

Effect of Prostate Inflammation on Ejaculate Indicators in Men of Various Ages from Infertility Couples

Elena G. Novikova^{1,2*} , Vera G. Selyatitskaya¹ , Igor I. Tityaev³ 

¹Federal Research Center of Fundamental and Translational Medicine, Novosibirsk, Russia

²Regional Medical and Diagnostic Centre Ltd., Novosibirsk, Russia

³State Budgetary Institution of Healthcare, Novosibirsk's Region, City Hospital No. 1, Novosibirsk, Russia

Email: *rmdc.nsk@gmail.com, ccem@centercem.ru, k.udalov@mail.ru

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Abstract

The question of the impact of chronic prostatitis on male fertility remains debatable. In our study, we proved that inflammation of the prostate affects the characteristics of a man's ejaculate. The inflammatory process negatively affects the reproductive characteristics of men of all ages, however, in the older age group, its clinical and laboratory manifestations are most pronounced.

Keywords

Spermatogenesis, Prostatitis, Inflammation, Male Infertility

1. Introduction

For many years, infertility remains a global problem not only in medical, but also in the social area, despite the rapidly developing reproductive technologies. Worldwide, it affects about 100 - 200 million couples of reproductive age. The male factor of infertility in a couple is about 50% in general statistics [1] [2] [3]. In the Russian Federation, the number of men with infertility more than doubled from 2000 to 2018 [4]. A group of authors from different countries in the work of 2017 presented statistical data on the number of spermatozoa in the ejaculate of men from different continents of the Earth for the period 1973-2011. More than 50% decreased fertility of men in North America, Europe, and Australia. These results strongly suggest a significant deterioration in male reproductive health, with serious consequences beyond fertility problems. Research into the causes and consequences of this decline is urgently needed [5].

Male infertility is considered to be a complex disease that has many causes,

ranging from genetic mutations to lifestyle, comorbidities, or iatrogenic problems [6].

Male fertility requires not only full-fledged spermatogenesis, but also coordinated functions of the genitourinary system organs and all accessory glands of the male genital tract [7]. However, the question of the relationship between genitourinary system diseases, in particular, between chronic inflammation of the prostate and impaired fertility, remains controversial [8] [9].

There are works that dispute the relationship between prostatitis and sperm characteristics [10]. According to several experts from the European Association of Urology [11], there is no convincing evidence that chronic prostatitis negatively impacts the sperm quality and causes male infertility.

On the contrary, according to other experts, the idea of the negative impact of inflammation of the prostate and other accessory glands of male urogenital tract on the ejaculate quality and, as a result, fertility, is completely justified [12]. The prevalence of male infertility associated with infection of the male genital tract ranges from 10% to 35% of the total number of infertile men [13]. If we include those men who have suffered from prostatitis at some point in their lives, the prevalence of infertility increases to 50%, and the condition becomes chronic in 10% of cases [14]. It can be assumed that for the diseases such as orchitis, epididymitis [15], and prostatitis [16], due to the prolonged infection and inflammation of the male genital tract, the spermatozoa are directly exposed to activated leukocytes and their products during a long period of their maturation. It is likely that leukocytes in seminal fluid not only impair spermatozoa motility and reduce their ability to fertilize, but also can induce apoptosis in human spermatozoa [14]. The inflammation can disrupt the physiological functions of the prostate, and change the properties of the secret produced by its epithelial cells that make up about 30% of the ejaculate volume [17]. According to Robertson *et al.* [18], the key contribution of prostatic fluid to the maintenance of male fertility is related to its role as a trigger of molecular pathways involved in ejaculation, sperm activation and capacitation, as well as in the stimulation of gene expression, cellular changes and tissue remodeling in the female reproductive tract and the immune system.

The effect of inflammation on the ejaculate characteristics in the age aspect has not been sufficiently studied, although it appears to be important in connection with the trend towards late fatherhood: over the past few decades the age of fathers increased in many countries [19]. For example, in Russia, according to RosStat, the proportion of fathers aged 35 to 45 increased from 25% in 1999 to 40% in 2009 [20].

The aim is to study the effect of inflammation of the prostate gland on the ejaculate characteristics of men of different ages from infertile couples.

2. Materials and Methods

The work performed was based on the informed consent of the subjects and in

accordance with the ethical standards of the Declaration of Helsinki (WMA, Edinburgh, Scotland, 2000), see also explanatory note on p. 29, approved by the WMA General Assembly (Washington, 2002) [21].

The study included 266 men aged 20 to 45 who consulted with an andrologist on the problem of pregnancy absence in their couple. Patients were examined according to the generally accepted scheme based on the recommendations of the Russian Society of Urology 2020 [22], including collecting information on complaints, anamnesis data, general examination, and physical methods. Spermological analysis of the subjects was carried out by the International network of independent clinical diagnostic laboratories in Novosibirsk "CITILAB" taking into account the WHO recommendations of 2010 [23] using the analytical system JEM-1400, JEOL (Japan); analytical sensitivity of test systems—100 nm, analytical specificity of test systems—1 - 100 cells. The general properties of the ejaculate, the concentration and number of spermatozoa, their mobility and morphology according to Kruger, the concentration of leukocytes and the microbiology of the ejaculate for opportunistic microflora were evaluated. Additionally, a MAR test was performed. DNA fragmentation in spermatozoa was studied by the TUNEL method.

Transrectal ultrasound examination of the prostate, seminal vesicles, and bladder was carried out using the Sonoscape S20 Pro ultrasound unit (China).

All patients were interviewed using the scale of symptoms of chronic prostatitis and pelvic pain syndrome in men according to the US National Institutes of Health (NIH-CPSI).

Criteria for inclusion in the study: infertility in a couple for more than a year of regular sexual life without contraception; the presence of the ejaculate characteristics changes at the spermogram compared with the reference values; the absence of a female factor of infertility.

Criteria for non-inclusion in the study: the presence of sexually transmitted infections, positive tests for HIV, syphilis, and hepatitis; chromosomal abnormalities, cryptorchidism, and oncological diseases, including those in the anamnesis.

3. Methods of Statistical Data Processing

The Shapiro-Wilk test was used to assess the type of parameters distribution. Due to the absence for most parameters of a normal distribution of their values, the results of the analysis are presented as a median, 25 and 75 percentiles. Qualitative values of the parameters are presented as observed frequencies and percentages. Multiple comparisons of parameter values between subgroups were performed using the 2-tailed Kruskal-Wallis h-test, critical level of statistical significance: $p < 0.05$.

Processing and graphical presentation of data was carried out using computer programs Statistica 12.0 from StatSoft Corporation (USA) and Microsoft Office Excel, 2017 (USA).

4. Results

For all examined infertile men the average percentage of viable spermatozoa was 60%, and the average percentage of spermatozoa with normal Kruger morphology was 2%.

At the first stage of the study all men ($n = 266$) were divided into 2 groups: under 35 years old ($n = 133$) and older than 35 years old ($n = 133$). Further, each group was divided into subgroups, considering the number of leukocytes in the ejaculate: with laboratory signs of the inflammatory process of the gonads in the form of leukospermia (>1 million/ml) and without these signs (leukocytes < 1 million/ml).

In the group of men under 35 years old, 44 subjects (33%) had leukospermia, 89 subjects (67%) had a normal number of leukocytes in the ejaculate. In the group of men older than 35 y.o. leukospermia was detected in 36 people accounting for 27%; without signs of inflammation—97 people, *i.e.* 73%, respectively. The mean age of men under 35 y.o., both without leukospermia and with leukospermia, did not differ significantly and amounted to 31 [29; 32] in both subgroups. The average age of men in the group over 35 y.o. without leukospermia was 38 [37; 41], and with leukospermia 41 years [38; 43], respectively.

On the leukocytes number in the ejaculate, a high significance of differences was shown between all subgroups. Thus, the number of leukocytes in the group of men under 35 y.o. without leukospermia was 0 million/ml [0; 0.5], with leukospermia—2.9 million/ml [1.3; 5.1], $p < 0.0001$; the number of leukocytes in the group of men older than 35 y.o. without leukospermia was 0.01 million/ml [0; 0.4] and 1.4 million/ml [1.1; 2.1], $p < 0.0001$.

Characteristics of the spermogram for all four subgroups are presented in **Table 1**. The volume of ejaculate did not differ between subgroups with/without leukospermia in the group of younger men, and in the group of older men the presence of leukospermia was associated with a decrease in ejaculate volume. The decrease in ejaculate volume was most noticeable in the subgroup of older men with leukospermia relative to the same subgroup of younger men.

Total sperm count also did not differ significantly between the subgroups with/without leukospermia in the younger group, although in the leukospermia subgroup this value tended to decrease; in the subgroup of older men the presence of leukospermia was associated with an almost threefold decrease in the total number of spermatozoa relative to the subgroup without leukospermia of the corresponding age. As a result, the sperm concentration was reduced in the subgroups with leukospermia relative to the corresponding subgroups without leukospermia in men of both groups.

For total sperm motility, as in the case of concentration, the value tended to decrease in subgroups with leukospermia relative to subgroups without leukospermia in the corresponding age groups. A similar situation was noticed in relation to the values of progressively motile spermatozoa, and in the subgroup with leukospermia of the older age group, this decrease was 60% and was found statistically significant.

Table 1. Values of spermogram parameters depending on the presence or absence of leukospermia in men of different age groups.

Index	<35 y.o. (n = 133)		>35 y.o. (n = 133)		p between groups and subgroups			
	L < 1 mln/ml	L > 1 mln/ml	L < 1 mln/ml	L > 1 mln/ml	1 - 2	1 - 3	2 - 4	3 - 4
	subgroup 1 n = 89 (67%)	subgroup 2 n = 44 (33%)	subgroup 3 n = 97 (73%)	subgroup 4 n = 36 (27%)				
Ejaculate volume, ml	3.2 [2.5; 4.3]	3.8 [2.2; 4.3]	3 [2.4; 4]	2 [1.5; 3.4]	1.0000	0.9895	0.0028	0.0205
Total sperm count in ejaculate, mln	96.8 [48; 158.4]	74.7 [14.4; 108]	91.2 [18.1; 190]	32.75 [6.8; 121]	0.1772	1.0000	1.0000	0.3045
Sperm concentration, mln/ml	28 [12.5; 53]	15.6 [2.8; 43]	27.32 [10; 65]	22 [3.82; 56.84]	0.3314	1.0000	1.0000	1.0000
Total motility, %	50 [35; 59]	38.5 [14.3; 58]	50 [35; 61]	39 [20; 55]	0.1020	1.0000	1.0000	0.5484
Progressively motile spermatozoa (PR), %	25 [14; 45]	20 [5; 33]	25 [13; 39]	14.5 [3; 30]	0.1940	1.0000	1.0000	0.0375
Non-progressively motile spermatozoa (NP), %	17 [10; 27]	12.2 [4.1; 25]	20 [10; 27]	20.65 [8; 30]	0.3997	1.0000	0.1877	1.0000
Totally immobile spermatozoa (IM), %	48 [34; 63.9]	53.35 [32; 80]	50 [35; 60.7]	45 [20; 63]	1.0000	1.0000	0.3874	1.0000
Viability, %	61 [59; 79]	60 [59; 69]	60 [59; 75]	60 [53; 69]	0.9727	1.0000	1.0000	0.7603
Kruger morphology	2 [1; 3.5]	2 [1; 5]	2 [1; 3]	2 [1; 5]	1.0000	1.0000	1.0000	1.0000

Note. L—number of leukocytes in seminal fluid, mln/ml.

The values of such characteristics as viability (%) and Kruger morphology did not differ between subgroups with/without leukospermia in men of different age groups.

Table 2 shows the results of evaluating the MAR test characteristics, the level of sperm DNA fragmentation, and the growth of opportunistic pathogens in the seminal fluid in men of different age groups, depending on the presence/absence of leukospermia. The MAR test characteristics (the percentage of spermatozoa coated with antisperm antibodies) did not differ between all subgroups, while the level of DNA fragmentation of spermatozoa was significantly larger in the subgroup of older men with leukospermia compared to the other subgroups (see **Table 2**). The growth of opportunistic pathogens in the titer $\geq 10^4$ KOE was significantly higher in both subgroups of men with leukospermia.

Opportunistic pathogenic microflora was presented by *Enterococcus faecalis*—28% in the group of men under 35 y.o. and 31% in the group over 35 y.o., there were no significant differences between the subgroups. The growth of *Escherichia coli* was detected in 11% of men in the group under 35 y. o. and in 14% of men in the group over 35 y.o.; *Staphylococcus epidermidis* and haemolyticus—in 10% of men under 35 y.o. and in 7% of men over 35 y.o. The growth of *Klebsiella pneumoniae* and *Streptococcus agalactiae* was detected in 3.8% of

subjects in both groups. There were no significant differences between the subgroups with leukospermia and without leukospermia in the increase of these indicators of opportunistic pathogenic microflora.

According to the results of ultrasound examination, the prostate volume was more than 25 cm³ in subgroups up to 35 y.o. in 23.5% and 22.7%, respectively. In the subgroups of men older than 35 years—34% and 72.2%, respectively. A significant difference between the older age subgroup and subgroup with leukospermia was found for both age groups. The ultrasound description of the seminal vesicles and bladder in the entire subject corresponded to the ultrasound norm. Prostate calcifications according to ultrasound examination were found in 36% of men under 35 y.o. without leukospermia and in 50% of subjects with leukospermia; in the group older than 35 y.o.—in 41.2% of men without leukospermia and in 77.7% of men with leukospermia, which was significantly higher compared to the group of older men without leukospermia. Consequently, in the group of older men with leukospermia, both volume and prostate calcifications occurred in more than 70% of subjects, which indicated a direct relationship between these characteristics. Results of ultrasound examination of the prostate gland in men of different age groups are presented in **Table 3**.

According to the results of the survey on the NIH-CPSI scale, clinical symptoms of chronic prostatitis were found only in men older than 35 years with leukospermia. The average total score in this group was 10.5 [0; 16].

Table 2. MAR test values, sperm DNA fragmentation, and growth of opportunistic microflora in seminal fluid.

Index	<35 y.o. (n = 133)		>35 y.o. (n = 133)		p between groups and subgroups			
	L < 1 mln/ml	L < 1 mln/ml	L < 1 mln/ml	L > 1 mln/ml				
	Subgroup 1 n = 89 (67%)	Subgroup 2 n = 44 (33%)	Subgroup 3 n = 97 (73%)	Subgroup 4 n = 36 (27%)	1 - 2	1 - 3	2 - 4	3 - 4
MAR test, IGG, %	0 [0; 0]	0 [0; 0]	0 [0; 0]	1 [0; 7]	1.0000	1.0000	0.3450	0.8250
DNA fragmentation, %	13.4 [9; 22]	9.4 [5; 22]	14 [10; 22]	23 [16; 29]	0.9352	1.0000	0.0286	0.1561
Growth of opportunistic microflora	28%	47.7%	35%	63.8%	0.0330	1.0000	1.0000	0.0240

Table 3. Results of ultrasound examination of the prostate gland in men of different age groups.

Index	<35 y.o. (n = 133)		>35 y.o. (n = 133)		p between groups and subgroups			
	L < 1 mln/ml	L > 1 mln/ml	L < 1 mln/ml	L > 1 mln/ml				
	Subgroup 1 n = 89 (67%)	Subgroup 2 n = 44 (33%)	Subgroup 3 n = 97 (73%)	Subgroup 4 n = 36 (27%)	1 - 2	1 - 3	2 - 4	3 - 4
Prostate volume > 25 cm ³	23.5%	22.7%	34%	72.2%	1.0000	0.8900	0.0006	0.0138
Prostate calcifications	36%	50%	41.2%	77.7%	0.5604	1.0000	0.0864	0.0018

In addition to the scale of prostatitis symptoms, all subjects were asked if there had been previous pregnancies related to that man, and what was the number of the child in marriage the spouses were planning. In the group of subjects under 35 y.o. 18% of men without leukospermia and 4.8% of men with leukospermia had had pregnancies in their marriage, while 89.9% of men without leukospermia and 100% of men with leukospermia planned the 1st child. In the group of men older than 35 years there were 59.8% of pregnancies without leukospermia and 64.7% of men with leukospermia; The 1st child was planned by 54.6% of men of the subgroup without leukospermia and 41.2% of men of the subgroup with leukospermia.

5. Discussion

In the course of the study the aims of the research were achieved, namely, additional data were obtained on the effect of prostate inflammation on the ejaculate characteristics of men of different age groups in infertile couples. In this study, the inflammatory process was not accompanied by clinical complaints specific for chronic prostatitis and its exacerbation in young men. Similar results were described in Frungieri M. B. *et al.* [24], where the authors showed that chronic inflammation was not always accompanied by clinical symptoms.

The inflammatory process had a negative effect on fertility in all subjects, but in the older age group, its clinical and laboratory manifestations were most pronounced. These manifestations may be indicators of aging, which, according to some researchers, is usually characterized by a chronic pro-inflammatory state and is considered a predictor of the pathogenesis and development of age-related diseases. The term “inflammation” is currently used to describe the increased inflammatory response that occurs with age. Aging and inflammation are two closely related processes. Many theories have been developed to establish whether inflammation is a cause of aging, or its consequence. However, no theory explains all aspects of aging, leading to the assumption that all multiple processes (*i.e.*, oxidative stress, mitochondrial damage, immune aging, endocrine aging, epigenetic modifications, and age-related diseases) contribute and that they are all interrelated with inflammatory processes [24].

Our results are also consistent with those of Yamamichi F. *et al.* (2017): in older patients, inflammation of the reproductive organs, pyuria and fever is observed more often than in younger patients [25].

Leukospermia reduces the number of progressively motile spermatozoa in men in the group over 35 years old, but we did not notice its significant effect on the viability and morphology of spermatozoa. In young men, the severity of the inflammatory process is clinically significantly lower than in men of the older group, and the negative consequences on ejaculate parameters in the older age group are more severe and statistically significant in terms of ejaculate volume, the total number of spermatozoa, their progressive motility, as well as the DNA fragmentation index in spermatozoa.

The growth of opportunistic microflora was found in a third of infertile men without leukospermia and significantly increased in its presence, regardless of age.

6. Conclusion

The results of the study demonstrate the negative impact of the inflammatory process on the fertility of men of all ages, however, in the older age group its manifestations are significantly more severe.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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