 26 hours after insemination respectively. Embryo grading was done on day 5 using Gardner embryo grading system.

Statistical analysis:
Categorical variables were presented in number (N) and percentage (%) and continuous variables were presented as mean ± SD (Standard deviation). Appropriate statistical methods for non-parametric data were done with the use of the Chi-square test. A p-value <0.05 was considered statistically significant. This data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Science (SPSS) version 16.0

Results : A total of 371 oocytes were assessed in the study, out of the 18% were morphologically normal oocytes, 20.7% has Centrally located granular cytoplasm, 7% has Smooth endoplasmic reticulum, 13.2 % has vacuoles, 9.4 % has Refractile body, 8% has Granular Perivitelline space, 2.6% has Large Perivitelline Space 3.7 %has SeptedZona, 2.9% has fragmented Polar Body. The Mean age of all patients included in this study is 31.1 ± 2.8. Morphological abnormalities of oocytes are divided into four groups. Group 1 Morphological normal oocytes, Group 2 oocytes with intracytoplasmic abnormalities, Group 3 oocytes withextracytoplasmic abnormalities,and group 4 oocytes with bothintracytoplasmic and extracytoplasmic abnormalities.

In Group 1 morphologically normal oocytes 46% blastocyst has excellent quality, 42% of blastocyst has average and 10% has poor quality.

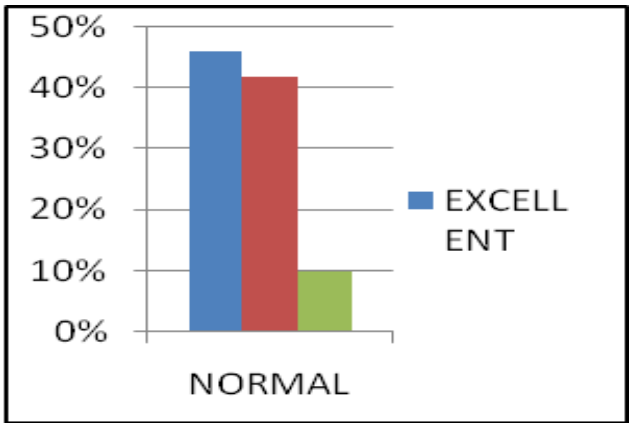


Figure 1.0- Group 1

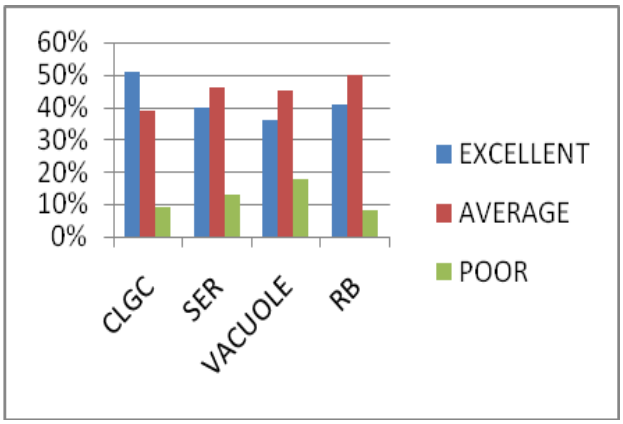
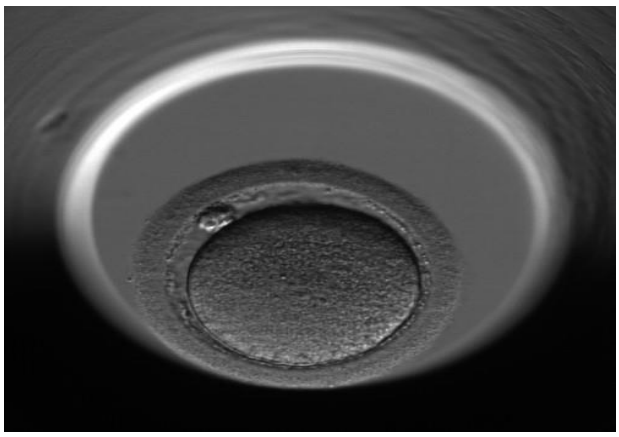


Figure 1.1: Group 2

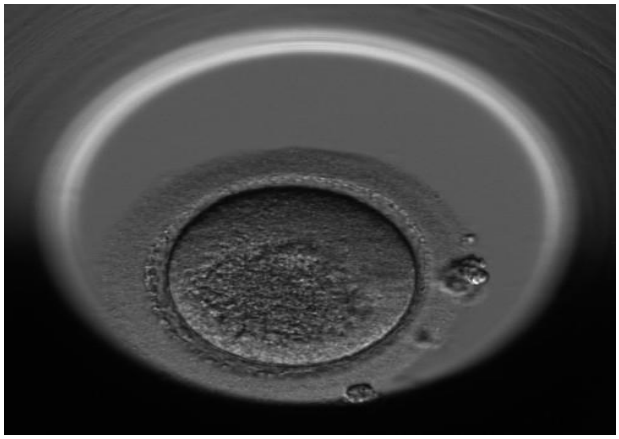
[figure 1.0- Group 1 (Normal) (Figure 1.1:Group 2 (Intracytoplasmic abnormalities)
(Gardner blastocyst grading system(excellent quality included-5AA,4AA.,
Average quality-4AB,4BB,4BA,3AB,3BB,3BA.,
Poor quality included- 4CC,3CC,4BC,3BC)

In Group 2 oocytes having intracytoplasmic abnormalities are divided into four subgroups are Centrally located granular cytoplasm, vacuoles, Smooth endoplasmic reticulum, and Refractile bodies. In CLGC 51% has

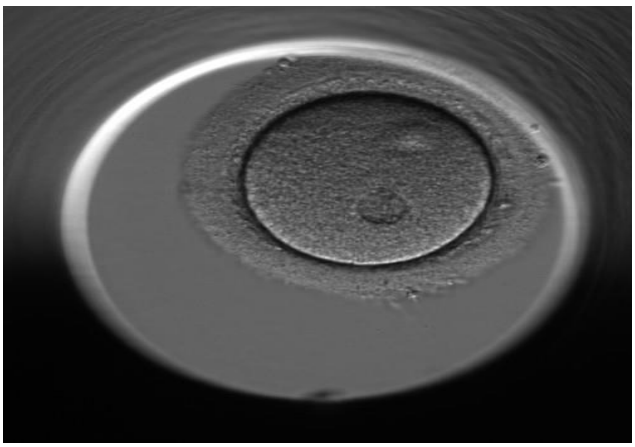
excellent quality blastocyst, Group 4 oocytes have both intracytoplasmic and extracytoplasmic abnormalities 17% has excellent quality blastocyst, 58% has average quality blastocyst and 23% has poor quality blastocyst. In CLGC 51% has excellent quality blastocyst, 39% has average and 9% has poor quality blastocyst. In SER 40% has excellent quality blastocyst, 46% has the average quality and 13% has poor quality. In vacuoles 36% has excellent quality, 45% has the average quality and 18% has poor quality blastocyst. In the Refractile body, 41% has excellent quality, 50% has the average quality and 8% has poor quality blastocyst.



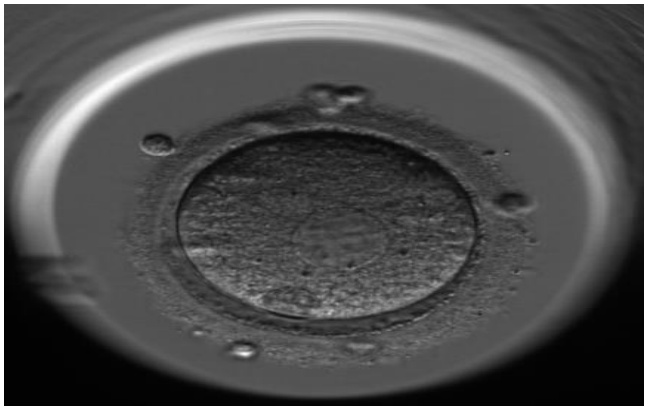
(Figure 1 – normal)



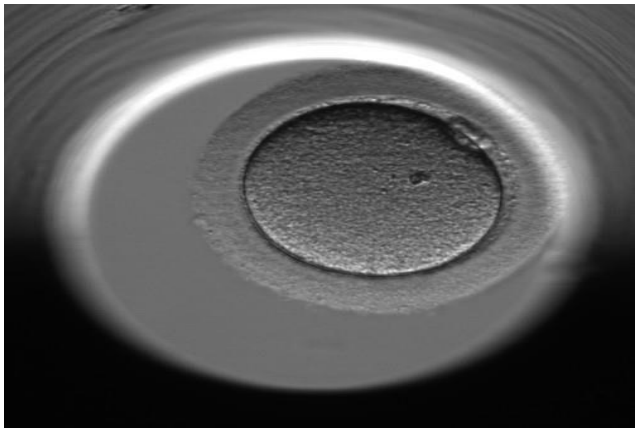
(Figure 2: CLGC)



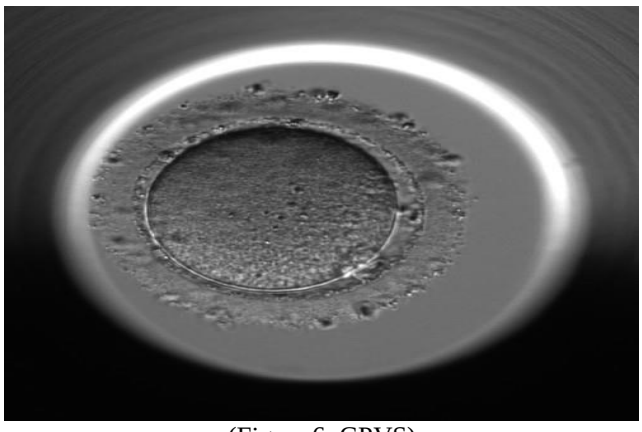
(Figure 3: Vacuolated oocyte)



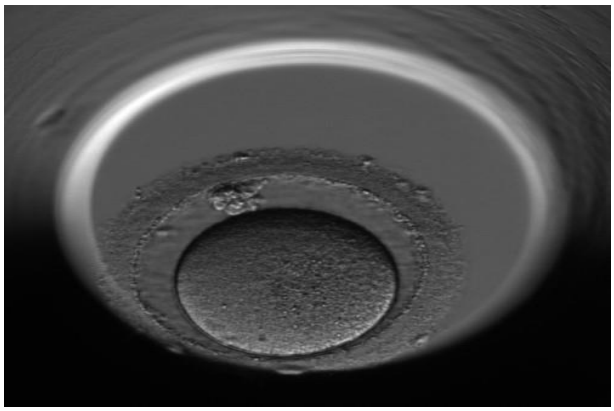
(Figure 4:SER)



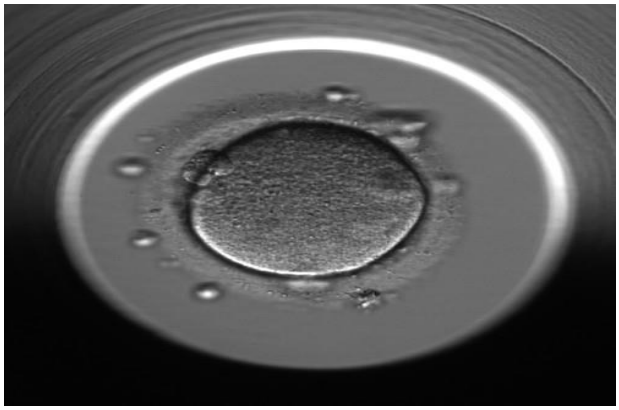
(Figure 5: RB)



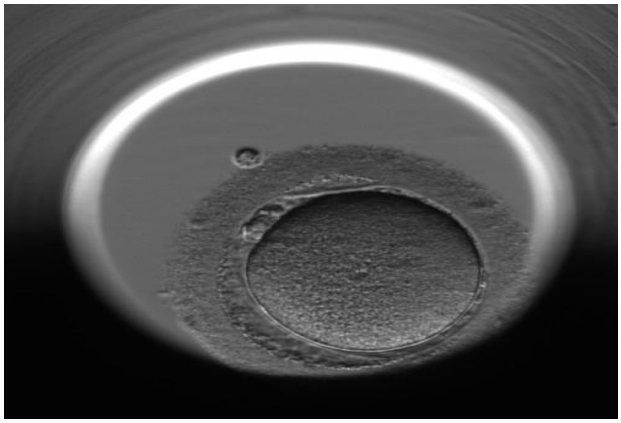
(Figure 6: GPVS)



(Figure 7: LPVS)



(Figure 8: FBP)



(Figure 9- Septed zona)

Table 1: Mean value of Group 1 and Group 2

Variable	Group 1	Group 2			
	Normal	CLGC	SER	VACUOLE	REFRACTILE BODY
M-II Oocyte	34 ± 19.4	39 ± 22.37	13.5 ± 7.6	25 ± 14.28	18 ± 10.24
Oocyte score	8.53 ± 0.50	7.70 ± 0.58	8 ± 0.48	7.7 ± 0.62	7.79 ± 0.47
Fertilization ratio	26.5 ± 15.15	29 ± 16.59	11.5± 6.49	19.5± 11.11	14 ± 7.93
Z-Score	1.82 ± 0.80	2.15 ± 0.89	1.77 ±0.75	1.81 ± 0.69	2.14 ± 0.94
Total Blastocyst	14.5 ± 8.22	17 ± 9.66	8 ± 4.47	11.5 ± 6.49	6.5 ± 3.6
KID Score	5.9 ± 2.44	4.96 ± 2.32	5.91± 2.51	6.37 ± 2.72	6.1 ± 2.11
Results are expressed mean (M) ± standared deviation (SD).					

(CLGC= Centrally located granular cytoplasm, SER= Smooth endoplasmic reticulum, RB= Retractile body)

Table 2: Mean value of Group 3 and Group 4

Variable	Group 3				Group 4
	GPVS	LPVS	SeptedZona	FPB	Multiple abnormality
M-II Oocyte	15.5 ± 8.80	5.5 ± 3.02	7.5 ± 4.18	6 ± 3.31	26.5 ± 15.15
Oocyte score	8.26 ± 0.52	8.4 ± 0.51	8 ± 0.37	8.09 ± 0.7	7.26 ± 1.12
Fertilization ratio	14 ± 7.93	4.5 ± 2.44	5 ± 2.73	4 ± 2.16	19.5 ± 11.11
Z-Score	1.92 ± 0.61	2 ± 0.70	1.6 ± 0.74	1.57± 0.53	2.13 ± 0.77
Total Blastocyst	7.5 ± 4.18	3.5 ± 1.87	2.5 ± 1.29	3.5 ± 1.87	9 ± 5.04
KID Score	7.06 ± 2.15	6.12 ± 1.63	5.7 ± 0.83	6.7 ± 1.83	6.46 ± 1.38
Results are expressed mean (M) ± standared deviation (SD).					

(GPVS= Granular perivitelline space, LPVS= Large perivitelline space, FPB= Fragmented Polar body)

Table 3: Comparison of Intracytoplasmic abnormality group 2 with the control group

Variable	Control Group		Group - 2		p-value
	Blastocyst	No Blastocyst	Blast	No Blastocyst	
No. Of oocytes	57		187		
Age	≤ 35		≤ 35		
CLGC	28/57 (49.1%)	29/57 (50%)	33/77 (42.9%)	44/77 (57.1%)	0.488
SER	28/57 (49.1%)	29/57 (50%)	15/26 (57.7%)	11/26 (42.3%)	0.489
Vacuole	28/57 (49.1%)	29/57 (50%)	22/49 (44.9%)	27/49 (55.1%)	0.700
RB	28/57 (49.1%)	29/57 (50%)	12/35 (34.3%)	23/35 (65.7%)	0.197
Results are expressed as n% A difference was considered significant (s) when (P) <0.05; (NS), not significant.					

(p >0.05 as a null hypothesis, CLGC= centrally located granular cytoplasm, SER= Smooth endoplasmic reticulum, RB= Refractile body)

Table 4: Comparison of Extracytoplasmic abnormality group 3 with the control group

Variable	Control Group		Group - 3		p-value
	Blastocyst	No Blastocyst	Blastocyst	No Blastocyst	
No. Of oocytes	57		65		
Age	≤ 35		≤ 35		
GPVS	28/57 (49.1%)	29/57 (50.9%)	14/30 (46.7%)	16/30 (53.3%)	1.000
LPVS	28/57 (49.1%)	29/57 (50.9%)	6/10 (60%)	4/10 (40%)	0.734
SeptedZona	28/57 (49.1%)	29/57 (50.9%)	4/14 (28.6%)	10/14 (71.4%)	0.233
FPB	28/57 (49.1%)	29/57 (50.9%)	6/11 (54.5%)	5/11 (45.5%)	1.000
Results are expressed as n% A difference was considered significant (s) when (P) <0.05; (NS), not significant.					

(p >0.05 as a null hypothesis, GPVS= Granular perivitelline space, LPVS= Large perivitelline space, FPB= Fragmented Polar body)

Table 5: Comparison of multiple abnormality (both intracytoplasmic&extracytoplasmic) abnormality group 4 with the control group

Variable	Control Group		Group - 4		p-value
	Blastocyst	No Blastocyst	Blastocyst	No Blastocyst	
No. Of oocytes	57		52		
Age	≤ 35		≤ 35		
Multiple abnormality	28/57 (49.1%)	29/57 (50.9%)	17/52 (32.7%)	35/52 (67.3%)	0.119

(p >0.05 as a null hypothesis)

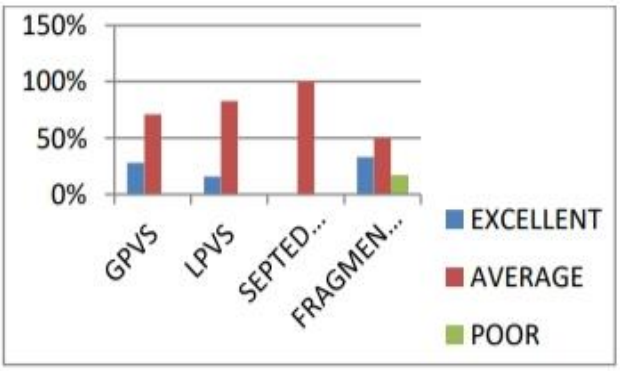


Figure-12:Group-3(Extracytoplasmic abnormalities), (Gardner blastocyst grading system(excellent quality included-5AA,4AA., Moderate quality-4AB,4BB,4BA,3AB,3BB,3BA., Poor quality included-4CC,3CC,4BC,3BC)

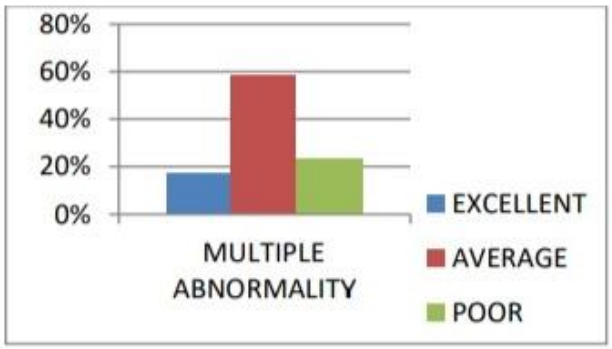


Figure13:Group 4(Multiple Abnormality))
(Gardner blastocyst grading system(excellent quality included-5AA,4AA., Moderate quality-4AB,4BB,4BA,3AB,3BB,3BA., Poor quality included-4CC,3CC,4BC,3BC)

In Group 3 oocytes having extracytoplasmic abnormalities are divided into four subgroups are granular perivitelline space, large perivitelline space, septedzona, and a fragmented polar body. In granular perivitelline space, 28% are excellent quality blastocyst, 71% are average quality blastocyst. In large perivitelline space, 16% has excellent quality and 83% has average quality blastocyst. In septedzona, 100% blastocyst is of average quality. In the fragmented polar body, 33% has excellent quality blastocyst,50% has average quality blastocyst and 16% has poor quality blastocyst.In Group 4 oocytes having both intracytoplasmic and extracytoplasmic abnormalities, 17% has Excellent quality blastocyst, 58% has Average quality blastocyst and 23% has poor quality blastocyst. **Excellent quality include (5AA, 4AA), Average quality include(4AB,4BB,4BA,3AB,3BB,3BA) and poor quality include (4CC,3CC,4BC,3BC,).**

Discussion: In this study, we found that oocyte abnormalities had no effect on fertilisation, cleavage rates, or blastocyst formation rate. Embryos generated from both morphologically normal and defective eggs formed blastocysts at equal rates. The rate of blastocyst formation in embryos produced from oocytes with septedzona and numerous defects was considerably lower than in other groups. According to Loutradis et al., the severity of the cytoplasmic abnormality influences fertilisation rates, embryo quality, and developmental potential. Although oocytes with dark cytoplasm, many vacuoles, or fragments in the cytoplasm had equivalent fertilisation capacity and produced embryos of similar quality, when triple cytoplasmic abnormalities such as dark cytoplasm, many vacuoles, and fragments in the cytoplasm were combined in the same oocyte, the fertilisation capacity and quality of the embryos decreased. The quality of the embryos was drastically reduced. Oocytes with cytoplasmic abnormalities, such as dark and granular cytoplasm, and refractile bodies, have low fertilisation rates, according to IVF results (Veeck, 1991). Oocyte morphological abnormalities have been linked to ovarian stimulation, the hormonal to which the gametes are exposed, and the oocyte's chromosomal makeup.

Conclusion: According to this study, there is no statistical difference between morphologically normal oocytes and oocytes with morphological abnormalities. Because the p-value for the Chi-square test is larger than 0.05, the null hypothesis is accepted and the alternative hypothesis is rejected. The rate of blastocyst formation in embryos generated from septedzona and other defects was significantly lower than in other groups.

Conflict of interest: The author has no conflict of interest to declare.

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AUTHORS PROFILE

Dr.Jayesh Amin – Clinical Director – WINGS IVF Group. Gujarat's chapter secretary for Indian Fertility Society (IFS).Educational Profile He has passed MBBS and MD from M.P. Shah Medical College, Saurasthra University. He has accomplished his fellowship for IVF and Embryology by LARS Johnson, Sweden and he is a member of Gujarat ISSAR Chapter, ESHERE, IAGE. Focus His prime area of interest include trouble shooting complex infertility, recurrent IVF failure cases, application of latest technologies in the field of IVF & role of genetics in IVF treatments.

Achievements

Dr. Jayesh Amin is a national and international faculty at various fertility conferences in India and abroad. Dr.Jayesh Amin has been awarded as a Times of India- IVF icon for 2018 & 2019, Excellence in Infertility treatments by the then Union Health Minister Shri.J.P.Nadda & Fertility Game Changer (West)-2019 by Economic Times.

Dr. Paresh Makwana - He did his Bachelor of Veterinary Science and Animal Husbandary and also Master of Veterinary Science from Anand Agricultural University. Pursued MBA in Healthcare Science from Sikkim Manipal University. One of finest embryologist in india. Biopsy expert, life time member of all Indian fertility society like, ACE,IFS,ISAR.

Focus

Andrology: Semen Analysis with all the parameters, Sperm preparation for IUI, Sperm freezing, PESA/TESA/Micro TESA sample preparation & ICSI.Embryology: IVM, IVF, ICSI, Oocytes staining, Laser Assisted Embryo Hatching, Blastocyst culture, Embryo vitrification and thawing, Grading of oocytes and Embryos, Embryo Transfer. Management: Selection of equipments, Maintaining Patient data Records, Quality Control of the lab, Designing of ART lab, Assisting in CME, Conference, Camp Organization, Website content preparation, Consent forms, brochure preparation, Economical stock maintenance for Media and disposables.

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