

# Correction of fertility disorders in patients with cryoglobulinemia

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## Article history

Submitted: Feb. 22, 2013

Accepted: May 24, 2013

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**Introduction.** Cryoglobulinemia can be among the causes of sperm bad quality. But conventional examination algorithm for patients with idiopathic oligozoospermia makes no provision for cryoglobulin determination in blood serum.

**Materials and methods.** We examined 55 patients with idiopathic spermatogenesis disorder. Each patient had cryoglobulin determined in blood serum. For this purpose, optical density of the patient's blood serum before and after its 7-day cooling at a temperature of 4°C was compared. Type of cryoglobulins was established by the method evaluation of serum optic density in different periods of cooling incubation (before and after cooling) using the curves of temperature resistance for comparison. Patients with cryoglobulinemia underwent intracutaneous immunization with autoleukocytes separated from heparinized venous blood.

**Results.** Cryoglobulins were revealed in 16 patients of all 55 examined (29.09%): in six patients' cryoglobulins of second type; in nine – third type and in one patient – cryoglobulins of first type were detected. In a control group, which consisted of 50 men is blood – donors' frequency of cryoglobulinemia was 2%.

Pathogenetic connection between cryoglobulinemia and disturbances in sperm quality is also supported by the fact that after autoleukocyte immunization in patients who positively responded to cryoglobulinemia treatment (14 persons of 16 or 87.5%) spermogram was found to be improved. Thus, in 12 patient number of spermatozoa. In all patients progressive motility and percentage of normal forms increased with spermatozoa concentration.

**Conclusions.** It is reasonable to include determination of cryoglobulins in blood serum for patients with idiopathic oligo- and zoospermia.

**Key Words:** idiopathic oligozoospermia ♦ cryoglobulinemia ♦ intracutaneous immunization with autoleukocytes

## INTRODUCTION

Different estimations of incidences of idiopathic oligo- and zoospermia vary from 25% to 80%. According to WHO data, which were received during examination of 7053 males with infertility, causes of fertility disorders, were not established in 75.1% [1]. It is known that idiopathic spermatogenesis disorder can be attributed to many diseases including vascular affections, diseases of central and peripheral nervous systems, hepatic and nephritic failures and

others. That is exactly why patients with idiopathic dysfunction of spermatogenesis should undergo thorough and comprehensive examination.

One of the most frequent reasons causing damage of vessels and other organs and systems of the body is cryoglobulinemia syndrome [2–7].

Frequent manifestation of cryoglobulinemia (in many cases the only clinically apparent one) is maltolerance of cold, though our clinical experience shows that doctors seldom ask patients about their well-being in chilly seasons of the year. Cryoglobulinemia

lins can be found even in people looking healthy externally.

Taking into consideration the wide range of cryoglobulin damaging action the aim of our investigation was to study possible connection between cryoglobulinemia and oligozoospermia. For this purpose, frequency of detection cryoglobulins in blood serum of the patients with idiopathic spermatogenesis disorder was determined, and the way of treatment of cryoglobulinemia affected the spermogram indices.

## MATERIALS AND METHODS

The investigation embraced 55 men with idiopathic oligo- and astenozoospermia at the age of 26 to 35 years. All patients were preliminarily examined as per recommendations of WHO [1, 8], but reasons of spermatogenesis disorder were not established.

Patients with established genetic reasons of infertility (Klinefelter syndrome, Hyhhako syndrome etc.) as well as the patients with other established or suggested reasons of infertility (varicocele, cryptorchism, urinogenital infections, various endocrine disturbances, different forms of normospermatogenic sterility, autoimmune processes et al.) were excluded from the investigation. Not included in the investigations were also patients with overweight, diabetes mellitus, tuberculosis, chronic diseases of kidneys and liver (viral hepatitis B and C among them), those with cardiovascular failure, alcoholics, and drug addicts, and with diseases in anamnesis which may lead to infertility. The states and diseases enlisted were diagnosed in compliance with standard approaches specifying clinical examination, biochemical and immunological investigations, instrumental methods of diagnosing. When infectious diseases were suspected, serological methods of diagnosis were used for detecting corresponding antibodies and antigens, among them a high-sensitive real-time polymerase chain reaction (PCR real time) was used.

Therefore, only practically healthy men with the only established pathology of stable spermatogenesis disturbance were included into the investigation. In all patients taking part in the study, cryoglobulins in the blood serum were determined by spectrophotometric method.

In order to detect cryoglobulins blood was taken from the peripheral vein in the volume of not less than 10 ml into a warm syringe (37°C), incubated at 37°C till clot formation. Some part of the separated serum was diluted with veronal-medinal buffer (pH 8.6) in the ratio of 1:10 and its optical density was determined by spectrophotometric method. (Immnochem-2100 photometric system, made by High Technology, USA,

was used, wave length 500 n.m.) The rest of the serum was incubated at 4°C over a period of 7 days, then it was also diluted in the buffer and optical density determined. If the difference in optic density of the serum before and after the cooling exceeded 10% the result was considered positive and expressed in relative value units (>10 RVU). Difference in optic density (%) was considered as a quantitative result of investigation and was expressed in relative units (for example, if difference in optic density of the serum before and after cooling was 15%, the result of investigation was 15 relative value units.

To check the idea that increased optical density of cooled serum is connected with cryoglobulins presence in it, the serum was warmed within an hour at 37°C. Decrease of optical density supported the results of investigation.

Type of cryoglobulins was established by the method evaluation of serum is optic density in different periods of cooling incubation (before and after cooling) using the curves of temperature resistance for comparison.

Control group to compare frequency of cryoglobulins revealed in patients and in population consisted of 50 men blood donors having children below 3 years of age.

Patients with cryoglobulins present in the blood serum received non-drug therapy of the cryoglobulinemia by means of intracutaneous immunization with autoleukocytes. [9–12].

Non-drug method of cryoglobulinemia treatment developed by us consists of two stages: isolation of leukocytes from peripheral blood and isoimmunization. The study was approved by the local Ethical Committee of Danylo Halytskyi Lviv National Medical University.

### Isolation of leukocytes

Leukocytes are isolated by settling patient's heparinized venous blood. For this purpose, 40–50 ml of venous blood is taken by a preliminarily warmed syringe (37°C) into a heparin-containing vial (Heparini-Richter) at 50 units of heparin per 10ml of blood (the volume depends on the number of leukocytes in 1 ml of blood). Then the blood is poured into test tubes 10ml each and incubated at an angle of 45° in thermostat at a temperature of 37°C during the period of 90 to 140 min. The plasma is very carefully drawn off avoiding mixing; leukocytes are washed twice in 5 to 10-fold volume of 0.9% solution of sodium chloride by centrifuging at 200 g. The autocytes suspension is administered by 0.1 ml into 8 to 10 points of the back skin (between the blade bones) using intracutaneous method of injection until orange-peel skin appears.

**Table 1.** *Spermatozoa concentration before and after cryoglobulinemia treatment*

Spermogram parameter										
Before treatment			After treatment							
Concentr. of spermatozoa, million/ml	Number of patients	%	Concentration of spermatozoa, million/ml							
			Up to 5		5–10		>10–<20		>20	
			Qty	%	Qty	%	Qty	%	Qty	%
Up to 5	8	66.67	0		0		1	12.5	7	87.5
>5–<10	4	33.33	0		0		1	25.0	3	75.0

In 10 to 15 days and 1 to 3 months after immunization, the content of cryoglobulins in blood serum was analyzed for the second time. Within the same time after immunization with leukocytes the ejaculate was investigated and determined the following indices of the spermogram: concentration of spermatozoa (million per milliliter), total quantity sperm count (million), progressive motility (%), normal forms (%). Investigations were performed using a sperm analyzer AFC–500–2 (made by “BIOLA NPF”, Russia). The results received were compared with findings of the analysis made before the treatment manipulation.

## RESULTS

Cryoglobulinemia was found in 16 (29.09%) men of 55 with idiopathic oligo- and astenozoospermia; in six patients, cryoglobulins of second type were revealed; in nine – third type and in one patient – cryoglobulins of first type were detected. In a control group, which consisted of 50 men blood – donor's frequency of cryoglobulinemia was 2%.

In 12 of them number of spermatozoa substantially increased, in most patients it exceeded 20 million per milliliter (Table 1). In all patients progressive motility and percentage of normal forms (100%) increased with spermatozoa concentration.

The data given in the above table testify to the fact that for some part of the patient's successful treatment of cryoglobulinemia, it is at the same time an efficient method for increasing spermatozoa concentration.

The stability of the achieved effect was different and during repeated examination after 1 to 3 months concentration of spermatozoa in five patients in 12 (41.67%) lowered though it was still higher than before treatment. Degradation of the spermogram indices coincided with restoration of the cryoglobulinemia initial level; concentration of spermatozoa in patients with prolonged remission of cryoglobulinemia concentration of spermatozoa exceeded 20 million/ml (7 patients).

In two patients of 14 (14.29%) who positively responded to cryoglobulinemia therapy quantity of

spermatozoa still before the treatment was in compliance with the totally accepted norms, but their progressive motility turned out to be insufficient. The treatment practically didn't have any effect upon the total spermatozoa concentration but the motility increased substantially.

Thus, in patient D., born 1985, total quantity of spermatozoa before and after the treatment was the same (28 million), but progressive motility increased from 13% to 33% ; in another patient (patient M., born 1974) quantity of spermatozoa increased inessentially (from 175 to 180 million), but motility index grew up noticeably (from 15 to 29%).

These patients' wives became pregnant within a short period after their husbands had been immunized with autoleukocytes. This testifies to the fact that increase of spermatozoa progressive motility is no less important an index of cryoglobulinemia treatment efficiency.

In two patients (of 16 with cryoglobulinemia; 12.5%) this attempt to achieve decrease of pathological cryoproteins concentration and sperm quality improvement was not successful.

## DISCUSSION

The data obtained indicate that in some patients there is a connection between cryoglobulinemia and disturbances in sperm quality, that's why detection of cryoglobulins should be included in algorithm of laboratory investigations. The mechanisms of cryoglobulins effects on spermatogenesis are still unknown, there is no information on this subject in available literature.

It is known that pathologic conditions that manifest with syndrome of cryoglobulinemia can promote indirectly sperm quality disorders, but we cannot exclude direct affects of cryoimmunoglobulins on condition of hematotesticular barrier and on morphologic peculiarities and functional activity of spermatozooids. Possibly the decrease of cryoglobulins concentration results in diminished damaging effects on spermatozooids. Obviously this problem

needs further investigation concerning the mechanisms of the curative action of the autoleukocyte intradermal immunization on autoimmune processes [13], precisely on cryoglobulins synthesis as described earlier [9, 11].

## CONCLUSIONS

1. High frequency of cryoglobulins detection in the blood serum of patients with idiopathic oligozoospermia and normalization of the spermogram indices

because of cryoglobulinemia treatment testify to the fact that it is reasonable to include the cryoglobulins detection procedure into the examination algorithm for male patients with fertility disorder.

2. Frequent detection of cryoglobulins in the group of carefully examined patients with idiopathic oligozoospermia points out possible connection between spermatogenesis disorder and in patient's past infectious disease (past infection) or chronic latent infection, as infectious diseases are considered to be the main cause of type 2 and type 3 cryoglobulins synthesis.

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