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Oocyte zona pellucida and meiotic spindle birefringence as a biomarker of pregnancy rate outcome in IVF-ICSI treatment

Dwójłomność osłonki przejrzystej i wrzeciona kariokinetycznego oocytu jako czynnik predykcji wskaźnika ciąż w leczeniu IVF-ET

Jolanta Świątecka[1], Tomasz Bielawski[2], Tomasz Anchim[1], Monika Leśniewska[1], Robert Milewski[3], Sławomir Wołczyński[1]

[1] Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok, Poland
[2] Department of Biology and Pathology of Human Reproduction in Bialystok, Institute of Animal Reproduction and Food Research, Polish Academy of Science, Olsztyn, Poland
[3] Department of Statistics and Medical Informatics, Medical University of Bialystok, Poland

Abstract

Objectives: *IVF-ICSI procedures are accompanied by a continuous search for predictors of ART outcome. The properties of zona pellucida (ZP) have been believed to reflect the history of oocyte cytoplasmic maturation. The meiotic spindle (MS) is crucial for chromosomal alignment and proper separation of the maternal chromosomes. There is data suggesting that birefringent ZP and MS can clinically predict the oocyte quality and developmental potential of an embryo.*

The aim of the study was to examine the possible effect of ZP birefringent properties and MS visualization and localization as valuable predictors of IVF-ICSI effectiveness.

Material and methods: *The prospective study was performed during a 16-month period. A total of 51 patients undergoing in vitro fertilization - embryo transfer (IVF-ET) treatment procedure with intracytoplasmic sperm injection (ICSI) were included. Controlled ovarian hyperstimulation (COH) was done using either a long n=32 (62.75%) or an antagonist protocol n=19. In the group of the 48 examined patients (aged 25-40), 46 ET were performed, resulting in 24 positive pregnancy tests and 19 (39.59%) clinical pregnancies. Oocytes were examined as follows: ZP birefringence autoscoring (OCTAX PolarAIDE), numeral autoscoring, thickness and clinical evaluation; MS visualization, if MS was visualized, localization of MS in relation to the polar body (PB).*

Results: *On day 3, 64.3% of the embryos were of good and 40.3% were of top quality. Visible differences, not statistically significant, were observed in the numeral score of ZP between oocytes selected and non-selected for ET. In cases when embryos were not of good or top quality, ZP score was higher (p=0.005 p=0.001). ZP manual evaluation indicated significantly stronger birefringence when pregnancy was not achieved (p=0.022). The rate of MS positive oocytes was the highest in the group with pregnancy, but it did not reach statistical significance (p= 0.471). The MS localization in relation to the PB was in most oocytes very close (<45o) in 70.9% and not different in the studied groups.*

Conclusions: *Unexpected polarization microscopy imaging and rating of ZP and MS cannot be a direct predictor of the IVF outcome.*

Key words: **zona pellucida** / **meiotic spindle** / **oocyte** / **birefringence** / **predictor** / / **IVF-ICSI outcome** /

Corresponding author:
Jolanta Świątecka,
Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok,
15-276 Białystok, Skłodowskiej-Curie 24A Poland,
Tel./fax.: +48 85 7468434,
e-mail: jswiatecka@wp.pl

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Streszczenie

Cel: *Stosując w leczeniu niepłodności procedury IVF-ICSI wciąż poszukujemy klinicznych predyktorów skuteczności terapii. Ustalono, że właściwości osłony przejrzystej (ZP) mogą odzwierciedlać historię dojrzewania cytoplazmatycznego oocytu, natomiast wrzeciono kariokinetyczne (MS) ma kluczowe znaczenie dla prawidłowego, równikowego ustawienia i separacji chromosomów matczynych. Dane literaturowe sugerują, że dwójłomność ZP i MS może stanowić czynnik korelujący z jakością oocytu i potencjałem rozwojowym zarodka. Celem badania była ocena wartości predykcyjnej obrazowania w świetle spolaryzowanym ZP i MS w prognozowaniu skuteczności leczenia IVF-ICSI.*

Materiał i metody: *W ciągu 16 miesięcy przeprowadzono badanie prospektywne obejmujące 51 pacjentek poddanych zapłodnieniu pozaustrojowemu z docytoplazmatyczną iniekcją plemnika. Kontrolowaną stymulację jajników przeprowadzono przy użyciu długiego protokołu u 32(62,75%) lub z antagonistą u 19 pacjentek. U 48 ostatecznie badanych pacjentek wykonano 46 transferów zarodków (ET) uzyskując 24 pozytywne testy ciążowe. Oceniono następujące parametry oocytów: automatyczny i numeryczny pomiar dwójłomności, szerokość ZP (program PolarAIDE OCTAX), ocena manualna dwójłomności ZP, wizualizacja MS, lokalizacja MS w stosunku do ciałka kierunkowego (PB).*

Wyniki: *Stwierdzono różnice w numerycznej ocenie dwójłomności ZP między grupami oocytów zakwalifikowanych i nie do transferu zarodka, nie stwierdzono jednak istotności statystycznej zmian. Manualna kategoryzacja ZP wykazała, znacznie silniejszą dwójłomność w grupach, w których nie uzyskano ciąży (p=0,022). Wizualizacja MS była najwyższa w grupie w której uzyskano ciąże, ale nie wykazano istotności statystycznej (p=0,471). Lokalizacja MS w stosunku do PB w większości oocytów zawarła się w 45o (70,9%) i nie różniła się w poszczególnych grupach.*

Wnioski: *Ocena dwójłomności ZP i MS nie może być bezpośrednim czynnikiem predykcyjnym skuteczności leczenia metodą IVF-ICSI.*

Słowa kluczowe: **osłonna przejrzysta** / **wrzeciono kariokinetyczne** / **komórka jajowa** / / **dwójłomność** / **czynnik predykcyjny** / **wynik IVF-ET** /

Introduction

Single ET is becoming an increasingly common practice in infertility treatment as it allows to avoid the main complication of assisted reproduction techniques, i.e. multiple pregnancy. The identification of predictive markers for the developmental potential of oocytes before fertilization has been one of the most frequently studied areas in assisted reproduction techniques. Until now, only a few predictive noninvasive markers for oocyte quality have been identified on the basis of morphologic criteria, which can be assessed using conventional microscopy.

The introduction of polarization light microscopy made it possible to visualize the subcellular structures in oocytes, such as the ZP and MS, noninvasively [1]. A probable relationship between ZP birefringence and the following embryo quality has been discussed in the literature [1, 2, 3, 4, 5].

The unresolved issue of ZP imaging is related to the biologic cause of the variation of birefringence. The morphology of ZP has been postulated to be an expression of oocyte cytoplasmic activity. Some reports indicate that the properties of the zona layers may reflect the history of oocyte cytoplasmic maturation, whereas different development stages and culture conditions may alter the architecture of ZP [1, 6].

Aneuploidy constitutes the most frequent cause of failure in human reproduction. It can result from inadequate cytoplasmic maturity and secondary abnormal division of the genetic material. The second possible reason may be incorrect distribution of the genetic material at appropriate cytoplasmic maturity. Finally, proper division while the quality of cytoplasm is abnormal has also been considered.

Visualization of MS in polarized light can be used not only for monitoring its position during ICSI, but it also allows to evaluate the spindle birefringence status and localization in relation to the PB. Birefringent spindle in human oocytes has been suggested to clinically predict the quality and age of oocytes [7].

The aim of the study was to investigate whether ZP birefringence, MS visualization and localization in relation to the PB may be valuable predictors of oocyte quality and effectiveness of IVF-ICSI treatment. Oocytes retrieved after controlled ovarian hyperstimulation before ICSI, and afterwards allocated into 3 consecutive groups: embryo transferred and pregnancy achieved, embryo transferred and pregnancy not achieved, non-selected for transfer, have been investigated.

Material and methods

The study was approved by the ethical and scientific committees of the Medical University of Bialystok, Poland. This prospective study was performed in the course of 16 months at the Department of Reproduction and Gynecological Endocrinology, Medical University of Białystok, Poland

A total of 51 patients undergoing *in vitro* fertilization- embryo transfer (IVF-ET) treatment procedure with intracytoplasmic sperm injection (ICSI), for the second time at the most, were included. We performed ICSI for all our patients as the procedure is more effective than IVF. Due to the fact that IVF treatment was non-refundable in Poland at the time of the study, we decided not to recommend classical IVF to patients [8]. Out of the 51 treated couples, 39 exhibited unexplained infertility, while 12 had a mild male factor (23.53%) ($10x10^6 \leq < 15x10^6$/ml sperm).

No cases of testicular sperm extraction procedures, severe endometriosis, polycystic ovary syndrome or patients deemed 'poor responder' in past protocols of ovarian stimulation, were included. Female age was: 25-40 years, median 34.0 (Q_1=30.0 Q_2=37.0), 20.8% age < 30, 33.4% 30 ≤ ≤34, 45.8% ≤35, BMI: median 22.9 (Q_1=21.2 Q_2=27.3), all patients were non-smokers.

15 patients had secondary infertility (9.80%). Estradiol level on the last day of the stimulation was 2125.77±1038.79pg/mL, number of oocyte cumulus per cycle: 8.94±4.24 , MII oocyte retrieved per puncture: 7.13±3.80. In order to collect oocytes, controlled ovarian hyperstimulation was done using either a long protocol n=32 (62.75%) or an antagonist protocol n=19. In the long protocol, down-regulation of the pituitary was achieved with the GnRH agonist Diphereline SR (Diphereline SR 3,75 IPSEN Pharma Triptorelinum). Stimulation was initiated with Gonal-f® (follitropin alfa for injection), the recombinant form of human follicle stimulating hormone (FSH) (Merck Serono Europe Ltd), 14 days after Diphereline SR. In the GnRH-antagonist protocol, FSH was started on day 2 of the cycle. In addition, a GnRH-antagonist (Orgalutran; Organon) was administered after 5–6 days of the stimulation, depending on the presence of a 12- to 13-mm follicle on ultrasound. Ovulation was induced with human chorionic gonadotropin (10.000 IU; hCG) (Pregnyl Organon, or Choragon Ferring Pharmaceuticals Ltd.) in all patients. Oocyte retrieval was done by the transvaginal ultrasound-guided method 36 hours after hCG administration.

The cumulus complex was flushed in a medium heated up to 37ºC in ambient atmosphere supplemented with HSA (G-MOPS Vitrolife, Sweden). All media used in embryo culture were equilibrated at 37ºC and 6% CO_2 atmosphere. After a 2-3 hour incubation time oocytes denudation was performed by enzymatic digestion of the cumulus complex using hyaluronidase (80 IU/mL; Sage In-Vitro Fertilization, USA). Denuded oocytes were placed in G-MOPS before Intracytoplasmic Sperm Injection (ICSI). The technical set-up for zona imaging of individual gametes resembled the one published by Montag et al. [3, 9]. Individual measuring was done noninvasively with an Olympus IX71 inverted microscope (Olympus, Vienna, Austria) equipped with 10, 20, and 40 Hoffmann interference optics, a circular polarization filter, and liquid crystal analyzer optics. The birefringence analysis including autocalibration was fully controlled by a polarization imaging software module (OCTAX Polar Aide; OCTAX Microscience GmbH, Altdorf, Germany) implemented in an imaging software system (OCTAX Eyeware).

228 cumulus oophorus were selected for the examination but 31 were subsequently excluded from the study due to inadequate oocyte maturation stage. The remaining 197 oocytes were examined as follows:

– ZP birefringence was evaluated on a 4-step scale, points 0^0,1^0,2^0,3^0, Figure 1,
– ZP autoscoring (OCTAX PolarAIDE system) was performed on a 3-step scale, points 0^0,1^0,2^0, Figure 2,
– ZP numeral auto scoring (OCTAX PolarAIDE system)
– MS visualization or lack of visualization, two groups were established: spindle detected (SpD subcategory) and spindle non-detected (SpND subcategory), Figure 3,
– when visualized MS localization in relation to the PB 4-step scale 1, 2, 3, 4 Figure 4.

1. An oocyte with a spindle forming at less than 30° (<30°) to PB.
2. An oocyte with a spindle forming at 30°-45° (30°≤ <45°) to PB.
3. An oocyte with a spindle forming at 45°-120° (45°≤ ≤120°) to PB.
4. An oocyte with a spindle forming at more than 120° (120°<) to PB.

Afterwards, the oocytes were divided into 3 groups:

– Group 1. (Gr. 1) Embryos selected for transfer and outcome – day 14 after ET: positive pregnancy test
– Group 2. (Gr. 2) Embryos selected for transfer and outcome – day 14 after ET: negative pregnancy test
– Group 3. (Gr. 3) Embryos not selected for transfer

Owing to the fact that most oocytes (4-step scale, 1+2) had a spindle located in a position close to the first polar body (<45^0) 70.9%, the ICSI was conducted after the oocytes were rotated to place the first polar body at 90^0 relative to the injection needle. ICSI in the SpND oocyte group was also performed in that manner. In oocytes with spindles located in other positions/another position, the ICSI was performed after the oocytes were rotated so that the injection needle avoided the spindles.

For the statistical analysis, chi-square test of independence was used to examine the relationship between qualitative attributes. The normality of the distribution of quantitative features was verified using the Shapiro-Wilk test and Kolmogorov-Smirnov test with the Lilliefors correction. There was no normal distribution of the analyzed variables. Comparing the quantitative variables without normal distribution, the Mann-Whitney U test was used to compare differences between two independent groups, and nonparametric ANOVA Kruskal-Wallis rank test with post-hoc multiple comparisons of mean ranks was used to compare the differences between more than two independent groups. The Spearman's rank-order correlation test was used to identify correlations between quantitative variables. Results were considered statistically significant at $p<0.05$. The calculations were based on the package Statistica 10.0 (StatSoft) and PASW Statistics 17.0 (Predictive Solutions).

Results

Table I. Visualization of meiotic spindle in oocytes from the three studied groups.
Group 1. Embryos selected for transfer and outcome - day 14 after ET: positive pregnancy test,
Group 2. Embryos selected for transfer and outcome- day 14 after ET: negative pregnancy test,
Group 3. Embryos not selected for transfer p=0.47.

	MS visualization		Total
	negative	positive	
Group 1	10 28.6%	25 71.4%	35 100%
Group 2	11 37.9%	18 62.1%	29 100%
Group 3	53 39.8%	80 60.2%	133 100%
Total	74 37.6%	123 62.4%	197 100%

A total of 35 oocytes constituted Group 1, where 21 transfers were done, 14 ET with 2 embryos and 7 with only 1 embryo per transfer.

Group 2 involved 29 ovarian cells; 20 ETs were done, 9 with 2 embryos and 11 with 1 embryo per transfer.

The 133 non-selected for ET oocytes accounted for Group 3.

On day 1, fertilization rate was 79.18% (156/197). On day 3, 98.7% (154/156) of the zygotes of the fertilized gametes had cleaved. 64.3% (99/154) of the embryos were of good quality (<10% fragmentation and 6-9 mononucleate blastomers), 88.6% (31/35), 79.3% (23/29), and 50.0% (45/90) in Groups 1, 2, 3 respectively (p<0.001). Among them, 40.3% (62/154) of the embryos were of top quality (<10% fragmentation and 7-9 mononucleate equal-sized and regular blastomers) 62.9% (22/35) 58.7% (17/29) and only 25,6% (23/90) in the studied groups, respectively (p<0.001) [3, 15]

In a group of the 48 examined patients, 46 ET were done (95.8%) on day 3, with 41 performed with the use of the examined embryos (85.1% and 89.13%, respectively). 14 days after ET, 24 positive serum pregnancy tests were obtained (50%), 21 after transfer of the studied oocytes (43.75%). The implantation rate was 35.9% (23/64), however 2 miscarriages reduced clinical pregnancy rate to 39.59% (19/48). Blastocyst cultures were continued until day 5, and blastocysts were achieved in 6.82% of cases, and then vitrified.

Determination of the zona pellucida birefringence

Numeral score of ZP birefringence accounted as follows: Gr.1. 39.53±34 (median 31.0), Gr.2. 37.86±31 (median 40.4), Gr.3. 52.27±39 (median 48.5). Although there were visible differences between groups 1 and 3, they did not reach statistical significance (p=0.098 ANOVA rank Kruskal-Wallis test), (Figure 5). No correlation between patient age and numeral ZP score was observed (p= 0.158 Spearman's rank-order correlation test), while weak negative correlation with BMI (p= 0.002, R=-0.22 Spearman's rank-order correlation test) was detected. Both, good 40.14±33 (median 36.0) and top quality 34.88±30 (median 27.8) embryos on day 3 indicated lower ZP birefringence score than the remaining embryos on day 3, 58.52±40 (median 66.3) 54.67±38 (median 61.8), respectively (p=0.005 p=0.001, respectively Mann-Whitney U test).

Autoscores obtained in all three groups, (Table IV) were nearly identical. No differences of statistically importance were observed between the three studied groups (p=498, Pearson Chi-quadrat test) between embryos of the good or top quality compared to the rest embryos of poor quality on day 3 (p=984, p=629), and when compared to age and BMI (p =0.206, p= 0.054 accordingly ANOVA rank Kruskal-Wallis test).

Finally, no effect of ZP birefringence status of both, automatically specified using OCTAX PolarAIDE system, numeral score or autoscore on embryo selection for ET, and the following implantation, was observed.

More comprehensive evaluation of ZP birefringence status, using a 4-step scale (Figure 1), indicated significant differences between the studied oocytes. In group 1, the percentage distribution was as follows: 14.3%, 34.3%, 20.0% and 31.4% respectively to points 1^0, 2^0, 3^0, and 4^0 on a 4-step scale. In groups 2 and 3 ZP birefringence was significantly stronger, and more than 70% of the oocytes turned out to be at 3^0 and 4^0 step of the scale (72.4% and 75.2% accordingly) (p=0.022 Pearson Chi-quadrat test) (Table III). Similar direction of changes was found when good and top quality embryos were analyzed. 60.6% of the good quality embryos and only 54.9% of the top quality embryos demonstrated strong ZP birefringence in comparison to the rest of the embryos on day 3: 81.8% and 77.1%, respectively (p=0.006 p=0.002 respectively Pearson Chi-quadrat test). No differences in ZP birefringence status were observed in relation to patient age and BMI (p=0.866, p=0.122 respectively ANOVA rank Kruskal-Wallis test).

Meiotic spindle evaluation

Out of the 197 evaluated oocytes, birefringent spindles were detected in 123 (62.4% SpD subgroup), and were not visualized in 74 (37.6% SpND subgroup; Figure 2) of cases. No differences were observed in relation to patient age and BMI (p= 0.860 and p= 0.282 respectively, Mann-Whitney U test).

The rate of SpD was the highest in group 1 (71.4%), when compared to groups 2 and 3 (62.1% and 60.2%, respectively), but without statistical significance (p= 0.471 Pearson Chi-quadrat test). (Table I).

The rate of SpD in good quality embryos on day 3 was nearly the same as compared to the remaining embryos of not good quality and amounted to 64.6% and 63.6%.6, respectively (p=0.900 Pearson Chi-quadrat test). In top quality embryos, the rate of SpD was the highest in the entire study (71.0% versus 59.8%), but failed to reach statistical importance (p=0.155).

The meiotic spindle localization in relation to the polar body was in most oocytes very close, forming at less than 30° (<30°) in 41.0% and at less than 45° (<45°) in 70.9% of cases. There were no statistically significant differences between the three studied groups of oocytes (p=906 Pearson Chi-quadrat test) (Table II) and no relation to age and BMI (p =0.439 p =0.159, respectively ANOVA rank Kruskal-Wallis test) was observed, either. Also, qualification of embryos to the good and top groups did not change the reported parameter (p=0.552 p=0.190, respectively).

Discussion

The improvement of oocyte quality and the identification of oocyte and then embryos with high implantation competence constitute the greatest challenges of assisted reproduction. On the other hand, single ET is increasingly used to avoid multiple pregnancy rates. Observational studies have demonstrated the strategy to have minimal influence on the overall pregnancy rate when applied in women with good prognosis [10, 11, 12]. Cell number and morphology remain to be the most widely used parameters for embryo selection before ET [13, 14].

The quality of oocyte can drastically affect the ART outcome, thus ZP and MS visualization before fertilization could be useful as prognostic markers [2]. ZP surrounding the oocytes before fertilization acted as a special barrier to sperm, but it could allow the merger of the oocyte and a single sperm to benefit fertilization. Afterwards, it changed its own chemical structure rapidly so as to prevent poly-sperm fertilization. ZP could protect early embryonic integrity and transportation. Endometrial lysine and blastocyst expansion caused zona thinning in order to prepare for implantation. Sometimes ZP failed to rupture, which would inhibit hatching, perhaps constituting one of the most important factors resulting in implantation failure [15].

Table II. Meiotic spindle position in relation to the polar body (in %), a 4-step scale as follows:
step 1. An oocyte with a spindle forming at less than 30° (<30°) to the polar body.
step 2. An oocyte with a spindle forming at 30°-45° (30°≤ <45°) to the polar body.
step 3. An oocyte with a spindle forming at 45°-120° (45°≤ ≤120°) to the polar body.
step 4. An oocyte with a spindle forming at more than 120° (120°<) to the polar body in the three studied groups of oocytes p=0.91.

	MS position in relation to the polar body				Total
	step 1	step 2	step 3	step 4	
Group 1	9 36.0%	9 3.60%	6 24.0%	1 4.0%	25 100%
Group 2	6 33.3%	7 38.9%	4 22.2%	1 5.6%	18 100%
Group 3	33 44.6%	19 25.7%	17 23.0%	5 6.8%	74 100%
Total	48 41.0%	35 29.9%	27 23.1%	7 6.0%	117 100%

Table III. Zona pellucida birefringence in a 4-step clinical expanded scale (details in Figure 4.) in the three studied groups of oocytes p=0.02.

	ZP birefringence on a 4-step scale				Total
	step 0	step 1	step 2	step 3	
Group 1	5 14.3%	12 34.3%	7 20.0%	11 31.4%	35 100%
Group 2	3 10.3%	5 17.2%	15 51.7%	6 20.7%	29 100%
Group 3	13 9.8%	20 15.0%	42 31.6%	58 43.6%	133 100%
Total	21 10.7%	37 18.8%	64 32.5%	75 38.1%	197 100%

Table IV. Zona pellucida birefringence autoscoring (OCTAX PolarAIDE system) on a 3-step scale, points 00,10,20, (details in Figure 2.) in the three studied groups of oocytes p=0,50

	ZP auto score on a 3-step scale			Total
	step 0	step 1	step 2	
Group 1	9 25.7%	0 0.0%	26 74.3%	35 100%
Group 2	7 24.1%	0 0.0%	22 75.9%	29 100%
Group 3	23 17.3%	4 3.0%	106 79.7%	133 100%
Total	39 19.8%	4 2.0%	154 78.2%	197 100%

Therefore, different parameters of ZP such as thickness variation, birefringence, birefringence uniformity and intensity, continue to be extensively studied but the literature data and their predictive value remain ambiguous.

In our study, it has been indicated that automatically evaluated ZP autoscore in most of the studied oocytes (> 70%) was very strong and allocated into the highest point on a 3-step scale. Additionally, we did not observe statistically significant differences between the studied groups.

Assuming that groups 1 and 2 (i.e. selected for transfer), especially group 1, included the best quality embryos, we postulate that automatically set autoscore of ZP is not a predictive factor of embryo quality and IVF outcome in clinical practice. Even more, that the comparison of autoscore detected in the best quality embryos with the remaining embryos on day 3 also revealed no significant differences. Surprisingly, the best embryos, classified as good and top on day 3, formed from MII oocytes with a much lower, statistically significant, ZP scores. In addition, numerically defined ZP birefringence score showed the highest value (both, presented as the median and as the mean) in the group of embryos not selected for transfer. However, there was no statistical significance of this difference. That may have been caused by significantly different numbers of embryos between groups 1 and 2 (selected for transfer) as compared with the non-selected group. Owing to the nature of IVF-ET treatment methods, avoidance of that difference is in fact not possible. However, it may suggest an inverse relationship between MII oocyte ZP score and the quality of the resulting embryo.

A slightly different situation occurred (but the direction of the change was similar) when the state of ZP birefringence was determined on a 4-level scale, more complex and detailed (ZP thickness, continuous glowing, uniformity of birefringence across

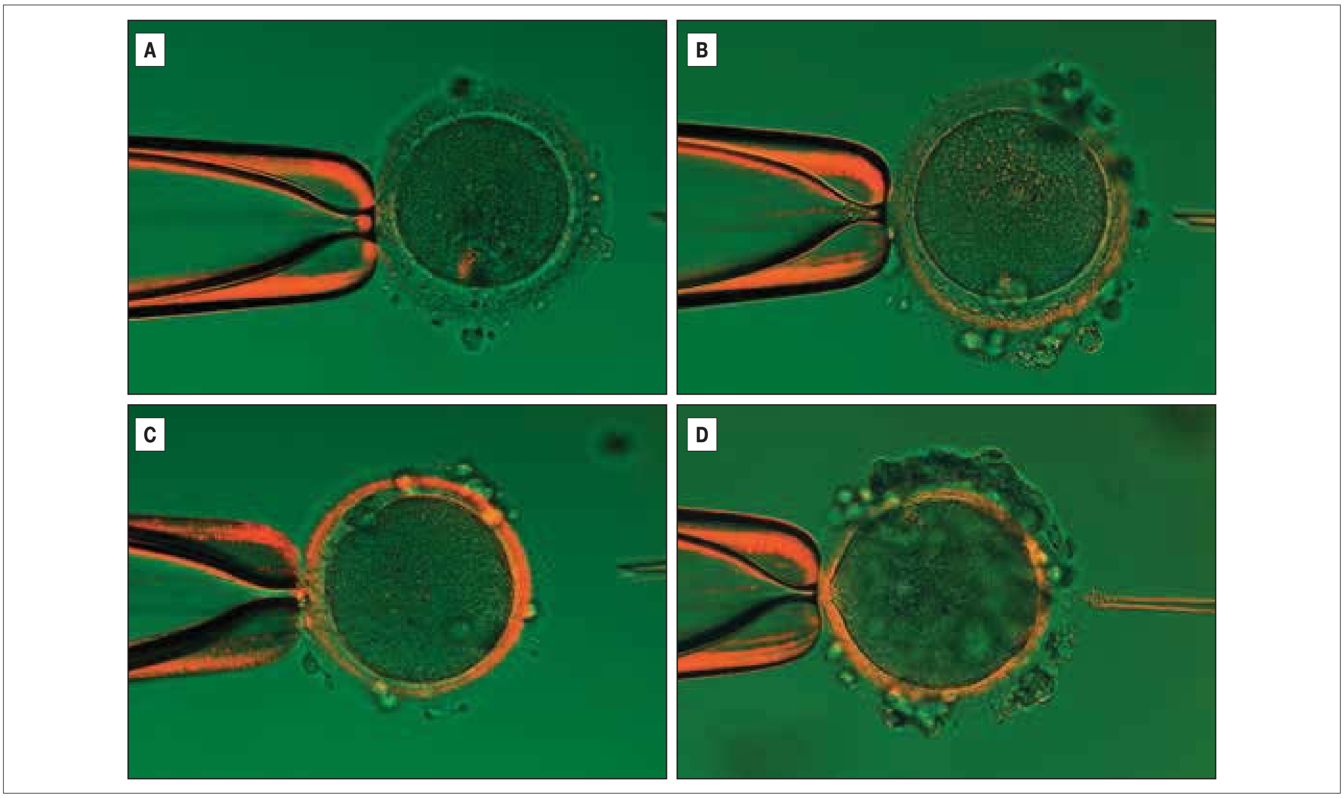

Figure 1. Zona pellucida birefringence on a 4-step clinical expanded scale, points. **A** – step 0°, **B** – step 1°, **C** – step 2°, **D** – step 3°.

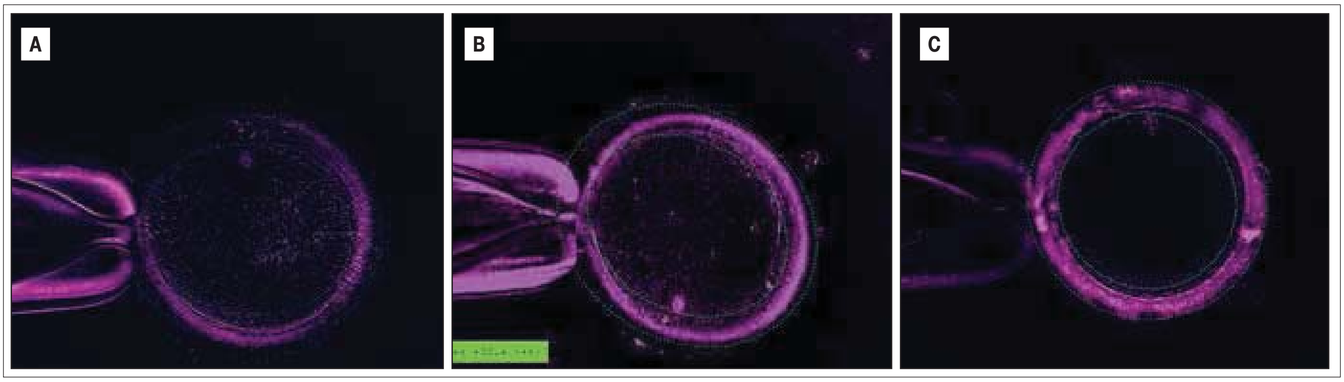

Figure 2. Autoscoring (OCTAX PolarAIDE system) on a 3-step scale, points. **A** – step 0°, **B** – step 1°, **C** – step 2°.

the entire layer and intensity). Regardless, ZP birefringence was strong in the all studied groups of oocytes (at least 50% of the oocytes were deemed eligible for steps 2 and 3 on the 4-level scale), and looked in detail as follows: in the group selected for transfer and resulting in a positive pregnancy test - 50%, in groups 2 and 3 - >70%. On the other hand, it is clear that about 50% of the oocytes in group 1 owned ZP with poor birefringence. Also, among the best embryos on day 3, almost half had ZP birefringence classified as low. Undoubtedly, that fact did not adversely affect the final outcome, i.e. a positive pregnancy test.

Finally, our findings are at odds with most of the literature data, reporting that higher zona pellucida birefringence is a positive predictor of oocyte and embryo quality, resulting in higher rate of conception [3, 4, 9, 16].

ZP thickness was the last parameter to be examined in our work. Betrand demonstrated that ZP thickness strongly influenced *in vitro* fertilization of human oocyte, as the thickness was different in fertilized and non-fertilized oocytes [17]. Jimenez reported that fertilization-failed oocytes have thicker ZP than embryos [13].

In our study, we did not find any significant differences in ZP thickness between the groups of oocytes with high and low cleavage and following implantation potential. Our findings, consistent with Sun, Hagemann, Lane, excluded the prognostic value of oocyte ZP thickness in assessing the quality of embryo [15, 18, 19, 20] On the other hand, Sun suggested that embryo ZP thickness variation, as prelude to blastocysts hatching, could be of some practical predictive value [13].

In metaphase II, unfertilized oocyte MS is crucial for normal chromosome alignment and separation of the maternal chromosomes during meiosis [21, 22]. Disruption of the MS results in rearrangement or scatter of chromosomes and may

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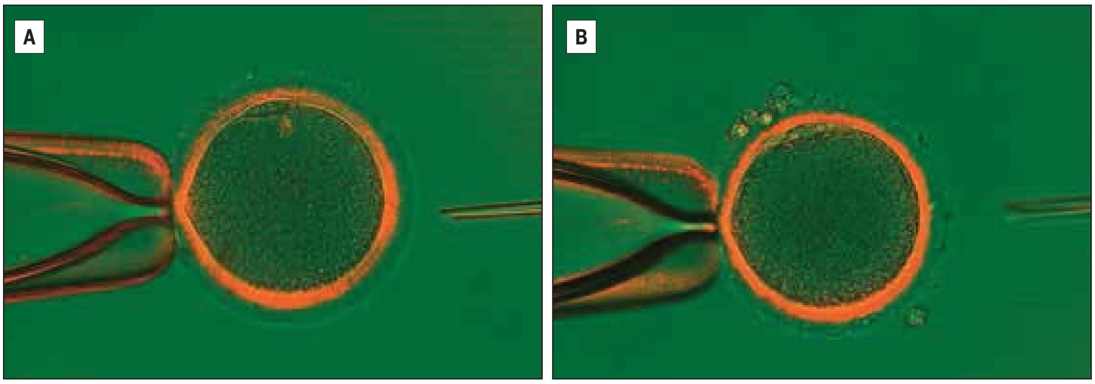

Figure 3. Meiotic spindle visualization or lack of visualization, two group were established:
A – spindle detected (SpD subgroup) and **B** – spindle non-detected (SpND subgroup).

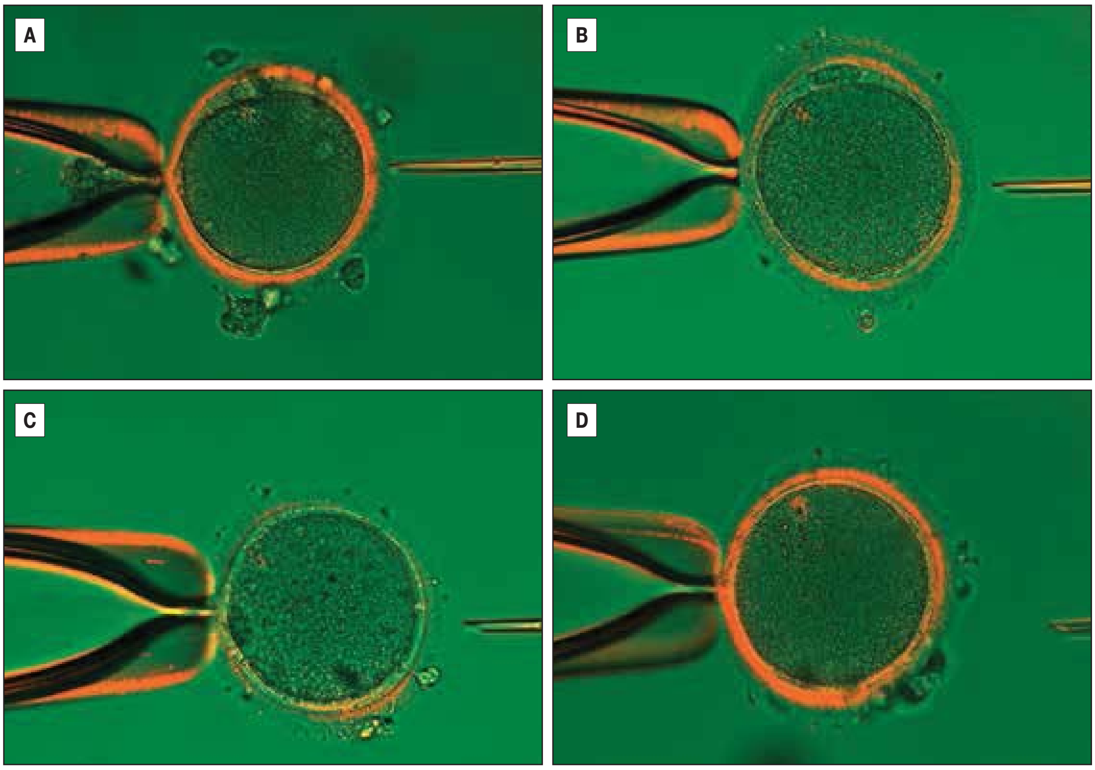

Figure 4. Visualized meiotic spindle localization in relation to the polar body on a 4-step scale.
A – step 1. An oocyte with a spindle forming at less than 30° (<30°) to the polar body. **B** – step 2. An oocyte with a spindle forming at 30°-45° (30°⩽ <45°) to the polar body. **C** – step 3. An oocyte with a spindle forming at 45°-120° (45°⩽ ⩽120°) to the polar body. **D** – step 4. An oocyte with a spindle forming at more than 120° (120°<) to the polar body.

contribute to aneuploidy after fertilization [23, 24, 25, 26, 27, 28]. Aneuploidy is one of the most common causes of abnormal fertilization in humans [29, 30].

In our work MS was visualized in more than 60% of the studied oocytes (60-71.4%). This is consistent with other data from the literature, where the authors reported positive result as follows: Wang in 81.7%, Kilani in 88%, Braga in 74.3% and Madaschi in 62.8% [2, 7, 10, 31]

The relationship between the occurrence of visible MS, fertilization rate, morphology of pronuclei, and the final clinical outcome in IVF-ET treatment are still inconclusive and continuously under debate.

Conclusion

In our study, spindle positive subcategory in the group where pregnancy was achieved was significantly higher than in the remaining ones, although without statistically significant difference. We did not observe meaningful, statistically significant differences in the MS localization with respect to the PB.

Probably, it will be possible to treat the birefringent spindle as a predictor of embryo capacity and quality after performing extended tests. One thing remains unquestionable, MS visualization during the ICSI process allows to avoid damage to the genetic material of the oocyte and is always desirable during the procedure.

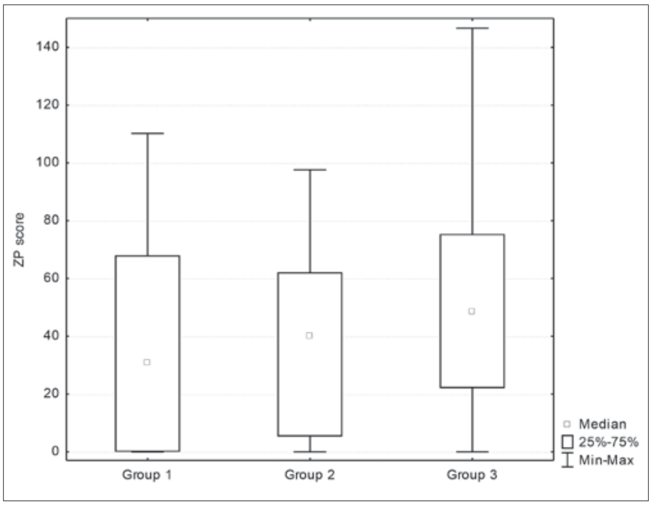

Figure 5. Zona pellucida birefringence numeral autoscore (OCTAX PolarAIDE system) in the three studied groups of oocytes p=0.09

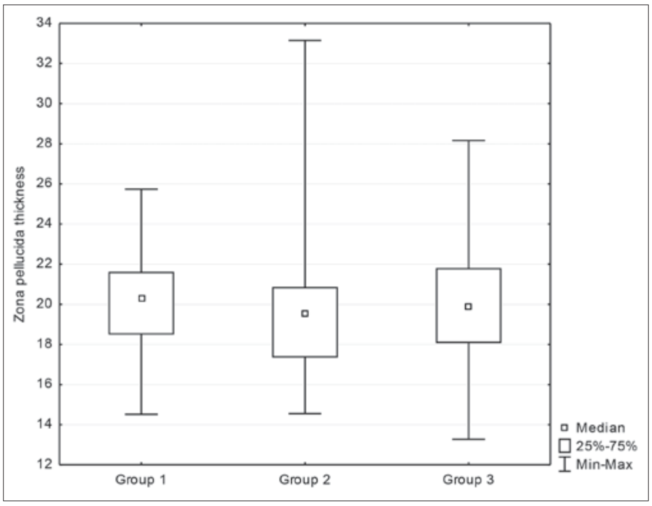

Figure 6. Zona pellucida thickness (OCTAX PolarAIDE system) in the three studied groups of oocytes p=0.66

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Oświadczenie autorów

1. Jolanta Świątecka – autor koncepcji, założeń protokołu, metody i analiza danych, analiza i interpretacja statystyczna - autor zgłaszający i odpowiedzialny za manuskrypt.
2. Tomasz Bielawski – autor koncepcji, założeń protokołu, metody i analiza danych.
3. Tomasz Anchim – metody i analiza danych.
4. Monika Leśniewska – autor koncepcji i założeń pracy.
5. Robert Milewski – analiza i interpretacja statystyczna.
6. Sławomir Wołczyński – autor koncepcji i założeń protokołu.

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