

Soluble human leukocyte antigen G in early human embryo cultures after assisted reproduction procedures

Živilė Čerkienė^{1,2},

Audronė Eidukaitė¹,

Audronė Usonienė²

¹VU Institute of Immunology,
Vilnius, Lithuania

²Fertility Clinic,
Vilnius, Lithuania

Human leukocyte antigen G (HLA-G) is a non-classical HLA class I molecule which can be expressed in the membrane-bound or soluble form and is well known for its tolerogenic properties. Increasing interest is now being addressed to the soluble forms, because they might have prognostic properties in the implantation and pregnancy process. The aim of our study was to evaluate a correlation between embryo cleavage, morphology and soluble human leukocyte antigen G (sHLA-G) levels, to estimate the impact of sHLA-G concentration on pregnancy outcome.

Materials and methods: The study was performed on a group of infertile patients with various infertility indications. *In vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedure were used for insemination. After 72 h, on the day of transfer, the embryo morphology was evaluated, embryo transfer (ET) according to embryo morphology was performed, the supernatants of the transferred embryos were collected and stored at -90°C . For the quantitative measurement of soluble forms of Human Leukocyte Antigen-G in cell culture supernatant we used the ELISA method. The ELISA kit from EXBIO Praha measures soluble HLA-G1 and HLA-G5 isoforms, plates are coated with mem-G9 MoAb and components are ready to use. The analysis of results was performed using the Statistics Package for Social Sciences SPSS 12.0.

Results: A total of 16 couples participated in this study. In all, 145 embryo culture supernatants were tested for sHLA-G. Soluble form of human leukocyte antigen G was detected in 12 (9.0%) embryo culture samples (range, 0.4–20.5 IU/ml). The production of human leukocyte antigen did not depend on the morphological criteria of the embryo. No significant differences were found between sHLA-G concentration in patients from the IVF and ICSI groups. sHLA-G levels did not differ significantly in the embryo cleavage rate and pregnancy outcome.

Conclusions: day 3 embryos secrete sHLA-G to the surrounding medium; the level of those soluble molecules can be detected using ELISA. The concentration of the human leukocyte antigen did not correlate with the number of blastomere and with the morphological criteria of the embryo. sHLA-G might be one of the markers of embryo implantation potential after IVF/ICSI procedures as a factor that helps to achieve and maintain pregnancy, but further investigations are required.

Keywords: sHLA-G, embryo, IVF, ICSI

INTRODUCTION

HLA-G is a non-classical HLA class I molecule that can be expressed in the membrane-bound or soluble form and is well known for its tolerogenic properties. Increasing interest is now being addressed to the soluble forms, because they might have prognostic properties in the implantation and pregnancy process.

Investigation of soluble human leukocyte antigen-G as a possible marker of embryo developmental potential started in 1996 when Juriscova et al. (1) reported data on total mRNA and protein in human embryos. In 1999, Menicucci et al. (2) evaluated the presence of sHLA-G molecules in supernatant cultures of early human embryos obtained by IVF. A similar study of 101 patients (3) showed that clinical pregnancy was obtained only if sHLA-G molecules were detected in culture supernatants of growing embryos.

Correspondence to: Živilė Čerkienė, Vaisingumo klinika, Laisvės pr. 64 A, Vilnius, Lithuania. E-mail: c.zivile@gmail.com

While evaluating the success of assisted reproductive technologies (ART) (implantation, pregnancy, birth rate) in general, they seem far from desired. Data from 75 individual groups presented in "The World Collaborative Report on *in-vitro* Fertilization and Embryo Replacement: Current State of the Art in January 1984" (4) showed the overall clinical pregnancy rate of 14.2%. Until now the probability of a successful pregnancy during an IVF cycle is approximately 18%, with a baby rate of at about 14%. These rates, which are not so very different from those that occur during natural cycles, vary throughout different IVF clinics throughout the world. So patients and specialists are extremely interested in the possibility to find new methods to raise the success rate. According to www.haveababy.com, the detection of soluble human leukocyte antigen *in vitro* is a revolutionary new test that seems highly predictive of the ability of individual embryos to successfully produce a pregnancy following *in vitro* fertilization (IVF). The one-year study authored by Sher et al. (5) in 2004 indicates that individually-cultured embryos producing sufficient concentrations of a marker, sHLA-G, give previously infertile women a better than 60% pregnancy success rate – double the current success rates for IVF. The presence of high levels of sHLA-G had a positive predictive value (greater than 70%) in women under the age of 39 and over 50% in women aged 39 to 44 years.

Selection of the best embryo for ET procedure is one of the most important tasks leading to success of the ART procedure, because embryo quality significantly correlates with implantation and pregnancy rates (6). Gardner and Sakkas in 2003 (7) suggested that by selecting specific embryos for transfer based on their individual sHLA-G expression, the pregnancy and implantation rates can be maximized while the number of embryos transferred can be reduced, thereby minimizing the incidence of high-order multiple pregnancies. In 2005, Noci et al. (8) published an article in which they proposed sHLA-G as a potential marker of embryo development and ELISA as a useful biochemical assay in addition to embryo morphology in embryo selection.

At present, embryo implantation is still poorly understood, and only about one sixth of transferred embryos give rise to a full-term infant. Taking into account that embryo selection is based only on morphological and cleavage criteria, though embryo viability is not strictly correlated with embryo quality, there is no doubt regarding the importance of creating a non-invasive technique for selection of the best embryo. sHLA-G may be used as a marker for embryo selection, because its production seems to be related to pregnancy outcome. Determination of the accurate pregnancy potential of each embryo before IVF transfer is significant for doubling the IVF success rate and reducing high-order multiple pregnancies.

The aim of our study was to evaluate a correlation between embryo cleavage, morphology and sHLA-G levels, as well as to estimate the impact of HLA-G concentration on pregnancy outcome.

MATERIALS AND METHODS

Patients

The study was performed on a group of infertile patients with various clinical indications for IVF or ICSI. To achieve the controlled ovary hyperstimulation, we used gonadotropin hormone agonists (GnRH a): Triptorelinum (Diphereline 3.75 mg, Beaufour Ipsen) and gonadotropin Folitropinum alfa (Gonal F, Serono). Starting GnRH agonist in the middle of the luteal phase of the previous menstrual cycle and from either the 3rd, 4th, or 5th day of the following menstrual cycle daily rFSH was added (9). Only clinical pregnancies were evaluated. The pregnancy was determined by serum HCG test and confirmed by finding the gestational sack in the uterus by ultrasound examination.

IVF

After the oocyte pickup procedure, the collected oocytes were cultured in dishes with IVF culture medium (Medicult, Denmark). The IVF or ICSI procedure was used for insemination depending on infertility indication. 16–18 h after insemination, fertilization observation under an inverted microscope (OLYMPUS IX 70) was performed. Zygotes were transferred into a fresh medium and cultivated separately in 4-well multidishes in 600 μ l of culture medium. Not fertilized oocytes were cultivated under the same conditions as zygotes, and their cultivating media were used for control. After 72 h, embryo morphology evaluation, ET according to embryo morphology and collection of supernatants were performed. Embryo quality (the number of blastomeres and embryo morphology) was assessed on the third day (72 h after insemination). Embryo morphology was scored according to Salumets et al. (10). Morphological criteria for embryo scoring included fragmentation and equines of blastomeres. Embryos were assessed as grade 4: no fragments and equal blastomeres; grade 3: < 20% fragmentation; grade 2: unequal blastomeres and / or 20–50% of fragmentation; grade 1: >50% fragmentation. All supernatants before sHLA-G testing were stored at -90°C .

sHLA-G ELISA

For the quantitative measurement of soluble forms of Human Leukocyte Antigen-G in cell culture supernatant we used the ELISA method. The ELISA kit from EXBIO Praha measures soluble HLA-G1 and HLA-G5 isoforms, plates are coated with mem-G9 MoAb and components are ready to use. In this sHLA-G ELISA, standards and samples are incubated in micro titration wells coated with mouse monoclonal anti-sHLA-G antibody. After washing, mouse monoclonal anti-human B2-microglobulin antibody labelled with horseradish peroxidase was added to the wells and incubated with the immobilized antibody-sHLA-G complex. Following another washing step, the remaining HRP-conjugated antibody was allowed to react with the substrate (H_2O_2) with tetramethylbenzidine). The reaction was stopped by adding an acidic solution, and absorbance of the resulting yellow product was measured spectrophotometrically at 450 nm.

The absorbance was proportional to the concentration of sHLA-G. A standard curve was constructed by plotting absorbance values versus sHLA-G concentrations of standards, and concentrations of unknown samples were determined using this standard curve.

Statistical analysis

Analysis of the results was performed using the Statistics Package for Social Sciences SPSS 12.0. The differences of sHLA-G concentration between the two groups were tested using the nonparametric Mann-Whitney U test. The differences among three or more groups were tested using the nonparametric Kruskal-Wallis test. The relationship among qualitative data was evaluated by the chi-square test or Fisher's exact test. The chosen level of statistical significance was 5% (the differences were considered statistically significant when the p value was less than 0.05).

RESULTS

In total, 16 patients were involved in this study and supernatants of 145 embryo cultures were investigated. Soluble form of human leukocyte antigen G was detected in 12 (9.0%) embryo culture samples (range, 0.4–20.5 IU/ml). None of the cultivating media of the unfertilized oocyte (control group) showed presence of sHLA-G. 37 embryos were received after ICSI procedure and 108 after IVF procedure. In the ICSI group, 4 embryos (10.8%) secreted measurable levels of sHLA-G to cultivation medium, and in the IVF group sHLA-G was detected in supernatants of 8 embryos (7.4%) (Table 1).

The embryos were divided into four quality groups according to the number of fragmentation and equability of blastomeres. 71 embryos represented group 4 (no fragments and equal blastomeres), 31 embryos were as-

Table 1. sHLA-G production in ICSI and IVF groups

	sHLA-G (IU/ml)	
	Detected	Undetected
ICSI	N = 4 (10.8%) Median 3.38 1 st quartile 0.77 3 rd quartile 9.80 Min 0.7 Max 11.1	N = 33 (89.2%)
IVF	N = 8 (7.4%) Median 3.58 1 st quartile 1.78 3 rd quartile 8.45 Min 0.4 Max 20.5	N = 100 (92.6%)

Note. sHLA-G secretion in ICSI and IVF groups was similar ($\chi^2 = 0.421$, $p = 0.517$). The concentration difference between the groups was not significant (Mann-Whitney U test = 15.0, $p = 0.865$).

cribed to group 3 (<20% fragmentation), 42 embryos to group 2 (unequal blastomeres and / or 20–50% of fragmentation) and only one embryo to group 1 (>50% fragmentation). Embryos of three quality groups secreted sHLA-G to cultivation medium (Table 2). 43 embryos were transferred into the uterus. of them seven, from the quality groups 4 and 2, secreted detectable amounts of sHLA-G (Table 3).

To combine soluble sHLA-G levels and cleavage rate, we grouped all embryos according to the number of blastomeres. Embryos with various numbers of blastomeres produced detectable amounts of sHLA-G. In the supernatants of four embryos with the highest number of

Table 2. Embryo quality and sHLA-G production

Embryo quality	sHLA-G-positive		sHLA-G-negative
	N = 12	Median	N = 133
4	5 (41.7%)	5.90	66 (49.6%)
3	3 (25.0%)	3.15	28 (21.1%)
2	4 (33.3%)	1.88	38 (28.6%)
1	0 (0%)	0	1 (8%)

Note. Effects of embryo quality on sHLA-G secretion were not significant ($\chi^2 = 0.399$, $p = 0.940$). The concentration differences among the groups were not significant (Kruskal-Wallis test = 2.103, $p = 0.349$).

Table 3. Embryo quality of transferred embryos and sHLA-G production

Embryo quality	sHLA-G positive		sHLA-G negative
	N = 7	Median	N = 36
4	4 (57.1%)	8.50	26 (72.2%)
3	0 (0%)	0	5 (13.9%)
2	3 (42.9%)	2.90	5 (13.9%)

Note. Effects of the quality of embryos chosen for transfer on sHLA-G secretion was not significant ($\chi^2 = 3.807$, $p = 0.149$). The concentration differences among the groups were not significant (Kruskal-Wallis test = 3.125, $p = 0.077$).

Table 4. sHLA-G production and number of blastomeres

No of blastomeres	sHLA-G-positive		sHLA-G-negative
	N = 12	Median or value	N = 133
2	1 (8.3%)	11.10	21 (15.8%)
3	1 (8.3%)	3.20	7 (5.3%)
4	1 (8.3%)	20.50	42 (31.6%)
5	3 (25.0%)	0.85	10 (7.5%)
6	1 (8.3%)	4.20	72 (21.1%)
7	1 (8.3%)	1.40	2 (15.0%)
8	4 (33.3%)	3.45	23 (17.3%)

Note. The association between embryo cleavage rate and sHLA-G secretion was not statistically significant ($\chi^2 = 9.334$, $p = 0.156$).

blastomeres we found a measurable amount of this antigen. Though eight cell embryos represent the most positive cases of sHLA-G synthesis (33.3%), the correlation between embryo cleavage rate and the production of this soluble antigen was not statistically significant (Table 4).

In order to evaluate the impact of soluble human leukocyte antigen on pregnancy outcome, after the ART procedure we divided the patients into pregnant and non-pregnant groups. In total, six of the 16 patients (37.5%) in this study became pregnant after IVF / ICSI procedure. For the transfer of a part of pregnant women (4 from 6 cases) embryos with detectable amount of sHLA-G were chosen, and two women got pregnant when no one of the transferred embryos produced sHLA-G. On the other hand, in the non-pregnant group for a half of women (5 from 10 cases) at least one transferred embryo synthesised sHLA-G to cultivation medium, but it was not enough for a successful outcome (Table 5). Comparing pregnant and non-pregnant groups, no significant difference was detected among pregnancy outcome within sHLA-G positive and negative subgroups (Fisher's exact test $p = 0.427$).

Table 5. sHLA-G values detected in culture medium of transferred embryos

Patient	sHLA-G (IU/ml)	Clinical pregnancy
1	0,0,0	no
2	4.0	no
3	0,0,5.9	no
4	0,0,0	yes
5	0,2.9,0	yes
6	0,0,4.2	yes
7	0,0,0	no
8	0.7,0,0	yes
9	0,0,0	no
10	0,0,0.4	no
11	0,0,0.7	no
12	0,0,0	no
13	0,0,11.1	no
14	0,0,0	yes
15	20.5,0,0	yes
16	0	no

DISCUSSION

A lot of data prove that early embryos secrete sHLA-G. This was confirmed in our study as well. The question is when embryos start producing detectable amounts of this soluble antigen: at a 2–4 cell or 6–8-cell stage. According to Sher et al. (5), the expression of sHLA-G in supernatants of individually cultured 46 h embryos seems to be a potentially valuable indicator of embryo competency. Noci et al. (8) also observed the time required to produce sHLA-G. On day 2 (48 h) only 8.9% of the supernatants showed sHLA-G secretion, on day 3

36.2% of the embryos produced a detectable amount of sHLA-G. According to our data 12 from 133 3rd day embryos (9.0%) released sHLA-G into the surrounding medium.

While dividing patients into IVF and ICSI groups, we decided to evaluate how the *in vitro* procedure influences sHLA-G production. Previously calculated data (8) from 138 IVF and 182 ICSI patients proved no significant differences between those two groups in sHLA-G secretion. This finding supports our report that the embryos obtained after IVF or ICSI produced detectable levels of sHLA-G and the secretion of this antigen did not depend on the method of insemination.

Embryo selection for transfer is one of the most important moments leading to success after IVF / ET procedure (11, 12). At present, embryo evaluation is based on morphological criteria such as the percentage of fragmentation and the size of blastomeres. Some embryologists add evaluation of zygote stage: lying of pronucleus (13), number of nucleoli (14) to step-by-step embryo grading. Others use the early cleavage method (15) for the determination of an embryo with best developmental potential. Though this well-known system of embryo scoring is rather simple to use, not invasive and well described, embryo viability is not always connected with cell quality (16, 17). Well-known data of Hardarson et al. (18) prove that the embryo culture period is a dynamic process and the morphology of the embryo can change rapidly. Taking this and other published data into account, scientists began to search for a more objective marker of embryo viability. It is necessary to mention the measurement of amino acid turnover by Brison et al. (19), glucose and pyruvate uptake during development of cells by Gardner and Leese (20) and measurement of respiration rates of individual embryos by Lopes et al. (21). Research works dealing with immunological markers of the embryo developmental potential can also be attributed to this kind of findings.

Preimplantation embryo development is regulated by an insulin-like growth factor (22) and the platelet-activating factor (23). The first evaluation of presence of soluble, non-classic HLA class I antigens in human embryo / culture and its possible association with embryo cleavage rate was performed in 1999 (2), though the later findings by Fuzi et al. (3) were controversial: the impact of sHLA-G to embryo cleavage depended on the stage of embryo development, at which sHLA-G screening was performed. No significant differences were observed between embryo cleavage and the presence of sHLA at either 24 or 72 h, whereas at 48 h the differences were significant. Measurements of sHLA-G in our study took place at 72 h embryo culture and the synthesis of this antigen did not correlate with embryo cleavage.

As regards the correlation between embryo morphology and the production of human leukocyte antigen, we have found that sHLA-G production is not related to the percentage of fragmentation and equability of blastomeres: embryos of best, good and poor quality produced

detectable amounts of sHLA-G. Earlier reported results correlate with our data that non-classic HLA class I antigens can be detected in human embryo culture of various quality, from top to poor, when the evaluation is based on (in our case – grade 4 and grade 2 respectively) morphological criteria.

It was suggested that HLA-G as a non-classical class I major histocompatibility complex molecule may be involved in the protection of the semiallogeneic human fetus during the implantation period, so it might have a direct impact on pregnancy outcome after IVF / ET procedure. According to Sher et al. (5), with transferring all sHLA-G-positive embryos the pregnancy rate reached 75% (38/51), versus 23% (13/56) when all transferred embryos were sHLA-G negative. According to our results, pregnancy was achieved in four from 6 patients when at least one embryo with a detectable amount of sHLA-G was chosen for transfer.

Though Noci et al. (8) report that absence of sHLA-G in embryo supernatants has a completely negative predictive value, our data showed that in seven women of the sHLA-G-negative group two pregnancies occurred.

CONCLUSIONS

To resume, day 3 embryos secrete sHLA-G to the surrounding medium; the level of those soluble molecules can be detected using the enzyme-linked immunosorbent assay. The human leukocyte antigen G did not correlate with the morphological criteria and blastomere number of the embryos. sHLA-G might be one of the markers of the embryo implantation potential after IVF / ICSI procedures as a factor that helps to achieve and maintain pregnancy, but further investigations are required.

Received 8 September 2006

Accepted 20 October 2006

References

- Juriscova A, Casper RF, MacLusky NJ, Mills GB, Librach CL. HLA-G expression during preimplantation human embryo development. *Developmental Biology* 1996; 93: 161–5.
- Menicucci A, Baricordi OR. HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *Eur J Immunol* 2002; 32: 311–5.
- Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, Bencini E, Menicucci A, Baricordi OR. HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *Eur J Immunol* 2002; 32: 311–5.
- Seppala M. The world collaborative reports on *in vitro* fertilization and embryo replacement: current state of the art in January 1984. *Ann N Y Acad Sci* 1985; 442: 558–63.
- Sher G, Keskinetepe L, Nouriani M, Roussev R, Batzofin J. Expression of sHLA-G in supernatants of individually cultured 46-h embryos: a potentially valuable indicator of “embryo competency” and IVF outcome. *Reprod Biomed* 2004; Online 9: 74–8.
- Usoniene A, Cerkiene Z, Usonyte A. Prognostic factors for clinical pregnancy using *in vitro* fertilization / intracytoplasmic sperm injection and embryo transfer procedures. *Acta Medica Lituanica* 2004;11(4): 34–9.
- Gardner DK, Sakkas D. Assessment of embryo viability: the ability to select a single embryo for transfer – a review. *Placenta* 2003; 24: 5–12.
- Noci I, Fuzzi B, Rizzo R, Melchiorri L, Criscuoli L, Diabizzi S, Biagiotti R, Pallegri S, Menicucci A, Baricordi OR. Embryonic soluble HLA-G as a marker of developmental potential in embryos. *Hum Reprod* 2005; 20(1): 138–146.
- Macnamee MC, Brinsden PR. Superovulation strategies in assisted conception. A Textbook of *in vitro* Fertilization and Assisted Reproduction. The Parthenon Publishing Group. 1999: 83–9.
- Salumets A, Suikkari AM, Mols T, Soderstrom-Anttila V, Tuuri T. Influence of oocytes and spermatozoa on early embryonic development. *Fertil Steril* 2002; 78(5): 1082–7.
- Terriou P, Sapin C, Giorgetti Hans E, Spach J.L, Roulier R. Embryo score is a better predictor of pregnancy than the number of transferred embryos or female age. *Fertil Steril* 2001; 75(3): 525–31.
- Sundstrom P. Good results of single embryo transfer after *in vitro* fertilization. The first follow-up report after the introduction of a new regulatory guideline. Establishment of a successful pregnancy following *in-vitro* fertilization with an endometrial thickness of no more than 4 mm. *Hum Reprod* 1998; 13(6): 1550–2.
- Kahraman S, Kumtepe Y, Sertyel S, Donmez E, Benkhalifa M, Findikli N, Vanderzwalmen P. Pronuclear morphology scoring and chromosomal status of embryos in severe male infertility. *Hum Reprod* 2002; 17(12): 3193–200.
- Scott LA, Smith S. The successful use of pronuclear embryo transfers the day following oocyte retrieval. *Hum. Reprod.*1998; 13 (4): 1003–13.
- Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. *Hum Reprod* 2001; 16: 2652–7.
- Graham J, Han T, Porter R, Levy M, Stilman R, Tucker M J. Day 3 morphology is a poor predictor of blastocyst quality in extended culture. *Fertil Steril* 2000; 74(3): 495–7.
- Balaban B, Urman B, Alatas B, Mercan C, Aksoy S, Isiklar A. Blastocyst stage transfer of poor-quality cleavage-stage embryos results in higher implantation rates. *Fertil Steril* 2001; 75(3): 514–8.
- Hardarson T, Lofman C, Coull G, Sjogren A, Hamberger L, Edwards RG. Internalization of cellular fragments in a human embryo: time-lapse recordings. *Reprod Biomed* 2002; Online 5: 36–8.
- Brison DR, Houghton FD, Falconer D, Roberts SA, Hawkhead J, Humpherson PG, Lieberman BA, Leese HJ. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. *Hum Reprod* 2004; 19(10): 2319–24.
- Gardner DK, Leese HJ. Non-invasive measurement of nutrient uptake by single cultured pre-implantation mouse embryos. *Hum Reprod* 1986; 1: 25–7.

21. Lopes AS, Ramsing N, Larsen LH, Raty M, Peippo J, Greve T, Callesen H. Correlation between oxygen respiration rates and morphology, sex, diameter and developmental stage of single bovine IVP-embryos. *Reprod Fert Develop* 2005; 17:151 (abst).
22. Liu HC, He Z Y, Mele C A, Veeck L, Davis O K, Rosenwaks Z. Expression of IGFs and their receptors is a potential marker for embryo quality. *Am J Reprod Immunol* 1987; 38(4): 237–45.
23. O'Neill C, Gidley-Baird AA, Ammit AJ, Saunders DM. Use of a bioassay for embryo-derived PAF as a means of assessing quality and pregnancy potential of human embryos. *Fertil Steril* 1987; 47: 969–75.

Živilė Čerkienė, Audronė Eidukaitė, Audronė Usonienė

TIRPUS ŽMOGAUS LEUKOCITŲ G ANTIGENAS ANKSTYVOSE EMBRIONŲ KULTŪROSE PO PAGALBINIŲ APVAISINIMO PROCEDŪRŲ

Santrauka

Įvadas. Tirpus žmogaus leukocitų antigenas (s HLA-G) yra neklasikinė HLA I klasės molekulė, kuri gali būti sujungta su membrana arba tirpi. Pastaraisiais metais dėmesys nukreiptas į tirpias formas, nes jos gali turėti reikšmės nuspėjant embrionų implantavimosi ir nėštumo galimybes. Mūsų tyrimo tikslas yra nustatyti ryšį tarp embriono kokybės, dalijimosi tempo ir tir-

pus žmogaus leukocitų G antigeno, taip pat įvertinti šių molekulių įtaką nėštumo dažniui.

Medžiagos ir metodai. Tyrime dalyvavusiems „Vaisingumo klinikos“ pacientams buvo atliktos pagalbinio apvaisinimo procedūros: dirbtinis apvaisinimas mėgintuvėlyje (IVF) arba spermijų injekcija į citoplazmą (ICSI). Remiantis morfologiniais kriterijais įvertinta embrionų kokybė. Surinkta embrionų auginimo terpė laikyta šaldiklyje –90 °C temperatūroje. S HLA-G embrionų auginimo terpėje nustatytas panaudojus komercinį ELISA rinkinį (EXBIO, Praha), matuojantį antigeno G1 ir G5 izoformas.

Rezultatai. Žmogaus leukocitų antigenas iširtas 145 embrionų auginimo terpėse, gautose atlikus pagalbinis apvaisinimo procedūras 16 pacientų. S HLA-G išskyrė 12 (9,0%) embrionų, tirpių molekulių koncentracija svyravo nuo 0,4–20,5 IU/ml. Žmogaus leukocitų G antigeno sintezė nesusijusi su embrionų morfologiniais kriterijais. Nėra patikimo ryšio tarp antigeno koncentracijos, embrionų dalijimosi tempo bei nėštumų dažnio.

Išvados. Nustatyta, kad trečios dienos embrionai išskiria s HLA-G į auginimo terpę, šių molekulių koncentracija gali būti nustatoma imunofermentiniu metodu. Embrionų išskiriamas žmogaus leukocitų G antigenas neturi įtakos embrionų kokybei ir blastomerų skaičiui. Tirpus žmogaus leukocitų antigenas galėtų būti vienu iš embrionų implantavimosi žymenų, tačiau reikalingi išsamesni tyrimai.

Raktažodžiai: žmogaus leukocitų G antigenas, embrionas, IVF, ICSI