UDC 616.697-07:616.69-008.6

# Features of spermatogenesis disorders in infertile men depending on the applied ART

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The paper represents the results of analysis of the spermograms of 420 infertile men from married couples, which applied for the fertilization to the Institute of Reproductive Medicine (IRM) (Kyiv) during 2013-2015 in order to restore the fertility. The features of the parameters depending on the programs of applied assisted reproductive technologies are found. They are complicated by the severity of the pathospermia. Based on the quantitative assessment in points, the most informative significant parameters of the spermogram that determine the choice of technology have been selected.

Key words: male infertility, reproductive technologies, spermogram.

## Особенности нарушений сперматогенеза у бесплодных мужчин в зависимости от применения вспомогательных репродуктивных технологий А.О. Куценко

В статье представлены результаты анализа спермограмм 420 бесплодных мужчин из супружеских пар, которые подали заявку на оплодотворение в Институт репродуктивной медицины (IRM) (Киев) в 2013-2015 г. с целью восстановления фертильности. Найдены особенности параметров в зависимости от программ прикладных вспомогательных репродуктивных технологий. Они осложняются тяжестью патоспермии. На основании количественной оценки в баллах были выбраны наиболее информативные значимые параметры спермограммы, определяющие выбор технологии.

Ключевые слова: мужское бесплодие, репродуктивные технологии, спермограмма.

### Особливості порушень сперматогенезу у безплідних чоловіків в залежності від застосування допоміжних репродуктивних технологій А.О. Куценко

У статті наведені результати аналізу спермограми 420 безплідних чоловіків із подружніх пар, які протягом 2013—2015 р. подали заявку на запліднення до Інституту репродуктивної медицини (ІРМ) (м. Київ) з метою відновлення фертильності. Виявлено особливості параметрів залежно від програм застосованих допоміжних репродуктивних технологій. Вони ускладнюються тяжкістю патоспермії. На підставі кількісного оцінювання в балах було обрано найбільш інформативні значущі параметри спермограми, що визначають вибір технології. Ключові слова: чоловіче безпліддя, репродуктивні технології, спермограма.

 $\Gamma$  he assisted reproductive technologies (ART) have become widespread in clinical practice over the years to restore fertility in infertile couples with male factor [2, 12, 15]. The limited capacity of the conservative, surgical therapy in solving the problem of fertilization, regardless of the reasons, determine the attractiveness of the modern technologies [4, 14, 16]. Indeed, this is about achieving the desired result in the absence of treatment for the disease itself. The need for this increases with the technological development of the country, depends on the socio-economic, environmental, and political problems that cause the tendency towards deterioration of male sperm, as noted by many authors [8, 10]. As pointed out by S.I. Gamidov et al. (2016), it is this factor that determines the success of pregnancy by 50% [1, 3, 9, 18]. In the fertilization procedure by means of ART, the leading element of efficiency is the number of sperms, the sampling technology representing a separate aspect, and, most importantly, their functional capacity [13, 19, 20]. Current sperm preparation technologies guarantee the selection of the normal, functionally active sperm.

However, the information on the severity of spermatogenesis disorders, features of pathospermia and their relation to the ART methods is fragmentarily represented in the publications. While today it is the issue of adequate selection of the patients, clear definition of the spermatogenesis parameters that is the most essential when choosing the optimal program and this remains one of the topical directions of scientific and practical developments [7, 21–23].

The objective: to research the features of spermograms of the men who applied the ART to restore fertility in the infertile married couples.

#### **MATERIALS AND METHODS**

The object of study and clinical material was the results of spermograms of 420 men with infertility, for which reason the married couples applied for the ART to the Institute of Reproductive Medicine (IRM) (Kyiv) during 2013-2015. The diagnoses were verified on the basis of examination data according to the existing clinical protocols. According to the type of provided ART programs, three groups were identified: 1st group – by insemination by male sperm (IMS), 140 pairs; 2nd group - by intracytoplasmic sperm injection (ISI), 180 couples, and 3rd group – by intracytoplasmic morphologically selected sperm injection (IMSI), 100 couples.

When assessing the results, in addition to the WHO standardized and accepted parameters, the data of examination of the reference group of 80 «conditionally» healthy men who were similar in age to the main groups have been used in terms of population relevance. According to the issue under consideration, it should be noted that electron microscopy was applied in the study of the ejaculate, which increased the accuracy of visualization of sperm morphology compared to the light-optical level. The TUNEL test was applied to study the level of DNA fragmentation. Men with obstructive azoospermia (66 persons) underwent biopsy of the appendix. In cases of non-obstructive azoospermia (43 persons) in 65,0±7,2%, the TESA test was used, and PESA test for the rest  $-35,0\pm7,2\%$  (p<0,05). In addition, a modified Student's t-test was applied to determine the informational «value» of spermogram parameters. As a result, quantitative assessment of quality indicators became possible. In addition, the signs with boundary values were

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#### СЕКСОЛОГИЯ И АНДРОЛОГИЯ

Table 1

# Causes of the male infertility

| <b>-</b>                           |      | Total |     | Study groups |       |     |      |            |     |             |       |     |
|------------------------------------|------|-------|-----|--------------|-------|-----|------|------------|-----|-------------|-------|-----|
| Type of pathozoospermia            | obo  | %     | m   | IMS, n=140   |       |     | IC   | CSI, n=180 | )   | IMSI, n=100 |       |     |
| pathozoosperinia                   | abs. |       |     | abs.         | %     | m   | abs. | %          | m   | abs.        | %     | m   |
| Asthenozoospermia                  | 53   | 12,6  | 2,3 | 53           | 37,9  | 4,1 | -    |            |     | -           |       |     |
| Oligozoospermia                    | 48   | 11,4  | 1,5 | 48           | 34,3  | 3,5 | -    |            |     | -           |       |     |
| Teratozoospermia                   | 26   | 6,2   | 1,1 | 26           | 18,6  | 3,2 | -    |            |     | -           |       |     |
| Oligo-astheno-<br>teratozoospermia | 185  | 44,0* | 2,4 | 13           | 9,2   | 2,4 | 120  | 66,7       | 3,5 | 51          | 51,0  | 4,9 |
| Azoospermia                        | 109  | 25,7  | 2,1 | -            | -     | -   | 60   | 33,3       | 3,5 | 49          | 49,0  | 4,9 |
| Total                              | 420  | 100,0 |     | 140          | 100,0 |     | 180  | 100,0      |     | 100         | 100,0 |     |

Note: \* - the difference between the values in the column is reliable; p<0,05.

Table 2

# Types of azoospermia

|                 |      | Total |     |      | Study groups |     |      |       |     |  |  |
|-----------------|------|-------|-----|------|--------------|-----|------|-------|-----|--|--|
| Туре            | aha  | 0/    |     | ICSI |              |     |      | IMSI  |     |  |  |
|                 | abs. | %     | m   | abs. | %            | m   | abs. | %     | m   |  |  |
| Obstructive     | 66   | 60,6* | 4,6 | 38   | 63,3*        | 6,2 | 28   | 57,1  | 7,0 |  |  |
| Non-obstructive | 43   | 39,4  | 4,6 | 22   | 36,7         | 6,2 | 21   | 42,9  | 7,0 |  |  |
| Total           |      | 100,0 |     | 60   | 100,0        |     | 49   | 100,0 |     |  |  |

*Note:* \* – the difference between the values in the column is reliable; p<0.05.

# Level of the gonadotropic hormones in men with azoospermia depending on its type

Table 3

| Indicators                     |          | obstructive                |                            | non-obstructive |                            |                            |  |  |  |  |  |
|--------------------------------|----------|----------------------------|----------------------------|-----------------|----------------------------|----------------------------|--|--|--|--|--|
| mulcators                      | Total    | 2 <sup>nd</sup> group ICSI | 3 <sup>rd</sup> group IMSI | Total           | 2 <sup>nd</sup> group ICSI | 3 <sup>rd</sup> group IMSI |  |  |  |  |  |
| FSH-normal value, 3,5-6,0 IU/I | 5,0±0,5  | 5,2±0,1                    | 4,7±0,9                    | 17,8±3,5        | 15,2±2,2                   | 20,3±2,5*                  |  |  |  |  |  |
| LH-normal value, 4,0-9,0 IU/I  | 6,6±1,0  | 6,1±0,3                    | 7,1±0,7                    | 13,4±3,2        | 11,8±1,1                   | 14,9±1,8*                  |  |  |  |  |  |
| Testosterone, 16,0-20,0 nmol/l | 18,0±0,8 | 18,3±0,8                   | 17,6±1,1                   | 12,0±2,4        | 13,2±1,2                   | 10,3±1,1*                  |  |  |  |  |  |
| FSH /LH                        | 2,6±1,0  | 2,2±0,9                    | 2,8±1,1                    | 3,2±0,7         | 2,8±0,3                    | 3,7±0,4 <sup>△*</sup>      |  |  |  |  |  |
| LH/ testosterone, U 100,0      | 20,5±1,2 | 18,3±1,3*                  | 22,9±0,8 <sup>Δ*</sup>     | 34,0±4,3*       | 31,0±2,1*                  | 37,0±1,8 <sup>△</sup> *    |  |  |  |  |  |
| inhibin-B, pg/ml               | 71,3±2,7 | 73,2±2,1                   | 69,5±2,8                   | 72,4±3,2        | 79,2±3,5                   | 65,1±3,9 <sup>△</sup>      |  |  |  |  |  |
| Estradiol, pg/ml               | 35,5±1,3 | 32,7±2,4                   | 38,2±2,9                   | 39,1±2,9        | 37,2±2,5                   | 39,9±3,1                   |  |  |  |  |  |

Note: \* – the difference between the indicators in the study groups is reliable; ^\* – the difference between the indicators is reliable; p<0,05.

identified for each of them. The basis for the calculations was the frequency of the presence of one or another sign in case of a negative result relative to such sign among the total number of observations.

The analysis of the obtained results will be presented in a comparative aspect according to the ART programs. It required statistical processing of the material, for which purpose the known variation statistics methods were involved. According to the parameters of laboratory examinations, the statistical series were compiled with values which were, first of all, assessed by a system of indicators: level, increase or decrease, growth or decrease rate, increase rate. The average values were found for summarization of the indicators by parameters. The fluctuation degree of the series and typical average values was characterized by a standard error. The known Student's t-test was calculated to determine the statistical validity of the comparative indicators.

#### **RESULTS AND THEIR DISCUSSION**

Based on the assumption that the spermatogenesis parameters play a leading role in solving the tasks that are facing prior to the selection of the ART method, as well as in achieving the effectiveness of the latter, we begin the structure of material presentation from the generalized data on the immediate causes of infertility. The Table 1 represents the types of patients' pathospermia depending on the ART programs.

The data of the Table 1 demonstrate that every fourth person had the most medically complicated pathology in the form of

azoospermia (109 – 25,7 $\pm$ 2,1%). All of them needed modern technology – most probably IMSI (49,0 $\pm$ 4,9% vs. 33,3 $\pm$ 3,5% ICSI). Oligo-astheno-teratozoospermia was found in 185 men (44,0 $\pm$ 2,4%) and clearly prevailed among others. It was found in 120 persons (64,9 $\pm$ 3,5%) from ICSI group, 51 persons (51,0 $\pm$ 4,9%) from IMSI group, and only 13 persons (9,2 $\pm$ 2,4%) were included into IMS program (p<0,05). The men with single changes in sperm (astheno-, oligo-, teratozoospermia) accounting for 30,2% (127) of the total number were selected for IMS method [17].

Next, we study the azoospermia cases. The Table 2 represents the data of its distribution by type.

As can be seen from the Table 2, the obstructive azoospermia is statistically represented by a large number of cases; the ratio remains among men from 2nd group, and in 3rd group the reliability is leveled, but the predominance remains. The cause of obstructive azoospermia in almost all patients was fibrosis of the seminiferous tubules due to chronic inflammatory process of the genitals. The main indicators of hormonal status (FSH, LH, testosterone, FSH/LH ratio, LH/testosterone; inhibin-B and estradiol) were within the standardized values. Another picture was found in non-obstructive azoospermia of the secretory-endocrine variant of infertility.

For known reasons, the special attention was paid to men with idiopathic infertility; there were 121 of them, representing 28,8%. Statistically fewer cases were in 1st group  $(24 \text{ of } 140 - 17,1\pm2,8\%)$ , and in 2nd and 3rd groups there were 58  $(32,2\pm3,4\%)$  and 39  $(39,0\pm4,7\%)$ 

Indicators of the spermogram of healthy and infertile men subject to study groups

|                                  |                  |                        | Study groups                         |  |  |  |  |  |
|----------------------------------|------------------|------------------------|--------------------------------------|--|--|--|--|--|
| Parameters                       | Healthy,<br>n=80 | Total,<br>n=420        | 1 <sup>st</sup> group –IMS,<br>n=140 | 2 <sup>nd</sup> group – ICSI,<br>n=180 | 3 <sup>rd</sup> group – IMSI,<br>n=100 |  |  |  |
|                                  | M±m              | M±m                    | M±m                                  | M±m                                    | M±m                                    |  |  |  |
| Volume, ml                       | 2,9±0,6          | 2,4±0,7                | 3,1±0,9                              | 2,5±0,7                                | *1,7±0,3                               |  |  |  |
| Concentration, mln/ml            | 81,0±13,2        | 31,5±10,6 <sup>△</sup> | 41,9±14,5                            | 34,5±12,8                              | **18,2±14,2                            |  |  |  |
| Total number of sperm cells, mln | 189,3±18,1       | 87,7±18,1 <sup>△</sup> | 119,3±20*                            | 83,7±10,5*                             | 64,0±23,5                              |  |  |  |
| Sperm motility, %, including     | 78,8±4,5         | 49,5±2,4 <sup>△</sup>  | 59,3±4,1                             | 52,2±3,7                               | △△38,0±4,8                             |  |  |  |
| category a, %                    | 22,6±5,2         | 9,6±2,0 <sup>△</sup>   | 12,8±3,6                             | 8,7±2,9                                | 6,1±3,8                                |  |  |  |
| category b, %                    | 43,6±6,2         | 25,0±3,0 <sup>△</sup>  | 25,5±4,7                             | 27,4±4,6                               | 23,7±6,8                               |  |  |  |
| category a+b, %                  | 66,2±5,9         | 34,7±3,3 <sup>△</sup>  | 38,3±5,3                             | 36,1±4,9                               | 29,8±7,4                               |  |  |  |
| category c, %                    | 12,5±4,2         | 10,8±2,1               | 11,6±3,5                             | 14,5±3,7                               | 8,0±4,4                                |  |  |  |
| category d, %                    | 21,2±5,1         | 50,5±3,5 <sup>△</sup>  | 40,7±5,4                             | 48,0±5,1                               | 62,0 <sup>ΔΔ</sup> ±7,8                |  |  |  |
| Alive forms, %                   | 76,3±3,7         | 56,1±2,4 <sup>△</sup>  | 66,9±3,9*                            | 46,1±3,4*                              | 33,0±4,7*                              |  |  |  |
| Morphologically normal forms, %  | 71,2±5,0         | 45,5±2,4 <sup>△</sup>  | 52,9±4,2                             | 46,1±3,7                               | **38,0±4,8                             |  |  |  |
| Head pathology, %                | 19,2±4,4         | 32,0±2,3 <sup>△</sup>  | 28,6±3,5*                            | 35,2±3,5                               | 39,0±4,8                               |  |  |  |
| Midpiece pathology, %            | 3,8±1,7          | 5,2±1,0                | 4,3±1,6                              | 5,0±1,6                                | 7,0±2,5                                |  |  |  |
| Tail pathology, %                | 5,0±1,9          | 6,7±1,1                | 5,3±1,0                              | 7,2±1,7                                | 7,5±1,8                                |  |  |  |
| DNA fragmentation, %             | 8,8±3,2          | 42,6±2,4 <sup>△</sup>  | 25,0±3,6*                            | 45,0*±3,7                              | 57,0±4,9*                              |  |  |  |

*Note*: \* – the difference between all indicators in the groups is reliable; \*\* – the difference between the indicators of 1st and  $3^{rd}$  groups is reliable; p<0,05;  $^{\triangle}$  – the difference between the indicators of healthy and infertile men is reliable; p<0,05;  $^{\triangle}$  – the difference for this indicator is reliable; p<0,05.

cases respectively, the difference is not significant. The tendency of predominance of the primary infertility was found, which was preserved in all study groups:  $57,1\pm4,2\%$ ;  $60,0\pm3,6\%$  and  $54,0\pm4,9\%$  [5].

The following is the most practically significant indicators of the gonadotropic hormones in patients with consideration to the type of azoospermia (Table 3).

The Table 3 confirms the abovementioned position regarding the absence of changes in hormone levels in case of obstructive azoospermia. In contrast, in case of non-obstructive azoospermia, the FSH and LH values are not only statistically higher than normal. The imbalance of their function, which causes a decrease in reproductive capacity, is also evidenced by indicators such as the ratio of FSH/LH, LH/testosterone, inhibin-B. In addition, a difference is found between the average figures of their values among men of 2nd and 3rd groups. A similar, however, opposite in direction situation is observed regarding the testosterone levels. The features indicating the impaired sperm secretion at the level of endocrine function of the testicles are found.

It is generally acknowledged that one of the leading methods of objective general assessment of the men's fertility status is classical semen analysis. The results of such study in men with infertility, including in the study groups, as well as among "conditionally" healthy similar population are presented in Table 4.

When interpreting the data of the Table 4, take note of the significant changes in parameters. We consider two indicators such as concentration and total sperm count at once in comparison. Both are considered significant in the literature, with the predominance of one or another according to different authors.

The data obtained testify to the validity of the difference in the values of indicators in infertile and healthy men. Moreover, it is also present in study groups. The patients with more severe parameters require more sophisticated ART. However, according to our data, the concentration of sperm in million of ejaculates changes more intensively and more clearly than their total number. Accordingly, the value decrease rate in men with infertility was 61% and 53,6%. A statistically significant difference is expectedly inherent in the values of the percentage of mobile spermatozoids. Moreover, among the total number of infertile men, it was in all forms (a, b, a + b, c, d).

However, in terms of groups, such a feature was only in the 3rd group in one category – there were significantly more immobile

forms. It should be noted that the actively mobile forms change most of all; their rate is more than half that in infertile men  $(9,6\pm2,6\% \text{ vs. } 22,6\pm5,2\%; \text{p}<0,05)$  and, at the same time, is more than half that in immobile forms:  $50,5\pm3,5\% \text{ vs. } 21,2\pm5,1\% \text{ (p}<0,05)$ .

The specific volume of alive sperm also acquired a statistically significant difference, which is typical of all three groups. Note the linear relationship between the percentage of mobile and alive spermatozoids. Among their morphologically changed forms, only the head pathology differed significantly among other structures (32,0±2,3% vs. 19,2±4,4% in healthy men). Moreover, the smallest such changes were found in patients of the 1st group (28,6±3,5% vs. 35,2±3,5% and 39,0±4,8% in 2nd and 3rd groups; p<0.05). The opposite pattern was found in the calculation of morphologically normal sperm. In this case, their smallest quantity was in 3rd group, and the largest one was in 1st group (38,0±4,8 vs. 52,9±4,2%; p<0,05). As a result, among all infertile men, the average value was 45,5±2,4% and was significantly lower than in healthy men (71,2±5,0%).

The high sensitivity and specificity of sperm DNA fragmentation proved by the authors [13] was confirmed by the results of the study. This indicator in infertile patients more than 4,5 times exceeded the value found in the clinical material of healthy persons, and was 46,8% higher than the standardized value ( $\leq$ 29%). The highest proportion of sperm DNA fragmentation was observed in 3rd group (57,0 $\pm$ 4,9%), the lowest one – in 1st group (25,0 $\pm$ 3,6%). The foregoing data, especially in terms of group variability, justify the need for an optimal approach to the selection of the ART method based on the personification principle.

The results of electronic microscopic examination of sperm also lead to the same conclusion. Compared to light microscopy, the method has more resolving powers, which makes the ultrastructures better visualized, and therefore, the pathological changes in the sperm structure are more frequently detected. The Table 5 presents the information obtained in terms of the study groups.

We comment the data of the Table 5. The percentage of pathological forms among the total population of sperm obtained from healthy males did not exceed 30.0% ( $28.7\pm5.0\%$ ) and was almost half that of infertile men ( $53.6\pm2.4\%$ ); p<0.05. Gradually, the value increases in each following group and reaches the value in 3rd group, which is statistically higher than the others ( $62.0\pm4.8\%$  vs.

#### СЕКСОЛОГИЯ И АНДРОЛОГИЯ

Table 5

# Morphological indicators of the spermogram of healthy and infertile men subject to study groups

|                                      | Car  | aditiono                       | llse |      |              |      |      |                                     |      | Stu  | ıdy gro                              | ups  |      |                                      |      |  |
|--------------------------------------|------|--------------------------------|------|------|--------------|------|------|-------------------------------------|------|------|--------------------------------------|------|------|--------------------------------------|------|--|
| Parameters                           |      | Conditionally<br>healthy, n=80 |      |      | Total, n=420 |      |      | 1 <sup>st</sup> group IMS,<br>n=140 |      |      | 2 <sup>nd</sup> group ICSI,<br>n=180 |      |      | 3 <sup>rd</sup> group IMSI,<br>n=100 |      |  |
|                                      | abs. | %                              | m    | abs. | %            | m    | abs. | %                                   | m    | abs. | %                                    | m    | abs. | %                                    | m    |  |
| Pathological forms                   | 23   | 28,7                           | 5,0  | 225  | 53,6         | 2,4∆ | 66   | 47,1                                | 4,2  | 97   | 52,9                                 | 3,7  | 6,2  | 62,0                                 | 4,8  |  |
| Head pathology, including            | 3    | 56,5                           | 10,3 | 164  | 72,9△        | 2,9  | 41   | 62,1                                | 5,9* | 71   | 73,2                                 | 4,4* | 52   | 83,9                                 | 4,6* |  |
| conical                              | 1    | 7,7                            |      | 2    | 1,2          | -    | -    | -                                   | -    | 2    | 2,8                                  | -    | -    | -                                    | -    |  |
| pear-shaped                          | 1    | 7,7                            | -    | 3    | 1,8          | -    | 1    | -                                   | -    | 3    | 4,2                                  | -    | -    | -                                    | -    |  |
| small                                | 2    | 15,4                           | -    | 2,1  | 12,8         | -    | 7    | 17,1                                | -    | 9    | 12,6                                 | -    | 5    | 9,6                                  | -    |  |
| amorphous                            | 2    | 15,4                           | -    | 19   | 11,6         | -    | 7    | 17,1                                | -    | 7    | 9,9                                  | -    | 5    | 9,6                                  | -    |  |
| vacuolated                           | 1    | 7,7                            | -    | 3    | 1,8          | -    | 1    | 2,4                                 | -    | -    | -                                    | -    | 2    | 3,8                                  | -    |  |
| small acrosomal area                 | 2    | 15,4                           | -    | 17   | 10,4         | -    | 6    | 14,6                                | -    | 9    | 12,7                                 | -    | 2    | 3,8                                  | -    |  |
| absent acrosome (globulozoospermia)  | 3    | 23,1                           | 11,6 | 91   | 55,5         | 3,8△ | 18   | 43,9                                | 7,7  | 38   | 53,5                                 | 5,9  | 35   | 67,3                                 | 6,5  |  |
| doubled                              | 1    | 7,7                            | -    | 8    | 4,9          | -    | 2    | 4,9                                 | -    | 3    | 4,2                                  | -    | 3    | 5,8                                  | -    |  |
| Midpiece pathology, including        | 8    | 34,8                           | 9,9  | 22   | 9,8△         | 1,9  | 1,4  | 21,2                                | 5,0  | 6    | 6,2                                  | 2,4  | 2    | 3,3                                  | 2,2  |  |
| bent                                 | 1    | 12,5                           | -    | 1    | 4,5          | -    | -    | -                                   | -    | 1    | 16,7                                 | -    | -    | -                                    | -    |  |
| asymmetric                           | 3    | 37,5                           | -    | 8    | 36,4         | -    | 6    | 42,9                                | -    | 2    | 33,3                                 | -    | -    | -                                    | -    |  |
| thick attachment                     | 1    | 12,5                           | -    | 2    | 9,1          | -    | 2    | 14,3                                | -    | -    | -                                    | -    | -    | -                                    | -    |  |
| thin                                 | 3    | 37,5                           | 17,1 | 11   | 50,0         | 10,6 | 6    | 42,9                                | 13,2 | 3    | 50,0                                 | 20,4 | 2    | 100,0                                | -    |  |
| Tail pathology, including            | 2    | 8,7                            | 5,8  | 39   | 17,3         | 2,5△ | 11   | 16,7                                | 4,5  | 20   | 20,6                                 | 4,1  | 8    | 13,0                                 | 4,3  |  |
| short                                | -    | -                              | -    | 8    | 20,5         | -    | 3    | 27,3                                | -    | 4    | 20,0                                 | -    | 1    | 12,5                                 | -    |  |
| bent                                 | -    | -                              | -    | 6    | 15,4         | -    | 2    | 18,2                                | -    | 3    | 15,0                                 | -    | 1    | 12,5                                 | -    |  |
| twisted                              | 1    | 50,0                           | -    | 13   | 33,3         | -    | 3    | 27,3                                | -    | 7    | 35,0                                 | -    | 3    | 37,5                                 | -    |  |
| stranded                             | 1    | 50,0                           | -    | 12   | 30,8         | -    | 3    | 27,3                                | -    | 6    | 30,0                                 | -    | 3    | 37,58                                | -    |  |
| Cytoplasmic droplet >1/3 of the head | 4    | 5,0                            | 4,5  | 31   | 7,4          | 1,7  | 8    | 5,7                                 | -    | 15   | 8,3                                  | -    | 8    | 8,0                                  | -    |  |

Note: \* – the difference between all indicators in the groups is reliable; \*\* – the difference between the indicators of 1st and 3rd groups is reliable; p<0,05;  $^{\triangle}$  – the difference between the indicators of healthy and infertile men is reliable; p<0,05;  $^{\triangle}$  – the difference for this indicator is reliable; p<0,05.

47,1 $\pm$ 4,2% and 52,9 $\pm$ 3,7% respectively in the 1st and 2nd group). In terms of the structure of pathological forms, the head defects predominate. They account for 56,5 $\pm$ 10,3% of the total number of healthy persons (23 of 80). The value of the indicator increases to 72,9 $\pm$ 2,9% in the study of sperm of infertile patients and is typical feature for each subsequent group (62,1 $\pm$ 5,9%, 73,2 $\pm$ 4,4% and 83,9 $\pm$ 4,65 in 1st, 2nd, and 3rd groups, respectively; p<0,05.

Among the diversity of variations of the head changes, they manifested themselves everywhere in the following forms: small, amorphous, with a small acrosomal area and absence of acrosome (globulozoospermia). The latter prevails in the head pathology structure, and reaches significant values with others.

The following observation is interesting. Against the background of the overwhelming damage to the sperm head, even in conditionally healthy men, a clear relationship in the frequency of changes in the neck and tail could not be found. However, the specifics are found in the study groups. In the 1st group, the pathology of the midpiece prevailed  $(21,2\pm5,0\% \text{ vs. } 6,2\pm2,4\% \text{ and } 3,2\pm2,2\%$  in 2nd and 3rd groups, respectively; p<0,05), and in other two groups the tail pathology prevailed. Among the varieties of midpiece changes, according to our data the most significant changes were thin and asymmetrical shapes; among the tail changes – twisted, doubled, short. In addition, it is worth to point out the observed trend towards increase in cases where cytoplasmic droplets is larger than 1/3 of the head of sperm in infertile men and which appears more often in the 2nd, 3rd groups as compared with the 1st group. This feature is an additional negative criterion,

which results in the sperm's inability to penetrate into oocyte and reduces the probability of fertilization.

In order to achieve the goal of clarifying the specifics of infertile men's spermogram, the informational significance of its main indicators and indicators reflecting their hormonal status was determined (Table 6).

It was found on the basis of the results of work that the most significant adverse signs include: the sperm concentration up to 15 mln/ml, the presence of pathological forms >90% and the FSH/LH ratio – their quantitative assessment was 6–6,4 points. Influential cases include estradiol (E2)  $\geq$ 35 pg/ml and inhibin B  $\leq$ 80 pg/ml – 4 points each. The indicators of motility (a + B), alive forms  $\leq$ 58%, and immature sperm  $\geq$ 90% have up to 3 points in terms of «significance» of the negative influence.

Thus, according to the results of study of ejaculate samples of the infertile men, the structure of pathozoospermia types was clarified. One out of four men has azoospermia, about half of cases have oligoasthenoteratozoospermia. The way of collecting material for ART in cases of non-obstructive azoospermia, which is substantiated by the frequency  $(44,2\pm7,5\%)$  of negative results remains important issue. The features of spermograms of infertile men are found, including depending on the applied ART methods. It has been observed that they are complicated by the severity of pathospermia. On the basis of the data of quantitative assessment (in points) of the most informative significant parameters of the spermogram, the determining parameters were chosen for selection of the technologies.

#### СЕКСОЛОГИЯ И АНДРОЛОГИЯ

 ${\it Table~6}$  The results of informational assessment of the main special indicators of the examination of infertile men who have applied for ART

| Nº | Factors                | Signs    | Points | Nº  | Factors          | Signs    | Points |
|----|------------------------|----------|--------|-----|------------------|----------|--------|
| 1. | Sperm concentration    | below 15 | + 6,0  | 7.  | FSH              | 1,5–12,4 | - 3,0  |
| 1. | (mln/ml)               | ≥ 15     | - 3,5  | 7.  | (IU/mI)          | ≥12,5    | + 3,1  |
| 2. | Motility 0/            | ≥ 25     | - 3,5  |     | FCII/III retie   | <2,5     | - 4,6  |
| ۷. | Motility %             | <25      | + 0,2  | 8.  | FSH/LH ratio     | ≥25      | + 6,4  |
| 3. | а+в                    | ≥ 50     | - 6,8  | 9.  | Estradiol (U2)   | <35      | - 3,0  |
| ٥. | а⊤в                    | <50      | + 2,8  | 9.  | pg/ml            | ≥35      | + 4,1  |
| 4. | Pathological forms (%) | ≤ 90     | - 8,3  | 10. | Inhibin B        | >80      | - 3,6  |
| 4. | Pathological forms (%) | > 90     | + 6,0  | 10. | pg/ml            | ≤80      | + 4,2  |
| 5. | Immedium anarm (0/)    | < 90     | - 2,0  | 11  | LH/ testosterone | ≤20      | - 0,5  |
| 5. | Immature sperm (%)     | ≥ 90     | + 2,0  | 11. | Units 100        | >20      | + 2,2  |
| 6. | Alivo formo (%)        | > 58     | - 3,7  |     |                  |          |        |
| 0. | Alive forms (%)        | ≤ 58     | + 3,0  |     |                  |          |        |

#### CONCLUSION

It was found that the overwhelming majority of infertile men  $(44,0\pm2,4\%)$  had oligoasthenoteratozoospermia, every fourth man  $(25,7\pm2,1\%)$  had azoospermia, among which  $60,6\pm4,6\%$  accounted for obstructive form, and the rest  $(30,3\pm2,2\%)$  had isolated changes in sperm.

The direct relationship between the severity of pathozoospermia and ART methods was observed: in the ICSI group, the oligoasthenoteratozoospermia is significantly more frequent  $-66,7\pm3,5\%$  vs.  $51,0\pm4,9\%$  for ICSI and  $9,2\pm2,4\%$  for IMS; in the IMSI group, azoospermia was  $49,0\pm4,9\%$  vs.  $33,3\pm3,5\%$  for ICSI and absent for IMS; in the IMS group in 90,8% cases the disorders were in the form of astheno-, oligo and teratozoospermia.

The sperm parameters of infertile men who have undergone the greatest changes and the degree of which is consistent with the com-

plexity of the applied ART methods have been determined. They include the sperm concentration, the level of which changes more intensively than their total number; statistically significant typical difference of motile forms of sperm. In addition, the pathological forms of sperm are twice as common as in healthy men and reach 62,0±4,8% for IMSI vs. 52,9±3,7% and 47,1±4,2% for ICSI and IMS, respectively. The structure of pathological forms is dominated by the defects of the head, the frequency of which is a typical feature of each group following in terms of complexity. However, a clear relationship in the frequency of changes in the midpiece and tail were not researched. The high sensitivity and specificity of sperm DNA fragmentation has been observed.

The quantitative assessment of the spermogram parameters was carried out, and the most informative significant parameters were found on its basis. The data obtained suggest a more precise definition of the criteria for selection of ART programs.

# Сведения об авторе

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Статья поступила в редакцию 23.09.2019