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Does autoimmunity play a role in the risk of implantation failures?

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Abstract

158 non-pregnant women with recurrent implantation failures after IVF/ET procedures were tested for peripheral blood autoimmune profile. The control group consisted of 76 patients after first successful IVF procedure and pregnancy outcome. The objective of this study was to investigate different autoantibodies peripheral blood profile after excluding anatomical, endocrinological, endometrial and genetic disorders and to estimate the risk of implantation failures. The study's including criteria were 1.indications for IVF/ET determined by male factor and unexplained infertility 2. absence of implantation after two consecutive cycles of IVF, ICSI or frozen embryo replacement cycles.
The presence of ANA in the sera increased the risk of RIF after ET/IVF procedures, especially in older patients. Patients with RIF have a higher frequency of the presence of autoantibodies ACA IgG, IgM and anti-β2GP I IgG in the sera than in patients with successful pregnancies after IVF/ET procedures.

INTRODUCTION

There has been a significant increase in fertility treatments by assisted reproduction techniques, especially *in vitro* fertilization (IVF). Immune disorders may have an important effect on IVF success and embryo transfer (ET). Recurrent implantation failure (RIF) is condition chracterized by repetetive unsuccessful cycles of IVF or intracytoplasmic sperm injection (ICSI) treatment. RIF should be defined as the absence of implantation after 2 to 6 consecutive cycles of IVF, ICSI or frozen embryo replacement cycles where the cumulative numer of transferred embryos was no less than four for cleavage-stage embryos and no less than two for blastocytes with all embryos being of good quality and of appropriate developmental stage with determination of imlantation by an increasing quantitative human chorionic gonadotropin (hCG) level (Margalioth *et al.* 2006). Particularly when transferred embryos are of good quality, recurrent implantation failure may be attributed to less than optimal embryo transfer technique and the presence of pathological lesions of the uterine cavity (Kwak-Kim *et al.* 2009; Lédée *et al.* 2016). Successful pregnancy implantation is related to adequate utetro-placental cirulation and immunological backgroud through mechanisms similar to recurrent miscarriages, as confirmed in the literature (Stern *et al.* 1998). The aim of this

ORIGINAL ARTICLE

article is to access autoimmune disorders by conducting peripheral blood analysis in patients who are undergoing IVF/ET treatment and have had implantation failures and to estimate the risk of RIF depending on the occurrence of autoantibodies.

MATERIALS AND METHODS

One hundred fifty-eight patients with a history of two and more implantation failures after IVF/ET procedures were evaluated. All patients had a normal ovarian reserve as maeasured by the levels of anti-műllerian hormone (AMH), follicle-stimulating hormone (FSH) and oestradiol.

The indications for IVF procedures were as follows:

- unexplained infertility
- male infertility.

Tha study was performed between January 2015 and January 2017. Patients were registered at the Department of Operative and Endoscopic Gynaecology at the Medical University of Lodz. The patients gave written consent for participation in the study, and the study was approved by the Ethics committee.

The control group comprised 76 women who had successfully gave birth at the first attempt of fresh ET. The indications for IVF procedures were the same as those for the study group.

The IVF/ET procedures were similar for all patients. Chromosomal abnormalities of the male and female were excluded after their karyotype analysis. Embryonic aneuploidy were evaluated using fluorescense in situ hybridization (FISH) for chromosomes 13, 16, 18, 21, 22, X and Y.

All the women were investigated to exclude the following causes of implantation failures:

1. anatomical causes, including congenital anatomic abnormalities of the uterus such as incomplete műllerian fusion and septum resorption, aquired abnormalities such as synechiae, fibroids, leiomyomas or endometrial polyps resulting from hysteroscopy and pelvic ultrasound or hydrosalpinx – resulting from laparoscopic treatment.
2. endometriosis as the result of laparoscopy
3. infectious factors such as Cytomegalovirus and Herpes simplex, infections such as Mycoplasma hominis, Ureaplasma urealyticum and Chlamydia trachomatis.
4. gene mutations in the genes coding for the proteins responsible for inherited thrombophilias such as mutations MTHFR (locus 1p36.3) for homocysteine C677T, A1298C, factor V Leiden mutation 1691GG (locus F5 1q23), mutation 20210G for prothrombin (locus F2 11211 – q12) and mutation for antithrombin III.
5. deficiences in protein S and protein C,
6. endocrinological causes - including: luteal phase insufficiency, polycystic ovary syndrome, insulin resistance, diabetes mellitus, hyperprolactinaemia and hyperandrogenism,
7. receptivity of the endometrium was estimated before first ET by endometrial biopsy during the cycle before the IVF cycle.

Immunological investigations included estimations of autoantibodies in peripheral blood, including the following antibodies: anticardiolipin (ACA) IgG and Ig M, anti-β2glycoprotein I IgG and IgM (β2GPI) antibodies, lupus anticogulant (LA), antinuclear (ANA) and antiplacental antibodies (APA).

Laboratory

Anti-β2-glycoprotein I antibodies (Anti-β2GP I) were detected using an IgG and IgM enzyme-linked immunosorbent assay (ELISA) Kit (IMMCO Diagnostic Inc., Buffalo, USA). The results were considered positive above the following cut-off values: >25 EU/mL for IgG and IgM. Medium and high values were considered positive above 40 GPL U/mL.

Lupus anticoagulant (LA): The concentration of serum LA was estimated using an LA screen and LA confirmation tests (Instrumentation Laboratory, Lexington, USA). Values were represented as normalized LA coefficients and were classified as follows: LA (–), <1.2; LA (+), 1.2–1.5; LA (++), 1.5–2.0; and LA (+++), >2.0. LA coefficients above 1.2 were considered positive.

The presence of serum anticardiolipin IgG/IgM antibodies (ACA) were quantified using immunoenzymatic ELISA assay. Standardized commercial Varelisa Cardiolipin Antibodies were used (Pharmacia Deutschland GmbH, Diagnostic Division, Germany). Absorbance of the samples was measured by a DS2 counter (Dynex Technology, Inc., USA) using the light of 450 nm wavelength. Results were presented in U/mL, and the adopted ranges of positive values were as follows: ACA IgG > 15 GPL U/mL, ACA IgM > 15 MPL U/mL. Medium and high values of anticardiolipin antibodies were considered positive above 40 GPL U/mL. Samples that tested positive for APLS initially were retested after 12 weeks according to the Sydney criteria (Miyakis *et al.* 2006).

Antinuclear antibodies (ANA) were estimated by indirect immunofluorescence using cultured laryngeal cancer HEP-2 cells as targets (EUROIMMUN, Lübeck, Germany). Sera that exhibited fluorescence at a dilution of 1:80 were considered positive.

Antiplacental antibodies (APA) were studied using indirect immunofluorescence together with placenta cells as targets (Euroimmune). Sera that exhibited fluorescence at a dilution of 1:10 were considered positive.

Statistical analysis

Risk of failure of IVF/ET procedures was modelled for cases (Y=1) and controls (Y=0) with model $h[P(Y=1|\mathbf{x})]=\alpha+\boldsymbol{\beta}^{T}\mathbf{x}$, where $\mathbf{x}$ is the matrix of immunological predictors and h is *logit* function. Results were adjusted to age of patients. Odds ratio (OR) was used

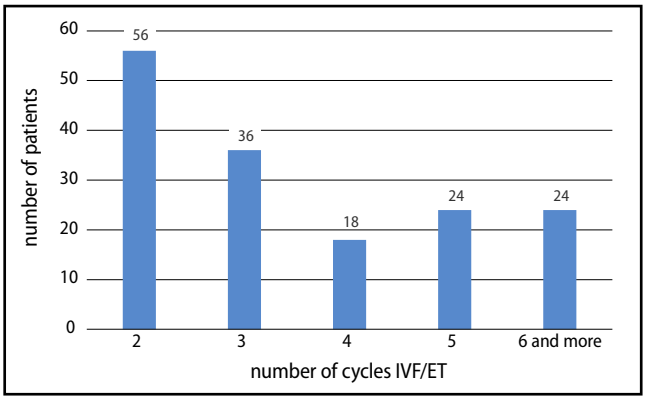

Fig. 1. The number of cycles of ET/IVF.

as a measure of the effect size Median and two quartiles (first and third, Q1 and Q3, respectively) were used as summary statics. The S_n statistic was computed as the measure of variability: $Sn=med\{med|x_i-x_j|; j=1...n\}$ (Rousseeuw *et al.* 1993).

RESULTS

The mean age in the group of patients with RIF after IVF/ET was 36 years (Q1=33, Q3=39) and in the control group was 34 years (Q1=31, Q3=37).

The number of cycles of IVF/ET in the tested patients is presented in Figure 1.

The most dominant group (56 patients) consisted of cases with two ET/IVF failures; the remaining tested patients underwent three to six or more cycles of ET/IVF procedures (from 18 to 36 patients).

Antinuclear antibodies were the most often detected autoantibodies in the sera of RIF patients (43.1%) than in the sera of the control groups (10.5%), with statistical significance (p=0.01). The other autoantibodies found in the patients' sera were β2-GPI, LA (8.8%) and IgG ACA (14.8%), with no statistical significance.

In the sera of the patients with RIF after IVF/ET procedures, the levels of ACA IgG, IgM and anti-β2GP I IgG were higher than in the control patients, with no statistical significance. We did not observe differences between cases and controls for antiplacental antibodies and lupus anticoagulant.

The estimated risk of RIF after IVF/ET procedures in patients with positive levels of ANA in the sera was 5.57-fold higher than that in women with negative levels of ANA. Considering the age of the patients, we found that one additional year of patient's age increases the risk of implantation failure after IVF/ET procedures by 13% (OR=1.13).

DISCUSSION

Abnormal immune responses are significantly increased in women who have had recurrent miscarriages (Kwak-Kim 2009; Jaslow *et al.* 2010). Implantation failure after IVF/ET procedures remains controversial, with different definitions of increase in the quantitative hCG level after embryo transfer (Rinehart 2007; Urman *et*

Tab. 1. The frequency of autoantibodies found in the sera of RIF patients.

Auto antibodies	Percent of positives estimated		OR	CI 95%		*p*-value
	Cases [%]	Controls [%]		Lower	Upper	
ACA IgG	14.8	4.8	3.48	0.43	28.40	0.12
ACA IgM	13.6	4.8	3.14	0.38	25.84	0.14
antiβ2GPI IgG	11.1	4.8	2.50	0.30	20.92	0.19
antiβ2GPI IgM	8.6	4.8	1.89	0.22	16.29	0.28
LA	8.6	14.3	0.57	0.13	2.41	0.77
ANA	**43.1**	10.5	**4.57**	1.25	16.73	**0.01**
APA	22.8	21.1	0.98	0.32	3.03	0.51

Tab. 2. Characteristics of cases and controls analysed in this study according to the autoantibody levels.

Autoantibodies					
Variable	**Group**	**Q1 (U/ml)**	**Median (U/ml)**	**Q3 (U/ml)**	**S_n**
ACA IgG	Cases	1.35	3.5	8.45	3.2
	Controls	1.35	1.7	4.9	1.1
ACA IgM	Cases	0.6	2.5	6.5	2.7
	Controls	1.2	2.2	4	1.7
antiβ2GPI IgG	Cases	3.05	6.6	10.8	5.7
	Controls	3.8	4.5	7.75	1.2
antiβ2GPI IgM	Cases	3.6	6.3	10.5	4.8
	Controls	7.5	11.1	12.4	3.8
LA	Cases	0.9	0.9	1	0.1
	Controls	0.9	0.9	1.1	0.1
Binomial variable		**Positive n_1**	**Negative n_2**	**N n_1+n_2**	**Positive %**
ANA	Cases	68	90	158	43.1
	Controls	8	68	76	10.5
APA	Cases	36	122	158	22.8
	Controls	16	60	76	21.1

Tab. 3. The risk of IVF/ET implantation failures independent of the ANA levels and patients' age.

Variable	OR	CI95%		*p*-value
Age increases at 1 year	1.13	0.986	1.29	0.04
ANA is present	9.67	1.79	52.23	<0.01
$X^2_{df=3}=21.84$; $p=0.00007$				

al. 2005). Despite the increasing number of gene mutations in the gene coding for the proteins responsible for thrombophilias, endometrial remodelling events indispensable to endometrial decidualisation and the good quality of transferred embryos, peripheral blood immunological balance is an independent required factor for successful pregnancy outcome (Chernyshov *et al.* 2016; Kwak-Kim *et al*. 2014; Mekinian *et al.* 2016; Polanski *et al*. 2014). The detection of different autoantibodies is necessary if there is no ongoing pathological activation of the immune system. The aim of our study was to identify whether different autoimmune profiles in infertile women who underwent IVF/ET procedures may influence the risk of implantation failures after excluding anatomical, genetic, endometrial, and endocrine disorders. The results show that this group is much more heterogenous concerning autoimmune profile than patients with successful pregnancy outcome after IVF/ET procedures.

In the sera of tested patients, ACA IgG observed in 14.8% of the patients and ACA IgM found in 13.6% of the patients were higher but not significantly than the levels of ACA found in the sera of the control group patients (4.8%). The same is true for the frequency of anti-β2GP I Ig G found in 11.1% of the tested patients and IgM found in 8.6% of cases compared to the control patients (4.8%), in that they did not differ significantly. The ratios of LA (8.6% vs 14.3%) and the levels of antiplacental antibodies (22.8% vs 21.1%) were comparable in both groups.

Antinuclear antibodies were found in 68 patients with RIF (43.1%) compared to 8 patients in the control group (10.5%), $p<0.05$. As presented previously (Malinowski *et al.*1994; Motak-Pochrzest 2013), antinuclear antibodies were found almost twice as frequently in patients who had recurrent miscarriages as in healthy women (18.7% vs.10.0%); moreover, we noted that the high incidence of ANA is characteristic for women with recurrent miscarriages of unknown aetiology. In the present study, the risk of pregnancy failure in patients with positive ANA in the sera was estimated as 4.57-fold higher than that in ANA-negative patients (CI 95%: 1.25; 16.73). In the observed tested group, we found that the presence of ANA correlated with an increase to a 9-fold higher risk of unsuccessful implantation after IVF/ET procedures in the older patients(OR=9.67) than in the control group ($p<0.01$).

CONCLUSION

The presence of ANA in the sera increases the risk of RIF after ET/IVF procedures, especially in older patients.

Patients with RIF have a higher frequency of the presence of autoantibodies ACA IgG, IgM, and anti-β2GP I IgG in the sera than in patients with successful pregnancies after IVF/ET procedures.

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