PROSPERITAS VESTRA FINIS NOSTRA!

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Human Oocytes in Assisted Reproduction - Systematization of Factors Affecting Quality, Development of a Methodology for Assessment and Analysis of Fertilization Enhancement Techniques

Extended abstract of a PhD thesis

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I. INTRODUCTION

In 2022, the world welcomed the birth of the 10 millionth baby conceived in laboratory conditions through the methods of assisted reproduction. Just a few years earlier, in 2018, a very important round anniversary was celebrated - 40 years since the birth of the first in vitro baby Louise Brown in 1978. For more than 4 decades, babies born with the help of assisted reproductive technologies have been increasing rapidly every year, and in some countries they are already more than 5% of the total number of children born annually. At the meeting of ESHRE (European Association of Human Reproduction and Embryology) in 2023 in Copenhagen, data were presented that worldwide more than 2 million in vitro procedures are performed annually, from which more than half a million children are born.

Much has changed since Edwards and Steptoe first successfully aspirated an oocyte, fertilized it in the laboratory and transferred it back into the uterus 40 years ago. Nowadays, embryology is one of the most modern and fastest-growing disciplines, and modern embryology laboratories are equipped with high-tech equipment. Dozens of scientific forums on both general and specialized topics are held every year, where the latest discoveries in the various areas of embryology - andrology, cryobiology, genetic diagnostics and engineering, as well as the latest developments of the companies producing laboratory products are presented equipment, food environments and specialized equipment.

Thanks to the rapid development of reproductive assisted technologies, in the last decade the individual approach has become established as a method of treatment for couples with infertility. Personalized medicine involves applying a variety of treatment methods and techniques depending on the specific cause of infertility. The widest field for the development and application of these additional methods is found in the embryological part of the in vitro cycle, where they are applied to spermatozoa, oocytes and embryos in order to improve their quality, fertility and implantation potential.

Various methods are applied to spermatozoa, which aim at selecting those with the most optimal morphology, the lowest percentage of fragmented DNA and the best motility

For embryos, the options for additional impact are the most diverse. Numerous nutrient media, specialized monitoring and handling equipment, genetic analysis tests, and a variety of other methodologies have been developed to improve their quality and high chance of implantation.

With oocytes, the possibilities are much more limited, mainly due to the highly limited number of gametes that can be obtained in one stimulation cycle. It is the increasing average age of women seeking treatment through assisted reproduction that emerges as the main limiting factor and cause of stagnation and plateau in the percentage of achieved successful pregnancies and live births in recent years.

This leads numerous clinical and scientific teams from around the world to focus their attention on methods for more precise analysis of human oocytes, development of techniques to improve their quality, implementation of a number of discoveries and innovations of modern medicine such as stem cell manipulations, PRP (pellet-rich plasma), mitochondrial transfer, growth factors, work with artificial intelligence.

Some of the applied methods have been repeatedly proven, confirmed and are already routinely introduced into clinical practice, another part is still in the process of clinical studies and every year their number is increasing.

The search for a way to better quality gametes for reproduction is a challenge that will be the main current focus in the coming years of the development of modern reproductive medicine and embryology.

II. AIM AND TASKS OF THE THESIS

II.1 AIM

THE AIM of the study is to make a comprehensive analysis of the accuracy of the factors affecting the quality and fertilization potential of human oocytes; to derive a graphical visualization, to propose an optimal system for non-invasive morpho-physiological evaluation of female gametes with a high predictive value for the subsequent fertilization, development of the resulting embryos and implantation and to investigate additional techniques to improve the fertilization potential.

II.2 TASKS

1. To investigate the influence of patient-specific factors on the number, maturity, fertilization and implantation potential of oocytes: age, body mass index (BMI), smoking, serum levels of follicle-stimulating hormone (FSH).

2. To investigate the influence of certain diseases - pelvic inflammatory diseases, endometriosis on the number, maturity, fertilization and implantation potential of the oocytes.

3. To investigate the influence of some laboratory techniques - intracytoplasmic sperm injection (ICSI) and cryopreservation on the number, maturity, fertilization and implantation potential of the oocytes.

4. To propose a systematization of the factors affecting oocyte quality, tabulated based on the analysis of literature data and to propose a new addition to the classification for higher precision.

5. To propose a new approach for the systematization of factors affecting oocyte quality, generated through directed work with artificial intelligence.

6. To propose a new oocyte evaluation system including both morphological features and functional manifestations during fertilization with high informative and predictive value for fertilization, quality of embryos obtained and the final outcome of assisted reproductive technologies (ART).

7. To analyze the effect of applying additional laboratory methods (assisted oocyte activation and polarization microscopy) on fertilization results and the percentage of clinical pregnancies achieved.

III. MATERIALS AND METHODS

III. 1. CLINICAL MATERIAL

III.1. 1. General characteristics of the working material

Clinical contingent

To fulfill the set tasks and achieve the set goal, 9,816 patients were included in the study, who underwent a cycle of infertility treatment with their own oocytes through controlled ovarian hyperstimulation for the purposes of assisted reproduction and reached a follicular puncture, as a result of which a total of 67,187 oocytes were collected , subject of the present study.

Location of the survey

The study was conducted in the in vitro department and the embryological laboratory of SAGBAL "Dr. Shterev", Sofia, in close cooperation with a clinical laboratory.

Study period

The study was conducted within a nine-year period and included patients who underwent ART treatment through SAGBAL "Dr. Shterev" for the period from January 2013. until December 2021 The analysis is retrospective in nature, with the data extracted using hospital computer software - JOYSTICK healthcare management system ("Smart Software Systems" - certified with ISO/IEC 27001:2017 and ISO 9001:2015). From January 2017 until December 2020 a prospective evaluation and follow-up of oocytes undergoing the ICSI procedure was performed in order to propose an optimal morpho-physiological evaluation system.

Unit of study

The study included all oocytes obtained from patients with an indication for assisted reproductive treatment and who underwent controlled ovarian hyperstimulation (COH), minimal stimulation or natural cycle follicle puncture by ovulation timing.

Depending on the factors that are investigated and evaluated, the patients and their oocytes included in the present study are divided into several groups, and specific tasks are solved for each of them.

III. 1.2. Basic groups of the working material

Factors affecting oocyte quality

Regarding the factors affecting the quality of the oocytes and analysis of their accuracy, for each individual factor the patients were divided into several groups, in which the average number of retrieved oocytes after follicular puncture, the average number of mature oocytes, the percentage of fertilization, the percentage of canceled cycles and number of clinical pregnancies.

Inclusion criteria:

- Can be evaluated numerically
- There are literary data about their influence
- They can be divided into different categories
- Can be compared with a control group
- They have established reference values
- The literature on their influence is contradictory or subject to debate
- During the period of conducting the experiment, they are a constant value
- The results can be compared both among themselves in the different subcategories and with a control group
- There is a sufficient number of cases to make a representative study
- Data can be extrapolated from electronic medical records by setting the desired parameters
- All patients who underwent follicular puncture resulting in the collection of between 0 and 46 cumulus-oocyte complexes
- Use of own oocytes to trace the correlation between the factor and oocyte quality
- Use of donor sperm for fertilization purposes
- Use of partner spermatozoa with normal sample parameters (normozoospermia) or mild to moderate deviations from the norm (mild to moderate asthenozoospermia, mild to moderate oligozoospermia) and morphology above 3% according to Kruger.
- Signed informed consent for the processing of personal data by patients
- Signed informed consent for the relevant manipulation by the patients

Exclusion criteria:

- Patients in whom hormonal stimulation has stopped, due to poor ovarian response, lack of follicular growth, thin uterine lining, etc.

- Patients in whom spontaneous rupture of the follicles occurred immediately before performing the follicular puncture

- Women, oocyte donors

- Severe abnormalities of the sperm sample on the day of follicular puncture (severe oligoasthenozoospermia, necrospermia, cryptozoospermia) and morphology below 3% according to Kruger

- Spermatozoa obtained from the testicles surgically

- Unsigned informed consent

Age

In order to confirm or reject the leading role of the age factor, we analyzed the medical records of 9816 patients who underwent an assisted reproduction procedure and reached a follicular puncture. According to the age factor, they are divided into 4 groups, defined by the European IVF monitoring of the European Association of Human Reproduction and Embryology - group 1: under 30 years; group 2: 31-34 years; 35-39 years; group 4: over 40 years (Fig. 1A).

Body Mass Index (BMI)

In order to determine whether there is a relationship between BMI and the number of retrieved oocytes and fertilization, we divided 4882 women into 4 main groups, defined according to WHO criteria, 1995. The groups are: group 1: BMI up to 18.49; group 2: from 18.5 to 24.99; group 3: from 25 to 29.99 and group 4: over 30 BMI. For this purpose, we calculated BMI for each patient for whom age and weight data were available using the formula: $BMI=KG/M^2$ (Fig. 1 B).

Smoking

We studied the results of 989 patients, in whom anamnestic data were taken whether they smoked or not, to consider whether there is an impact on the number and quality of oocytes in Bulgarian women aiming for pregnancy and using nicotine products during the treatment process. Depending on this, they are divided into 2 groups: group 1: smokers; group 2: non-smokers (Fig. 1 C).

Basal FSH levels

In order to determine whether high serum levels of follicle-stimulating hormone, measured in the early follicular phase, significantly affect oocyte quality, data were extracted from 411 patients who had their FSH levels measured by a blood laboratory test between days 3 and 5 of menstrual cycle before proceeding with an in vitro procedure. According to most literature sources analyzing markers of ovarian reserve depletion, 3 groups are distinguished depending on the hormone values: group 1: up to 8.5 IU/l, group 2: from 8.51 to 11.99 IU/l, group 3: over 12 IU /l (Fig. 1 D).

Impact of endometriosis

In order to study the relationship between the pathophysiological mechanism of the disease and the number and quality of oocytes, we examined the results of patients diagnosed with endometriosis who sought treatment by assisted reproduction in the clinic. Data were collected on 472 women diagnosed with endometriosis (Fig. 1E) as the leading factor for infertility in the couple. For a control group, the results of 573 patient couples with a male factor were compared.

Influence of the tubal factor

We analyzed the results of 2,201 patients treated by an in vitro procedure with a diagnosis of tubal sterility to account for how different inflammatory and adhesion processes affect oocytes and their quality. As a control group, data were compared with those of male factor patients (Fig. 1F).

Impact of the ICSI procedure

In order to definitively reject the importance of the ICSI procedure as a factor that had a strong influence in the initial years after its introduction, we calculated the fertilization results in 2897 cycles. In 666 of them, a classical IVF procedure was applied, and in 2231 - ICSI manipulation (Fig. 1G).

Effect of cryopreservation

In order to assess whether this factor still has clinical significance in Bulgarian patients, we summarized the data for a total of 213 cycles - 1154 oocytes. Fresh oocytes were used in 162 of the cycles and thawed in 62 (Fig. 1H).

Factor management and graphical representation

In order to present the most complete and clear summary picture of the factors that have a bearing on oocyte quality, we performed an integrative analysis of the scientific literature, as well as with artificial intelligence (AI) with subsequent extrapolation of the data in a user-friendly tabular form.

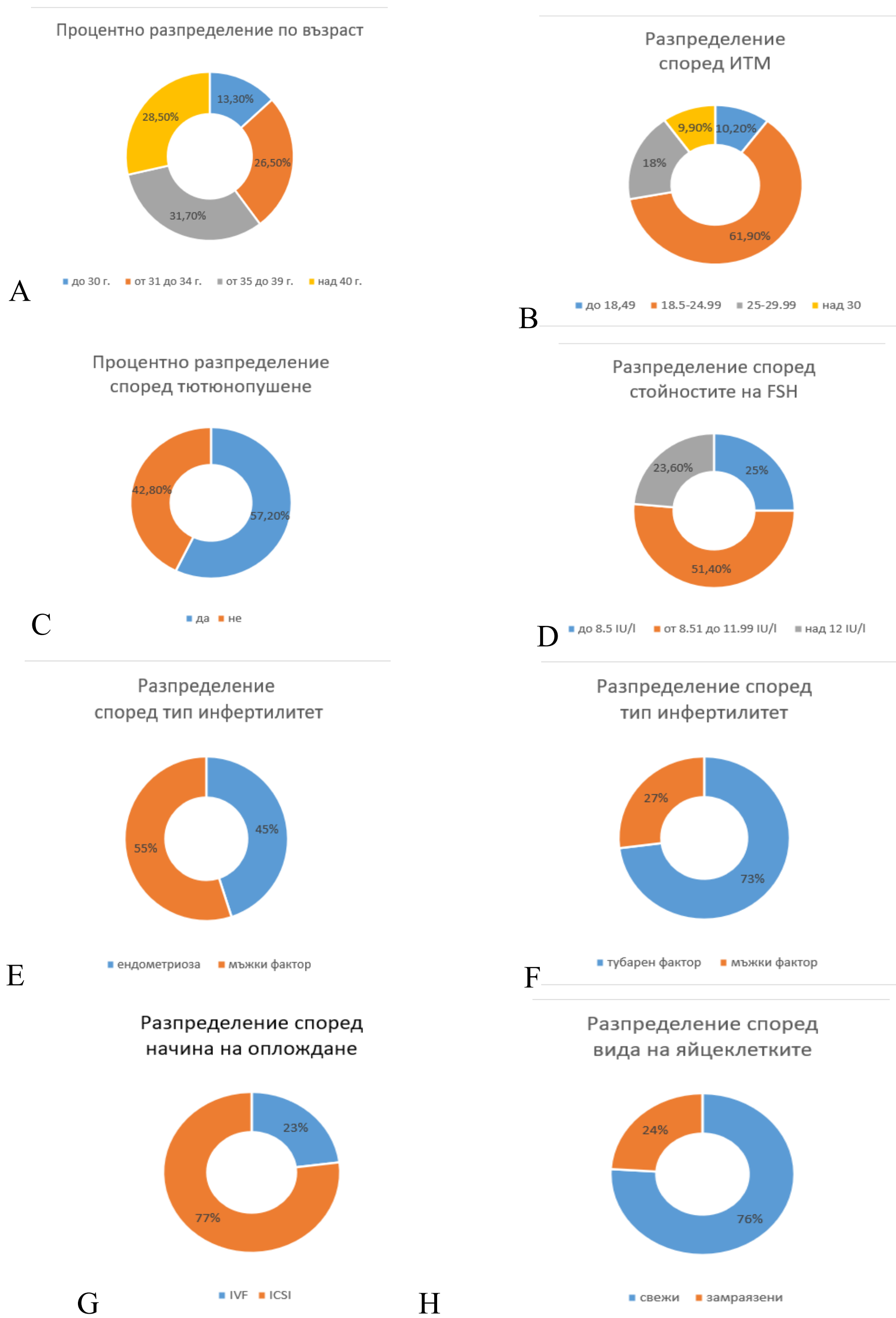


Fig. 1. Distribution of groups depending on the studied factors

Assessment of oocyte quality

According to the ESHRE Good Laboratory Practice recommendations, when performing an ICSI fertilization procedure, it is necessary to accurately document the quality of the oocytes. However, in the day-to-day work of embryologists, this is not a standard procedure and is not routinely applied. There is also no popular and easily applicable oocyte scoring system. In order to propose an optimal oocyte evaluation system based not only on their morphological features but also on their functional characteristics, we analyzed data from the procedures of 468 pairs of patients. Over a 4-year period (January 2017 - December 2020), a total of 4127 oocytes were prospectively tracked and graded. Only mature, metaphase II oocytes (MII) amenable to fertilization by ICSI were evaluated.

Techniques increasing the fertilizing potential of oocytes

Assisted oocyte activation

In order to determine the effect of applying calcium activation in Bulgarian patients with previous unsuccessful attempts related to problems in fertilization, the data of 337 mature MII oocytes were tracked within 74 ICSI cycles.

In order to compare the results, the cycles were divided into 2 main groups:

Group 1 – 30 cycles in which Ca2+ ionophore was used as an additional technique to improve fertilization results.

Group 2 - 44 cycles with low or absent fertilization with no applied calcium activation - control group.

Spindle view (SV)

In order to determine whether the visualization of the division spindle using polarization microscopy can help improve the results, we followed the application of this technique in a selected group of patients with indications for its implementation. As a control group, we used women meeting the same criteria in whom this additional technique was not applied.

Group 1 – 24 patients aged ≥ 40 years with between 1 and 3 mature oocytes retrieved, undergoing ICSI procedure and spindle view applied

Group 2 – 72 patients aged ≥ 40 years with between 1 and 3 mature oocytes retrieved, undergoing ICSI procedure without spindle view

III.2. METHODS

III.2.1. Methods of primary selection of patients:

Anamnestic methods

On their first visit to the clinic, patients fill out an information card containing basic information about the patient, which is then entered into the electronic hospital system JOYSTICK healthcare management system ("Smart Software Systems" - ISO/IEC 27001:2017 and ISO 9001:2015) and the patient's electronic medical record is created. All subsequent tests, procedures and interventions are documented in the electronic file.

Diagnostic methods

Based on the information obtained from the initial consultation, additional diagnostic tests are prescribed to clarify the causes of infertility:

Imaging methods

Methods for establishing tubal patency

Laboratory methods

In a clinical laboratory (MDL "Cibalab" EOOD) through blood sampling, hormones related to reproductive function are examined - basal serum levels of FSH [mIU/ml], LH [mIU/ml], Prol [ng/ml], E2 [pg/ml], AMX [ng/ml], Inhibin B [pg/ml], TSH [mIU/l].

Methods of semen analysis:

In the case of the male of the couple, a simple spermogram is done to test the male factor. It is performed in two main ways - manually by microscopy (Nikon Eclipse Ei R, Nikon Microscope Solutions, Japan) or by an automatic computer analysis system (SCA® CASA system, Microptic, Hamilton Thorn, USA). The conclusion is given according to WHO criteria, 1999.

III.2.2. Hormonal stimulation methods for ART purposes

When starting a treatment cycle, ovarian stimulation of the woman's ovaries is performed.

Methods of conducting KOH

Stimulation of the ovarian cycle involves the administration of oral and/or injectable medications according to established treatment protocols in order to induce superovulation.

Manipulation methods

Follicular puncture (FP) was performed under ultrasound guidance by attaching a metal guide with a 16-18 G aspiration needle, 330 mm long (COOK Medical, USA; Wallace, Cooper Surgical, Dennmark) to the vaginal transducer of the ultrasound machine for aspiration. of the follicular contents.

III.2.3. Methods of in vitro fertilization of gametes

Evaluation and manipulation of gametes begins with the admission of follicular aspirates to the embryology laboratory for analysis of the contents.

Methods for evaluation and selection of cumulus-oocyte complexes.

In a laminar box (IVFTech Sterile 180, Denmark) under a constant flow of sterile air, the aspirated follicular fluid was transferred to petri dishes (Nunc™ Cell Culture/Petri Dishes, Thermo Fisher Scientific, USA) and under a stereo microscope (Nikon SMZ1270, Japan) were identified cumulus-oocyte complexes (COCs). These SOCs containing vitellus were washed several times in bicarbonate buffered medium (G-IVF PLUS, Vitrolife, Sweden) and placed for incubation in a CO2-incubator with a dual gas mixture (SANYO MCO-5AC, PHC group, Japan).

Methods for evaluation and selection of mature oocytes

After 3-5 hours of incubation, SOCs that will be subjected to ICSI procedure are denuded using nutrient medium containing hyaluronidase (Hyase 10X, Vitrolife, Sweden). Only the mature oocytes of the MII stage are prepared for fertilization.

Methods of fertilization of oocytes

Fertilization is carried out by 2 main methods - with the so-called In classical in vitro fertilization (IVF), pretreated and isolated spermatozoa are added to the oocytes without removing their cumulus masses, while in ICSI fertilization all mature oocytes undergo intracytoplasmic injection with a single spermatozoon. The injection was performed on an inverted microscope (Zeiss Observer a1 ax10, Germany) equipped with a micromanipulator (Eppendorff Transfer Man4, Germany).

III.2.4. Own methods for morpho-physiological assessment

In order to propose a new, optimal and informative system for the two-factor evaluation of oocytes, both their morphological characteristics and their physiological (functional) manifestations were evaluated during the injection procedure. The new binary scoring system is called OVOSCORE.

Morphological evaluation methods

Morphological evaluation was performed by microscopic observation. The main morphological features followed are those reported to be relevant to the genetic constitution of the oocyte and the implantation potential of the embryo it generates.

Functional assessment methods

During the oocyte injection procedure, the functional characteristics of the oocyte were documented.

Scoring system

Oocytes received two separate evaluations, based on their morphological characteristics and their functional characteristics during the ICSI procedure.

Assessment by morphological markers:

• Grade A: oocytes without visible morphological defects

• Grade B: oocytes with 1 visible morphological defect

• Grade C: ova with 2 or more simultaneously present defects

Assessment of functional parameters:

• Grade 1: intussusception and resistance to pipette penetration; normal rupture of the ovule; viscous cytoplasm with a large elongate "funnel" (1/4 to 1/6 of oocyte volume)

• Grade 2: intussusception; low resistance; easy tearing of the ovule and viscous cytoplasm with a small short-lived funnel

• Grade3: no intussusception; no resistance; sudden rupture of the ovule; no viscosity; no injection funnel

III.2.5. Methods to increase the fertilization rate

Calcium activation

In order to achieve higher fertilization rates, we used, in addition to standard laboratory procedures, ready-to-use medium GM508 CultActive of GYNEMED, Germany according to the manufacturer's protocol.

Visualization of the meiotic spindle

For this purpose, we used specialized equipment equipped with software - a poloscope (Oosight™, Hamilton Thorn, USA), with which it was possible to observe (in the cases in which it was formed) the position of the meiotic spindle.

III.2.6. Methods of factor analysis and management

Analysis of literary sources

Five databases and reference lists (Medline, PubMed, Scopus, Cochrane Library, Scholar) were searched for relevant studies published between 2000 and 2023. Two independent researchers performed an integrative review of the literature sources and selected 115 of them as the most relevant. relevant to the topic.

Methods of systematization through artificial intelligence

In order to present the factors that are the subject of the present study in an informative and systematized form, we used the product Chat Generative Pre-trained Transformer (ChatGPT) of the company OpenAI, L.P. with its premium version GPT-4 from March 2023. created with a high medical focus.

III.2.7. Statistical methods

Primary data selection

Statistical data processing

IV. RESULTS AND DISCUSSION

After the analysis of the clinical material and application of the methods, including statistical ones, we formed the obtained results in graphical and tabular form and subjected to analysis. The studies are systematized in 4 main sections:

1. Analysis of the influence of factors related to the lifestyle of the patients (age, BMI, smoking and FSH values), some diseases (endometriosis and pelvic inflammatory diseases) and laboratory procedures (ICSI, cryopreservation) on the number, maturity and the fertilizing potential of the harvested oocytes and the resulting results such as clinical pregnancies obtained.

2. Analysis, systematization and proposal for management of the factors influencing the quality of oocytes by means of transformation of the information in tabular form, as well as presentation of a new classification generated on the basis of directed work with artificial intelligence.

3. Proposal for a new OVOSCORE oocyte evaluation system, with high informative and predictive value, based on morpho-physiological characteristics and analysis of results and its effectiveness in terms of fertilization, embryo quality and implantation.

4. Analysis of the effectiveness of additional laboratory procedures to increase the chance of oocyte fertilization after applying the methods of calcium ionophore activation and visualization of the meiotic spindle using a poloscope.

IV.1. Analysis of the influence of factors related to the lifestyle of the patients (age, BMI, smoking and FSH values), certain diseases (endometriosis and pelvic inflammatory diseases) and laboratory procedures (ICSI, cryopreservation) on the number, maturity and fertility potential of harvested oocytes and resulting results such as clinical pregnancies obtained

For the analysis and evaluation of the factors included in the present study, the results of 9,816 IVF/ICSI cycles performed over a 7-year period at SAGBAL "Dr. Shterev" since January 2013, when specialized hospital software with embryological section until December 2019. Due to the fact that between 1000 and 1500 cycles of ART treatment are performed annually in the IVF department and a great deal of data is accurately filled in pre-created sample forms, it was possible to select and extract a number of parameters and analyze their correlation for number patients with a high representative value and good homogeneity of the studied subgroups.

For the purpose of the study, we evaluated the influence of each factor by itself and the results of its influence on the average number of retrieved oocytes, the average number of mature oocytes that can undergo fertilization, the fertilization index and the percentage of pregnancies achieved to assess the power of its prognostic value.

Age

In order to investigate this factor in Bulgarian patients, we distinguished 4 age groups according to the criteria adopted by the European IVF Monitoring to ESHRE. From the distribution of the number of cycles in the different age groups, it is established that the largest share of patients is in the age group 35-39 years (n= 3107), followed by that of women over 40 years old (n= 2800). Younger patients aged 31-34 years were fewer in number (n= 2114) and the youngest were 18 to 30 years old (n= 1795).

After performing follicular puncture under US control, a total of 67,187 cumulus-oocyte complexes were obtained from these 9,816 attempts. On the table 1 shows the distribution of all oocytes in the different age categories:

Table 1. Basic statistical indicators of the total number of oocytes by age group

Total NO oocytes					
age	**n**	**min.**	**max.**	**average**	**SE**
< 30	18871	0	42	10,513	0,149
31 - 34	17930	0	45	8,482	0,127
35 - 39	19168	0	43	6,169	0,0911
➢ 40	11218	0	34	4,006	0,0739

From the statistical analysis done by the Kruskal-Wallis test, it was found that there was a clear statistical difference between the studied groups ($p< 0.05$). There is a clear tendency to decrease the number of retrieved oocytes with increasing age (Fig. 2). The study shows that there is an inverse correlation between the parameters age and average number of retrieved oocytes after FP. With increasing years in each age group, a decrease in the number of oocytes is observed, which is also reflected in fig. 3, showing the number of patients in the different age groups and the data on the average number of oocytes in the respective groups:

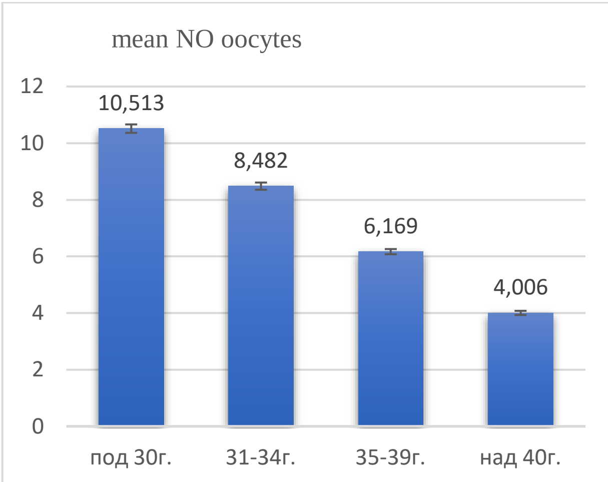


Fig. 2. Ratio between the average number of retrieved oocytes in the different age groups

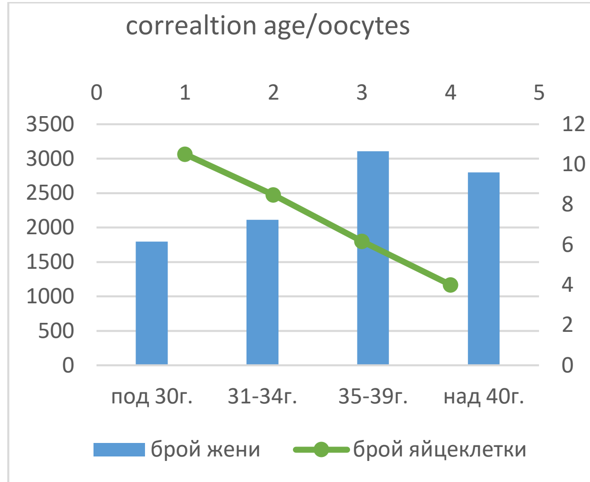


Fig. 3. Number of women in age groups and the average number of oocytes retrieved in each group

Multiple comparisons using Dunn's method showed differences between all pairs of age groups ($p<0.05$).

When analyzing the cycles in which there are no oocytes suitable for fertilization, significant differences are again found in the individual age groups. (Table 2).

From the numerical comparison between the groups, a clear trend is revealed again - in patients over 40 years old, the risk of the entire experience ending without reaching a gamete fertilization procedure due to a lack of suitable oocytes is over 18%. In young women in the age groups up to 30 years and 31-34 years, it varies between 4 and 6% on average due to the presence of women with poor ovarian response, endometriosis and/or premature ovarian failure (PCA) in these groups.

In fig. 4 shows the number of mature oocytes in the different age groups:

Table 2. Percentage of cycles in which there are no oocytes and/or mature oocytes

age	< 30г.	31-34г.	35-39г.	➢ 40г.
no oocytes (%)	1,28	1,7	4,35	8,2
no NM (%)	2,42	4,13	6,56	10
Total % cyles	**3,7**	**5,83**	**10,91**	**18,2**

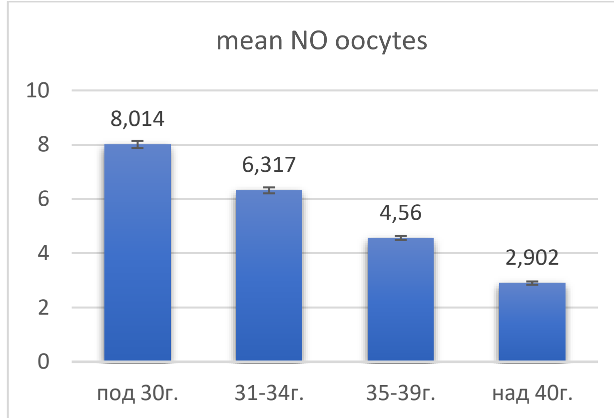


Fig. 4. Average number of MII oocytes in the different age groups

The graph demonstrates a trend for a decreasing number of fertilized oocytes as the age of the treated women increases, maintaining an inverse correlation between age and number of oocytes. Regarding the percentage ratio of mature oocytes to the total number, a significant difference ($p<0.05$) was again observed in the groups, expressed when comparing the group of 40+ year-old patients with the other age groups.

From the comparative analysis, we found that not only the average number of mature oocytes has a decreasing trend with increasing age, but also their proportion directly to the total number of retrieved oocytes is also lower in older women.

Regarding fertilization, the following results were registered: group up to 30 years. - 75% fertilization, group 31-34 years - 74%, group 35-35 years - 74% and group over 40 years - 73%. By means of Pearson's chi-squared test, reliable differences were established in the group of women over 40 years of age. A strong significant difference was reported in the mean percentage of fertilized oocytes between the group of women over 40 compared to those under 30 ($p < 0.0001$) and borderline compared to the other 2 groups: ($p = 0.0595$) for the age group 31-34 and ($p = 0.0563$) for the 35-39 age group, respectively.

When tracking the cycles until the final outcome of the treatment in relation to the age factor, we reached the following results, demonstrated in the table. 3:

Table 3. Basic data on the outcome of the treatment

Age group	под 30г.	31-34г.	35-39г.	над 40г.
Cancelled after PF				
number	174	308	675	913
%	10%	15%	22%	33%
Embryo transfer				

number	946	1210	1892	1618
%	53%	57%	61%	58%
Embryo transfer and freeze				
number	349	319	288	100
%	19%	15%	9%	3%
freeze				
number	326	277	252	169
%	18%	13%	8%	6%

The data show that a significantly higher percentage of attempts made by women over 40 years of age do not reach embryo transfer (ET) and/or freezing. The difference was significant compared to all other groups ($p < 0.001$). Also, the percentage of embryos suitable for freezing is significantly lower and shows an inverse correlation. The relative proportion of women with a recent transfer was comparable in the four groups.

When comparing the results of achieved clinical pregnancies, statistically different are also observed (Fig. 5).

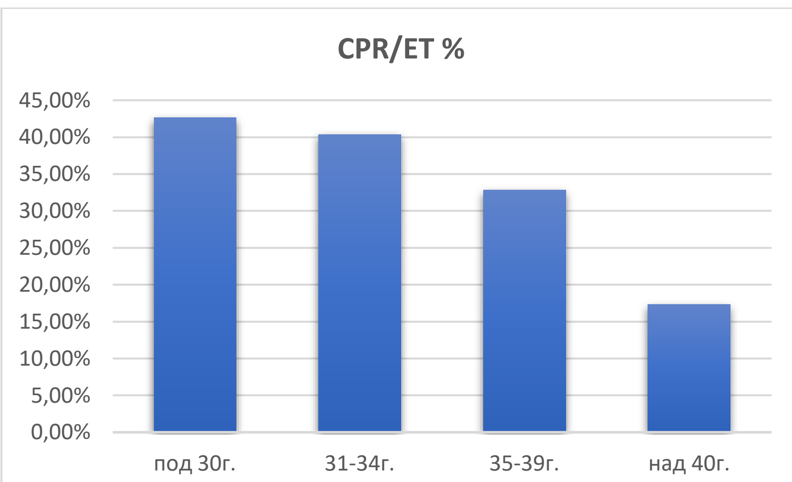


Fig. 5. Clinical pregnancies versus embryo transfer

The demonstrated data show comparable treatment results in women up to 35 years of age. In the 35-39 age group, however, a significantly lower percentage of achieved pregnancies was observed ($p < 0.001$) compared to the first 2 groups. In the group of women over 40, pregnancies were statistically significantly different compared to each of the other groups ($p < 0.001$).

Discussion:

The results generated after processing the data for over 67 thousand oocytes from Bulgarian patients correlate with the numerous literature reports connecting the age factor with the decreasing production of oocytes. The analysis of the number and percentage distribution of mature oocytes also confirms this hypothesis – the older the partner of a treated couple, the less fertile oocytes are obtained after hormonal stimulation. The age distribution also showed a relation to the fertilization rate, decreasing after 40 years of age.

The results of achieved clinical pregnancies confirm the globally accepted conditional cut-off for the age at which human oocytes begin a noticeable deterioration in quality and, respectively, the chances of pregnancy occurring - 35 years. In patients who seek treatment for infertility after the age of 40, the chance of achieving embryo implantation is even more reduced and decreases sharply with each passing year after 40.

Age as the main leading factor affecting oocyte quality is confirmed by the present study.

BMI

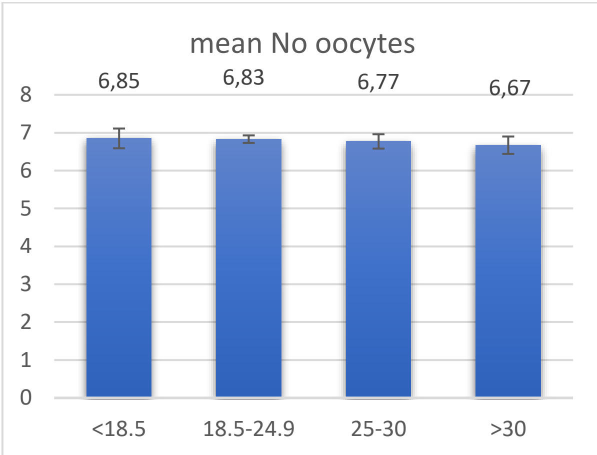
In order to investigate the specific effect on the oocytes, their quality and fertilization potential, we analyzed the results of a total of 4,882 women from partner couples who underwent ART treatment, for whom height and weight data were entered at the start of the in vitro fertilization procedure. The patients were distributed as follows: in group 1 (BMI below 18.5) – 498 women, group 2 (from 18.5 to 24.9) – 3021 women, group 3 (25 - 29.9) – 789 women and group 4 (over 30) – 484 women.

A total of 33,205 oocytes were obtained after the KOH and FP (Table 4).

Table 4. Basic statistical data on harvested oocytes in different BMI categories

Total No oocytes					
BMI	n	min.	max	average	SE
<18.5	3406	0	43	6,85	0,26
18.5-24.9	20620	0	45	6,83	0,1
25-30	5950	0	36	6,77	0,19
>30	3229	0	28	6,67	0,23

The non-parametric Kruskal-Wallis test showed no statistical differences in the mean number of oocytes retrieved between the different pairs of groups (Fig. 6). Regarding the mean number of MII oocytes suitable for fertilization, multiple comparison analysis with Dunn's test also found no significant differences. Analyzing the data on the percentage ratio of mature oocytes to the total population of harvested oocytes, a tendency to decrease their proportions was observed in the group with obesity (Fig. 7), but no statistically significant difference was found.



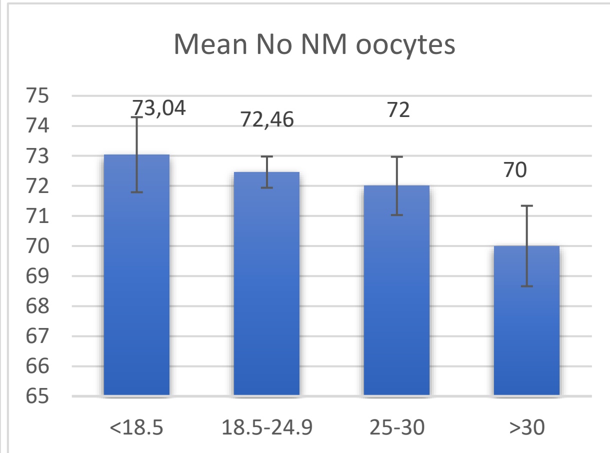


Fig. 6. Ratio between the average number of retrieved oocytes in the different age groups

Fig. 7. Proportion of retrieved MII oocytes relative to the total number of retrieved oocytes

When comparing the cancellation rate (CR) after follicular puncture - the percentage of cycles in which no oocyte was retrieved or no mature oocyte was retrieved varied between 9-11% in the different groups and no significant difference was found between the groups .

The percentage of fertilization is also comparable in the individual BMI categories and is in the values of 74-75%.

The analysis of the final results of the in vitro cycle showed a similar distribution in the percentages of the exit of the cycles in the different groups. When analyzing the frequency of pregnancies achieved (Fig. 8), significant differences were found in some of the groups:

In the group of underweight patients, positive results were found in 32.37% of them, resulting in 109 clinical pregnancies. The highest values were registered in the normal weight group - 745 pregnancies and 34.38% KB/ET. In overweight women, 183 positive blood tests and 29.60% successful cycles were reported. The lowest number was observed in the obese group – 28.8% implantations out of 344 embryo transfers.

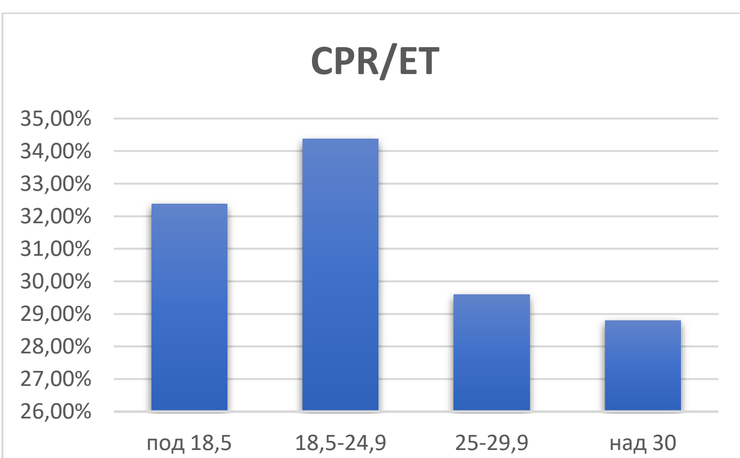


Fig. 8. Clinical pregnancies versus embryo transfer

In a comparative analysis between individual groups, no statistical difference was reported between the group of women with underweight (group 1) and normal weight (group 2) ($p = 0.4714$). The percentage of clinical pregnancies was significantly lower between group 2 and the overweight group (group 3) ($p = 0.0256$), as well as between group 2 and the obese group (group 4) ($p = 0.0412$). Although multiple comparison by Dunn's test did not find a statistical difference between groups 3 and 4, a clear inverse correlation was observed between BMI values and percentage of successful implantations.

Discussion:

The indicators that directly indicate the quality and fertilization potential of the oocytes - average number, proportion of MII, fertilization index, do not show a direct correlation dependence on the BMI values of the patients. The only significant differences that were found in the present study were in the results of successful pregnancies, supported by earlier studies by our team. The conclusions drawn by the present study are that increased levels of adipose tissue do not directly affect oocyte quality and the reason for the lower rates of pregnancy achieved in overweight women should be sought in the compromised receptivity of the endometrial mucosa.

Smoking

The study included 557 women who noted in their information card that they were smokers. 416 non-smoking women in similar age ranges were selected as a control group.

The results when counting the number of harvested oocytes are presented in table 5:

Table 5. Basic statistics on the average number of oocytes retrieved

Total NO oocytes					
smoking	**n**	**min**	**max**	**average**	**SE**
yes	3174	0	27	5,7	0,19
no	2272	0	30	5,48	0,23

From the Mann-Whitney statistical test, no statistical differences were found between the two cohorts in the mean number of oocytes retrieved (Fig. 9).

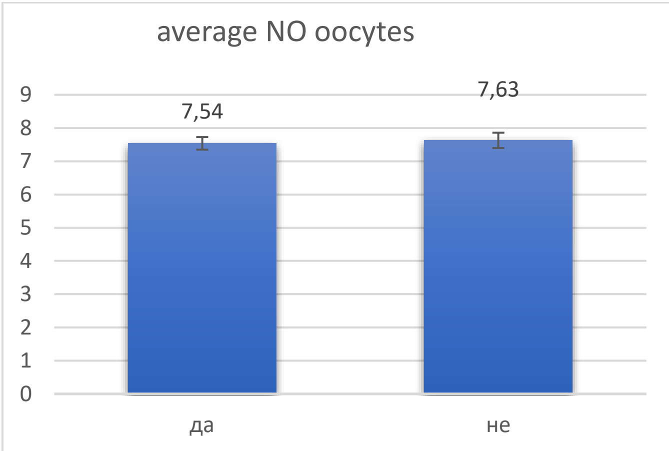


Fig. 9. Ratio between the average number of retrieved oocytes in the two groups

Regarding the mean number of mature MII oocytes, no statistically significant differences were also found. The data from the two compared groups are homogeneous and comparable in this indicator, as well as in relation to the average percentage of mature oocytes from the total cohort. Fertilization results also showed similar characteristics – 75% in both groups.

The analysis of the final results of the treatment cycle demonstrated homogeneity in both groups. Similar are the results of the achieved clinical pregnancies demonstrated in fig. 10:

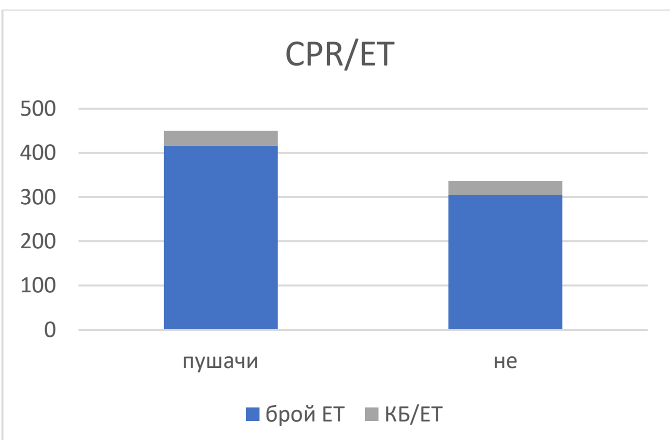


Fig. 10. Clinical pregnancies versus embryo transfer

No statistical difference was found in boron and the percentage of successful pregnancies achieved.

Discussion:

The present study found no significant differences in the oocytes of female smokers compared with a control group. No differences were detected regarding the mean values of the number of retrieved oocytes, as well as the number of fertile oocytes. Their fertilization data as well as the final results of the ART procedure also demonstrated similarity. There was also no difference in the proportions of women who became pregnant, regardless of whether they smoked or not.

The findings of this study correspond with a recently published study from the University of Cambridge, in which the research team found an effect on some morphological characteristics of oocytes in the tobacco product group, but did not report significant differences in ovarian response, fertilization and implantation outcomes. However, the authors emphasize that it is important that patients trying to achieve pregnancy be counseled and informed about the many negative effects of smoking on health and potentially on the developing fetus.

FSH

In this study, we analyzed the results of 411 patients for whom there were data on the basal serum FSH levels at the start of hormonal stimulation for the purposes of ART, divided into 3 groups depending on the specific values: group 1: (below 8.5 IU/ l) n= 103, group 2: (from 8.51 to 1.99 IU/l) n= 211, group 3: (above 12 IU/l) n= 97.

When considering the average number of harvested oocytes after COX, the results demonstrated in the table were obtained. 6. Statistical analysis using the Kruskal-Wallis test revealed significant differences between the number of oocytes in group 1 versus group 3 ($p<0.05$), as well as between group 2 versus group 3 ($p<0.05$) (Fig. 11):

Table 6. Basic statistical data on the harvested oocytes in the different groups

Total NO oocytes					
FSH	**n**	**min**	**max**	**average**	**SE**
< 8,5	697	0	27	6,77	0,48
8,51-11,99	1579	0	27	7,48	0,43
> 12	448	0	26	4,62	0,44

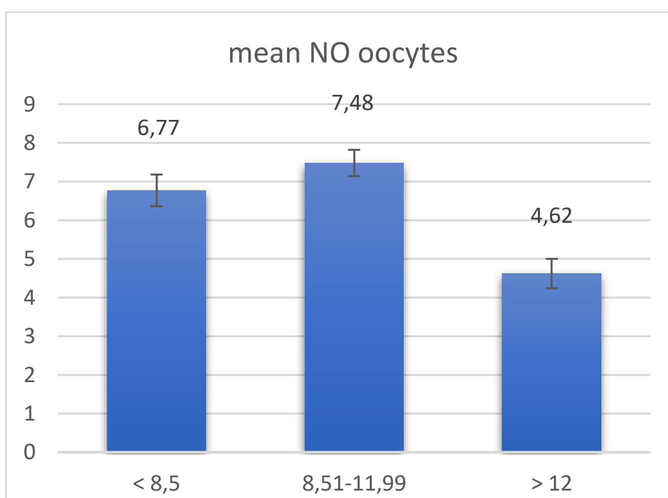


Fig. 11. Ratio between the average number of retrieved oocytes in the different groups

The data regarding the proportion of mature oocytes in the different groups are similar - the tendency for a reduced number of oocytes at elevated FSH levels above 12 IU/l is confirmed.

Regarding the relationship between the age of the treated women and their FSH values, the analyzed data and the results are presented graphically in Fig. 12:

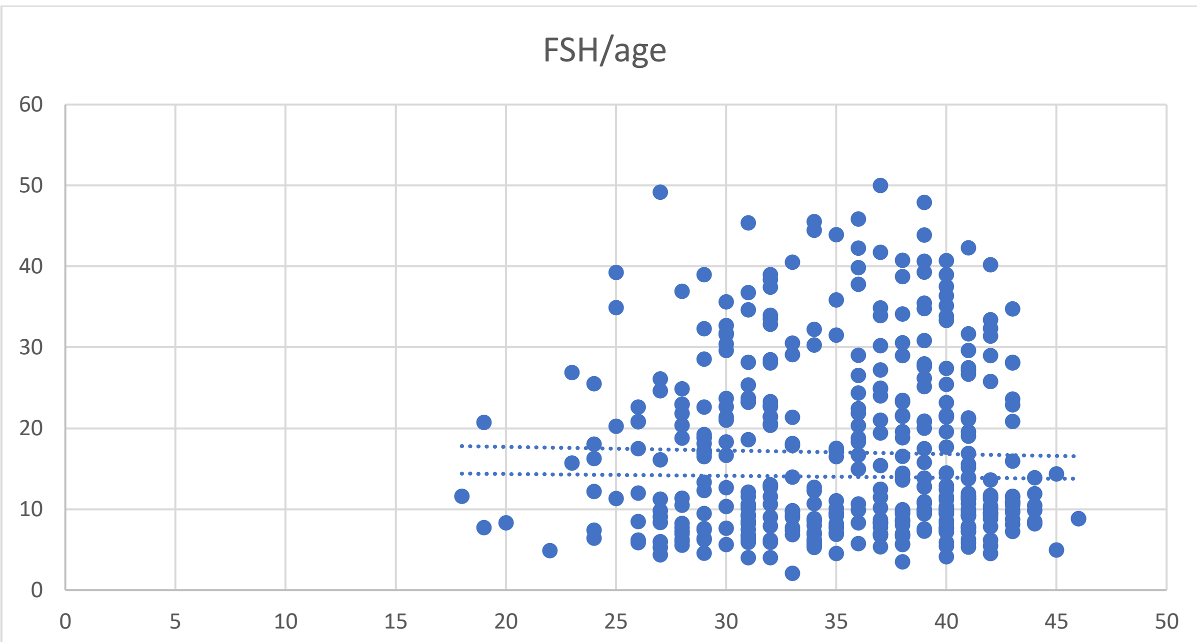


Fig. 12. Correlation dependence between age and FSH values.

Analysis of the parametric correlation coefficient between these 2 factors reported an interdependence between the increasing age of the patients and the values of their basal serum FSH levels.

The final results of the treatment show some regular differences in the individual studied groups. Although significance was not reported, there was a directly proportional relationship between the percentage of canceled cycles and increasing FSH values. In the group of patients with more than 12 IU/l, almost 1/3 of the cycles ended without a single developing embryo suitable for transfer or freezing. These results are also confirmed when the implantation index is calculated (Fig. 13):

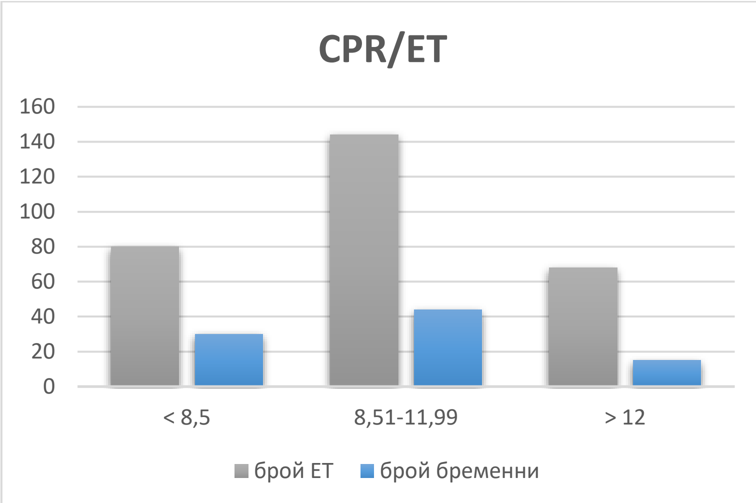


Fig. 13. Clinical pregnancies versus embryo transfer

In the group up to 8.5 IU/l, the highest rates of achieved pregnancies were reported - 37.5%. The results were lower in the second group (from 8.51 to 1.99 IU/l) – 30.56% and the worst treatment results were achieved in the group above 12 IU/l FSH – 22.10%. The results demonstrated are a regular result of the overall cohort analysis and reach significance in treatment outcomes between group 1 and 3 ($p = 0.0431$).

Discussion:

Existing literature data that support the thesis that high serum FSH levels negatively affect both oocyte morphology (high percentage of dark, granular oocytes) and lead to lower results when registering pregnancies fully correspond with the results obtained in our study. As a cut-off limit, we could outline values above 12 IU/l, where significant differences are reported both in terms of the number of harvested oocytes and in terms of pregnancies achieved. Based on our data, we could confirm the role of FSH values not only as a reliable marker of ovarian reserve, but also as a biological factor affecting gamete quality.

Endometriosis and tubal factor

To determine whether there is a correlation between oocyte production, fertilization and implantation potential in women affected by endometriosis and pelvic inflammatory disease (PID), we investigated the achieved ART treatment outcomes of women with diagnosed endometriosis and PID and compared them with patient pairs with a male factor as a control group (Table 7). The result of the statistical analysis done by the Kruskal-Wallis test was that there was significance found in the mean number of retrieved oocytes between the endometriosis group and the control group, and also between the TVB group and the control group ($p<0.01$). When comparing the two studied groups, statistical differences were also observed - in women with endometriosis, a significantly lower number of oocytes obtained after stimulation was reported ($p<0.01$) (Fig. 14).

Table 7. Basic statistical data on the harvested oocytes in the different groups

Total NO oocytes					
	n	**min**	**max**	**mean**	**SE**
Endometriosis	2471	0	28	5,24	0,21
Tubal factor	15693	0	45	7,1	0,24
Control	4408	0	32	7,7	0,23

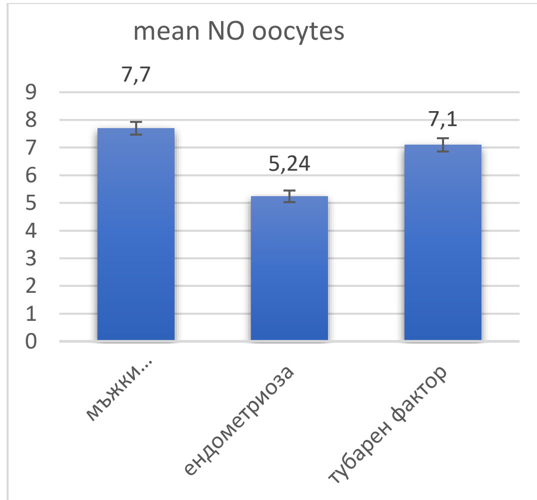


Fig. 14. Ratio between the average number of retrieved oocytes in the different groups

The results are similar when we compare the proportion of the average number of fertilizable oocytes retrieved. Again, a statistical difference was found both when comparing the separate two groups with the control and between the studied groups themselves ($p<0.01$).

Regarding the final outcome of the treatment, the three groups - the two studied and the control group - demonstrated comparable data regarding how the procedure ended. The percentage of canceled cycles both in the group of women with endometriosis and in the group with TBB was higher and approached values of ¼ of all punctures performed, however, no statistical dependence was found when comparing with the control group.

When analyzing the data from achieved pregnancies, the following dependencies were established, demonstrated in fig. 15:

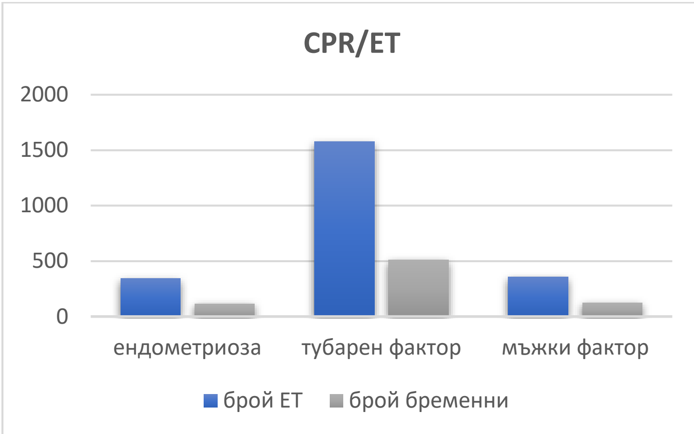


Fig. 15. Clinical pregnancies versus embryo transfer

The results of this study show that despite a significantly lower number of retrieved oocytes in patients with inflammatory diseases of the reproductive system, the achieved clinical pregnancies (CB) were comparable in the three groups. The percentage of successful implantations for tubal factor was 32.26%, for the endometriosis group it was 33.70% without a significant difference (p = 0.6055). The highest percentage of implantations was found in the control group - 34.83% KB/ET, but it was not statistically significant either in relation to the endometriosis group (p = 0.7530) or in relation to the tubal factor (p = 0.3507).

Discussion:

The results that our team obtained when processing the data on the various factors of sterility are diverse, but in their main essence they fully correspond to the conclusions of the literature review published in 2017. in the Journal of Ovarian Research by Prof. Raoul Orvietto's team at Tel Aviv University Hospital, Israel (192). In the paper, the authors note that inflammatory processes in the pelvis alter the normal follicular fluid environment, but this process has more biological than clinical significance for women aiming to become pregnant. In a number of publications that are cited, the authors found numerous changes in the morphological status of the oocytes harvested after stimulation, but when the final results of implantations and pregnancies were analyzed, the data were extremely contradictory. Our analysis of treatment outcome in Bulgarian patients demonstrates similarity with the main findings regarding the number of retrieved oocytes and supports these publications that found no clinically significant differences in pregnancy rates.

ICSI procedure

In order to consider whether the ICSI procedure is still a relevant factor, we processed the results of a total of 2897 cycles in which only one of the two procedures was applied. The average number of oocytes retrieved, as well as the average age, are comparable to make the results as comparable as possible (Table 8).

Table 8. Basic statistics on the harvested oocytes in the different groups

Total NO oocytes								
	n	min.	max.	mean	SE	FR	Damage	No fertilization
ICSI	17368	5	16	7,7	0,46	75%	2,5%	22,5%
IVF	5234	6	14	7,8	0,47	74%	NA	26%

When comparing the average percentage of fertilized oocytes, no significant differences were reported ($p = 0.1443$). Unfertilized oocytes were 22.5% and 26% for the ICSI and IVF procedures, respectively. Degenerated oocytes after injection (damage) are 2.5%.

In terms of end results, the data also show similarity between the two procedures. No significant differences were reported in terms of the percentage of cycles canceled due to non-fertilization or non-developing embryos. The data on established pregnancies after applying both procedures are demonstrated in fig. 16.

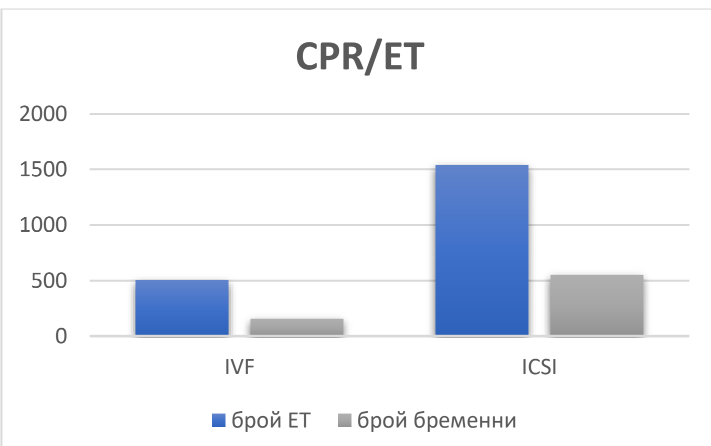


Fig. 16. Clinical pregnancies versus embryo transfer

Clinical pregnancies were registered in a higher percentage when ICSI was applied as a fertilization method (35.7%) compared to cases where the IVF technique was chosen (31.2%). Despite these differences, statistical analysis did not report significant differences in the results achieved between the two procedures ($p = 0.0661$).

Discussion:

Data from our study demonstrate a negligible low percentage of degenerated gametes after micromanipulation, which is fully compensated by the high fertilization index. The choice of ICSI as a method of fertilization is also associated with a precise selection of both oocytes and sperm, and in recent years it has become the preferred method of work in embryology laboratories worldwide. Modern publications fully confirm our results and remove this laboratory procedure from the list of factors that affect the quality of oocytes and the final result of treatment.

Cryopreservation

In order to investigate whether frozen oocytes have the same competence as fresh ones, we reported the results of 62 procedures in which frozen oocytes were used and compared them with 162 with fresh ones.

After thawing according to protocol, 208 survivors were recorded out of 213 thawed - 98% survival rate (SR). The main data are presented in the table. 9:

Table 9. Basic statistics

Total No oocytes					
	n	**min.**	**max.**	**mean**	**SE**
fresh	1154	1	14	5,5	0,29
frozen	213	1	11	5,3	0,27

Reported fertilization rates are 72% for frozen oocytes and 75% for fresh oocytes, respectively. Despite the higher values for fresh oocytes, no significant differences were detected after statistical treatment using Pearson's chi-squared test (p = 0.3560). The results of the final outcome of the treatment (Fig. 17) are also similar - higher values of achieved pregnancies in the procedures with fresh oocytes - 35.83% and 31.91% in the case of frozen ones, and again no significance was established (p = 0.3560).

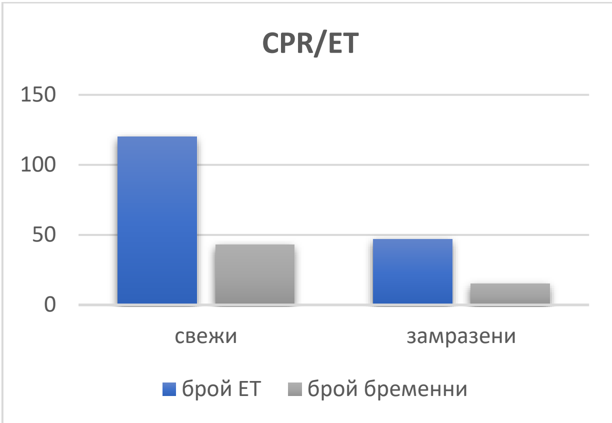


Fig. 17. Clinical pregnancies versus embryo transfer

Discussion

Publications in recent years have favored vitrification as a technique and found no differences in the treatment of patients with fresh or frozen oocytes. Other contemporary publications report no effect of vitrification on oocyte quality. Our results fully correspond with the literature data on the subject and are proof of the excellent working methodology and the good training and experience of the embryologists and cryobiologists in Bulgaria.

SUMMARY:

In terms of the main parameters discussed in this dissertation, the different factors studied influence different aspects of the oocyte recruitment, retrieval and fertilization cycle.

An analysis of the average number of retrieved oocytes reported a lower yield in proportion to the increase in the age of the treated patients, which was most pronounced in women over 40 years of age. Another influencing factor is serum FSH values above 12 IU/l, as well as in patients diagnosed with endometriosis or TVB.

Reciprocal are the data concerning the oocytes suitable for fertilization. The influence of the same factors was found when processing the data for the mean number of mature oocytes, with age again emerging as the factor of greatest importance for the studied parameter.

Regarding fertilization, relative homogeneity is reported for most of the studied factors, with the exception of women who are over 40 years of age - they were found to have statistically lower results and a lower number of embryos obtained from fertilized oocytes.

Analyzing the data on how the assisted reproduction procedure ended, an important indicator is the cancellation rate - at what percentage of the performed procedures did not reach transfer and/or freezing due to lack of mature oocytes, lack of fertilization or lack of developing embryos.

The most distinct differences with a strong statistical dependence were observed in the age factor - significant differences were found between all groups ($p<0.001$). A correlation was also found in patients with FSH values above 12 IU/l, in which the risk of the entire cycle being canceled was higher, compared to women in the group with FSH < 8.5 IU/l ($P = 0.0372$).

Another important indicator that can be tracked is clinical pregnancies achieved. In women over the age of 35, the chances of achieving successful implantation are significantly lower. The result in patients with FSH values above 12 IU/l is also normal. Here, a new factor appears for the first time – women with high BMI values have lower pregnancy rates. When considering this factor, however, the influence of high levels of excess adipose tissue on mucosal receptivity must be taken into account, as well as the lack of statistical differences in the number of retrieved oocytes and the fertilization index in the different BMI groups.

The generalization that could be made on the basis of the examined 9816 Bulgarian patients and 67187 oocytes is that age stands out as the leading factor having the most significant influence on the quality and fertility potential of the oocytes. Another important factor with a leading role is the indicators of the follicle-stimulating hormone, when its serum values exceed 12 IU/l (Fig. 18).

ОБОБЩЕНИЕ

ФАКТОРИ	БРОЙ Я	БРОЙ NM	ОПОЖДАНЕ	CANCELLATION RATE	БРЕМЕННОСТ
ВЪЗРАСТ	✓	✓	✓	✓	✓
ИТМ					✓
ТЮТЮНОПУШЕНЕ					
FSH	✓	✓			✓
ЕНДОМЕТРИОЗА	✓	✓			
ТУБАРЕН ФАКТОР					
ICSI					
КРИОКОНСЕРВАЦИЯ					

Fig. 18. Relationship between the investigated factors and oocyte competence

IV.2. Analysis, systematization and proposal for management of the factors influencing the quality of oocytes by means of transformation of the information in tabular form, as well as presentation of a new classification generated on the basis of directed work with artificial intelligence.

For the purpose of the present study, we analyzed data from the most recent publications in the scientific literature to supplement, update and convert into an easy-to-use tabular form the two main types of factors affecting human oocyte competence. For greater accuracy, our team offers an additional subdivision of factors with external impact.

In addition to this popular division into 2 main groups, we also present 1 more new classification based on data from artificial intelligence work

The original division of factors with external and internal impact undergoes continuous updates due to the active work of various teams in this direction. After integrative analysis of 115 publications until 2023. including from 5 scientific databases, our team transformed the literature data into a tabular view of the main factors with external and internal influence (Table 10), for which there is evidence of a potential effect on oocyte competence. In addition to the basic division into 2 groups, we suggest for greater precision and additional subdivision for factors with external impact:

- Factors with an indirect effect on oocytes - until the follicular puncture

- Factors with a direct impact - after the follicular puncture

Table 10. Factors affecting oocyte quality

FACTORS AFFECTING OOCYTE QUALITY	
FACTORS WITH INTERNAL IMPACT	FACTORS WITH EXTERNAL IMPACT
	EXTERNAL FACTORS WITH INDIRECT IMPACT
AGE	COH
POF	CHEMOTHERAPY
BMI	AIR POLLUTION
SMOKING	MEDICATIONS
ALCOHOL	OXIDATIVE STRESS
DRUGS	SURGERY
CAFEINE	PROFESSIONAL RISKS
PHYSICAL ACTIVITY	TOXINS
NUTRITION	PRP
SUPLEMENTS	STEM CELLS
STRESS	MITOCHONDRIA TRANSFER
FSH	HYPERBARIC OXIGENATION
SLEEP QUALITY	PHYSIOTHERAPY
ENDOMETRIOSIS	GROWTH HORMONE
TUBAL FACTOR	NUCLEAR TRANSFER
PCOS	

OHSS	**EXTERNAL FACTORS WITH DIRECT IMPACT**
AUTOIMUNNE DISEASES	**DENUDATION**
CARCINOMAS	**ICSI**
GENETIC MUTATIONS	**CRYOCONSERVATION**
МЕТАБОЛИТЕН СИНДРОМ	**LABORATORY CONDICIONS**
CHRONIC ILNESS	**INCUBATOR CONDITIONS**
	MEDIA CONDITIONS
	EPIGENETIC INFLUENCE

Systematization of factors through artificial intelligence

In order to get a detailed and complete picture of the factors that influence the quality of human oocytes, we used the product ChatGPT (Open AI) with its premium version - GPT-4, implemented in the month of March 2023. Through a series of specifically targeted questions, topics and commands GPT-4 generates a completely new and different classification of factors, grouping them thematically into 9 separate categories (Fig. 19):

1. Age

This is the factor with the most key importance and the greatest influence on the number and quality of oocytes. The chance of achieving a successful pregnancy mainly depends on it

2. Ovarian reserve

Reduced ovarian reserve is associated with reduced chances of achieving pregnancy even in younger patients due to the severely limited number of oocytes that are retrieved after ovarian stimulation

3. Lifestyle

A number of choices a person makes in their daily life, as well as some harmful habits, can significantly affect both the quantity and quality of their oocytes. The following factors have the most significant influence:

- Weight and BMI

- Smoking

- Alcohol abuse

- Use of narcotic substances and hallucinogens

- Unbalanced nutrition, malnutrition and high-fat diets

- Lack of physical activity or excessive physical activity

- Abuse of caffeinated drinks and products

- Stress

4. Genetic factors

A number of inherited and de novo mutations could have a significant impact on an individual's fertility, associated with reduced ovarian reserve, suboptimal response to stimulation and production of gametes with an unbalanced karyotype

5. Medical conditions

The most significant diseases that have a bearing on oocyte quality are:

- PCOS

- Endometriosis

- Autoimmune diseases

- Chronic illnesses

- Previous pelvic surgery

- Cancers, chemotherapy and radiotherapy

- Infections and inflammations of the reproductive tract

6. Hormonal status

Of great importance for fertility and the quality of oocytes are the normal values of sex hormones such as LH, FSH, TSH, progesterone, prolactin, estradiol and especially anti-Müllerian hormone. It is also important for the thyroid gland to function normally and for its hormones to be within reference limits.

7. Ovarian stimulation

The correct selection of the ovarian stimulation protocol and the appointment of optimal daily doses are key to the optimal outcome of oocyte retrieval after follicular puncture. Excessively high doses, in turn, are associated with a risk of OHSS

8. Influence of environmental factors

In their daily life, people are exposed to a number of toxic and harmful environmental influences. The most important and related to fertility are:

- Toxins disrupting endocrine function (bisphenol A and phthalates)

- Polluted air

- Workplace hazards (chemicals, pesticides, heavy metals, radiation)

9**. Assisted reproductive technologies**

The extraction of gametes and their manipulation in laboratory conditions are associated with a number of risks for their destruction or improper development due to suboptimal handling and incubation conditions. The most critical points are:

- Follicular puncture and gamete processing

- Cryopreservation

- Incubator conditions

- Laboratory conditions

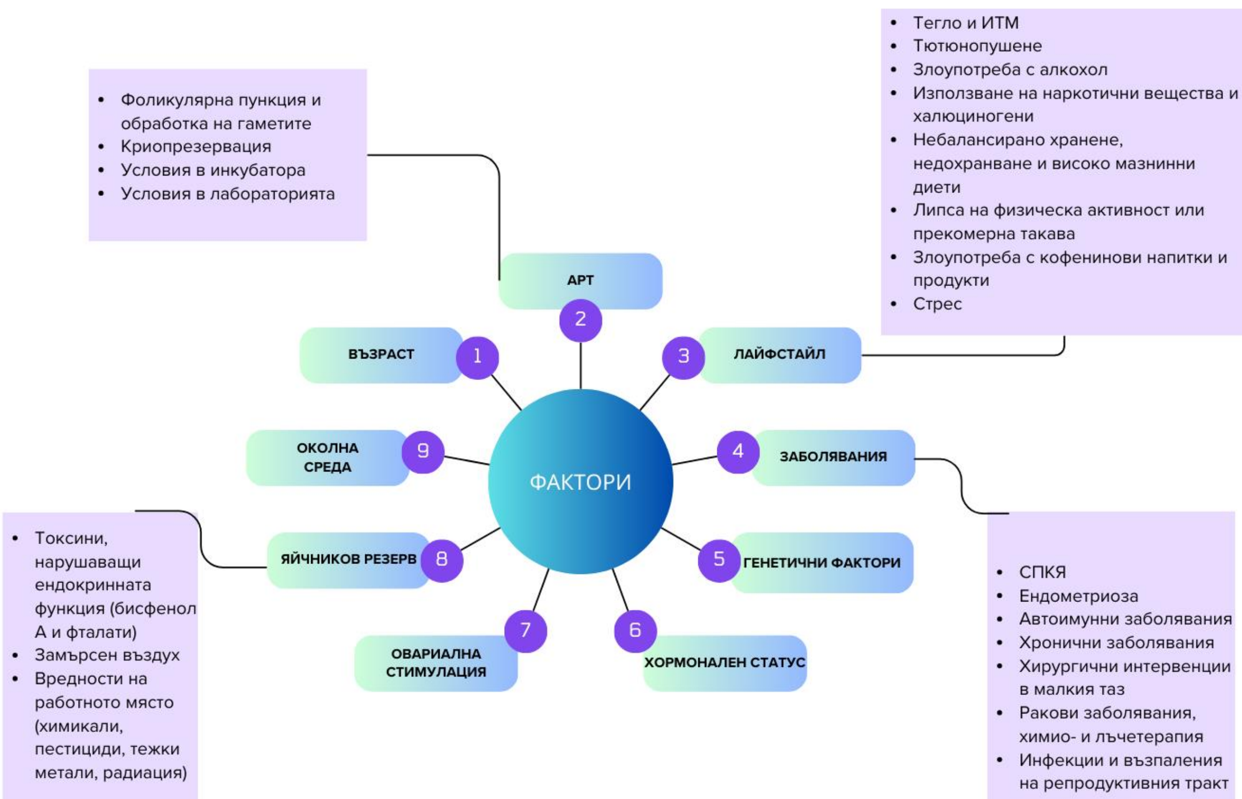


Fig. 19. Summary visualization of AI-generated factors affecting oocyte quality

SUMMARY:

The management of factors through systematization and implementation in an easy-to-navigate tabular form is part of the modern methods for preparing a comprehensive and complete picture of the reproductive problems of the specific patient couple and precisely preparing an individual therapeutic plan.

The aggregation and classification of factors with a potential influence on oocytes gives good predictability in counseling and treatment of infertility patients regarding the expected ovarian response to stimulation, the number and quality of oocytes, their fertilizing potential, as well as the expected chances of achieving pregnancy. At the same time, it also provides an opportunity to correct or eliminate a number of factors in order to increase the chances of treatment success accordingly. These are mostly lifestyle factors, as well as more closely monitored control of certain diseases related to fertility, undergoing surgical interventions if necessary before starting the procedure, etc.

The use of modern technological achievements, and artificial intelligence in particular, is of critical importance for the future of assisted reproductive technologies and medicine in general. In assisted reproduction as a modern and high-tech discipline, new technologies are always adopted and utilized at a rapid pace. Work is underway in various directions where the use of this modern technology can help improve treatment outcomes for patients with infertility. The used model successfully generates a classification in which all the factors included have literature data on potential influence on female gametes and are present in the classical classification. AI's approach to grouping is different compared to classical systematization - in the classification proposed by artificial intelligence, factors are grouped by similarity of their origin.

IV.3. Proposal for a new OVOSCORE oocyte evaluation system, with high informative and predictive value, based on morpho-physiological characteristics and analysis of results and performance and in terms of fertilization, embryo quality and implantation.

During in vitro fertilization, oocyte quality is usually assessed visually by morphological criteria under an inverted microscope. The main disadvantage of the method is that it is subjective because it is based on purely qualitative criteria, it depends on the operator and requires years of training. In a 2022 paper, a team of researchers from several scientific institutions in France reviewed the literature on the subject and concluded that morphological assessment remains the main and most accessible method of analysis. In parallel, the ESHRE recommendations for good laboratory practice do not specify any specific system for evaluating the quality of oocytes, but emphasize the importance of accurately reflecting their morphological features. This requires each laboratory to choose how to reflect oocyte quality in patient records, or whether such information should be present at all.

At SAGBAL "Dr. Shterev", all information regarding the couples' treatment cycle is reflected accurately and in detail in their medical records, and for this reason our team created its own evaluation system - OVOSCORE, the data from which are analyzed in the present study.

We analyzed data from 468 cycles in which 4127 cumulus-oocyte complexes were obtained. 3083 of them were fertile and were evaluated according to the OVOSCORE system, tracking the results of fertilization, blastocyst formation and, in cases where it was possible to report the percentage of pregnancies achieved.

After morphological evaluation by inverted microscope and evaluation of functional characteristics by micromanipulator injection, all oocytes were distributed into the different groups and subgroups demonstrated in Fig. 20.

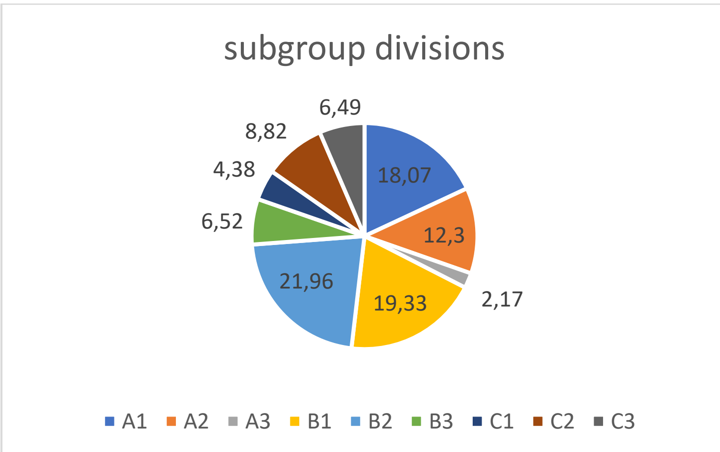


Fig. 20. Percentage distribution of oocyte subtypes

With the highest frequency are those characterized as type B (47.81%) by the embryological team, and among them the most common subtypes are B1 (19.33%) and B2 (21.96%) and in a significantly lower percentage B3 (6.52%). A lower percentage was assessed for type A oocytes (32.5%), which according to the working team's high criteria must be "perfect" and meet absolutely all definitions of a good oocyte. Of these, during the ICSI procedure, the largest percentage (18.07% of the total population) demonstrated a normal physiological response to penetration with the injection pipette and was classified as the highest class A1, 12.3% showed a slightly decreased turgor and elasticity and were evaluated as A2 and the lowest proportion of all subgroups included the type A3 oocytes (2.17%), in which excellent morphological characteristics and cytoplasm with disturbed ultrastructural composition were observed, which did not demonstrate compactness during fertilization . The smallest relative share is occupied by type C oocytes (19.69%), in which there are clearly distinguishable 2 or more severe morphological defects. With them, in accordance with their external indicators, the most common types are C2 (8.82%) and C3 (6.45%), and with a relatively rare frequency C1 (4.38%).

Age and oocyte quality

The proportional distribution of the number of oocytes in the different age groups outlines an increasing share of gametes with morphological deviations as the average age of the treated women increases. After the application of the double model for evaluation by OVOSCORE, the summary picture demonstrates synchronicity between the deteriorated morphological and physiological characteristics in aging oocytes (Table 11).

Table 11. Correlation between oocyte type and subtype and age

Type oocytes	**Age groups**			
	30	**31-34**	**35-39**	**40**
A1	193	155	159	50
A2	152	129	75	22
A3	31	20	14	2
общо	**376**	**304**	**248**	**74**
B1	218	162	148	68
B2	249	216	154	58
B3	73	58	50	20
общо	**540**	**436**	**352**	**146**
C1	33	45	37	20
C2	97	78	63	34
C3	53	63	59	25
общо	**183**	**186**	**159**	**79**

When comparing the proportions of ova in the different age groups, the Critical Chi-square cut off test found a significant relationship (p= 0.0212) between the group of the youngest under 30 and the group of the oldest over 40 women in the proportion of the percentage of excellent quality type A oocytes.

The analysis showed homogeneity between all age groups with respect to type B oocytes.

Reliable differences were found when comparing the proportions of ova rated worst in terms of their morphological manifestations. A strong statistical difference was again reported between the youngest and oldest groups of patients (p= 0.0012), with a positive correlation between increasing mean age and the number of gametes with distinct dysmorphism (Fig. 21).

The data obtained from the statistical analysis confirm the hypothesis that there is an inextricable link between oocyte aging at the genetic, ultrastructural and morphological levels. Although there is not a lot of research on the subject, this contention follows the logic that the accumulation of changes at the molecular level affects the entire organelle and cell, and our analysis reinforces this. As a cut-off limit, based on the obtained results, we can indicate the age of 40 years, after which the proportion of oocytes with various dysmorphisms increases significantly, and this correlates with the results of ART treatment.

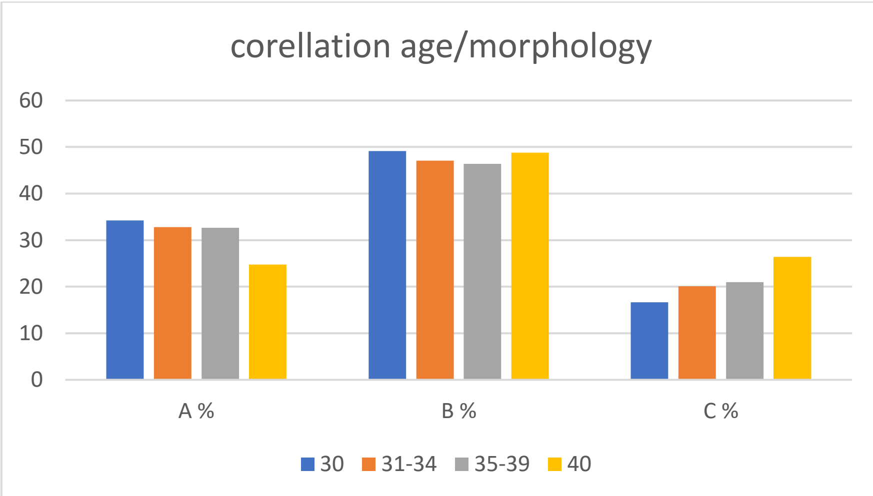


Fig. 21. Correlation of the main groups and age

Fertilization results

In order to establish whether there is a reliable relationship between fertilization index and morpho-physiological characteristics of oocytes, we analyzed the groups and subgroups defined by the OVOSCORE system in terms of their fertilization results.

Average fertilization rates defined by the Vienna Consensus range between 70 and 80%. From the statistical analysis done by the Kruskal-Wallis test, it was found that there was a clear statistical difference between the studied groups. There is a distinct tendency to decrease the proportion of fertilized oocytes with a deterioration in their morphological quality (Table 12).

Table 12. Fertilization results of oocyte subtypes

	yes	no	Total No	% yes	% no
A1	509	48	**557**	**91,382406**	**8,617594255**
A2	340	37	**377**	**90,185676**	**9,814323607**
A3	49	18	**67**	**73,134328**	**26,86567164**
B1	493	103	**596**	**82,718121**	**17,28187919**
B2	524	153	**677**	**77,400295**	**22,59970458**
B3	114	87	**201**	**56,716418**	**43,28358209**
C1	97	38	**135**	**71,851852**	**28,14814815**
C2	180	92	**272**	**66,176471**	**33,82352941**

C3	71	129	**200**	**35,5**	**64,5**

The results are similar when evaluating the functional parameters - the less viscous the cytoplasm and the inelastic the membrane, the higher the proportion of unfertilized gametes. In one of the subgroups - C3, in which the worst morpho-functional parameters are present, the quota of unfertilized oocytes is greater than the fertilized ones.

Statistical processing of the data in the subgroup of A type oocytes did not reveal a significant relationship between A1, A2 and A3 type oocytes. However, in the remaining 2 groups – B and C, significant differences are reported when comparing the subgroups ($p<0.05$) (Fig. 22).

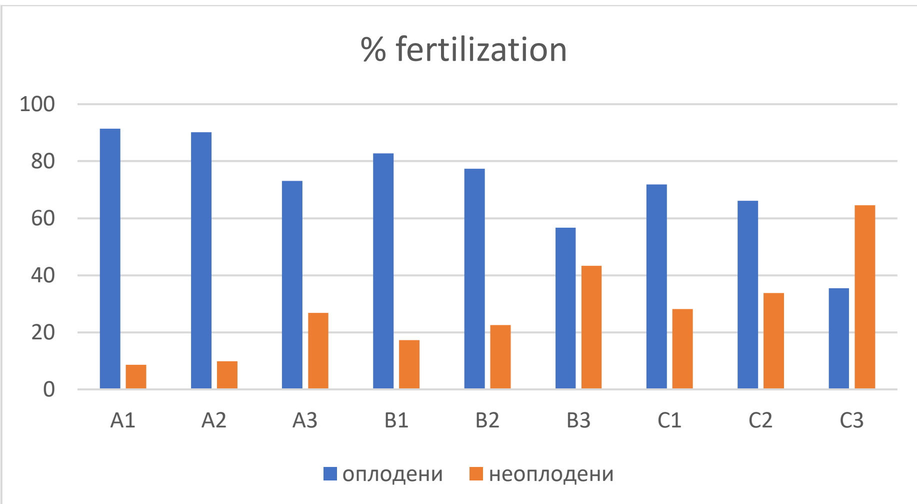


Fig. 22. Fertilization in subgroups

The presented results indicate that the indicators related to the fertilization potential of the oocytes show a clear negative trend with the deterioration of the morpho-physiological indicators of the gametes.

Degeneration after ICSI

An important indicator when considering fertilization is the percentage of degenerated gametes after the manipulation. To establish a potential relationship between oocyte morpho-physiological characteristics and the risk of degeneration after ICSI, we tracked and compared the percentages of degenerated oocytes after manipulation.

On the table 13 presents the numerical and percentage expression of the fertilization of the oocytes in the different subgroups and the proportion of degenerates after the manipulation:

Table 13. Percentage distribution of fertilized, unfertilized and degenerated oocytes after injection

	fert	No fert	damage	total	% damage
A1	509	46	2	557	**0,36**
A2	340	33	4	377	**1,1**
A3	49	9	9	67	**13,43**
B1	493	93	10	596	**1,68**
B2	524	122	31	677	**4,58**
B3	114	49	38	201	**18,91**
C1	97	33	5	135	**3,7**
C2	180	67	25	272	**9,19**
C3	71	50	79	200	**39,5**

Analysis of the results showed a high proportion of degeneration in oocytes whose physiological indicators were assessed as category 3 – these are oocytes with low cytoplasmic density and inelastic membranes that are easily ruptured upon penetration of the injection pipette and do not offer resistance. Within subgroups, significant differences were found regarding degenerated oocytes.

There are no significant differences between subgroups A1 and A2 ($p = 0.1704$), but when compared with A3, a strong statistical difference is found in both subgroups ($p< 0.001$). The data are similar when calculating reliable dependencies in subgroups B1, B2 and B3. In contrast, in Group C, significant differences were reported in each of the subgroups compared to the others by Dunn's multiple comparison.

To account for the additional influence of the morphological factor on the prognosis of oocyte degeneration, we compared the subgroups A3, B3 and C3 and found a lack of significance between A3 and B3 ($p = 0.3413$) and the presence of a distinct difference when comparing each of the two subgroups with C3 ($p < 0.0001$) (Fig. 23).

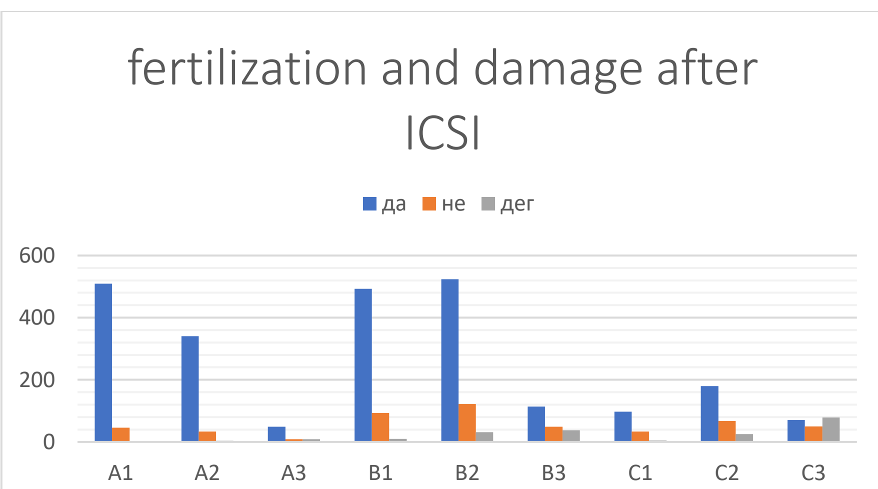


Fig. 23. Fertilization and degeneration after injection

Although isolated, there are publications that have investigated the topic and associated fertilized oocytes with the presence of a significantly larger ICSI funnel, compared to degenerated ones. It is the physiological characteristics that have the greatest relevance to the successful performance of the manipulation. Our study confirms the above data and highlights the importance of physiological characteristics of oocytes as a predictive marker of their fertilizing potential and risk of degeneration. As a complement to the published data, we can add our results, in which the combination of the physiological characteristics of the oocyte with its morphological quality by means of the OVOSCORE system gives a score with a high prognostic value for fertilization and degeneration after ICSI.

Blastocyst formation

Only a fraction of the embryos generated by ART methods reach the blastocyst stage. To make a connection between oocyte morpho-physiological parameters and the probability of blastocyst formation, we analyzed the data to count how many of the fertilized oocytes reached this stage when cultured until day 5.

In group A, the blastocyst proportion reaches 74.16%, in group B it is 43.22% and in group C it drops to 27.24%. A multiple comparison between all groups showed a significant difference ($p < 0.0001$).

Regarding the subgroups, the test conducted using Marasculio's procedure for comparing multiple proportions did not find significant differences within the boundaries of the group itself. Significant differences were found when comparing the A1, A2 and A3 subgroups with each other subgroup ($p < 0.0001$) (Fig. 24).

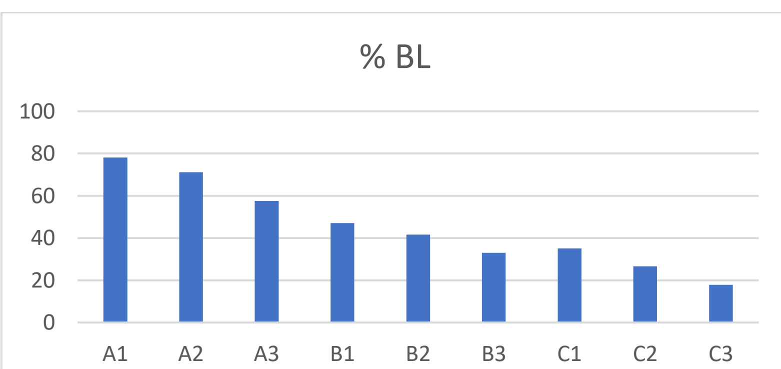


Fig. 24. Percentage of blastocysts obtained in subgroups

The present study confirms the reported data on the correlation between the characteristics of the female gamete and the capacity of the embryo and emphasizes the leading role of the morphological criterion as a prognostic factor.

Blastocyst quality

In addition to the quantitative measurement of the percentage of blastocyst formation obtained from the cohort of zygotes, an important indicator of blastocyst potential is their qualitative assessment. One of the goals of our research is to link the morpho-physiological characteristics of the oocytes and the quality indicators of the obtained blastocysts.

In subgroup analysis, it was found that in A3, B3 and C3 not a single hatching blastocyst was reported. The proportions of blastocyst quality remain comparable and only in subgroups C1, C2 and C3 is the proportion of early Bl1 blastocysts significantly higher compared to the other subgroups (<0.05) (Fig. 25).

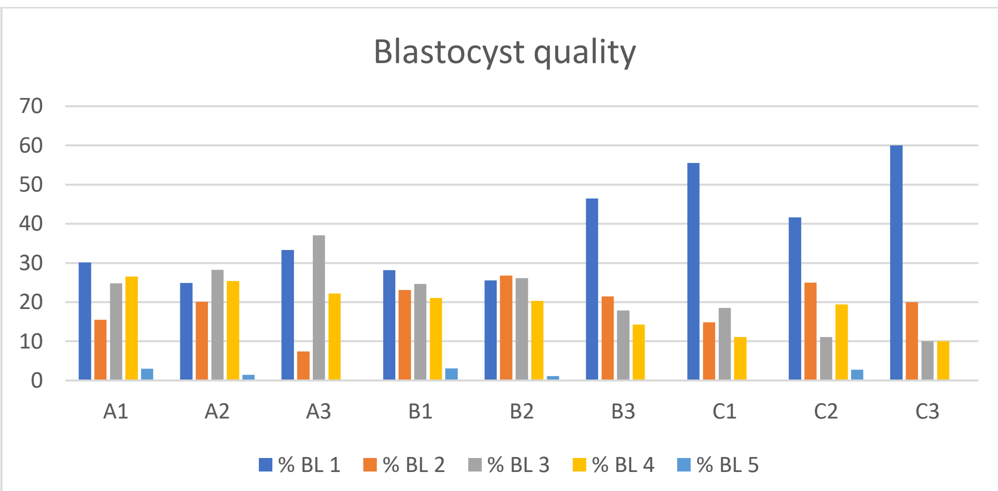


Fig. 25. Distribution of blastocysts by quality in oocyte subtypes

Our results confirm the literature data on the primary importance of morphological assessment and add as an additional predictive factor for blastocyst quality the functional parameters of the oocyte during fertilization.

Implantation results

Only a small fraction of the initially harvested oocytes reach embryo transfer. Analysis was possible in an even smaller percentage of these cycles – only in cases where we had 0% or 100% implantation.

The data from the resulting pregnancies in the three main morphological groups demonstrated in Fig. 63 show a clear tendency to decrease the number of successful pregnancies with increasing morphological dysmorphism. The percentage of successful pregnancies in group A reached 66.5% KB/ET. For group B it is 23.88% and in group C it drops to 11.39%. Statistical processing showed strong significant differences between groups A and B ($p < 0.0001$), as well as a significant difference between B and C ($p = 0.0185$). A strong significant difference ($p < 0.0001$) was also found between group A and C as expected.

When looking at the pregnancies achieved in the different subgroups, the trend remains the same (table 14). In subgroup B3 and C3, not a single resulting pregnancy was registered. Although due to the small number of oocytes that fall into the different subgroups, a statistical analysis is difficult to make, the results that are obtained are convincing and regular.

Table 14. Number and percentage of implantations received

implantation					
	yes	no	total	% yes	% no
A1	85	39	**124**	**68,5484**	**31,4516**
A2	47	28	**75**	**62,6667**	**37,3333**
A3	7	3	**10**	**70**	**30**
B1	33	70	**103**	**32,0388**	**67,9612**
B2	20	78	**98**	**20,4082**	**79,5918**
B3	0	21	**21**	**0**	**100**
C1	2	22	**24**	**8,33333**	**91,6667**
C2	7	33	**40**	**17,5**	**82,5**
C3	0	15	**15**	**0**	**100**

Data from our study support publications finding a correlation between oocyte quality and cycle outcome. The detailed studies carried out on cytoplasmic viscosity, ovule elasticity and the presence of injection funnels provide additional data that functional characteristics also have a bearing on the final result and can be included as an additional factor with good informational value to the overall evaluation of the oocyte.

SUMMARY:

The recommendations of the European Association for Good Laboratory Practice recommend that oocyte quality be documented when performing insemination procedures without specifying a specific system for this. A number of authors studying oocytes emphasize the correlation between oocyte quality, embryo quality and success rate of the procedure, but at the same time note that the lack of a unified system causes each work team to apply their own methods of evaluating gametes and different ways of documenting them . According to a survey conducted at the XVII Congress of the Bulgarian Association for Sterility and Reproductive Health (BASRZ), only in single in vitro laboratories in Bulgaria the quality of oocytes was documented, and no specific assessment systems were specified.

A study shows that while compromised oocytes have a statistically lower chance of forming a good embryo, their quality sister gametes are not affected by this factor. Therefore, choosing high-quality oocytes can increase the success rate of fertilization and the subsequent development of the embryo.

• The OVOSCORE Ovulation Evaluation System is easy to use, non-invasive and requires no time or additional technique for its application.

• Meets ESHRE recommendations for documenting oocyte quality without time-consuming and redundant data entry into the computer database or on paper.

• It has a high informative value and is a good prognostic marker for subsequent fertilization, embryo quality and implantation.

• Enables the tracking of the oocyte to the embryo and is part of the embryo selection methods through the so-called de-selection, or tracing the history of the embryo (embryo history).

• The system easily identifies giant oocytes and aggregation of smooth endoplasmic reticulum, which are recommended to be transferred only as a last resort in the absence of other embryos.

• OVOSCORE provides information on the quality of the gametes and is a useful tool for the reproductive specialist in preparing a subsequent treatment strategy after a failed attempt - depending on the results and in the case of a history of poor quality oocytes application of additional procedures - visualization of the dividing spindle, Ca ++ activation, time-lapse tracking, etc.

• After several unsuccessful attempts in advanced reproductive age over 40 years, exhausted ovarian reserve, a decisive factor in the therapeutic plan can be the documented quality of the oocytes - in the presence of mainly assessed as type C, a discussion with the patients about the option of switching to a donor is possible program as offering higher chances of achieving pregnancy.

• It proves conclusively the relationship between the morpho-physiological characteristics of the oocyte and its potential for fertilization and competence of the generated embryo. The

accumulation of more than 1 dysmorphism significantly lowers its outlook as a result of the cumulative effect.

As a conclusion, the fundamental importance of the accurate assessment of not only embryos but also oocytes as part of good laboratory practice can be emphasized in order to select, track and evaluate quality oocytes as an important tool for more successful embryo selection in order to achieve more high frequency of clinical pregnancies.

IV.4. Analysis of the effectiveness of applying additional laboratory methods to increase the chance of oocyte fertilization after applying the methods of calcium ionophore activation and visualization of the meiotic spindle using a poloscope.

In recent years, a number of additional techniques have been developed to improve outcomes in patient couples where a previous treatment cycle had an established problem in the gametes or embryos, reducing their chances of success. With oocytes, there are limited possibilities for work due to their limited number and impossibility of selection. The most frequently applied methods are activation with calcium ionophore medium and visualization of the meiotic spindle with a poloscope.

Assisted oocyte activation

To investigate the effectiveness of the applied additional technique, we analyzed the data of patient pairs who had completed 74 treatment cycles, divided into 2 groups depending on whether the technique was additionally applied or not.

In Group 1, a total of 175 mature MII oocytes underwent fertilization. On day 1, 49 of them were fertilized. The average fertilization rate in the group was 24.7%.

In group 2, 162 oocytes of normal maturation were injected, which were then incubated for 15 minutes in $Ca2+$ ionophore medium. 99 of them showed signs of fertilization, the average rate for this group being 65%. ($p < 0.0001$).

In 18 of the couples (19 cycles) in Group 1, a complete lack of fertilization was reported and, accordingly, no transfer was performed. The result was repeated in 2 of these pairs and in an experiment with applied calcium activation (3 cycles).

Adequate development of the embryos and their passage through each developmental stage was monitored and evaluated over the following days. In 7 of the experiments in the control group and in 1 of the calcium activation group, the transfer was canceled due to slow division and stationing of the embryos.

Embryo transfer and pregnancies

Out of all 44 conducted treatment cycles in group 1, only 18 (41%) successfully achieved embryo transfer. Clinical pregnancies were established in 2 of the couples (11.1%) that ended in spontaneous abortion in the first trimester of pregnancy.

In group 2, 26 transfers were realized out of 30 attempts (83.3%) after additional activation of the oocytes subjected to ICSI. 16 pregnancies (61.5%) were reported, of which 14 were normally developing, and 2 ended with absence of cardiac activity and early abortion. In 2 of the patient couples, there are remaining embryos with good characteristics that are frozen and in them pregnancy occurs after thawed embryo transfer (Table 15)

Table 15. Basic data on the outcome of the treatment

	Total No	No cycles wit fert	No cycles with ET	pregnancy	CPR/ET %
Group 1	44	25	18	2	**11.1%**
Group 2	30	27	26	16	**61.5%**

In summary, of 28 patient couples who had between 2 and 5 attempts at infertility treatment within a 3-year period, 16 of them had a clinical pregnancy (57%) that developed normally - in 14 of the cases from fresh and in 2 of the cases from thawed transfer. 2 twin pregnancies were detected, 1 of which with identical twins. Recurrence of a result with no fertilization was reported in 2 partner couples who were referred to medical-genetic counseling and discussed options for using donor gametes.

The results found in the conducted study categorically confirm the already published data on the successful application of assisted oocyte activation in the treatment of patients with infertility. An improvement was observed not only in the percentage of fertilized oocytes, but also in the quality of the obtained embryos - in the cycles in which the Ca2+ ionophore was used, only one was canceled due to inadequate division of the embryos. The positive effect of this methodology suggests its wider application in the embryological laboratory. The method can be used both alone and in combination with other techniques to achieve an optimal result.

Spindle view

To assess the effectiveness of the technique, we summarized the data of 24 patients over the age of 40 with up to 3 mature oocytes harvested and compared the results with a similar group of 72 women who did not have this technique applied.

In 15 (32%) of the oocytes, despite the presence of a second polar body indicating cytoplasmic maturity, the characteristic structure showing the already formed dividing spindle was not visualized (Table 16). In 31 of these, visualization was successful, with 22 oocytes (48%) having a normally positioned division spindle in the region below the first polar body, and 9 (20%) showing lateral displacement.

Table 16. results of visualization with a poloscope

visualization SV		No visualization
Normal position	**displacement**	
22	9	15

ICSI and fertilization

All mature oocytes, regardless of whether the meiotic spindle was visualized or not, were subjected to the ICSI procedure, and for those in which displacement was detected, the injection pipette was positioned at 90 degrees to the spindle site. At the 18th hour, 73% fertilization was reported in the study group and 68% in the control group. Despite the better results, no statistical difference was found (p = 0.6475).

Embryo transfer and pregnancies

In the study group, ET was performed in 20 of the cases (83%), while ET was performed in 57 of the cycles (79%) in the control group. There were 3 clinical singleton pregnancies in the spindle view group and 6 singleton pregnancies in the control group (Fig. 26).

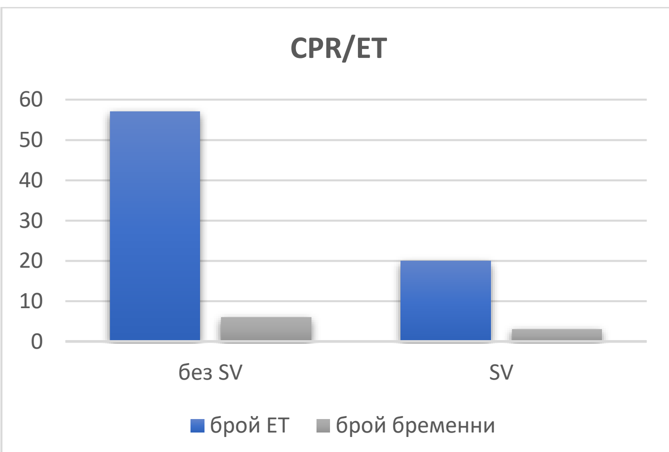


Fig. 26. Clinical pregnancies versus embryo transfer

Despite the higher percentage of pregnancies obtained (15% KB/ET of the study group and 10.53% KB/ET of the control group), no statistical better success rate was reported by Pearson's chi-square test analysis (p = 0.5949).

Visualizing the meiotic spindle in the laboratory before proceeding to a fertilization procedure could provide information on whether the oocyte has formed a stable division structure and where it is located spatially. There is a lack of definitive data both in the publications on the subject and in our research about the effectiveness of the application of this additional methodology. A limiting factor is the high price of specialized equipment and software. However, any modern embryology laboratory must be equipped with the latest technical means to be able to meet the needs of more complex and specific cases of patients who turn to for help and treatment.

SUMMARY:

The additional techniques that are offered beyond the standard in vitro fertilization are increasing with the advancement and development of modern technologies. Some of them have lost their relevance over the years, but others have proven their high efficiency and are an indispensable part of the treatment of couples in which pregnancy does not occur through conventional treatment methods. Researchers are constantly looking for new methods that will be the next big step towards improving the results of in vitro procedures, which have been declining in recent years due to the increasing average age of patients. Recently, all eyes and hopes are connected to the application of artificial intelligence in ART technologies, and although for now it is mainly used for non-invasive highly specialized evaluation and selection, work with it continues in the direction of improving the quality of oocytes and preventing chromosomal errors leading to aneuploidy embryos.

No matter how many additional methods exist and are offered in embryological laboratories, their effectiveness can be relied upon only when there are strictly defined indications for their application. That is why each case must be carefully considered by the team of reproductive specialists and embryologists and the approach must be strictly individual in order to expect optimal treatment results.

CONCLUSION

The oocyte is a unique and highly differentiated cell that is responsible for both creating and activating and controlling the embryonic genome, maintaining essential processes such as cellular homeostasis, metabolism and cell cycle progression in the early embryo. During oogenesis, the oocyte must accumulate the components necessary to maintain the early metabolism and physiology of the embryo, as well as components necessary to complete meiosis, initiate cell cycle progression, and direct events in the early stages of development of the newly formed organism. The oocyte must combine the two haploid genomes into a single embryonic genome, activate the transcription of that genome at the right time, and activate the appropriate set of genes to be transcribed. Oogenesis is critically dependent on the proper functioning of multiple highly specialized molecular mechanisms. Any disturbance of these subtle processes, provoked by various factors, can compromise the quality of the oocytes, which can lead to long-term consequences related to the abnormal progression of the subsequent phases of meiosis, reduced fertility, poor embryo quality and epigenetic defects that affect the long-term health of the offspring.

The analysis and differentiation of the factors that have a negative impact on the quality of the oocytes can serve as a basis for identifying a number of measures for the prevention of negative consequences for a woman's reproductive health.

Age stands out as by far the most significant factor, having a critical impact on all stages of the ART treatment cycle and a major prognostic criterion predetermining the final outcome. Postponing pregnancy until later in life carries a number of risks, both from difficulty in achieving implantation and pregnancy, as well as from a high incidence of spontaneous abortions in the first trimester of pregnancy and an increased risk of chromosomal abnormalities in the fetus.

A number of other factors, such as obesity, smoking, poor diet, inflammatory diseases also have a partially negative impact, and their cumulative effect significantly reduces the chances of successful reproduction.

Oocyte competence is the determinant of ART treatment success, as oocytes provide virtually all cellular building blocks, including mitochondria, required during embryogenesis. An accurate reflection of their quality during the fertilization procedure can largely predict the potential of each oocyte, as well as serve as a starting point for a subsequent treatment plan and the application of additional methods to increase the success rate after an unsuccessful in vitro experiment.

A holistic approach and personalized medicine are widely used in the treatment of female factor infertility patients. Identifying the factors that have a negative or positive impact on oocyte quality allows the treatment team to make the most appropriate recommendations and choose an optimal treatment plan for the couple. The reduction or complete refusal of a number of harmful habits, a change in the food and movement regime, appropriate supplementation would greatly change the predictions of success from the in vitro procedure. Detailed coverage of gamete and embryo quality puts a new tool in the hands of the treating specialist, what could be adjusted and upgraded if a new cycle of stimulation and fertilization is needed.

However, despite the achievements of modern medicine, the percentage of successful pregnancies and births has been relatively low for years, and this has motivated teams from all over the world to do numerous studies and tests, to test different methods and techniques, and to develop innovative equipment in order to overcome the plateau of success and reproductive medicine to move to the next level of its development.

The analysis and results summarized in this dissertation underline the truly remarkable qualities of the ovum, which is endowed with universal abilities to combine its own components and genetic material with that of the sperm entering it and give rise to a unique new organism and give rise to foreground its fundamental importance for the entire treatment process.

CONCLUSIONS

1. The age of the woman stands out as a prognostic factor with the highest reliability. In all age groups, an inverse correlation was reported between the average number of retrieved oocytes and the age of the treated patients.

2. No correlation was found between the number of oocytes and BMI values. Statistically lower pregnancy detection results are reported when overweight ≥ 25 kg/m^2 or obesity ≥ 30 kg/m^2.

3. Smoking is a factor that does not directly affect oocyte quality, and women who smoke do not report lower results after COC.

4. Serum FSH levels are a biological factor influencing oocyte competence. Values above 12 IU/l can be taken as a limit, where reliably lower results are found both in terms of the amount of harvested oocytes and the percentage of successful implantations.

5. Endometriosis affects oocyte production. A statistically higher number of oocytes are obtained in women who are clinically healthy compared to those with endometriosis.

6. Some of the laboratory factors related to the viability of oocytes and their fertilization potential in the past, such as the ICSI procedure and cryopreservation, are already losing their relevance in the conditions of the modern embryological laboratory.

7. The most common factors in the scientific literature could be managed by extrapolation into a convenient and easy-to-use tabular form. The management of factors by means of tabular visualization is a tool that directs the consulting specialist to an individual therapeutic approach depending on their presence and significance in the particular patient.

8. Factors with an internal influence can be divided into 2 subgroups - with an indirect influence (up to the follicular puncture), which do not directly influence the oocyte itself, and those that have a direct influence after the oocytes are aspirated outside the woman's body and directly onto them various manipulations are performed.

9. Through a series of targeted commands and questions, the AI generates a new classification of factors differentiated into 9 separate categories. All established factors are reflected in the classification created by AI, their aggregation approach is different from the standard one, which separates them into 2 large categories.

10. The OVOSCORE oocyte scoring system reliably reflects oocyte potential and competence. Embryos obtained from oocytes rated as good quality have better characteristics and chances of implantation.

11. Assisted oocyte activation by calcium ionophore is a suitable method for increasing fertilization capacity. A significantly higher percentage of fertilization is achieved by applying this methodology, which is also reflected in a higher proportion of pregnancies achieved.

12. Visualization of the dividing spindle in patients over 40 years old. with reduced ovarian reserve can give a reliable picture of the quality of their gametes. The application of the methodology does not lead to a significant increase in the success rate, but it can be a decisive factor when counseling the patient couple to switch to a program with donor oocytes.

CONTRIBUTIONS

1. For the first time in our country, the topic of the quality of the oocytes of Bulgarian patients treated by ART methods has been thoroughly developed.

2. For the first time, data is processed for such a large cohort of Bulgarian patients – 9016, and the results for 67,187 oocytes with a high representative value are analyzed.

3. The relationship between a number of factors, such as age, basal FSH levels, some diseases and the competence of oocytes has been proven - data that can serve to optimize the therapeutic approach.

4. The expired relevance of factors such as the ICSI procedure and cryopreservation in the modern embryology laboratory in accordance with the advancement of medicine and technology in the last decade is demonstrated.

5. A contribution is the study of the frequency of obtaining mature MII oocytes after hormonal stimulation, and the positive correlation with the total number of retrieved oocytes and factors related to lifestyle, various diseases, etc. has been proven.

6. An original contribution is the proposal to manage factors related to the potential of oocytes by extrapolating them in a convenient and easy-to-view tabular form, which would serve

reproductive specialists in the process of preparing a treatment strategy depending on the individual characteristics of the patient .

7. For the first time, work is being done on the topic of factors influencing oocyte competence using artificial intelligence.

8. An original contribution is the proposed new classification of factors generated by working with artificial intelligence.

9. An original contribution is the proposed new oocyte scoring system OVOSCORE, based on morphological features and physiological manifestations during fertilization with a high predictive value for fertilization and embryo quality.

10. Correlation and synchrony between morphological, physiological and molecular processes in the aging oocyte was investigated and established.

11. Some additional techniques have been investigated, having a positive effect on the fertilizing capacity of oocytes, which may underlie the therapeutic plan to optimize the outcome of ART in patients with previous failures.

THESIS RELATED PUBLICATIONS

1. The human ovum - historical review and development of assisted reproduction - I. Antonova, M. Yunakova, N. Magunska, T. Milacic, D. Ivanov - magazine GP news, no. , 2020

2. Patient-specific factors affecting the quality and fertilization potential of oocytes - Antonova I., Yunakova M., Magunska N., Boyadzhiev A., Dyulgerova-Nikolova D., Ivanov D., Journal of Reproductive Health, no. 33/2022

3. ART in Europe, 2018: results generated from European registries by ESHRE - European IVF Monitoring Consortium (EIM), for the European Society of Human Reproduction and

Embryology (ESHRE), C Wyns, C De Geyter at al.; Human Reproduction Open, Volume 2022, Issue 3, 2022, hoac022,

4. Oocyte cryopreservation for future reproduction - D. Dyulgerova – Nikolova, I. Antonova, L. Valkova, T. Milachich, T. Timeva, Embryology journal, (volume 13, issue 1/2023)

5. ART in Europe, 2019: results generated from European registries by ESHRE, - European IVF Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE), Jesper Smeenk, Christine Wyns, Christian De Geyter, Markus Kupka at al. Human Reproduction, 2023;, Volume 38, Issue 12, December 2023, Pages 2321–2338

6. Smoking as a factor affecting the quality and implantation potential of oocytes - Antonova I., Yunakova M., Yaneva G., Ivanov D., magazine Reproductive Health, no. 35/2024

7. Eating habits and female reproductive health - I. Antonova, magazine GP news, no. 3/2024

8. Lifestyle factors affecting human reproduction - I. Antonova, Medinfo magazine, no. 1/2024

9. Body mass index as a factor influencing oocyte competence and implantation potential distribution and results in 4,882 women undergoing fertility treatment - Antonova I., Ivanov D., Yaneva G., Magunska N., Duylgerova-Nikolova D., Yunakova M., Shterev A., Acta medica, volume 52 (2024), issue 2

THESIS RELATED PARTICIPATION

1. Body mass index and results of ART in Bulgarian patients; XIX Congress of BASRZ, Borovets, Bulgaria - plenary lecture

2. The oocyte or the endometrium? Who has the leading role for the impaired implantation rate in morbidly obese women?; 18th World Congress of the Academy of Human Reproduction, - Dublin Ireland – oral presentation

3. Atretic oocytes-frequency and factors which increase their production, Yunakova, M., I. Kostov, N. Magunska, and I. Antonova.; HUMAN REPRODUCTION, vol. 36, pp. 411-411. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND: OXFORD UNIV PRESS, 2021

4. Calcium ionophore activation improves ICSI outcome in patients with history of fertilization failure" 19th World Congress of the Academy of Human Reproduction - Venice, Italy - oral presentation