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Cytoplasmic abnormalities of mature oocytes have a significant effect on fertilization in Bologna poor responders

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Background/aim: We aimed to investigate whether oocyte morphologic abnormalities affected cycle outcome in poor responder infertile women who fulfilled the Bologna criteria.

Materials and methods: Data were obtained from infertile couples who underwent ICSI and embryo transfer at the Zeynep Kamil Maternity and Children's Health Training and Research Hospital Assisted Reproduction Unit in İstanbul, Turkey. They were allocated to two groups: Group A, poor responders, and Group B, normal responders. All morphological abnormalities of oocytes retrieved were reviewed and grouped as cytoplasmic abnormalities or extracytoplasmic abnormalities. All morphological features were compared between the poor and normal responder groups. ICSI cycle outcomes were described as biochemical pregnancy, fertilization rate, number of top-quality zygotes, top-quality zygote rate, number of embryos transferred, and number of top-quality embryos transferred. The relation between each morphological feature and ICSI outcomes was investigated in each group.

Results: The results showed no difference between the groups in terms of morphological features of oocytes. The relation between ICSI cycle outcomes and each oocyte morphological feature was compared in the poor and normal responder groups. Presence of cytoplasmic morphological abnormality was found to significantly correlate with the fertilization rate (P = 0.019) in poor responders.

Conclusion: Our data show that oocyte cytoplasmic abnormalities significantly reduce fertilization achievement in poor responders.

Key words: Fertilization, intracytoplasmic sperm injection, oocyte morphology, poor responder

1. Introduction

Delay in childbearing causes a rise in the demand for assisted reproductive technologies (ARTs). Infertility clinics increasingly encounter women with low ovarian reserve and poor ovarian response (POR) to conventional ovarian stimulation (1). In 2011, the first systematic definition of poor responder women was developed by the European Society of Human Reproduction and Embryology and published as the Bologna criteria (2).

Etiological factors associated with a poor response to controlled ovarian hyperstimulation (COH) are advanced age, iatrogenic or accidental ovarian damage, previous ovarian surgery, serious endometriosis, and numerical and/or structural chromosomal aberrations (3–5). Currently, obtaining enough oocytes in the ART cycles of poor responder infertile women is the most important challenge. Another problematic issue is the deterioration of oocyte quality concomitant with diminishing ovarian reserve (6). Good-quality oocytes are important for the proper regulation of the intraoocyte signaling cascade, completion of fertilization events, and early embryonic development (7). The correlation between oocyte morphology and intracytoplasmic sperm injection (ICSI) outcomes (i.e. fertilization rate, embryo development, implantation rate, and clinical pregnancy) has remained a controversial issue for a long time. The effect of morphologic abnormalities of oocytes on fertilization has unfortunately remained unclear (8–10).

Oocyte quality in poor responder women and its consequence in ICSI cycles was the main concern in this

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study. We aimed to investigate whether there is a difference between normal and poor responder women in terms of morphologic abnormities of oocytes and whether these abnormalities have any effect on clinical and laboratory outcomes in poor responder women who fulfill the Bologna criteria.

2. Materials and methods

Data were obtained from ART cycles of infertile couples included in the ICSI and embryo transfer program from January 2014 to December 2015. This retrospective cohort study was conducted at the Assisted Reproduction Unit of the Zeynep Kamil Maternity and Children's Health Training and Research Hospital, İstanbul, Turkey. Local ethics committee approval was obtained for the study (approval number: 10/12/2015-106).

2.1. Patient characteristics

Infertile women were allocated into two groups: Group A, poor responders (PRs) and Group B, normal responders (NRs). Women with male partners with azoospermia (no spermatozoa in the ejaculate) or severe oligozoospermia with a sperm count of <5.0 million/mL ejaculate fluid were excluded from the study. PR women were selected according to the Bologna criteria: 1) age of ≥40 years or having any other risk factors; 2) a previous POR (cycle cancelled or ≤3 oocytes with conventional stimulation protocol); or 3) abnormal ovarian tests (antral follicle count of <5–7 follicles or anti-Müllerian hormone (AMH) level of <0.5–1.1 ng/mL). Patients who fulfilled two or more of the Bologna criteria were included. Poor responder patients did not receive any adjuvant therapy. If infertile women had day 3 FSH of <10 IU/L, E2 of <60 ng/mL, AFC of 7–10, AMH of 1–3.5 ng/mL, and a normal response to previous ovarian stimulation (5–15 oocytes), then they were categorized as NRs. Age (mean age <35 and ≥35 years), day 3 FSH and E2 levels, and antral follicle count were defined as the first evaluation parameters.

2.2. Ovarian stimulation protocols

Controlled ovarian stimulation protocols administered to patients were 1) flexible gonadotropin-releasing hormone (GnRh) antagonist protocol, 2) microdose flare-up protocol, 3) minimal stimulation protocols with clomiphene citrate, and 4) GnRh agonist long protocol.

In all protocols, once three follicles reached 17 mm in diameter or two follicles reached 18 mm in diameter, ovulation was triggered with recombinant human chorionic gonadotropin (rec-hCG) (250 μg, Ovitrelle, Serono, İstanbul, Turkey). Oocyte pick-up was performed using transvaginal ultrasound-guided double lumen needle aspiration 36 h after rec-hCG injection.

Stimulation properties were defined based on COH protocols, total gonadotrophin doses, E2 level at hCG day, and number of retrieved total and MII oocytes.

2.3. Oocyte preparation and morphology assessment

Retrieved oocytes were routinely incubated in culture medium for at least 2 h; these were then incubated with 80 IU/mL hyaluronidase for 20–30 s, and cumulus cells surrounding the oocytes were carefully denuded mechanically by gentle pipetting. Before sperm injection, the morphology of all available mature oocytes was assessed for quality using an inverted microscope at 200× or 400× magnification.

Morphologic abnormalities of oocytes were grouped as cytoplasmic and extracytoplasmic abnormalities. Cytoplasmic abnormalities were defined as those with inclusion and refractile bodies, cytoplasmic granulation, and vacuoles and smooth endoplasmic reticulum clusters.

Cytoplasmic granulations were also divided into two groups according to density: 1) central and 2) dense central granulation.

Cytoplasmic vacuoles were divided into three groups according to the location and density: 1) heterogeneous, 2) peripheral, and 3) crowded (dense) peripheral vacuoles.

Extracytoplasmic abnormalities were defined as those with abnormal shapes, fragmented first polar body, large perivitelline space, granulation in the perivitelline space (perivitelline debris), dark zona pellucida, and thin zona pellucida.

The ICSI procedure was performed with mature oocytes (3–4 h after retrieval) according to the technique described by Palermo et al. (11).

2.4. Fertilization assessment

Fertilization was assessed at approximately 16–18 h after the ICSI procedure, which was confirmed by the presence of two pronuclei (2PN) and extrusion of the second polar body.

2.5. Embryo assessment

Embryos were graded according to the ESHRE İstanbul Consensus Workshop on Embryo Assessment based on the observed morphology of embryos every morning (12). The embryo/embryos were transferred on or after day 3 when they reached their optimal stage.

The number of transferred embryos was determined in accordance with the Turkish law on reproductive technologies. Following oocyte retrieval, the luteal phase was supplemented with vaginal progesterone (Crinone 8% gel, Serono) twice a day and continued until menstruation or until 8 weeks of gestation if pregnancy was achieved.

2.6. ICSI cycle outcome assessment

Analyzed ICSI outcomes included the number of fertilized oocytes, total zygotes, top-quality zygotes, top-quality zygote rate (day 1, grade 1, 2PN/total 2PN), days required for the embryos to reach the transfer stage, embryos transferred, top-quality embryo (TQE) transferred, and biochemical pregnancy. Fertilization rate was described as the number of 2PN stage zygotes/number of oocytes

injected with sperm approximately 16 h after the ICSI procedure (13).

Biochemical pregnancy was confirmed by a serial rise in serum human chorionic gonadotropin (hCG) concentrations 12 days after the embryo transfer.

2.7. Statistical analysis

All morphological features and their subgroups belonging to mature oocytes were compared between the normal and PR groups. The relationship between each morphological feature and first evaluation parameters, stimulation cycle properties, and ICSI outcomes was evaluated in each group. Results are presented as mean ± standard deviation for numeric variables. Proportions (%) were used for categorical variables. Statistical analysis was performed using the NPAR1WAY procedure (SAS System 9.1, SAS Institute Inc., Cary, NC, USA). Statistical analyses were performed using R statistical software. The parametric Student t-test or nonparametric Mann–Whitney U test was used for the comparison of mean values. Proportions were compared by chi-square or Fisher exact test according to the expected frequency. $P < 0.05$ was considered statistically significant.

3. Results

Eighty-nine PRs and eighty-five NRs were enrolled in this study. Overall, the analysis included 134 PR infertile women defined according to the Bologna criteria undergoing their last ovarian stimulation for an ICSI cycle. Twenty-four of them had a cancelled cycle because of no response to stimulation protocol, at a rate of 17.9% (24/134). Twenty-one of them were excluded from the analysis due to absence of mature oocytes retrieved. Finally, 89 PR women from whom mature oocytes were obtained in their last ICSI cycles were included in this study. The cancellation rates including failed fertilization and arrest of embryo growth were found as 21% (n = 19/89) and 6.7% (n = 6/89), respectively, in the PR group.

First evaluation parameters, COH cycle properties, and ICSI outcomes were compared between the PR and NR groups. The variables did not satisfy the normality assumption within each group; hence, the Mann–Whitney U test was employed to compare the group medians. Fertilization rate did not show any significant difference between the two groups. In the NR group, other ICSI cycle outcomes were found to be significantly higher than those in the PR group. The results are presented in Table 1.

In the NR group, the COH protocols used were flexible GnRh antagonist and microdose flare-up protocol in 95% and 5% of patients, respectively. In the PR group, ovarian stimulation protocols used were flexible GnRh antagonist protocol (47%), microdose flare-up protocol (33%), and minimal stimulation protocols with clomiphene citrate (20%).

In total, data of oocyte morphology were obtained from 284 mature oocytes retrieved from PR patients and 696 mature oocytes retrieved from NRs. We compared the percentage of morphological abnormalities of oocytes between the two groups and found no difference between the groups (Table 2).

We also evaluated the relationship between each morphological abnormality of oocytes and first evaluation parameters and COH cycle properties in the two groups. None of the morphologic features were individually related to any of the parameters. The results for the two groups are presented in Table 3.

We evaluated fertilization in terms of quality and quantity. The presence of cytoplasmic morphological abnormalities significantly correlated with fertilization rate ($P = 0.019$) in the PR group. The number of TQEs transferred was also significantly related to extracytoplasmic abnormalities in the NR group. In the PR group, the number of TQEs transferred was not statistically evaluated because only a few TQEs were obtained (Table 4).

4. Discussion

In this study, we compared PR and NR women in terms of morphologic features of oocytes obtained from COH cycles. We expected higher morphological abnormalities in PRs than in NRs; however, our results showed no difference in terms of morphological features of oocytes between them.

Although the standardized definition of POR has been established, the mechanism leading to ovarian deficiency has been only partially determined and not completely understood. Each etiological factor causing the depletion of the ovarian pool may have a different impact on oocyte quality and outcomes of ART cycles (14,15). The homogeneity of the population, risk factors in the first evaluation criteria, AFC and AMH threshold values, and impact of oocyte quantity versus quality on treatment outcomes of ART cycles are criteria that require revision for defining PRs (16,17). In this study, to obtain a homogeneous group, PRs were defined based on the Bologna criteria; however, we cannot eliminate the heterogeneity of etiology due to the retrospective design of the study. Hence, oocyte quality of PRs was evaluated irrespective of their etiological factors. The stratification of PR women, taking into consideration their etiological factors, could be applied to resolve this issue. Hence, the comparison of the oocyte quality between NR and PR groups could be more clearly established. Few studies have reported morphologic abnormalities of oocytes in PRs.

In one study, Nichi et al. reported that PR infertile women aged <35 years have similar morphological abnormalities of oocytes as NR women of the same age

Table 1. First evaluation parameters, stimulation cycle characteristics, and ICSI outcomes of infertile women in poor and normal responder groups.

	PR (n = 89) Mean ± SD	NR (n = 85) Mean ± SD	P-value [a]
Age (years)	35.64 ± 4.55	31.72 ± 4.23	0.0001
Day 3 FSH (pg/mL)	11.76 ± 5.81	5.92 ± 1.63	0.0001
Day 3 E_2 (pg/mL)	46.16 ± 26.11	43.63 ± 12.03	0.2919
Day 3 AFC (no.)	4.99 ± 1.79	13.39 ± 3.57	0.0001
Total gonadotropin dose (IU)	3544 ± 1124	1931 ± 672	0.0001
E2 at HCG time (pg/mL)	1024 ± 659	1631 ± 757	0.0001
Total oocytes retrieved (no.)	3.2 ± 1.89	8.19 ± 3.51	0.0001
Total MII oocytes retrieved (no.)	2.4 ± 1.3	6.55 ± 3.03	0.0001
Oocytes fertilizing (no.)	1.62 ± 1.28	4.34 ± 2.65	0.0001
Fertilization rate	0.54 ± 0.41	0.56 ± 0.23	0.903
Day reached by embryo	1.9 ± 1.17	3.04 ± 0.89	0.0001
No. of total embryos	1.28 ± 1.14	3.65 ± 2.17	0.0001
No. of top-quality embryos	0.57 ± 0.80	1.56 ± 1.443	0.0001
Top-quality embryo rate	0.33 ± 0.42	0.42 ± 0.29	0.01
No. of top-quality embryos transferred	0.12 ± 0.39	0.71 ± 0.67	0.0001
No. of embryos transferred	0.98 ± 0.74	1.31 ± 0.49	0.002
Pregnancy, no. (%)	16 (18%)	34 (40%)	0.002[b]

E2 = Estradiol; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; AFC= antral follicle count.

[a] P-value calculated using Wilcoxon test of independence.

[b] P-value calculated using chi-square test.

group (8). De Sutter et al. reported that the quality of oocytes belonging to young PR women is not inferior to that of young NR women (18).

With increasing age, follicular atresia increases, and follicular selection becomes less stringent. Epigenetic changes caused by advanced maternal age also have deleterious effects on oocyte quality, including oocyte maturation, chromosomal segregation, epigenetic modifications, and mitochondrial functions (19). It is expected that a significant correlation exists between the increase in the age of POR women and in morphological abnormalities. In contrast, our data showed that in PR infertile women, morphological abnormalities are not related to age.

During COS cycles, it is reported that high doses of gonadotropins create a supraphysiological hormonal environment that has detrimental effects on the female gamete and/or on the regulators of the follicular environment and cumulus corona cells, which thereby alters the morphology of oocytes (7,20). Our results showed that there was no relationship between the administered gonadotropin doses or applied COS protocol and morphological features of oocytes in the PR group.

Fertilization processes are cornerstones in ART procedures and are divided into six major consecutive steps (cumulus cell penetration, sperm/oocyte binding and penetration, sperm/oocyte fusion, oocyte activation, sperm processing, and PN formation). Following sperm/oocyte fusion, consecutive fertilization events, such as oocyte activation, cortical granule reaction, and completion of the second meiotic division, occur in the ooplasm (21). These steps are dependent on the quality/quantity of mitochondria and endoplasmic reticulum organelles (22) and biochemical remodeling of endogenous protein resources (e.g., histones and glutathione) in the cytoplasm (23). The first three fertilization steps are accepted to be mechanical events and can be eliminated using the ICSI procedure, and the last three steps in the cytoplasm are closely related to the maturation and developmental potential of oocytes.

In this study, we excluded male partners with azoospermia or severe oligozoospermia to eliminate the contribution of abnormal sperm morphology in fertilization. Therefore, the relationship between

Table 2. The percentage of oocyte morphologic abnormalities in poor and normal responder groups.

Morphologic abnormalities	Poor responders (n = 89)	Normal responders (n = 85)	P-value [a]
Cytoplasmic abnormalities	66 (74%)	69 (81%)	0.3534
Inclusion body	23 (27%)	24 (28%)	0.8892
Refractile body	2 (2%)	2 (2 %)	1 [b]
Cytoplasmic granulation	45 (46%)	54 (55%)	0.986
Central granulation	38(43%)	41 (48%)	0.5611
Dense central granulation	7 (9%)	13 (15%)	0.2967
Cytoplasmic vacuoles	21 (54%)	18 (46%)	0.3569
Heterogeneous vacuoles	5 (6%)	2 (3%)	0.4779
Peripheral vacuoles	17 (19%)	14 (16%)	0.7986
Dense peripheral vacuoles	0	3 (4%)	0.2281 [b]
Smooth endoplasmic reticulum cluster	5 (6%)	6 (7%)	0.9372
Extracytoplasmic abnormalities	56 (63%)	48 (56%)	0.476
Abnormal shape	4 (67%)	2 (33%)	0.6822 [b]
Fragmented first polar body	15 (42%)	21 (58%)	0.2753
Large perivitelline space	21 (64%)	12 (36%)	0.1613
Granulation of perivitelline space	16 (67%)	8 (33%)	0.1562
Dark zona pellucida	10 (40%)	15 (60%)	0.24
Thin zona pellucida	14 (74%)	5 (26%)	0.0659

[a] P-value calculated using chi-square test.
[b] P-value calculated using Fisher exact test.

morphology abnormalities of oocytes and fertilization rates could be clearly understood.

In the first instance, we individually analyzed all morphologic features and then grouped them according to their locations as cytoplasmic and extracytoplasmic. We found that none of the individually investigated morphologic features showed any correlation with fertilization rate and other ICSI outcomes in the PR and NR groups. The presence of cytoplasmic morphological abnormalities was found to be significantly related to lower fertilization rates compared with their absence in the PR group. This study revealed that in PR women, the total morphological abnormalities in cytoplasm have a significant effect on fertilization achievement, whereas individually each of them has no effect on fertilization. These data for PRs, defined according to the Bologna criteria, are virtually absent in the literature. In the NR group, none of the morphologic abnormalities, individually or as a group, correlated with ICSI outcomes.

However, the significance of cytoplasmic and extracytoplasmic abnormalities for treatment outcomes remains debatable (24,25). Cytoplasmic vacuoles, centrally located granularity, and SER clusters have been reported as intracytoplasmic morphological abnormalities correlated with abnormal IVF/ICSI outcomes (9,26).

Cytoplasmic maturation requires proper relocation of organelles, synthesis of proteins and carbohydrates, and posttranslational modifications of mRNAs, some of which cannot be readily visualized by light microscopy. This makes the judgement of oocyte quality difficult (27).

Our data were obtained from morphological features of oocytes evaluated using conventional light microscopy. Using more effective methods to assess oocyte quality may provide more evidence-based knowledge regarding morphological variations or abnormalities of oocytes. It is increasingly evident that only simple visualization is not an adequate assessment of oocyte quality, and noninvasive methods are required to more efficiently assess oocyte maturation. The assessment of glucose, lipid, and amino acid stores of oocytes has also been evaluated for potential new markers to delineate the oocyte quality (28). Metabolomics is a biological endpoint marker of cellular processes that determines whether these processes are completed. New assessment strategies involving the omics (i.e. genomics, transcriptomics, proteomics, and metabolomics) profile of follicular fluid and cumulus cells

Table 3. The relationship between cytoplasmic and extracytoplasmic morphologic features of oocytes and clinical characteristics in poor and normal responder groups.

		Poor responders						Normal responders					
Presence		Cytoplasmic abnormalities			Extra cytoplasmic abnormalities			Cytoplasmic abnormalities			Extracytoplasmic abnormalities		
		Absence	P-value	Presence	Absence	P-value	Presence	Absence	P-value	Presence	Absence	P-value	
Age, years, mean ± SD		36 ± 4.5	34.3 ± 4.4	0.312[a]	35.5 ± 4.6	35.8 ± 4.4	0.425[a]	31.3 ± 4.3	33.4 ± 3.3	0.07[a]	31.7 ± 4	31.7 ± 4.5	0.925[a]
Age, years, n (%)	<35 years	21 (64%)	12 (36%)	0.321[b]	20 (61%)	13 (39%)	0.91[b]	72 (77%)	21 (23%)	0.887[b]	54 (58%)	39 (42%)	0.736[b]
	≥35 years	45 (80%)	11 (20%)		36 (64%)	20 (36%)		63 (78%)	18 (22%)		50 (62%)	31 (38%)	
Induction protocol, n (%)	Pr1	34 (81%)	8 (19%)	0.574[b]	28 (67%)	14 (33%)	0.523[b]	67 (83.7%)	13 (16.2%)	0.124[b]	46 (57.5%)	34 (42.5%)	0.544[b]
	Pr2	20 (69%)	9 (31%)		16 (55%)	13 (45%)		-	-		-	-	
	Pr3	12 (67%)	6 (33%)		12 (67%)	6 (33%)		2 (50%)	2 (50%)		1 (25%)	3 (75%)	
D3 AFC, number, mean ± SD		4.8 ± 1.6	5.3 ± 2.1	0.421[a]	4.6 ± 1.8	5.5 ± 1.6	0.081[a]	13.4 ± 3.7	13.2 ± 2.9	0.923[a]	13.3 ± 3.4	13.4 ± 3.7	0.95 [a]
E2 at hCG day, pg/mL, mean ± SD		1058.2 ± 675.1	929.4 ± 615.9	0.152[a]	1147.4 ± 705.1	817 ± 519.7	0.027[a]	1642.1 ± 749.3	1588 ± 815.2	0.657[a]	1695.4 ± 753	1549.5 ± 765.5	0.325[a]
Total gonadotrophin doses, IU		3476.8 ± 1139.93	3740.2 ± 1078.9	0.345[a]	3629.02 ± 1130.72	3402.2 ± 1116.19	0.207[a]	1885.1 ± 629.4	2133.7 ± 825.9	0.304[a]	1985.4 ± 626.9	1862.5 ± 730.1	0.346[a]

AFC = Antral follicles count; E2 = estradiol; hCG = human chorionic gonadotropin; Pr: stimulation protocol; Pr 1: flexible gonadotropin-releasing hormone (GnRh) antagonist protocol, Pr 2: microdose flare-up protocol flexible gonadotropin-releasing hormone (GnRh) antagonist protocol, Pr 3: minimal stimulation protocol with clomiphene citrate.

[a] P-value calculated using Wilcoxon test of independence.

[b] P-value calculated using chi-square test.

Table 4. The relationship between cytoplasmic and extracytoplasmic morphologic features of oocytes with outcomes of ovarian hyperstimulation protocols in poor and normal responder groups.

	Poor responders						Normal responders					
	Cytoplasmic abnormalities		P-value	Extracytoplasmic abnormalities		P-value	Cytoplasmic abnormalities		P-value	Extracytoplasmic abnormalities		P-value
	Presence	Absence		Presence	Absence		Presence	Absence		Presence	Absence	
Fertilization rate	0.4 ± 0.4	0.7 ± 0.4	*0.01[a]	0.5 ± 0.4	0.5 ± 0.4	0.582[a]	0.5 ± 0.2	0.5 ± 0.3	0.163[a]	0.5 ± 0.2	0.6 ± 0.2	0.214[a]
Day 1, number of 2PN stage zygotes	1.3 ± 1.2	1.2 ± 0.9	0.723[a]	1.3 ± 1.1	1.2 ± 1.08	0.577[a]	3.8 ± 2.2	2.8 ± 1.7	0.087[a]	3.3 ± 1.9	4 ± 2.3	0.135[a]
Day 1, top-quality zygote rate	0.3 ± 0.4	0.4 ± 0.4	0.564[a]	0.3 ± 0.4	0.3 ± 0.4	0.846[a]	0.4 ± 0.2	0.4 ± 0.3	0.153[a]	0.4 ± 0.3	0.4 ± 0.2	0.748[a]
Number of embryos transferred	1.02 ± 0.7	0.8 ± 0.6	0.428[a]	1.04 ± 0.7	0.8 ± 0.7	0.331[a]	1.3 ± 0.4	1.3 ± 0.6	0.584[a]	1.2 ± 0.4	1.3 ± 0.4	0.372[a]
Number of top-quality embryos transferred	-	-		-	-		0.7 ± 0.6	0.7 ± 0.6	0.768[a]	0.5 ± 0.6	0.8 ± 0.6	0.05[a]
Pregnancy, n (%)	11 (69%)	5 (31%)	0.99[b]	10 (62%)	6 (38%)	0.899[b]	28 (82%)	6 (18%)	0.879[b]	19 (56%)	15 (44%)	0.991[b]

*P < 0.005.

[a] P-value calculated using Wilcoxon test of independence.

[b] P-value calculated using chi-square test.

have also been applied to ARTs (29). Advances in these areas will contribute to the evaluation of oocyte quality and increase the predictive power of oocyte quality for infertility treatment outcomes and obstetric consequences.

This study reveals that PR women with cytoplasmic abnormalities have lower fertilization rates, although they have similar morphological abnormalities as NRs. Overall, to obtain a healthy embryo, the first goal must be to obtain a healthy oocyte. Our quest to improve ICSI outcomes in PR women must include an understanding of the biological processes of oocytes and optimizing these processes by creating a suitable microenvironment with oocyte-specific culture medium as well as modifying the stimulation protocols.

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