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Blood type ABO and the cytokine profile of follicular fluid in women undergoing IVF/ET

Short title: Blood type ABO and the cytokine profile of follicular fluid

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ABSTRACT

Objectives: ABO blood type was hypothesised to be related to a number of infertility processes. There is still an open debate on ABO blood group’s incompatibility and infertility.

It was associated with ovarian reserve in women with subfertility. There is still not enough information on the influence of blood type and the immunology of follicular fluid (FF).
Material and methods: 78 patients were selected, who underwent in vitro fertilization (IVF) between April 2021 and January 2022. FF samples from each individual patient were taken on the day of ovarian puncture and stored at -80°C until immunological assessment. Concentration of chosen interleukins - IL-1α, IL-2, IL-4, IL-5, IL-6, IL-8 IL-10, IL-15, IL-1β, IL-18, IFN, LIF, TNFα, GCSF and PIBF-1 were measured using commercially available ELISA kits.
Results: All assessed cytokines were present in the FF of exanimated patients. The concentration was compared to the blood type ABO of all women undergoing in vitro fertilization. No statistical relevance was found between blood type ABO and the concentration of GCSF, PIBF1, LIF, IL-15, IL-5, IL-8, IL-1 alfa, IL-1 beta, INF gamma, IL-2HS, IL-4HS, IL-6HS, IL-10HS in the FF obtained during ovarian puncture ($p > 0,05$). There was no statistically significant correlation between blood type ABO and the quality of embryo, and the positive pregnancy test in patients undergoing IVF/ET.
Conclusions: The blood type ABO does not influence the wide cytokine profile of FF obtained during ovarian puncture in women with infertility of different origin, as well as embryo quality and pregnancy rate.
Key words: follicular fluid; cytokines; blood type ABO; embryo quality; pregnancy

INTRODUCTION

ABO blood type was hypothesized to be related to a number of infertility processes. There is still an open debate on ABO blood group incompatibility and infertility. It was associated with ovarian reserve in Chinese women with subfertility [1, 2]. ABO blood type was also considered a factor in predicting the chances of successful IVF/ET [3].

There is still not enough information on the influence of blood type and the immunology of follicular fluid (FF). In this study, we analyzed the ABO blood type and selected cytokines concentration in FF. During ovarian puncture in IVF qualified women FF was obtained. The connection between cytokine profile of FF, blood type ABO and the selected parameters of different stages of IVF-ET procedure was explored.

MATERIAL AND METHODS

The study group consisted of patients that underwent in vitro fertilization (IVF) in the Fertility Center in Bydgoszcz, Poland, between April 2021 and January 2022. A total of 78 patients were selected in order of qualification for IVF, after meeting the inclusion and exclusion criteria, which were described in a previous publication in 2022 [4].

All qualified patients suffered from infertility, defined as the inability to achieve pregnancy after one year of regular intercourse and underwent detailed medical interview, physical examination and gynecological assessment of the primary reason for infertility. Patients suffering from other serious or chronic diseases or those taking medication that could have an influence on the results of the study were excluded.

All immunological assessments were performed in collaboration with the Clinic of Allergology, Clinical Immunology and Internal Diseases, Collegium Medicum in Bydgoszcz, Poland. The analysis of data and interpretation of obtained results were prepared in collaboration with the Department of Clinical Immunology and Allergology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.

Gynecological assessment

All patients underwent detailed medical interviews, physical examination, gynecological and cytological examination, ultrasound assessment of the reproductive system, determination of vaginal biocenosis and pH. The serum estradiol, prolactin, progesterone, AMH, FSH, electrolytes were assessed, as well as morphology, APTT and blood type.

All fertility procedures were carried out in accordance with the currently applicable standards of the Polish Society of Reproductive Medicine and Embryology (*PTMRiE)* and the European Society of Human Reproduction and Embryology.

The hormonal stimulation and in vitro fertilization were described in detail in a previous publication [4]. In short, it was conducted in one menstrual cycle, with the administration of gonadotropin, followed according to procedure, by a gonadotrophin-releasing hormone antagonist and recombinant human chorionic gonadotrophin (rhCG, Merck) after ultrasound follicular assessment. Oocyte retrieval was performed transvaginally. FF was obtained to sterile tubes (Falcon) and then transferred to petri dishes. Using a stereoscopic microscope, cumulus-oocyte complexes were found and transferred to the OC plate (Falcon) with the MHM-C (Irvine Scientific) medium. After cutting the cumulus oocytes were placed in an incubator (K-systems) at 37°C in CSC-C media droplets (Irvine Scientific) coated with oil (Vitrolife) on IFV 35mm (Nunc) plates. Fertilization of oocytes was performed using the Olympus IX inverted microscope with Hoffman contrast equipped with

RI's Integra Ti micromanipulation equipment, with sperm on 50x9mm (Falcon) plates in MHM-C drops coated with oil using micropipettes (ICSI Micropipettes, holding Micropipettes, ORIGIO® Micropipettes).

Follicular fluid samples from each individual patient were taken on the day of ovarian puncture and stored at -80°C until immunological assessment.

Oocyte fertilization took place between 39 and 41 hours after picking up. Fertilization assessment was performed at 17+/-1 hours. The 3rd day embryo assessment, according to Gardner and Schoolcraft criteria, took place at 68+/-1 hours after fertilization, assessment in the 5^{th} day took place at 116+/-1 hours after fertilization. The quality of the embryos was assessed by an embryologist on the 3^{rd} and 5^{th} day after fertilization [4, 5]. Clinical confirmation of pregnancy was based on blood serum B-hCG concentration between the10^{th} and the 15^{th} day after transfer.

Immunoassay

The follicular fluid, which was obtained during the ovarian puncture, was stored in -80°C until the assessment.

Laboratory analysis

All interleukin concentrations were prepared according to manufacture instruction in Immunological Laboratory of The Clinic of Allergology, Clinical Immunology and Internal Medicine, by highly qualified personnel. The detailed methodology was described previously [4]. In general, the concentration of IL-2, IL-4, IL-6, IL-10 and IFNγ was measured using commercially available High Sensitivity (HS) ELISA kits (Diaclone, Medix Biochemica Group). The concentration of IL-1α, IL-1β, IL-5, IL-8 and IL-15 was measured using commercially available ELISA kits (Diaclone, Medix Biochemica Group). The concentration of IL-18, LIF, TNFα, GCSF and PIBF-1 was measured using commercially available ELISA kits (Cloud-Clone Corp.)

Bioethics Committee and statistical analysis

This study was received approval from the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Bioethical Committee. The assigned classification number was KB 334/2021. All patients gave informed, written consent to participate in the study.

The Mann-Whitney test was used to compare quantitative variables between two groups, while the Kruskal–Wallis test (followed by Dunn post-hoc test) was used for more

than two groups. The relationship between two quantitative variables was assessed using the Spearman's coefficient of correlation. Univariate logistic regressions were used to analyze the impact of selected variables on dichotomous outcome (positive/negative pregnancy test). Odds ratios (OR) with 95% confidence intervals were shown. The significance level for all statistical tests was set to 0.05. R 4.1.2. and MS Excel 365 was used for computations.

RESULTS

The study group consisted of women with a mean age of 34,26+/-4 years old (Table 1). The body weight was in general within normal. All women met the inclusion and exclusion criteria. The diagnosed reasons of infertility have been described before [4].

PIBF1 concentration and cytokine profile of follicular fluid in all patients were measured. The results of immunoassay are presented in Table 2.

All assessed cytokines were present in the follicular fluid of exanimated patients. The concentration was compared to the blood type ABO of all women undergoing IFV. No statistical relevance was found between blood type ABO and the concentration of GCSF, PIBF1, LIF, IL-15, IL-5, IL-8, IL-1 alfa, IL-1 beta, INF gamma, IL-2HS, IL-4HS, IL-6HS, and IL-10HS in the FF obtained during ovarian puncture ($p > 0,05$) (Table 3).

Successful IVF/ET is defined in the study as a positive pregnancy test. Pregnancy was confirmed between the 10th and the 15th day after ET, by measuring blood serum B-HCG). In the analyzed group, IVF/ET was successful in 24 cases (30.8%). There was no statistically significant correlation between blood type ABO and positive pregnancy tests in patients undergoing IVF/ET (Table 4).

Additionally, the assessed quality of embryo did not have a connection with ABO blood type either, with similar results for all assessed blood types (Table 5).

DISCUSSION

The influence of ABO blood type on human fertility was discussed since early 1950s. Several aspects were taken into account, including the blood type of the fetus and the mother, the heterogenicity of the fetus and its influence on live birth [5]. There is evidence that ABO blood group has an influence on haemostasis, as documented by the relationship between ABO blood type and von Willebrand factor (VWF), and hence factor VIII (FVIII) in the blood serum [6]. It was found that individuals with blood group O have approximately 25% lower VWF plasma levels compared with those with non-O blood groups. In a research based on the

blood donors that were attending the transfusion center in Italy from January 2010 to September 2014, it was found that the group B was 2.17% more frequent in pregnant women and 2.04% more frequent in female blood donors with children than in female blood donors without children [7]. The results go in line with a study by Shami et al., who found that B-positive mothers were more successful in reproduction [8]. Recently, during the COVID 19 pandemic, the connection between the concentration of von Willebrand factor (VWF), blood type and the risk of severe SARS-COV2 infection was analyzed. There was evidence that group O may be associated with a lower risk of SARS-CoV-2 infection and group A may be associated with a higher risk of SARS-CoV-2 infection along with severe disease [9]. Currently, the aspect of IVF/ET success rate and AB blood type is of interest. In the study by Pereira et al. in 2017, a total of 2329 patients were included, with the distribution of blood types as follows: A = 38.5%; B = 17.0%; AB = 5.2%; and O = 39.3% patients. The adjusted ORs for live birth did not show any statistical significance depending on the individual blood type. What is more, the patients with different blood type did not differ in their childbirth weight or gestational age at delivery [10].

Spitzer et al. in a retrospective observational single-center study analyzed 1889 IVF cycles carried out for 7 years (2005–2012). The maternal age and ABO blood type was compared with the number of cumulus oocyte complexes and metaphase II oocytes, fertilization rate, pregnancy rate and birth rate. The mean number of MII oocytes and 2PN stages and IVF outcome measured in terms of pregnancy rate and birth rate were similar for all blood type groups [11].

In case of cytokine profile of follicular fluid obtained during in vitro fertilization, there is an increasing amount of information on its influence on human fertility. It is known that pro-inflammatory cytokines are crucial in the maturation process of the ovarian follicle, in addition to the process of embryo implantation [12]. In our previous research, we found that the higher PIBF1 concentration in FF may indicate a greater possibility of successful IVF due to the higher number of top-quality embryos. IL-1 beta concentration was found to be lower in the FF of patients with successful IVF. Therefore, PIBF1 and IL-1 beta in FF could be candidates for a marker of successful IVF [4]. It was previously proven that the cytokine profile of FF changes during ovarian ageing is clinically characterized by a diminished ovarian reserve [13].

In the current follow up analyses, we established that the blood type AB0 does not influence the wide cytokine profile of FF obtained during ovarian puncture in women with infertility of

different origin, qualified to IVF/ET. We found that there was no significant correlation between ABO blood type and the cytokine profile of FF, embryo quality and pregnancy rate. The results on pregnancy rate are in line with previous research on ABO and IVF/ET results [10, 14]. In a prospective cohort study conducted in 497 women undergoing IVF/ET, Di Nisio et al. confirmed the lack of a significant association between non-O blood type and clinical outcomes of IVF [14]. However, some studies pointed out that there might be a significant difference between the successful rate of IVF/ET and blood type. 30,717 couples were included in a retrospective cohort study. All patients underwent IVF cycles (2010–2019). Bao et al. [15] found that there was a statistically significant positive association between the combination of female blood type AB and male blood type AB with biochemical pregnancy, clinical pregnancy and live birth rate. This issue requires further investigation.

To our knowledge, this is the first study to investigate the connection between ABO blood type and the cytological profile of FF obtained during ovarian puncture in patients preparing for IVF/ET. Despite several recent publications, the knowledge of follicular fluid immunology in the context of reproductive pathology is superficial. Understanding the roles of individual cytokines in reproductive pathology might be crucial in predicting the effectiveness of fertility procedures and in searching for the markers of successful IVF/ET. The concentration of pro- and anti-inflammatory cytokines both in blood serum and FF is of interest and might influence the results of fertility procedures, clinical course of pregnancy and term delivery. The imbalance between Th1/Th2 cytokine profile seems crucial for lack of correct implantation and pregnancy losses. Kuroda et al. [16] found that Th1 cell levels and the Th1/Th2 cell ratio were significantly higher in the women who needed at least ≥ 4 embryo transfer cycles to conceive and who had ≥ 2 pregnancy losses. Makhseed found that in pre-term delivery women, there were significantly higher levels of the type 1 cytokines, interferon (IFN)-gamma and interleukin (IL)-2 in the blood serum. In the normal pregnancy group, there was significantly greater production of the type 2 cytokines, IL-4, IL-5 and IL-10 [17].
An interesting study by Sarapik et al. [18] found that the cytological profile of human FF might differ depending on the origin of infertility. In general, higher levels of MIP-1α were found in women diagnosed with polycystic ovarian syndrome, higher levels of IL-23, INF-γ and TNF-α in endometriosis, lower levels of IL-β and INF-α were correlated with tubal infertility and low IL-18 concentration was characteristic of unexplained infertility.
Khadem et al. [19] found that a higher concentration of IL-1 in the endometrial secretion, aspirated before performing the oocyte collection, was associated with successful chemical

pregnancies (p = **[???]**.00) in 76 women undergoing IVF/ICSI. The cytokine analysis of peritoneal fluid also shows relevant changes in patients with endometriosis, because endometrial lesions start an inflammatory reaction represented by the overproduction of prostaglandins (PGE2), metalloproteinases (MMP-2, -3, -9), cytokines (IL-1β, IL-8, IFN-γ, TNF-α, MCP-1 and MIF) and adhesive molecules (ICAM-1, VCAM-1) [20].

In our previous study, IL-2 and IL-6 concentrations in FF were positively correlated with the numbers of COC-1 and MII [4].

In an interesting work by Havrylyuk et al. [21], 27 healthy controls and 55 infertile male patients had their seminal plasma and blood taken for cytokine assessment. In general, statistically significant elevated levels of TGF-β1 were found in infertile males with idiopathic infertility. What is more, statistically significant elevated levels of IL-10, IL-18 and IFN-γ were found in blood serum samples of a group of idiopathically infertile males, compared to the male healthy control group. Interestingly, in this group of infertile males, the IL-1β level in seminal plasma was significantly decreased while IFN-g was significantly increased [21].

The examples above show that the cytokine profile of blood serum and FF might have an influence on the maturation, development and implantation of embryo, as well as on the course of the pregnancy.

The limitation on all studies based on human FF is that the FF analysis requires its collection by ovarian puncture. Therefore, the knowledge about the cytokine composition of FF concerns mainly animal models, and human FF is collected via various gynaecological procedures. Another limitation is that is a relatively small and diverse research group, with infertility of different origins. Further studies are required to establish if there is in fact no correlation between ABO blood type and cytokine profile of FF.

CONCLUSIONS

Despite several recent publications, the knowledge of follicular fluid immunology in the context of reproductive pathology is superficial. In the current research, we established that the blood type ABO does not influence the wide cytokine profile of FF obtained during ovarian puncture in women with infertility of different origin, as well as embryo quality and pregnancy rate. Further studies on less diverse population are required in search for good markers of successful IVF/ET.

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Data availability statement

Data are available on request

Ethics statement

The study was approved by the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Bioethical Committee and was assigned a classification number: KB 334/2021. All patients gave informed, written consent to participate in the study.

Author contributions

RA — conceived and designed the analysis, wrote the paper, collected the data, study design; NUS — conceived and designed the analysis, wrote the paper, study design; KL — performed

immunoassay; VCh — conceived and designed the analysis; AH — conceived and designed the analysis; DA conceived and designed the analysis; WC — conceived and designed the analysis; ZB — supervision, study design; MD — supervision, study design.

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Not applicable.

Conflict of interest

No conflict of interest to declare.

Supplementary material

Not applicable.

Table 1. General characteristic of the study group.

Parameter		**Total (N = 78)**
Age [years]	mean ± SD	34.26 ± 4.53
	Median	33
	Quartiles	31–37.75
Age	Up to 34 years	45 (57.69%)
	35 years and more	33 (42.31%)
BMI [kg/m²]	mean ± SD	23.52 ± 3.7
	Median	22.47
	Quartiles	20.86–25.39
Weight [kg]	mean ± SD	65.23 ± 11.46
	Median	62.5
	Quartiles	57–71
Blood group	0	23 (29.49%)
	A	32 (41.03%)
	AB	11 (14.10%)
	B	12 (15.38%)

Table 2. Cytokine concentration in follicular fluid of patients.

Parameter	N	Missing	Mean	SD	Median	Min	Max	Q1	Q3
PIBF1 [ng/mL]	78	0	1.85	1.61	1.46	0.1	9.91	0.66	2.5
L-5 [pg/mL]	78	0	2.23	3.35	0	0	17.02	0	5.07
GCSF [pg/mL]	78	0	7.42	0.99	7.29	5.94	11.2	6.73	7.78
PIBF1 [ng/mL]	78	0	1.85	1.61	1.46	0.1	9.91	0.66	2.5
LIF [pg/mL]	78	0	20.12	17.24	16.99	13.65	132.78	15.72	18.31
IL-15 [pg/mL]	78	0	46.73	3.67	46.52	38.22	57.23	44.67	49.02
IL-5 [pg/mL]	78	0	2.23	3.35	0	0	17.02	0	5.07
IL-8 [pg/mL]	78	0	181.41	62.97	172.74	24.13	335.23	132.1	216.63
IL-1 alfa [pg/mL]	78	0	23.49	6.74	23.02	0	58.98	20.66	25.89
IL-1 beta [pg/mL]	78	0	23.01	12.68	22.73	0	60.74	13.92	32.02
INF gamma [pg/mL]	78	0	15.82	16.1	12.13	0.75	100.8	9.57	14.83
IL-2HS [pg/mL]	78	0	1.92	2.25	1.45	0	17.32	1.29	1.73
IL-4HS [pg/mL]	78	0	2.3	1.73	1.97	1.21	13.28	1.78	2.23
IL-6HS [pg/mL]	78	0	7.77	6.88	6.04	0	50	3.33	9.89
IL-10HS [pg/mL]	78	0	8.48	2.52	8.14	4.11	20.21	7.11	9.32

Table 3. Blood type ABO and the concentration of cytokines in FF.

Parameter		Blood type ABO				p
		0 (N = 23)	A (N = 32)	AB (N = 11)	B (N = 12)	
GCSF [pg/mL]	mean±SD	7.39 ± 1.01	7.37 ± 0.93	7.71 ± 1.34	7.36 ± 0.82	p = 0.939
	median	7.18	7.29	7.36	7.35	
	quartiles	6.65–7.8	6.84–7.75	6.8–8.52	6.71–7.63	
PIBF1 [ng/mL]	mean ± SD	1.5 ± 1.98	1.8 ± 1.21	2.38 ± 1.42	2.14 ± 1.9	p = 0.096
	median	1.01	1.67	2.5	1.44	
	quartiles	0.51–1.78	0.77–2.61	1.1–3.4	0.94–3.09	
LIF [pg/mL]	mean ± SD	22.61 ± 24.11	21.01 ± 17.55	16.49 ± 1.13	16.35 ± 2.2	p = 0.115

Parameter		Blood type ABO				p
		0 (N = 23)	A (N = 32)	AB (N = 11)	B (N = 12)	
	median	17.45	17.42	16.36	15.92	
	quartiles	15.99–18.51	15.76–18.82	15.95–17.13	14.86–16.81	
IL-15 [pg/mL]	mean ± SD	47.35 ± 2.98	46.9 ± 3.82	46.87 ± 2.21	44.94 ± 5.14	p = 0.245
	median	46.66	46.5	46.7	44.6	
	quartiles	45.27–49.75	44.61–49.41	45.21–48.32	42.37–46.97	
IL-5 [pg/mL]	mean ± SD	2.47 ± 3.39	2.53 ± 3.89	1.38 ± 2.36	1.76 ± 2.6	p = 0.678
	median	0	0	0	0	
	quartiles	0 - 5.41	0 - 5.2	0 - 2.51	0 - 5.04	
IL-8 [pg/mL]	mean ± SD	186.66 ± 62.68	188.18 ± 67.19	155.82 ± 43.07	176.75 ± 67.97	p = 0.418
	median	179.88	183.9	131.78	156.74	
	quartiles	138.92–214.42	138.8–242.2	126.94–180.84	131.17–215.11	
IL-1 alfa [pg/mL]	mean ± SD	24.97 ± 8.17	23.79 ± 6.58	22.59 ± 4.41	20.69 ± 5.5	p = 0.336
	median	23.32	23.39	21.58	22.26	
	quartiles	21.06–24.93	21.32–27.13	19.98–25.75	18.38–23.84	
IL-1 beta [pg/mL]	mean ± SD	25.51 ± 12.83	21.66 ± 12.55	21.11 ± 17.19	23.58 ± 7.9	p = 0.571
	median	26.6	20.14	22.29	22.73	
	quartiles	14.37–37.2	13.77–30.36	10.03–26.65	17.35–29.16	
INF gamma [pg/mL]	mean ± SD	19.21 ± 20.41	15.68 ± 17.04	10.36 ± 3.69	14.71 ± 9.71	p = 0.254
	median	13.53	11.63	10.48	12.27	
	quartiles	10.64 - 18.31	9.16 - 14.31	8.25 - 13.31	11.38 - 14.38	
IL-2HS [pg/mL]	mean ± SD	2.42 ± 2.33	1.93 ± 2.86	1.37 ± 0.49	1.46 ± 0.41	p = 0.385
	median	1.54	1.46	1.41	1.39	
	quartiles	1.36–2.01	1.24–1.74	1.34–1.57	1.31–1.47	

Parameter		Blood type ABO				p
		0 (N = 23)	A (N = 32)	AB (N = 11)	B (N = 12)	
IL-4HS [pg/mL]	mean ± SD	2.58 ± 2.09	2.32 ± 2.03	1.97 ± 0.27	2.04 ± 0.4	p = 0.562
	median	2.06	1.94	1.93	2.01	
	quartiles	1.88–2.35	1.76–2.19	1.76–2.15	1.82–2.38	
IL-6HS [pg/mL]	mean ± SD	6.36 ± 3.58	8.19 ± 9.03	8.69 ± 6.2	8.5 ± 5.93	p = 0.783
	median	5.28	6.34	9.2	7.22	
	quartiles	4.48–7.63	3.06–9.39	3.37–10.22	3.28–12.85	
IL-10HS [pg/mL]	mean ± SD	8.6 ± 1.9	8.53 ± 3.17	8.25 ± 1.59	8.32 ± 2.52	p = 0.829
	median	8.36	7.54	8.37	8.75	
	quartiles	7.72–9.18	6.39–9.43	7.35–9.41	6.36–9.72	

p — Kruskal–Wallis test

Table 4. Correlation between blood type ABO and the positive pregnancy test in patients undergoing IVF/ET was not statistically significant.

Blood type ABO	Positive pregnancy test	OR	95% CI		p
0 (N = 17)	6 (35.29%)	1	ref.		
A (N = 27)	11 (40.74%)	1.26	0.359	4.428	0.718
AB (N = 10)	5 (50.00%)	1.833	0.374	8.984	0.455
B (N = 10)	2 (20.00%)	0.458	0.073	2.89	0.406

p - univariate logistic regression.

Table 5. The relation between ABO blood type and the embryo quality was not statistically significant.

No of embryo		**Blood type ABO**				
		0 (N = 23)	**A (N = 32)**	**AB (N = 11)**	**PB (N = 12)**	
Top quality embryo	mean ± SD	2.13 ± 1.18	2.03 ± 1.09	2.55 ± 1.57	1.75 ± 0.75	p = 0.647
	median	2	2	2	2	
	quartiles	1–3	1–3	1–3.5	1–2	
Non-top quality embryo	mean ± SD	0.7 ± 1.11	0.31 ± 0.59	0.45 ± 0.69	0.33 ± 0.49	p = 0.399
	median	0	0	0	0	
	quartiles	0–1	0–0.25	0–1	0–1	
Top + non-top quality embryo	mean ± SD	2.83 ± 1.67	2.34 ± 1.29	3 ± 2.05	2.08 ± 0.79	p = 0.547
	median	3	2	2	2	
	quartiles	2–3	1.75–3	1–4.5	2–2	
Non-viable embryo	mean ± SD	1.78 ± 1.51	1.28 ± 1.33	1.64 ± 1.8	1.33 ± 0.98	p = 0.664
	median	2	1	1	1.5	
	quartiles	0–3	0–2	0–3.5	0.75–2	

p — Kruskal–Wallis test