PROSPECTIVE STUDY TO ESTIMATE THE ROLE OF DIFFERENT INFERTILITY FACTORS IN PREDICTION OF UNSUCCESSFUL IVF OUTCOME

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INTRODUCTION

Female infertility is a global health issue affecting nearly 48 million women [1]. Infertility is defined as the inability to conceive after a year or more of frequent unprotected sexual contact [2]. Infertility is said to be unexplained when its common causes, such as absent ovulation, poor sperm quality, or tubal pathology, have been ruled out after standard fertility tests [3]. Female factors such as chronic anovulation, blocked fallopian tubes or endometriosis, or male factors such as sperm abnormalities, obstructions, ejaculatory dysfunction, or unexplained causes are the main factors that prevent the possibility of pregnancy in women with infertility. Subfertility refers to a delay in conceiving. Other factors such as advanced age, endometriosis, diabetes mellitus, ovarian dysfunction, polycystic ovarian syndrome, and previous genital tract infection can all contribute to subfertility [4].

Recent research has looked at the role of autoimmune variables in implantation in women undergoing *in vitro* fertilization (IVF) [5–7]. Antiphospholipid antibodies are more commonly found in women planning IVF; they affect live birth rates, miscarriage rates, and pregnancy outcomes [8–10]. Heat shock protein 60 (HSP60) is a protein chaperone that aids in protein transfer and refolding. The role of HSP60 in sperm capacitation and sperm-oocyte membrane binding facility, HSP60 impact on the IVF rate and cleavage rate in mouse embryos was studied by Z. Abdi et al. [11]. This study found that a low dose of HSP60 had a good effect on two-cell embryo development but had no effect on fertilization rate. At greater doses of HSP60 had a negative effect on fertilization and cleavage rates [11, 12].

Several publications have recently proven that chronic endometriosis is ubiquitous in infertile women, particularly those with recurrent implantation failure during IVF [13, 14]. Failure of IVF is highly frustrating for both patients and professionals. Lower pregnancy rates can be expected in these patients even in subsequent IVF/ICSI (Intra-Cytoplasmic Sperm Injection) cycles [15, 16].

Chlamydia trachomatis infection is a significant sexually transmitted disease associated with tubal infertility and increased salpingitis episodes that lead to tubal obstruction [17–19]. Many investigations have found that serologic evidence of prior chlamydial infection is strongly linked to tubal infertility [20–24]. Chlamydial infection also reduced the likelihood of a favorable outcome for IVF [25]. Individuals with a positive *Chlamydia trachomatis* serology are more likely to develop pelvic inflammatory disease (PID) [26, 27]. Recent investigations have found a substantial link between antibody action against the chlamydial HSP60 (CHSP60) and ectopic pregnancy [28]. CHSP60 is a heat shock protein homolog of the GroEl family [29, 30]. This protein family is highly conserved in both eukaryotes and prokaryotes [31, 32].

Idiopathic infertility has no recognized etiology; nonetheless, potential factors such as a disturbed oxidant balance may play a role. An excess of reactive oxygen species has been seen in the follicular fluid of women with idiopathic infertility [33]. Exposure to environmental factors, endocrine disruptions, and hormonal imbalances may explain a significant portion of infertility [34]. In this prospective study, we attempted to explain the role of various hormonal, immunological, and functional factors that positively affect the IVF outcomes.

Research objective: to study the role of various infertility/immunological factors, particularly HSP60 and GroEl, as a prognostic factor in the favorable IVF outcome in women receiving infertility treatment.

MATERIALS AND METHODS

It was a prospective study of all women with infertility who are undergoing treatment or diagnosed with infertility. Following the proposed research study, samples were collected from 106 unselected females presenting for their first infertility examination at the Gynecology Department, Infertility Center, O.O. Bogomolets National Medical University (Kyiv, Ukraine) between September 2019 and February 2021.

54 women received conventional treatment for infertility and 52 women received conventional therapy with immunocorrection therapy starting from the day of embryo transfer (5% intravenous IgG 400 mg/kg only once, enoxaparin 20 mg subcutaneously daily and aspirin 100 mg daily till the implantation result).

Patients of two groups underwent ICSI techniques for IVF. HSP60 and GroEl levels were measured in all of them at the time of admission, after treatment (before embryo transfer), and after embryo transfer. The patient's medical records

and operative reports were reviewed, and characteristics such as age, duration of infertility, additional infertility problems, previous assisted reproductive technology treatments, prior surgeries, and preoperative symptom profile were recorded. Surgical findings and procedures were documented, and any missing data was gathered. Basal body temperature charting, ultrasound study, hysteroscopy, necessary blood test, hormonal profile, clotting profile, microbial exam/culture and serology, colposcopy, laparoscopy, and cervical *C. trachomatis* screening with ELISA were performed for all women.

Diagnosis of tubal infertility established by hysterosalpingography and/or laparoscopic test that found distal tubal blockage or by laparoscopic detection of peritubular adhesions. No laparoscopic assessment was performed when a complete reciprocatory distal tubal obstruction was diagnosed by hysterosalpingography. Microimmunofluorescence tests of Wang and Grayston were used to study all collected samples for IgG and IgM antibodies to *C. trachomatis* [35] using formalin fixed primary bodies that have been detoxed.

Inclusion criteria:

- known infertility;
- informed consent;
- women with tubal infertility, age less than 44 years or more than 22 years, ovulatory dysfunction, *C. trachomatis* seropositivity, endometriosis, ectopic pregnancy, history of miscarriage, spontaneous abortion, IVF failure, women with routine gynecological check-up.

Exclusion criteria:

- male infertility factor;
- patients with decompensated cardiovascular disease, diabetes mellitus, liver, thyroid gland, and lung diseases; patients who had cancer and tumors or had a course of antiestrogen drugs; patients with alcohol and nicotine addictions, cognitive disorders; patients with diseases of other organs and systems in the decompensating stage, which could have impact the outcome;
- infectious diseases of extreme severity (HIV infection, tuberculosis, syphilis, viral hepatitis B and C);
- acute infectious process during the study;
- women with a history of autoimmunity, oncology, or hypersensitivity;
- subjects with TORCH-infections and suffering from other autoimmune diseases.

Blood serum were tested at 1:8 dilution and tittered to the endpoint at double dilutions. Seropositivity for HSP60 and GroEl was defined by microimmunofluorescence titer 1:8 [36]. ELISA with recombinant HSP60 and GroEl expressed as a mixed protein with glutathione-S-transferase as an antigen was used to assess the HSP60 and GroEl antibodies [37, 38]. In addition to being diluted at 1:500, patient serum was incubated with recombinant antigen bound to 96 well microtiter plates. Horseradish peroxidase-conjugated goat anti-human IgG was added, and after determining the optical density of each, standardized absorbance was measured at 405 nm with a time exposure of 10 minutes. All ELISA-positive serum was confirmed by immunoblotting with recombinant HSP60 and GroEl as antigens. Serum was tested blindly and without the knowledge of a clinical diagnosis.

The chi-squared test or Fisher's exact test was used to compare groups. Odds ratios (OR) with 95% confidence intervals (CI) are also computed to help the diagnostic precision of both serologic tests for their ability to predict tubal disease as the cause of infertility in patients who came for infertility assessment. The calculation of possibility ratios allows comparing the diagnostic importance of tests in different populations based on infection frequency. The positive test ratio is calculated as sensitivity / (100 – specificity). A positive possibility of 2 to 5 indicates a poor clinical test, 5 to 10 indicates a good scientific test, and > 10 indicates an excellent clinical test. The negative test ratio was calculated as sensitivity / (100 – specificity). A negative ratio of 0.5 to 0.2 indicates an unsatisfactory examination, a positive ratio of 0.2 to 0.1 indicates a good clinical assessment, and a value of 0.1 indicates a superb clinical assessment [39].

The statistical package EZR 1.54 (graphical interface to R statistical software v. 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) was used in the results analysis [40].

The study was approved by the Commission on Bioethical Expertise and Ethics of Scientific Research at the O.O. Bogomolets National Medical University (protocol No. 126, November 13, 2019). All participants signed informed consent to participate in the study prior to the survey.

RESULTS

Group 1 had an average age of 34.1 ± 3.4 years, and group 2 had an average age of 33.3 ± 5.4 years. The vast majority of those who took part were white, non-Hispanic, married, seeking treatment with a partner, and college-educated. The distribution of the infertility causes among patients with infertility (n = 106) was as follows: 58.5% – endocrine factor (including ovulation disorders), 50.9% – morpho-anatomical causes, 23.58% – tubal factor, 14.15% – autoimmune factor. The impact of factors characteristics on the implantation process was determined. The characteristics of individuals included in this study are shown in Table 1.

Table 1. Individual characteristics of both groups

Factorial sign	Group 1 (n = 54)	Group 2 (n = 52)	p
Age, years	34.1 ± 3.4	33.3 ± 5.4	0.361
Height, cm	166.4 ± 7.5	165.1 ± 5.7	0.322
Weight, kg	69 (54–82)	65 (56.5–73)	0.493
Body mass index, kg/m^2	24.6 (20.8–27.1)	23.8 (20.5–26.0)	0.702
Menarche, age	14 (12–15)	13 (12–14)	0.772
Cycle, days	31 (26–34)	28 (27–28.5)	0.026
Menstruation, days	5 (4–6)	5 (4–5)	0.277
Hirsutism scores	5 (3–7)	4 (2.5–5)	0.019
Coitus, times per week	3 (2–4)	2.5 (2–3.5)	0.239

Note: t-test (in the case of a normal distribution) and Mann-Whitney test (in the case of a non-normal distribution) were used. Me (QI–QIII) (no normal distribution).

The study found no differences in age, height, weight index, menarche, menstrual duration, or coitus between the two groups (p > 0.05). The average length of the menstrual cycle was 31 days (26–34 days) in group 1 and 28 days (27–28.5 days) in group 2, p = 0.026. The severity of hirsutism was 5 points

(3–7 points) in group 1, and 4 points (2.5–3.5 points) in group 2 patients, p = 0.019. According to Table 1, the average values of factor traits in patients with infertility are within normal limits. For further analysis of the impact of factor traits on the risk of implantation failure, the severity of hirsutism and the menstrual cycle duration were considered, as these factor traits differ between the two groups.

The following obstetric and gynecological history factors were examined to assess the differences between groups 1 and 2, as shown in Table 2.

Table 2. Obstetric and gynecological characteristics of both groups, n (%)

Factorial sign		Group 1 (n = 54)	Group 2 (n = 52)	p
Pregnancy	0	28 (51.9)	27 (51.9)	0.933
	1	9 (16.7)	8 (15.4)	
	2	5 (9.3)	8 (15.4)	
	3	9 (16.7)	7 (13.5)	
	4	1 (1.9)	1 (1.9)	
	6	2 (3.7)	1 (1.9)	
Parity	0	54 (100.0)	49 (94.2)	0.115
	1	0 (0.0)	3 (5.8)	
Artificial abortion	0	40 (74.1)	46 (88.5)	0.268
	1	8 (14.8)	3 (5.8)	
	2	5 (9.3)	2 (3.8)	
	3	1 (1.9)	1 (1.9)	
Pregnancy loss, miscarriage	0	36 (66.7)	37 (71.2)	0.673
	1	11 (20.4)	10 (19.2)	
	2	3 (5.6)	3 (5.8)	
	3	1 (1.9)	1 (1.9)	
	4	2 (3.7)	0 (0.0)	
	5	1 (1.9)	0 (0.0)	
	6	0 (0.0)	1 (1.9)	
Ectopic pregnancy	0	47 (87.0)	41 (78.8)	0.366
	1	6 (11.1)	6 (11.5)	
	2	1 (1.9)	4 (7.7)	
	3	0 (0.0)	1 (1.9)	
Cycle regularity	yes	39 (72.2)	41 (78.8)	0.501
	no	15 (27.8)	11 (21.2)	
Menorrhagia	yes	11 (20.4)	8 (15.4)	0.615
	no	43 (79.6)	44 (84.6)	
History of laparoscopy	yes	23 (42.6)	22 (42.3)	> 0.999
	no	31 (57.4)	30 (57.7)	
History of hysteroscopy	yes	14 (25.9)	12 (23.1)	0.822
	no	40 (74.1)	40 (76.9)	
History of laparotomy	yes	6 (11.1)	7 (13.5)	0.773
	no	48 (88.9)	45 (86.5)	
History of embryo transfer	0	30 (55.6)	30 (57.7)	0.999

Note: Fisher's exact test or chi-square test (for 3 or more gradations of the trait) were used. Me (QI–QIII) (no normal distribution).

The analysis of factor traits (Table 2) revealed no statistically significant differences between groups 1 and 2 (p > 0.05 in all cases). The data in Table 1 and Table 2 show no differences between the study groups, except for severity and duration of the menstrual cycle. Thus, the data represents the study of the clinical significance of the proposed treatment and diagnostic algorithm for infertility patients.

28 (51.85%) patients in group 1 and 27 (51.92%) patients in group 2 were never pregnant and thus had primary infertility, while 26 (48.14%) and 25 (48.07%) patients in groups 1 and 2 had secondary infertility. None of the patients in group 1 had a history of childbirth, but 3 (5.8%) women in group 2 had a history of childbirth. Patients in group 1 did not achieve results in 29 (53.7%) cases after treatment, while women in group 2 did not achieve results in 21 (40.4%) cases.

In all 106 patients we used the method of building and analyzing one-factor logistic regression models to identify factors associated with the risk of not achieving results (not getting pregnant).

Furthermore, serum antibodies to HSP60 and its bacterial homologue GroEl were determined in 2 groups of infertile patients before treatment, before embryo transfer, and after successful/unsuccessful implantation. The data is presented in optical density units (Table 3).

Table 3. Antibody levels to HSP60 and GroEl in two groups

Factorial sign	Group 1 (n = 54)	Group 2 (n = 52)	p
HSP60_1, O.D.	0.403 (0.325–0.526)	0.397 (0.313–0.523)	0.91
HSP60_2, O.D.	0.385 (0.295–0.486)	0.374 (0.296–0.488)	0.818
D HSP_1_2, O.D.	-0.0295 (-0.0410 – -0.0120)	-0.0240 (-0.0405–0.0105)	0.353
HSP60_3, O.D.	0.367 (0.295–0.469)	0.357 (0.289–0.482)	0.667
D HSP_2_3, O.D.	-0,0430 (-0,0680 – -0,0150)	-0,0405 (-0,0765–0,0145)	0,523
GroEl_1, O.D.	0.414 (0.358–0.485)	0.340 (0.270–0.502)	0.081
GroEl_2, O.D.	0.386 (0.342–0.485)	0.331 (0.250–0.466)	0.093
D GroEl_1_2, O.D.	-0.0190 (-0.0330 – -0.00700)	-0.0205 (-0.0370–0.0110)	0.862
GroEl_3, O.D.	0.382 (0.338–0.485)	0.322 (0.212–0.447)	0.165
D GroEl_1_3, O.D.	-0.0250 (-0.0500 – -0.0130)	-0.0225 (-0.0645–0.0125)	0.85

Note: t-test (in the case of a normal distribution) or Mann-Whitney test (in the case of a non-normal distribution) was used. D stands for difference, and O.D. stands for optical density.

The analysis found no differences between the group's levels of antibodies to HSP60 and GroEl in all blood samples. Following that, the study examined at the dynamics of antibody levels in 2 groups. Figure 1 depicts the dynamics of HSP60 changes in groups 1 and 2.

In group 1 there was a HSP60 decrease (p < 0.001 according to Friedman's criterion for repeated measurements), with the highest (p < 0.05) value of this indicator observed at the start of treatment and the lowest (p < 0.05) – for the third measurement. Similarly, there was a HSP60 decrease (p < 0.001 according to Friedman's test for repeated measurements) in group 2, with the highest (p < 0.05) value observed at the start of treatment

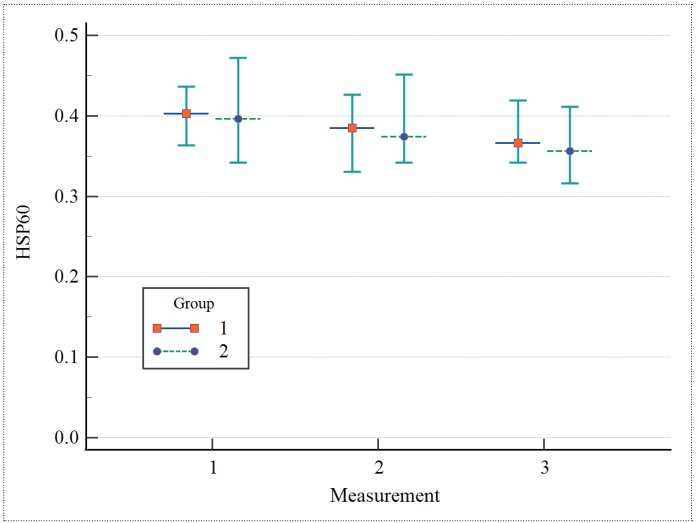

Figure 1. Dynamics of HSP60 changes in groups 1 and 2 (median value and 95% CI)
Note: HSP60 levels are given in units of optical density.

and the lowest ($p < 0.05$) on the third measurement. Treatment of the underlying infertility cause in both groups results in a HSP60 decrease.

The next step was to determine GroEl levels. The dynamics of change in GroEl are depicted in Figure 2.

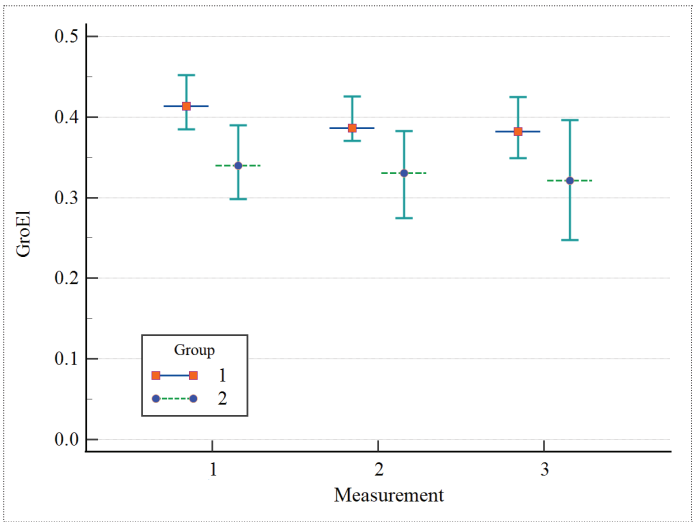

Figure 2. Dynamics of GroEl changes in 1 and 2 groups treated with different methods (median value and 95% CI)
Note: GroEl levels are given in units of optical density.

In group 1 there was GroEl decrease ($p < 0.001$ according to Friedman's test for repeated measurements), the highest ($p < 0.05$) value was observed at the start of treatment, the lowest ($p < 0.05$) – for the third measurement. Similarly, there was GroEl decrease ($p = 0.001$ according to Friedman's criterion for repeated measurements) in group 2, with the highest ($p < 0.05$) value at the start of treatment and the lowest ($p < 0.05$) on the second and third measurements. Thus, treatment of the cause of infertility in 2 groups results in a decrease in GroEl levels with and without immunocorrective therapy.

HSP60 and GroEl are strong predictors of infertility risk. The area under curve (AUC) technique was used to examine the association between these indicators and the risk of failure. The results of this analysis are shown in Table 4.

Table 4. Analysis of ROC curves predicting the risk of treatment failure for HSP60 and GroEl

Factorial sign	AUC (95% CI)	Significance of differences in AUC for 0.5, p
HSP60_1	0.59 (0.49–0.68)	0.113
HSP60_2	0.68 (0.59–0.77)	< 0.001
dHSP_1_2	0.81 (0.72–0.88)	< 0.001
GroEl_1	0.77 (0.68–0.85)	< 0.001
GroEl_2	0.82 (0.73–0.89)	< 0.001
dGroE_1_2	0.78 (0.69–0.85)	< 0.001

Note: HSP60_1 and GroEl_1 – antibodies levels before treatment, HSP60_2 and GroEl_2 – antibodies levels before embryo transfer, d-difference.

Thus, there was a moderate association between the risk of treatment failure and HSP60 for the second measurement, GroEl for the first measurement, and GroEl reduction from the first to the second measurement (Table 4). Furthermore, there was a strong association between the risk of treatment failure and the decrease in HSP60 from the first to the second measurement and GroEl in the second measurement. Association between a change in the level of antibodies to HSP and successful implantation was examined. As a result, Figure 3 depicts the ROC curve (receiver operating characteristic curve) of predicting the risk of implantation failure with HSP60 treatment success rate from the first to the second measurement.

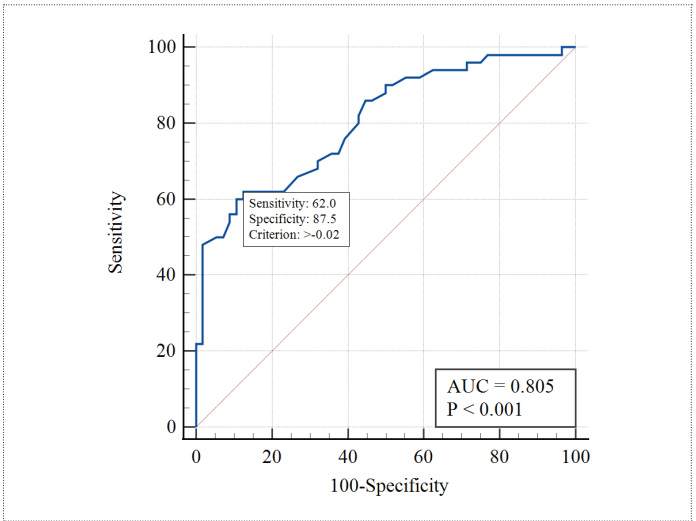

Figure 3. ROC curve of operational characteristics of predicting the risk of implantation failure after treatment based on the decrease in HSP60 from the first to the second measurement

Optimal threshold (according to Youden Index) of this test was HSP60 decrease from the first to the second measurement by less than 0.02 units of optical density. The risk of treatment failure is predicted – its sensitivity is 62% (95% CI 47.2–75.3), the specificity is 87.5% (95% CI 75.9–94.8), positive prognostic value is 81.6% (95% CI 68.2–90.2), negative prognostic value is 72.1% (95% CI 64.1–78.8). Association between changes in the level of antibodies to GroEl and successful implantation was investigated.

The ROC curve for predicting the failure risk of GroEl treatment for the second measurement is shown in Figure 4.

Optimal threshold (according to Youden Index) of this test was GroEl value for the second measurement more than

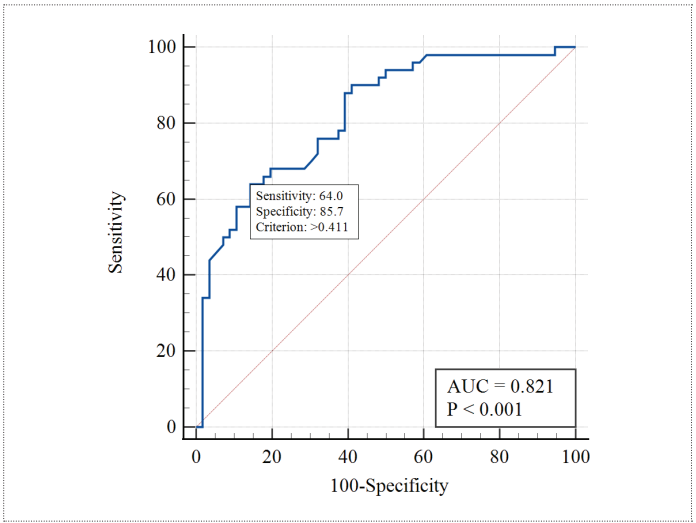

Figure 4. ROC curve of the risk prediction of treatment failure according to GroEl for the second measurement

0.411 units of optical density. The risk of treatment failure is predicted – its sensitivity is 64% (95% CI 49.2–77.1), specificity is 85,6% (95% CI 73.8–93.6), positive prognostic value is 80,0% (95% CI 67.1–88.7), negative prognostic value is 72.7% (95% CI 64.5–79.7).

The strong association of GroEl at the third measurement with the risk of treatment failure was revealed (Fig. 5).

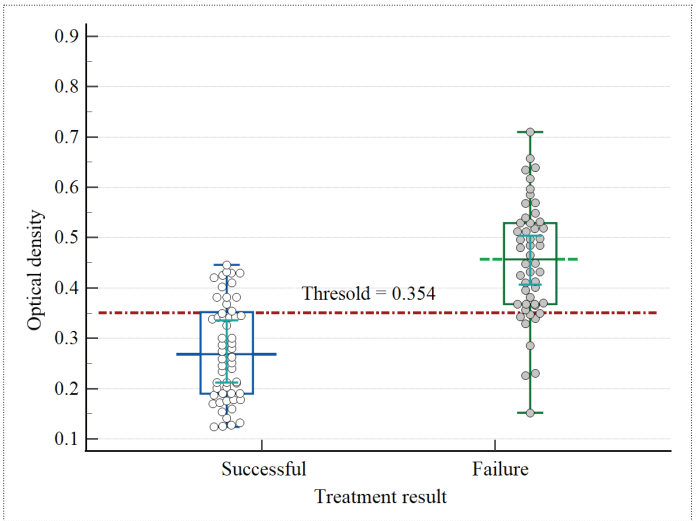

Figure 5. GroEl for the third measurement
Note: the median value and 95% CI are given, Min and Max values of the first and third quartiles.

The figure shows that most patients for whom treatment success was not achieved a high GroEl on the third measurement. GroEl = 0.354 units of optical density when determining the best threshold. 75.9% of patients with a GroEl score on the third measurement that did not exceed this threshold became pregnant; 82.7% of patients with a GroEl score on the third measurement that exceeded this threshold did not become pregnant. As a result, dynamic of antibody levels to HSP60 and GroEl has prognostic value in terms of embryo transfer, duration of treatment, or search for the infertility cause.

Furthermore, we examined the relationship between different risk factors and implantation failure. A selection of factor features for all variables was performed to generalize the analysis in the multifactor model of logistic regression. Akaike criterion was used in the selection of significant features (Table 5).

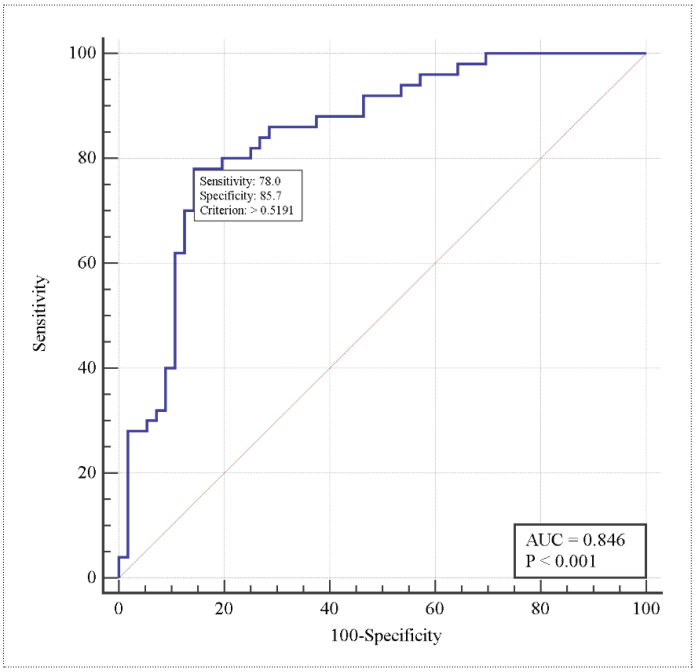

Figure 6. The curve of the 12-factor model for predicting the risk of treatment failure

Thus, the analysis revealed signs associated with an increased risk (OR at $p < 0.05$) of implantation failure: age, number of sexual intercourses per week, D-dimer level, antibodies to HSP60_1 (before treatment). Short stature patients were found to have a higher risk of treatment failure ($p < 0.05$). Most importantly, with standardization by other factors, immunocorrective therapy reduces risk of implantation failure in comparison with conventional treatment (OR = 0.26, 95% CI 0.09–0.76, p = 0.014).

DISCUSSION

The primary goal of this research was to examine autoantibodies concerning gynecology and reproductive immunology. We studied HSP60 and GroEl to see how they affect the outcome of a normal pregnancy. Our study emphasized the importance of both of these autoimmune factors in implantation, highlighting their potential role in the reproduction process. Autoantibodies and early embryo mortality play a critical role in the implantation failure. In this study, antibodies to HSP60 and GroEl were found in high concentrations in the serum of infertile women. The treatment of the infertility cause in both groups results in a significant decrease in HSP60 and GroEl levels. The study found no differences in age, height, body mass index, menarche, menstrual duration, or coitus between the two groups ($p > 0,05$).

Selection of women, particularly those who meet the inclusion and exclusion criteria for this study, is one of the study's strengths. This research study is a suitable representative evaluation for infertility risks evaluation in Ukrainian population

Table 5. Significant features of implantation failure

Factorial sign		Group 1 (n = 54)	Group 2 (n = 52)	p
Age, years		34.1 ± 3.4	33.3 ± 5.4	0.361
Height, cm		166.4 ± 7.5	165.1 ± 5.7	0.322
Hirsutism scores		5 (3–7)	4 (2.5–5)	0.019
Coitus, times per week		3 (2–4)	2.5 (2–3.5)	0.239
Pregnancy loss, miscarriage	0	36 (66.7)	37 (71.2)	0.673
	1	11 (20.4)	10 (19.2)	
	2	3 (5.6)	3 (5.8)	
	3	1 (1.9)	1 (1.9)	
	4	2 (3.7)	0 (0.0)	
	5	1 (1.9)	0 (0.0)	
	6	0 (0.0)	1 (1.9)	
D-dimer, mg/l		0.406 (0.285–0.461)	0.305 (0.195–0.41)	0.005
Anti-Mullerian hormone, ng/ml		1.325 (0.71–2.03)	1.425 (0.92–2.05)	0.615
Ureaplasma urealyticum	no	45 (83.3)	42 (80.8)	0.803
	yes	9 (16.7)	10 (19.2)	
Anomaly of the uterine cavity	no	20 (37.0)	18 (34.6)	0.841
	yes	34 (63.0)	34 (65.4)	
Hysteroscopy	no	16 (29.6)	15 (28.8)	> 0.999
	yes	38 (70.4)	37 (71.2)	
HSP60_1, O.D.		0.403 (0.325–0.526)	0.397 (0.313–0.523)	0.91

Note: HSP60_1 – antibodies level before treatment, O.D. – optical density.

Table 6. Analysis of the 12-factor model of logistic regression predicting the risk of implantation failure

Factorial sign	Coefficient of the model, b ± m	Significance from 0, p	OR (95% CI)
Age, years	0.18 ± 0.08	0.022	1.19 (1.02–1.39)
Height, cm	-0.15 ± 0.06	0.013	0.86 (0.77–0.97)
Hirsutism scores	-0.31 ± 0.18	0.088	–
Number of sexual intercourses per week	0.70 ± 0.24	0.003	2.01 (1.27–3.20)
Pregnancy loss, miscarriage	0.39 ± 0.24	0.098	–
D-dimer, mg/l	-2.53 ± 1.67	0.131	–
Anti-Mullerian hormone, ng/ml	0.85 ± 0.34	0.014	2.3 (1.19–4.58)
Ureaplasma urealyticum	1.32 ± 0.72	0.067	–
Anomaly of the uterine cavity	1.22 ± 0.65	0.058	–
Hysteroresectoscopy	-1.04 ± 0.66	0.118	–
HSP60_1, O.D.	4.81 ± 2.27	0.035	122 (1.4–10500)
Group 2 vs group 1	-1.34 ± 0.55	0.014	0.26 (0.09–0.76)

Note: HSP60_1 – antibodies level before treatment. O.D. – units of optical density.

in Kyiv, and it also contributes to researches on the potential effects on women with infertility associated with autoimmune processes.

The study's limitations included the prospective case-control design, which limited data collection. The sample size was also very limited because only those with established infertility, informed consent, tubal infertility, age less than 44 years or more than 22 years, history of miscarriage, spontaneous abortion, IVF failure, and women with routine gynecological check-ups.

The information was gathered only from 3 institutes. There are frequently organizational biases in providing medical diagnosis and treatment, and replicating this research study with multiple research work.

This fact remains a critical issue for IVF researchers who are attempting to implant the vast majority of embryos. Implantation failure could be caused by reduced uterine receptivity, embryonic defects, sperm defects, recurrence of the embryo or hardening zones, or a combination of factors (thin endometrium, altered expression of adhesive molecules, and possible immune factors). Our recent research discovered that the bacterial analogue of HSP60 impacts normal pregnancy outcomes, and we also describe the most specific and sensitive analytical technique for determining it [41].

According to our findings, HSP60 and GroEl are the risk factors for developing pregnancy-related pathologies. Clinical measurement of these autoantibodies can be

implicated as an essential laboratory screening parameter before implantation in expectant individuals to increase the ratio of positive pregnancy outcomes. The prophylactic level of antibodies to HSP60 and GroEl can provide information for forecasting expected reproductive outcomes. Raising awareness of this screening among health care professionals and timely diagnosis and management will increase the likelihood of a high implantation success ratio in expectant individuals. The vast majority of genital chlamydial infection, as well as other infections in women, are asymptomatic. If these infections left untreated they can spread all the way to the top of the genital system, causing PID and reproductive pathologies [9].

One murine research study found that HSP60 has no effect on fertilization at low doses. In contrast, at high doses, it has a negative impact on fertilization and implantation [42]. Twelve-factor model revealed the positive and negative impacts on IVF success. Twelve-factor model revealed the positive and negative impact on IVF success. This model can be useful in predicting the effect of general characteristics of individuals on the risk of implantation failure. According to this study, having a high concentration of HSP60 and GroEl has a negative impact on the outcome of a normal pregnancy. Further immunochemical research on this parameter is required. Given the exploratory nature of the findings, we await for future research studies to confirm our findings.

CONCLUSIONS

In the prospective control study, we found that HSP60 and GroEl are significant risk factors for infertility. HSP60 and GroEl levels in all collected serum samples were measured by ELISA at the time of admission, after treatment (before embryo transfer), and after embryo transfer. The serum of infertile women contained a high concentration of HSP60 and GroEl. The treatment of the infertility cause in both groups results in a significant decrease in HSP60 and GroEl levels. The majority of patients who did not achieve treatment success had a high GroEl on the third measurement. As a result, determination of the optimal threshold value showed GroEl = 0.354 optical density units. 75.9% of patients with a GroEl score that did not exceed this threshold in the third measurement became pregnant; 82.7% of patients with a GroEl score that exceed this threshold in the third measurement did not become pregnant. These immunological parameters and risk assessment can be used as prognostic factors to predict positive IVF outcomes in patients undergoing infertility treatment. In twelve-factor model some parameters (age, number of sexual intercourses per week, D-dimer level, antibodies level to HSP60_1 (before treatment)) increase the risk of implantation failure and some factors (height, immunocorrective therapy) decrease.

Conflict of interest

The authors report no personal, scientific and financial conflicts of interests.

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PROSPECTIVE STUDY TO ESTIMATE THE ROLE OF DIFFERENT INFERTILITY FACTORS IN PREDICTION OF UNSUCCESSFUL IVF OUTCOME

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Research objective: in a prospective controlled study to investigate the role of HSP60, GroEl and other infertility factors as predictors of successful IVF outcome.

Materials and methods. 106 female patients were divided into two groups: 54 individuals who received conventional treatment for infertility (using ICSI techniques for IVF) and 52 individuals who received conventional therapy with intravenous IgG, enoxaparin and aspirin. All collected blood samples were tested for HSP60 and GroEl antibodies using immunofluorescence and ELISA techniques at the time of admission, after treatment (and before embryo transfer), and after embryo transfer. We analyzed the factors that can be helpful as prognostic parameters to estimate the risk of implantation failure.
Results. The risk of implantation failure is predicted when HSP60 level decreases from the first to the second measurement by less than 0.02 optical density units, with a sensitivity of 62% (95% confidence interval (CI) 47.2–75.3), and a specificity of 87.5% (95% CI 75.9–94.8), the positive predictive value was 81.6% (95% CI 68.2–90.2), the negative predictive value was 72.1% (95% CI 64.1–78.8). The GroEl value for the second dimension was more than 0.411 optical density units, which suggests a risk of treatment failure with a sensitivity of 64% (95% CI 49.2–77.1) and a specificity of 85.6% (95% CI 73.8–93.6), the positive predictive value was 80.0% (95% CI 67.1–88.7), the negative predictive value was 72.7% (95% CI 64.5–79.7). The highest ($p < 0.05$) value was observed at the beginning of treatment, and the lowest ($p < 0.05$) – during the third measurement.
Treatment of the underlying cause of infertility led to a decrease in HSP60 and GroEl levels, which ensured a positive *in vitro* fertilization result. It was found that HSP60 and GroEl have a strong association with embryo implantation. The risk of implantation failure was strongly associated with twelve factors, the area under the curve (AUC) was 0.85 (95% CI 0.76–0.91).
Conclusions. HSP60 and GroEl are good prognostic factors for predicting a successful IVF outcome in patients undergoing infertility treatment. The measurement of these parameters during the initial infertility examination may help in the immediate diagnosis of autoimmune infertility. Embryo implantation is a multifactorial process. The risk of implantation failure should be evaluated with multiple factors (twelve factors).

Keywords: infertility, GroEl, heat shock protein HSP60, IVF, ELISA, implantation failure.

ПРОСПЕКТИВНЕ ДОСЛІДЖЕННЯ ОЦІНЮВАННЯ РОЛІ ЧИННИКІВ БЕЗПЛІДДЯ У ПРОГНОЗУВАННІ НЕВДАЛОЇ СПРОБИ ЗАПЛІДНЕННЯ *IN VITRO*

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Мета дослідження: у проспективному контрольованому дослідженні вивчити роль HSP60, GroEl та інших чинників безпліддя як предикторів успішного результату запліднення *in vitro*.
Матеріали та методи. 106 пацієнток було розділено на дві групи: 54 жінки отримували традиційне лікування безпліддя (з використанням методів інтрацитоплазматичної ін'єкції сперматозоїдів для запліднення *in vitro*); 52 жінкам, окрім традиційної терапії, призначали внутрішньовенно IgG, еноксапарин та аспірин. За допомогою методів імунофлюоресценції та імуноферментного аналізу всі зібрані зразки крові були перевірені на наявність антитіл до HSP60 та GroEl під час надходження, після лікування (до перенесення ембріонів) і після перенесення ембріонів. Проаналізовано чинники, які можуть бути корисними як прогностичні параметри для оцінювання ризику невдачі імплантації.
Результати. Ризик невдачі імплантації прогнозується при зниженні концентрації HSP60 від першого до другого вимірювання менш ніж на 0,02 одиниці оптичної щільності, з чутливістю 62% (95% довірчий інтервал (ДІ) 47,2–75,3) і специфічністю 87,5% (95% ДІ 75,9–94,8), позитивне прогностичне значення становило 81,6% (95% ДІ 68,2–90,2), негативне прогностичне значення – 72,1% (95% ДІ 64,1–78,8). Значення GroEl для другого вимірювання сягало понад 0,411 одиниці оптичної щільності, що передбачає ризик неефективності лікування з чутливістю 64% (95% ДІ 49,2–77,1) і специфічністю 85,6% (95% ДІ 73,8–93,6), позитивне прогностичне значення становило 80,0% (95% ДІ 67,1–88,7), негативне прогностичне значення – 72,7% (95% ДІ 64,5–79,7). Найвище ($p < 0,05$) значення спостерігалося на початку лікування, а найнижче ($p < 0,05$) – під час третього вимірювання.
Лікування основної причини безпліддя привело до зниження рівня HSP60 і GroEl, що зумовило позитивний результат екстракорпорального запліднення. Виявлено, що HSP60 і GroEl мають тісний зв'язок з імплантацією ембріонів. Ризик невдачі імплантації твердо асоціювався з дванадцятьма чинниками, площа під кривою AUC становила 0,85 (95% ДІ 0,76–0,91).
Висновки. HSP60 і GroEl є хорошими прогностичними чинниками для прогнозування успішного результату запліднення *in vitro* в пацієнток, які проходять лікування безпліддя. Вимірювання цих параметрів під час первинного обстеження безпліддя може допомогти в негайній діагностиці аутоімунного безпліддя. Імплантація ембріона – багатофакторний процес, а ризик невдачі імплантації слід оцінювати з урахуванням множинних факторів (12 чинників).

Ключові слова: безпліддя, GroEl, білок теплового шоку HSP60, запліднення *in vitro*, імуноферментний аналіз, невдача імплантації.

ПРОСПЕКТИВНОЕ ИССЛЕДОВАНИЕ ОЦЕНКИ РОЛИ ФАКТОРОВ БЕСПЛОДИЯ В ПРОГНОЗИРОВАНИИ НЕУДАЧНОЙ ПОПЫТКИ ОПЛОДОТВОРЕНИЯ *IN VITRO*

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Цель исследования: в проспективном контролируемом исследовании изучить роль HSP60, GroEl и других факторов бесплодия как предикторов успешного результата оплодотворения *in vitro*.
Материалы и методы. 106 пациенток были разделены на две группы: 54 женщины получали традиционное лечение бесплодия (с использованием методов интрацитоплазматической инъекции сперматозоидов для оплодотворения *in vitro*); 52 женщинам наряду с традиционной терапией назначали внутривенно IgG, эноксапарин и аспирин. С помощью методов иммунофлюоресценции и иммуноферментного анализа все собранные образцы крови были проверены на наличие антител к HSP60 и GroEl во время поступления, после лечения (до переноса эмбрионов) и после переноса эмбрионов. Проанализированы факторы, которые могут быть полезны в качестве прогностических параметров для оценки риска неудачи имплантации.
Результаты. Риск неудачи имплантации прогнозируется при понижении концентрации HSP60 от первого до второго измерения менее чем на 0,02 единицы оптической плотности, с чувствительностью 62% (95% доверительный интервал (ДИ) 47,2–75,3) и специфичностью 87,5% (95% ДИ 75,9–94,8), положительное прогностическое значение составило 81,6% (95% ДИ 68,2–90,2), отрицательное прогностическое значение – 72,1% (95% ДИ 64,1–78,8). Значение GroEl для второго измерения составило более 0,411 единицы оптической плотности, что предполагает риск неэффективности лечения с чувствительностью 64% (95% ДИ 49,2–77,1) и специфичностью 85,6% (95% ДИ 73,8–93,6), положительное прогностическое значение – 80,0% (95% ДИ 67,1–88,7), отрицательное прогностическое значение – 72,7% (95% ДИ 64,5–79,7). Наивысшее ($p < 0,05$) значение наблюдалось в начале лечения, а самое низкое ($p < 0,05$) – во время третьего измерения.
Лечение основной причины бесплодия привело к снижению уровня HSP60 и GroEl, что обеспечило положительный результат экстракорпорального оплодотворения. Установлено, что HSP60 и GroEl имеют тесную связь с имплантацией эмбрионов. Риск неудачи имплантации прочно ассоциировался с двенадцатью факторами, площадь под кривой AUC составила 0,85 (95% ДИ 0,76–0,91).
Выводы. HSP60 и GroEl являются хорошими прогностическими факторами для прогнозирования успешного результата оплодотворения *in vitro* у пациенток, проходящих лечение бесплодия. Измерение этих параметров при первичном обследовании бесплодия может помочь в немедленной диагностике аутоиммунного бесплодия. Имплантация эмбриона – многофакторный процесс, а риск неудачи имплантации следует оценивать с учетом множественных факторов (12 факторов).

Ключевые слова: бесплодие, GroEl, белок теплового шока HSP60, оплодотворение *in vitro*, иммуноферментный анализ, неудача имплантации.