PREDICTING THE BLASTOCYST DEVELOPMENT RATE DURING ASSISTED REPRODUCTIVE TECHNOLOGIES BASED ON SEMEN MICROBIOTA

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Obtaining enough good and excellent quality embryos is one of the key factors for achieving pregnancy using assisted reproductive technologies. This work was aimed at developing a mathematical model for predicting good and excellent quality embryos based on semen microbiota assessment in normozoospermia. The study included 127 men whose semen was used for in vitro fertilization (IVF). Patients were divided into 2 groups depending on the proportion of good-quality blastocyst developed on the 5th day of culturing (good-quality blastocyst development rate, GBDR). The 1st group included 57 patients with GBDR ≥ 40%, the 2nd group included 70 patients with GBDR < 40%. All patients' semen was assessed at the day of fertilization. Semen parameters were evaluated in accordance with the WHO standards and semen microbiota composition was determined by means of real-time PCR. Discriminant analysis was used for development of the prognostic model. We developed a method for predicting efficiency of the embryological IVF stage in normozoospermia: EGO-Pro-N prognostic index (Embryos of GOod and Excellent quality Prognosis in Normozoospermia). If the EGO-Pro-N value is greater than 0.212, the probability of receiving GBDR ≥ 40% is low. Conversely, if the EGO-Pro-N value is less than or equal to 0.212, the probability is high. Sensitivity and specificity of the method were 71.9% and 70.0% respectively, accuracy was 70.9%. The developed model allows us to predict good and excellent quality embryos based on comprehensive semen microbiota assessment in normozoospermia before IVF.

Keywords: semen microbiota composition, prognosis, ART effectiveness, IVF, real-time PCR, discriminant analysis

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Compliance with ethical standards: the study was approved by the Ethics Committee of Ural State Medical University, Federal State Budget Educational Institution of Higher Education under the Ministry of Health of the Russian Federation (Protocol № 7 of September 20, 2019). All patients signed the informed written consent to participation in the study.

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ПРОГНОЗИРОВАНИЕ ЭФФЕКТИВНОСТИ ЭМБРИОЛОГИЧЕСКОГО ЭТАПА ВСПОМОГАТЕЛЬНЫХ РЕПРОДУКТИВНЫХ ТЕХНОЛОГИЙ ПО МИКРОБНОМУ СОСТАВУ ЭЯКУЛЯТА

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Один из ключевых факторов наступления беременности при использовании вспомогательных репродуктивных технологий (ВРТ) — получение достаточного количества эмбрионов хорошего и отличного качества. Целью работы было разработать математическую модель для прогнозирования качества получаемых эмбрионов на основании результатов оценки микробного состава эякулята, используемого для оплодотворения в программах ВРТ при нормозооспермии. Эякулят 127 мужчин использовали в процедуре экстракорпорального оплодотворения (ЭКО). В зависимости от доли бластоцист хорошего и отличного качества, сформировавшихся на 5-й день культивирования (good-quality blastocyst development rate, GBDR), пациенты были разделены на две группы. В первую включены 57 пациентов с GBDR ≥ 40%, во вторую — 70 пациентов с GBDR < 40%. Всем пациентам в день оплодотворения проведено исследование параметров спермограммы по стандарту ВОЗ и определен состав микробиоты эякулята методом количественной ПЦР в реальном времени. С помощью дискриминантного анализа разработан способ прогнозирования эффективности эмбриологического этапа ВРТ у пар, планирующих процедуру ЭКО, при показателях эякулята, соответствующих критериям нормозооспермии, с расчетом прогностического индекса «Эмбрионы хорошего и отличного качества. Прогноз нормозооспермия» (ЭХО-Про-N). Если значение ЭХО-Про-N > 0,212, вероятность получения GBDR ≥ 40% низкая, если ЭХО-Про-N ≤ 0,212, вероятность высокая. Показатели чувствительности и специфичности составили соответственно 71,9% и 70,0%, эффективность способа — 70,9%. Разработанная прогностическая модель дает возможность прогнозирования получения GBDR ≥ 40% на основании комплексной оценки микробиоты эякулята.

Ключевые слова: микробиота эякулята, прогнозирование, эффективность ВРТ, ЭКО, ПЦР в реальном времени, дискриминантный анализ

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The demand in assisted reproductive technologies (ART) is continuously growing. At the same time, according to the National Registry of the Russian Association of Human Reproduction, the effectiveness of *in-vitro* fertilization (IVF) treatment programs does not exceed 34.8% in terms of embryo transfer rate [1]. Obtaining the sufficient amount of good and excellent quality embryos is one of the key factors for achieving pregnancy using ART [2]. Predicting the amount of good-quality embryos would allow us to optimize patient preparation and select the best management option for the embryological stage. Currently specialists are actively searching for embryo quality markers based on various data. This data includes metabolomic, proteomic, transcriptomic semen parameters, follicular fluid, and embryo culture medium [3]. At the same time, the impact of semen microbiota on the ART results, as well as on the quality of obtained embryos, requires further study.

IVF is performed in non-sterile conditions [4]. Despite meticulously following aseptic measures, at most we are able to decrease the level of microbial contamination in IVF labs and in embryo cultures. Gametes (semen and oocytes) used for culturing embryos usually come from sources containing bacteria in most cases [5]. According to molecular genetic analyses, semen microbiota can be represented by complex microbial communities. These communities are made up of different bacterial genera and phyla, including obligate and facultative anaerobic bacteria, even in patients without inflammation and with normal semen parameters [6–8]. There is a correlation between detecting specific groups of opportunistic microorganisms (OMs) in semen samples and the decrease of semen parameters [9, 10].

Excluding pathogens causing sexually transmitted infections (STIs) in urethral or semen samples is currently the only stage of preparing male patients for the ART programs [11]. Presence of other microorganisms, which could be both, the cause of subclinical inflammation in the urogenital tract (UGT) and the cause of ART protocol failures, is not tested.

Bacterial contamination during embryo culture is a serious problem faced in all IVF laboratories [12, 13]. According to previous reports, the frequency of bacterial contamination is less than 1%, however, its occurrence could lead to bad quality embryos, as well as to embryonic demise, thus to the ART treatment failure.

Semen microbiota is considered one of the main sources of bacterial contamination for culture media [12]. Despite meticulous processing, the obtained semen sample still contains microorganisms that could lead to contamination of the embryo culture medium. In classic IVF, oocytes are co-cultured with the processed semen for 16–20 hours in a CO2 incubator at a temperature of 37 °C. Such conditions are favorable for the development of microorganisms, primarily for obligate anaerobes. As a result, media may contain toxic bacterial metabolites. Negative effects of bacterial toxins can cause embryo cell fragmentation and embryonic death [13].

The effect of semen microbiota on the development of embryos should be evaluated more carefully in order to develop adequate measures to prevent unsuccessful outcomes of the embryological stage of ART.

The aim of this study was to develop a mathematical model for predicting the quality of the obtained embryos based on the results of the semen microbiota assessment in normozoospermia.

METHODS

Research design

A single-center cohort retrospective study included an assessment of the semen parameters and semen microbiota of 127 men who entered into the ART programs at «Garmonia» Medical Center (Yekaterinburg, Russia) from 2020 to 2021. The embryology key performance indicators were evaluated: fertilization rate, blastocyst development rate, and good and excellent quality blastocyst (with maximum potential for implantation) development rate.

Inclusion and exclusion criteria

Inclusion criteria: patient age 19–51 years; semen parameters meet the criteria of the normozoospermia; fertilization of eggs by classic IVF; consent to participate in the study; age of women 22–40 years; absence of genetic diseases in patients and their relatives. The study included programs with fresh oocytes using classic IVF. Exclusion criteria: use of unfrozen oocytes for fertilization; oocyte fertilization by intracytoplasmic semen injection (ICSI); clinical manifestations of UGT infectious and inflammatory diseases; STIs; intake of hormonal, antibacterial drugs during the last three months, genetic diseases in patients or their relatives; semen volume less than 2 ml; refusal of patients to participate in the study.

ART embryological stage

Oocyte-cumulus complexes were placed in the Flushing medium (Origio; Denmark). Sequential Fert medium (Origio; Denmark) was used for oocyte fertilization. After fertilization evaluation, the zygotes were placed in the SAGE 1-Step medium (Origio; Denmark), in which the embryos were cultured until day 5.

To prepare semen samples, SupraSperm (Origio; Denmark) density gradient sedimentation method was used, then the spermatozoa were washed twice in the SpermPrep buffer (Origio; Denmark), after which the "swim-up" method was used.

Assessment of embryo morphology

The quality of developing zygotes and embryos was individually assessed under a microscope approximately 18, 72 and 96 hours after fertilization. Embryo morphological evaluation was performed on the 5th day of development from the moment of fertilization.

Embryos of excellent and good quality on the 5th day of cultivation had the following morphological characteristics: adequate amount of densely packed cells in the intracellular mass, the trophectoderm contains many cells that have formed a dense epithelium; the blastocele occupies more than 80% of the embryo volume. The efficiency of the ART embryological stage was considered acceptable when at least 40% of blastocysts of good and excellent quality (good-quality blastocyst development rate — GBDR) from the total number of fertilized eggs were obtained: GBDR ≥ 40% [14].

Depending on the proportion of blastocysts of good and excellent quality formed at the embryological stage, patients were divided into two groups: the first included 57 patients with GBDR ≥ 40%, the second had 70 patients (GBDR < 40%).

Research methods

In both groups controlled ovarian hyperstimulation was initiated according to a short protocol using antagonists of recombinant and/or urinary gonadotropins at a daily dose of 150–300 IU. Human chorionic gonadotropin was used as an ovulation trigger 34–36 hours before the puncture. Follicle aspiration was performed under intravenous anesthesia.

Table 1. The detection rate of specific groups of microorganisms quantities exceeding the threshold value*

Groups of microorganisms	Detection rate				Significance p
	Group 1 (n = 57)		Group 2 (n = 70)		
	n	%	n	%	
Lactobacillus spp.	17	29.8	11	15.7	0.084
Gram-positive facultative anaerobes	24	42.1	23	32.9	0.356
Staphylococcus spp.	6	10.5	3	4.3	0.297
Streptococcus spp.	7	12.3	13	18.6	0.463
Corynebacterium spp.	21	36.8	16	22.9	0.116
Gram-negative facultative anaerobes	0	0	11	15.7	0.001
Haemophilus spp.	0	0	10	14.3	0.002
Pseudomonas aeruginosa / Ralstonia spp. / Burkholderia spp.	0	0	1	1.4	1
Obligate anaerobes	35	61.4	41	58.6	0.856
G.vaginalis	8	14	17	24.3	0.181
Megasphaera spp. / Veillonella spp. / Dialister spp.	10	17.5	18	25.7	0.291
Sneathia spp. / Leptotrichia spp. / Fusobacterium spp.	4	7	5	7.1	1
Bacteroides spp. / Porphyromonas spp. / Prevotella spp.	14	24.6	24	34.3	0.25
Peptostreptococcus spp. / Parvimonas spp.	8	14	11	15.7	1
Anaerococcus spp.	17	29.8	14	20	0.219
Eubacterium spp.	30	52.6	29	41.4	0.217
A.cluster	4	7	7	10	0.753
Enterobacteriaceae spp. / Enterococcus spp.	8	14	6	8.6	0.398
Mycoplasma	10	17.5	11	15.7	0.814
U. urealyticum	2	3.5	4	5.7	0.69
U. parvum	7	12.3	7	10	0.779
M. hominis	2	3.5	2	2.9	1

Note: * — for *U. urealyticum*, *U. parvum*, *M. hominis* groups, threshold values are > 0, for other groups of microorganisms ≥ 10^3 GE/ml.

Patient preparation and semen sampling were carried out in accordance with WHO recommendations on the collection of semen for microbiological studies (paragraph 2.2.4 of the WHO Guidelines) [15]. Ejaculatory abstinence for the period of 3–4 days was mandatory. Prior to semen collection, patients urinated and washed their external genitalia. Semen was collected through masturbation into a sterile container. Native semen was used for the study. The analysis of semen concentration and motility was carried out using the Biola AFS-500 analyzer ("Biola"; Russia). The morphology of spermatozoa was evaluated in stained preparations with a 1000x microscope magnification using an immersion lens. The LeucoScreen test (FertiPro; Belgium) was used to count peroxidase-positive leukocytes. The normozoospermia criteria were met by samples with semen volume of at least 1.5 ml, spermatozoa concentration of at least 15 million/ml, a total mobility of at least 40% or progressive mobility of at least 32%, normal spermatozoa morphology of at least 4%, a leukocyte count of no more than 1 million/ml, and the absence of increased viscosity. Semen microbial composition was determined using a quantitative real-time PCR (qPCR) using the Androflor real-time PCR detection kit (DNA Technology; Russia) and the DTprime detection thermal cycler (DNA-Technology; Russia).

The material for the study (0.5 ml of native semen) was collected on the day of oocyte fertilization in an Eppendorf tube containing 1 ml of buffer solution (PREP-PK kit; DNA Technology; Russia). DNA was isolated using PREP-NA extraction kit ("DNA Technology"; Russia). qPCR was performed according to the manufacturer's instructions.

Once the amplification is over, the special software (DNA Technology; Russia) automatically calculates the quantities (expressed in genome equivalents per 1 ml (GE/ml)) of the total bacterial load (TBL) and each of the detected OM in a given sample. Positive signals for the target microorganisms were detected before the 35th amplification cycle, which is equivalent to the microbial load of least 10^3 GE/ml and more. The proportions of individual species and groups of bacteria were calculated relative to the sum of all bacteria detected in the sample. The Androflor real-time PCR detection kit is designed to determine TBL and the level of 19 OM groups (*Lactobacillus spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, *Corynebacterium spp.*, *Haemophilus spp.*, *Pseudomonas aeruginosa / Ralstonia spp. / Burkholderia spp.*, *Enterobacteriaceae / Enterococcus spp.*, *Gardnerella vaginalis* (*G. vaginalis*), *Eubacterium spp.*, *Sneathia spp. / Leptotrichia spp. / Fusobacterium spp.*, *Megasphaera spp. / Veillonella spp. / Dialister spp.*, *Bacteroides spp. / Porphyromonas spp. / Prevotella spp.*, *Anaerococcus spp.*, *Peptostreptococcus spp.*, *Atopobium cluster* (*A. cluster*), *Mycoplasma hominis* (*M. hominis*), *Ureaplasma urealyticum* (*U. urealyticum*), *Ureaplasma parvum* (*U. parvum*), *Candida spp.*), the presence of obligate pathogens (*Neisseria gonorrhoeae*, *Chlamydia*

Table 2. The average quantities of *Haemophilus spp*. and GNFA in groups 1 and 2 and the accuracy of these indicators in predicting a favorable outcome of the ART embryological stage

Microorganism group	Median (5th and 95th percentile)		p	The AUC value (95% CI)
	Group 1	Group 2		
Haemophilus spp., GE/ml	0 (0–0)	0 (0–103.8)	0.003	0.571 (0.472–0.671)
GNFA, GE/ml	0 (0–0)	0 (0–103.8)	0.002	0.579 (0.479–0.678)

trachomatis, *Mycoplasma genitalium* and *Trichomonas vaginalis*); and the level of human genomic DNA (as a control of taking biological material).

Methods of statistical analysis

Statistical data processing was carried out using R-version 4.2.2 (build 2022-10-31) and StatPlus:mac 8.0.3 (AnalystSoft; USA). The normality of the distribution for tested parameters was checked by the Shapiro–Wilk test. The median with 5th and 95th percentiles was indicated to describe the distribution of variables. The Mann–Whitney U test was applied to compare the medians between groups 1 and 2, whereas the two-tailed Fisher's exact test was used to compare the frequencies between the groups. All differences were considered statistically significant at $p < 0.05$.

To develop the regression equation, which formed the basis of the prognostic model for obtaining at least 40% of embryos of good and excellent quality, the method of discriminant analysis with the calculation of canonical discriminant function coefficients (CDFC) was used. ROC analysis was performed to evaluate the accuracy of the proposed predictive model. The optimal cutoff value was determined by the Youden's index.

RESULTS

Clinical characteristics of the patients

Initially, the groups were compared by age, height, weight and patient's medical history. The groups were comparable by age of men (35.0 (28.8–43.2) vs. 34.0 (28.5–45.0) years old in group 1 and 2, respectively; $p = 0.664$) and age of women (34.0 (26.0–40.0) vs. 34.0 (28.0–40.0) years old in group 1 and 2, respectively; $p = 0.507$). In terms of body mass index, the differences were not significant ($p > 0.05$). There was no significant difference in medical history (cardiovascular pathology, diseases of the urinary or respiratory tract, or nervous system, endocrinopathies, pathology of the gastrointestinal tract and autoimmune diseases) between the two groups ($p > 0.05$). Thus, the groups were clinically comparable.

Embryological stage data

The number of MII mature oocytes was 559 and 606 in group 1 and group 2, respectively. At the same time, the average number of oocytes obtained during puncture in patients of groups 1 and 2 did not differ (medians were 9 and 8, $p = 0.250$). As a result of IVF, 458 zygotes were obtained in group 1 and 442 in group 2, the average number of zygotes obtained did not differ between groups (medians were 6 and 5.5 for groups 1 and 2, respectively; $p = 0.207$). The fertilization rate, the blastulation rate and the GBDR were calculated for each patient. The fertilization rate did not differ in group 1 and group 2 patients (medians were 85.7% and 80.0%, respectively; $p = 0.471$), while significant differences were found in the blastulation rate and GBDR. The blastulation rate was 75.0% in group 1 and 37.5% in group 2 ($p < 0.001$); the GBDR was 72.7% in group 1 and 27.7% in group 2 ($p < 0.001$).

Analysis of semen microbial composition

Bacterial DNA (TBL) was absent or detected in an amount of less than 10^3 GE/ml in 5 (8.8%) of 57 samples of group 1 and 5 (7.1%) of 70 samples of group 2 ($p = 0.752$). In positive samples, from 1 to 13 groups of microorganisms were simultaneously detected in above the threshold values. The detection rate of specific groups of bacteria in above the threshold values is shown in Table 1.

Haemophilus spp. and, as a result, the group of gram-negative facultative anaerobes (GNFA) to which these bacteria belong, were detected less frequently in the group 1 samples. Detection rate of other groups of OMs did not significantly differ between groups 1 and 2. Taking into account statistically significant differences in the detection rates of Haemophilus spp. and GNFA between groups 1 and 2, we tried to assess quantitative differences for these microorganism groups and their accuracy in predicting a favorable outcome of the ART embryological stage, but received the unsatisfactory results (Table 2).

None of the individual semen microbiota parameters of demonstrated acceptable accuracy in predicting a favorable outcome of the ART embryological stage. Due to the variety of combinations in which the individual groups of OMs were found, it was decided to apply discriminant analysis to calculate a prognostic index that takes into account all significant parameters of semen microbiota.

Discriminant analysis

To predict GBDR ≥ 40% for patients whose semen parameters met the criteria of normozoospermia, we developed a prognostic index EGO-Pro-N (Embryos of GOod and Excellent quality Prognosis in Normozoospermia)

To develop a prognostic index a discriminant analysis was carried out in the obtained matrices of laboratory parameters for patients of the analyzed groups.

To determine the contribution of each individual microorganism to forming the probability of obtaining GBDR ≥ 40% and to develop a prognostic index, we ranked the parameters using the calculation of the CDFC.

The EGO-Pro-N index was calculated using the following formula:

EGO-Pro-N = 0.22 × X1 – 0.26 × X2 – 0.59 × X3 + 0.25 × X4 + 0.26 × X5 + 0.21 × X6 – 0.4 × X7 + 0.15 × X8 + 0.09 × X9 + 0.15 × X10 – 0.25 × X11 – 0.33 × X12 – 0.17 × X13 + 0.51 × X14 + 0.9 × X15 — 0.28 × X16 – 0.47, where

X1 — total bacterial load (TBL),
X2 — *Lactobacillus spp.*,
X3 — *Staphylococcus spp*.,
X4 — G. vaginalis,
X5 — Megasphaera spp. / Veillonella spp. / Dialister spp.,
X6 — *Sneathia spp*. / *Leptotrichia spp*. / *Fusobacterium spp*.,
X7 — *U. urealyticum*,
X8 — *U. parvum*,
X9 — *A. cluster*,
X10 — *Bacteroides spp*. / *Porphyromonas spp*. / *Prevotella spp*.,

X11 — *Anaerococcus spp.*,
X12 — *Peptostreptococcus spp./ Parvimonas spp*.,
X13 — *Eubacterium spp*.,
X14 — *Haemophilus spp*.,
X15 — *P. aeruginosa / Ralstonia spp. / Burkholderia spp*.,
X16 — *Enterobacteriaceae spp*. / *Enterococcus spp*.

If the EGO-Pro-N value is > 0.212, it can be predicted that the probability of GBDR ≥ 40% is low, if EGO-Pro-N ≤ 0.212, it is high.

The average value of EGO-Pro-N in groups 1 and 2 was –0.470 (–2.430; –0.580) and 0.365 (–0.933; –2.260), respectively. The differences are statistically significant ($p < 0.001$). Graphically, the EGO-Pro-N index value is shown in Fig. 1.

The ROC curve for the EGO-Pro-N index is shown in Fig. 2. The area under the curve (AUC) value is 0.777 (95% CI: 0.670-0.856), which is considered as acceptable discrimination in predicting models.

The optimal cutoff value for the EGO-Pro-N index was 0.212. This threshold made it possible to predict the production of GBDR ≥ 40% with a sensitivity of 71.9% and a specificity of 70.0% with 70.9% overall accuracy (Table 3).

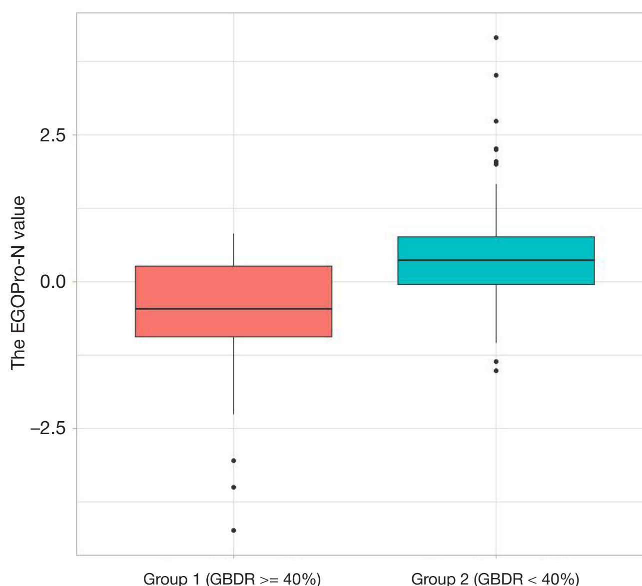

Fig. 1. The EGO-Pro-N index values in groups 1 and 2

DISCUSSION

The present study included couples who participated in ART protocols, and whose semen parameters met the criteria of normozoospermia. According to current clinical guidelines, the presence of STI pathogens was excluded in both partners at the stage of preconception care [11]. Such semen samples are considered ideal for IVF, and in routine clinical practice, an extended study of semen microbiota is not carried out. Our study showed that most of the used semen samples contained bacterial DNA in an amount of at least 10^3 GE/ml. Up to 13 groups of microorganisms in various combinations were simultaneously identified in positive samples. The presence of bacteria in semen has been shown previously in numerous studies [4, 6, 8, 10, 16], our observations once again confirm this fact.

We were interested in semen microbiota in terms of its potentially negative impact on the outcome of the ART embryological stage. In this study we were able to demonstrate the difference in semen microbiota composition between the samples with acceptable proportion of good quality blastocysts on the 5th day (GBDR ≥ 40%) and those with insufficient number of embryos. The latter group was characterized by the presence of GNFA (mainly Haemophilus spp.). At the same time, in cases where the efficacy of the embryological stage was satisfactory, there was a tendency to more frequent detection of Lactobacillus spp. and Corynebacterium spp., but the difference was not significant.

Given these observations, we made an attempt to develop prognostic model based on the semen microbiota parameters, which could be used to assess its quality even at the stage of preconception care. Attempts to predict the effectiveness of the embryological stage of ART have been carried out repeatedly using data from proteomic, transcriptomic and metabolomic analyses of semen, follicular fluid and culture media [2, 3]. According to some data, 93 types of semen proteins can be functionally associated with the formation of a zygote and the next stages of embryo development at the embryological stage of ART [16]. Potential protein markers in semen have been identified that could predict the outcome of in vitro ART in couples with idiopathic infertility [17]. The presence of Alphaproteobacteria in semen samples with normozoospermia reduced the likelihood of obtaining good and excellent quality embryos [4]. However, these observations did not lead to the development appropriate predictive models.

The developed mathematical model with the calculation of the prognostic index EGO-Pro-N can be used at the stage of preconception care before the ART procedure, especially in cases when the expected number of oocytes is low, and each embryo of good and excellent quality is of great value.

It is important to clarify that bacterial contamination of culture media with embryos was registered only in cases of oocyte fertilization using classic IVF. In ICSI programs, when a single spermatozoon is injected into an oocyte using micro-tools, such cases have not been registered [18]. This is probably due to the fact that in the case of ICSI, fewer viable bacteria may be introduced into the culture medium. Thus, in order to increase the effectiveness of the IVF program, in case of a negative prognosis for obtaining a sufficient number of good-quality embryos, ICSI may be recommended.

CONCLUSIONS

The developed prognostic model makes it possible to predict the acceptable GBDR based on semen microbiota investigation in semen samples used for IVF. The semen microbiota evaluation in patients planning to undergo the ART procedure

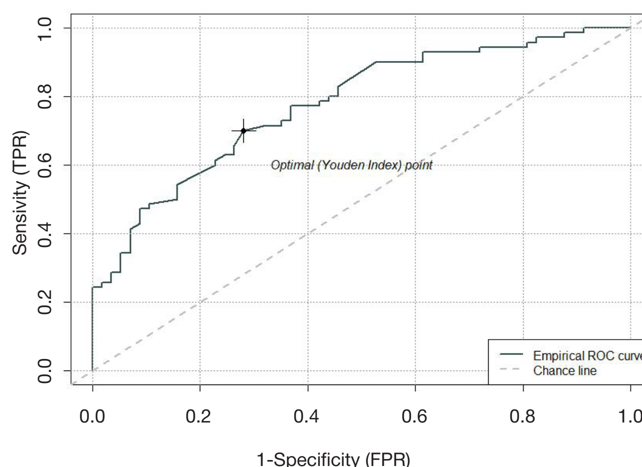

Fig. 2. ROC curve for the EGO-Pro-N index

Table 3. Sensitivity and specificity of the EGO-Pro-N index (classification matrix)

Group/ Prognosis	1	2	Total	The number of correct prognoses, %
1	41	16	57	71.9
2	21	49	70	70
Total	62	65	127	70.9

can be performed at the stage of preconception care, including the cases when the semen parameters meet the criteria of normozoospermia. The use of the proposed EGO-Pro-N index justifies the development of an individual treatment plan for the ART patients to obtain the optimal number of high-quality embryos. The proposed method can be used in routine clinical practice, it does not require significant material costs and organizational efforts.

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