Reproductive Medicine; *Endocrinology and Infertility*

Role of Oocyte Morphological Abnormality Rates on the Embryo Development and Implantation

Bulent Emre BILGIC[1], Cigdem YAYLA ABIDE[2], Enis OZKAYA[2], Tayfun KUTLU[2], Sule AYLA[1], Ilhan SANVERDI[2], Gulden TUNALI[1], Erdal YUCEL[2], Savas KANBUR[3]

Istanbul, Turkey

ABSTRACT

OBJECTIVE: The aim of this study was to assess the impact of oocyte morphological abnormality rates on embryo development and implantation.

STUDY DESIGN: Oocyte morphological abnormalities including oocyte size, elliptical shape, vacuole, plain polar body, fragmented polar body, large perivitellin space, perivitellin debris, central granulation, dense central granulation, inclusion body, thick zona pellucida, clusters of smooth endoplasmic reticulum, easy needle insertion and dark cytoplasm were determined for each oocyte. Rates of these oocyte morphological anomalies were determined for each case and the impact of rates on the cycle outcome was analyzed.

RESULTS: Similar oocyte morphology abnormality rates were observed between cycles with and without successful embryo implantation. On the other hand, both fragmented polar body and vacuole rates were found to be significantly higher in cycles with Grade 2 embryo transfer. These rate differences were remained significant after adjustment for the age and basal FSH level.

CONCLUSION: None of the oocyte morphological features was found to have significant impact on ART outcome.

Keywords: Oocyte morphology, Embryo implantation, Fertilization, ICSI

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Introduction

Some sperm morphology abnormalities were shown to result in failure of fertilization (1). From this point of view some investigations have been conducted to figure out whether it was same for the oocytes or not. Previous report indicated no relationship between the oocyte morphology and fertilization after ICSI (2). Following studies showed failed implantation of embryos derived from morphologically abnormal oocytes (3). Further studies on this issue revealed no relationship between the oocyte morphology and decreased fertilization rate or unfavorable embryo quality. Additionally, authors found similar clinical pregnancy and implantation rates compared with the group of patients with normal oocyte morphology (4). More recently Rienzi et al reported a systematic review, and authors concluded "no clear tendency in recent publications to a general increase in predictive value of morphological features was found. These contradicting data underline the importance of more intensive and coordinated research to reach a consensus and fully exploit the predictive potential of morphological examination of human oocytes" (5).

Therefore, the aim of this study was to assess the impact of some oocyte morphological parameters on IVF/ICSI cycle outcome.

Material and Method

The study material consisted of 277 ICSI cycles with an indication of unexplained infertility. A total of 1546 mature oocytes were included in the analysis. The data was retro-

[1] *University of Health Sciences Zeynep Kamil Women and Children's Health Training and Research Hospital Department of Histology and Embryology, and* [2]*Department of Obstetrics and Gynecology, Istanbul*
[3] *Gedik University, Health Sciences Faculty, Department of Occupational Health and Safety, Istanbul*

Address of Correspondence: *Cigdem Yayla Abide*
University of Health Sciences Zeynep Kamil Women and Children's Health Training and Research Hospital, 34668 Istanbul, Turkey
cigdemabide@gmail.com

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spectively gathered from hospital files and electronic database in ART unit of Zeynep Kamil Women and Children's Health Training and Research Hospital between August 2016 to January 2017. Study protocol was approved by institutional review board and signed consent was obtained from each participants. Women with their first cycle and without any male factor or any systemic disorder who underwent ICSI cycle with antagonist protocol were included in this study. On the second day of the menstrual cycle, 150-225 IU of rFSH (Gonal-F, Merck-Serono, Geneva, Switzerland) were administered and follicular growth was monitored using transvaginal sonography. The dosage of rFSH was adjusted from day 5 of stimulation according to the ovarian response. Antagonist (Cetrorelix, Merck-Serono, Geneva, Switzerland) was administered when the follicular size was 12 mm with a dose of 0.25 mg/day. After the follicular size reached 18 mm, 250 µg of rhCG was administered, and follicular puncture was performed after 34-36 hours by 17-gauge double-lumen needle (Cook Ireland Ltd., Limerick, Ireland). Next, 8% vaginal progesterone gel (Crinone gel 8%; Merck-Serono, Geneva, Switzerland) was applied twice daily. ICSI was applied for each oocyte obtained by follicular puncture. Elective transfer of single grade-1 or 2 embryo was performed either at cleavage (day 3) or blastocyst (day 5) stage, according to the developmental characteristics of the embryo. The embryos were graded for morphology according to Staessen et al. (6). Grade 1: even and homogeneous blastomeres without fragmentation; Grade 2: even and homogeneous blastomeres with, 20% fragmentation; Grade 3: uneven and non-homogeneous blastomeres with 20–50% fragmentation; Grade 4: uneven and non-homogeneous blastomeres with 50% fragmentation.

Serum levels of the β-subunit of hCG (β-hCG) were measured after 2 weeks. If they were more than or equal to normal levels (5 IU/L), the patient was considered to have successful implantation.

The morphology of the oocyte was assessed under an inverted microscope at 3200 or 3400 magnification (Nikon Diaphot 300 with Hoffman modulation contrast) and the oocytes were assessed for some morphological characteristics as follows: Oocyte size, elliptical shape, vacuole, plain polar body, multiseptate polar body, large perivitellin space, perivitellin debris, central granulation, dense central granulation, inclusion body, thick zona pellucida, clusters of smooth endoplasmic reticulum, easy needle insertion and dark cytoplasm.

Normal fertilization was assumed for oocytes, which formed two pronuclei and two polar bodies.

The ratio of oocytes with specific morphological abnormality was calculated over mature oocytes retrieved and the ratios were compared between different embryo grades and cycles with and without embryo implantation. Additionally, oocyte morphological features of the transferred embryo were assessed whether any of the individual parameter has any effect on implantation or not.

Statistical analysis

Data are presented as mean and standard deviation. For statistical analysis, Student's t-test and Mann- Whitney- U were used to compare continuous variables. Fisher's exact and Chi-square tests were used to compare the characteristics of the oocytes retrieved and their abnormalities and embryo implantation. Correlation analysis was used show associations between variables. ANCOVA was used to calculate adjusted means. $P < 0.05$ was considered statistically significant.

Results

There were significant differences between groups with and without embryo implantation in terms of basal FSH, total gonadotropin dose, number of total and mature oocyte number ($P<0.05$, Table 1).

Similar oocyte morphology abnormality rates were observed between cycles with and without successful embryo implantation. Comparisons were remained to be insignificant after adjustment for the basal FSH level and total number of oocytes ($p>0.05$). On the other hand, both fragmented polar body and vacuole rates were found to be significantly higher in cycles with Grade 2 embryo transfer ($p< 0.05$). These differences became insignificant after adjustment for basal FSH level and total number of oocytes ($p>0.05$). Oocyte morphology anomaly rates comparison of cycles with and without embryo implantation was summarized in table 2. While comparison of cycles resulted with grade 1 or grade 2 embryo transfer was shown in table 3. The individual characteristics of oocyte of transferred embryo were not significantly different between two embryo grades and groups with and without embryo implantation ($p>0.05$). There were significant correlations between the age with the rate of individual morphological abnormalities such as plain polar body (r= -0.143, $p=0.017$), fragmented polar body (r=0.172, $p=0.004$) and dense central granulation rates (r=0.187, $p=0.002$). While basal FSH was significantly correlated with fragmented polar body (r=0.175, $p=0.003$), wide perivitellin space (r=0.206, $p=0.001$) and central granulation rates (r=0.134, $p=0.025$).

Over all embryo implantation rate was 31 % with single embryo transfer and pregnancy rates were similar in groups with grade 1 and 2 embryo transfers (31% vs. 30%, $p>0.05$).

Serum FSH, total gonadotropin dose and number of total oocyte number adjusted means of rates for specific oocyte morphological abnormalities remained insignificant between groups with and without embryo implantation ($p>0.05$)

Table 1: *Comparison of some demographic and cycle characteristics of groups with and without successful embryo implantation*

	EI	N	Mean	Std. Deviation	*p* Value
Age (years)	NEG	192	33.7	4.8	
	POS	85	33.4	7.8	NS
FSH (mIU/mL)	NEG	192	7.3	3.2	
	POS	85	6.4	1.9	<0.05
Estradiol (pg/mL)	NEG	192	53.1	48.08	
	POS	85	51.3	50.08	NS
AFC	NEG	192	8.8	4.331	
	POS	85	8.8	3.950	NS
Total Gonadotropin Dose	NEG	192	2682.5	1196.04	
	POS	85	2185.3	1119.1	<0.05
Peak Estradiol (pg/mL)	NEG	192	1663.9	1010.2	
	POS	85	1721.03	1282.9	NS
Number Of Total Oocytes	NEG	192	6.5	4.1	
	POS	85	9.04	4.6	<0.05
Number Of Mature Oocytes	NEG	192	5.005	3.3	
	POS	85	6.8	3.5	<0.05

NEG: Negative, POS: Positive

Table 2: *Oocyte morphological abnormality rates between groups with and without successful embryo implantation*

Rates (%)	EI	N	Mean (%)	Std. Deviation	P Value (MWU)
Small Oocytes	POS	85	1.08	5.4	
	NEG	192	1.00	5.47	NS
Elliptical Shape	POS	85	1.48	6.92	
	NEG	192	1.13	8.13	NS
Vacuoles	POS	85	1.49	5.92	
	NEG	192	1.70	7.51	NS
Plain Polar Body	POS	85	0.11	0.98	
	NEG	192	0.08	0.81	NS
Fragmented Polar Body	POS	85	10.81	15.72	
	NEG	192	16.68	23.77	NS
Wide Perivitellin Space	POS	85	5.39	13.47	
	NEG	192	1.36	22.11	NS
Perivitellin Debris	POS	85	3.68	12.86	
	NEG	192	6.99	17.003	NS
Central Granulation	POS	85	9.45	12.5	
	NEG	192	16.55	24.46	NS
Dense Central Granulation	POS	85	4.26	13.17	
	NEG	192	6.45	18.21	NS
Inclusion Body	POS	85	6.99	11.85	
	NEG	192	11.45	21.75	NS
Thick Zona Pellicuda	POS	85	4.00	9.62	
	NEG	192	6.23	17.28	NS
SER	POS	85	2.44	8.03	
	NEG	192	1.99	9.81	NS
ENI	POS	85	3.89	9.77	
	NEG	192	5.32	14.82	NS
Dark Cytoplasm	POS	85	1.08	5.42	
	NEG	192	2.87	12.9	NS

SER: Smooth endoplasmic reticulum clusters, ENI: Easy needle insertion, EI: Embryo implantation, NEG: Negative, POS: Positive, MWU: Mann Whitney-u

Table 3: *Oocyte morphological abnormality rates between groups Grade 1 and Grade 2 embryo transfers*

Rates (%)	EG	N	Mean Rate (%)	Std. Deviation	*p* Value (MWU)
Small Oocytes	2	227	1.25	5.99	
	1	50	.0000	.00000	NS
Elliptical Shape	2	227	1.41	8.51	
	1	50	0.43	2.16	NS
Vacuoles	2	227	1.99	7.75	
	1	50	0	0	<0.05
Plain Polar Body	2	227	0.11	0.95	
	1	50	0	0	NS
Fragmented Polar Body	2	227	16.78	23.25	
	1	50	6.24	9.05	<0.05
Wide Perivitellin Space	2	227	10.12	20.79	
	1	50	06.85	16.03	NS
Perivitellin Debris	2	227	06.20	15.94	
	1	50	4.93	15.79	NS
Central Granulation	2	227	15.54	22.65	
	1	50	9.06	16.14	NS
Dense Central Granulation	2	227	6.73	18.32	
	1	50	1.47	4.93	NS
Inclusion Body	2	227	10.74	19.98	
	1	50	7.11	15.99	NS
Thick Zona Pellucida	2	227	6.02	16.62	
	1	50	3.38	6.98	NS
SER	2	227	2.41	10.14	
	1	50	0.86	2.99	NS
ENI	2	227	4.99	13.05	
	1	50	4.38	15.36	NS
Dark Cytoplasm	2	227	2.63	12.2	
	1	50	0.90	3.79	NS

SER: Smooth endoplasmic reticulum clusters, ENI: Easy needle insertion, EG: Embryo grade

Discussion

In this study, we tried to assess the relationship between some oocyte morphological abnormalities with the embryo quality and embryo implantation. Our data showed that, although some parameters have an impact on the embryo quality, embryo implantation was not affected by the presence of any of these oocyte morphological features. Among all these parameters, previous studies showed abnormal cytoplasmic morphology to be associated with increased rates of aneuploidy (7). However, no relationship was shown between the cumulus–oocyte complex morphology and the fertilization, embryo cleavage and clinical pregnancy rates in a study by Rattanachaiyanont et al. Ebner et al. (8,9) confirmed this result, but authors showed dense central granulation of oocytes to have negative effect on fertilization and blastocyst rates. According to the studies on morphological features of zona pellucida, no effect of thick zona pellucida was observed on fertilization rates, pronuclear morphology, embryo development and clinical pregnancy (10,11). On the other hand, Bertrand et al. (12) showed thinner zona pellucida is associated higher fertilization rates. Several conflicting results have been proposed by several studies (13,14). Another morphological parameter, perivitellin space, was assessed by some studies, and several studies reported no correlation between increased perivitellin space and further developmental characteristics (2,15,16). However, a significant relationship between the size of perivitellin space, presence of granulation and subsequent embryo quality was shown by Chamayou et al. (17). More recently published study proposed large perivitellin space to have significant impact on fertilization rates (11). Again several conflicting results have been reported with regard to the effect of polar body morphology on the several steps of ART (18,19).

An ovoid shape of the oocyte, was reported to be associated with delays in in vitro parameters (9). Additionally, dark and/or granular cytoplasm was investigated by several studies, which resulted in variable conclusions (20,21).

Furthermore, there are also variable conclusions with regard to the effect of the presence of vacuoles and/or cytoplasmic inclusions, smooth endoplasmic reticulum clusters on the ART outcome (11,21,22).

Pronuclear morphology and embryo quality were shown to be affected when there was central granulation or centrally located granular cytoplasm in the oocyte (9,11). Furthermore, decreased survival and impaired in vitro development after cryopreservation of embryos derived from oocytes with central granulation was reported by Balaban et al. (23). In our study population, most commonly seen oocyte morphology abnormalities were fragmented polar body and central granulation, they were seen in 16% of cases without embryo implantation, despite a big difference between groups, the difference did not reach statistical significance. On the other hand, lowest rate of abnormality rate was observed for the plain polar body.

Both Viscosity of the cytoplasm and the resistance of the cell membrane at ICSI were found to have significant effect on fertilization, embryo quality, blastocyst rates and fertilization (24). None of the above mentioned studies reported the overall rate of oocyte abnormality among all the collected mature oocytes, this is the first study assessing the effect of overall rate of oocyte morphological abnormality rate among all the collected metaphase II oocytes on the top embryo quality and the implantation rates independent from the individual characteristics of oocyte of transferred embryo. Our data showed that although presence of vacuoles and fragmented polar body were shown to have significant impact on the embryo quality, embryo implantation was not affected by the rates of each morphological anomaly among all the collected metaphase II oocytes.

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