

# Inhibition of cryoglobulin synthesis as a method of positive influence on spermogram indices (clinical observations)

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**Introduction** Cold-shock precipitating proteins – cryoglobulins damage various vessels and, depending on the localization of damages, may lead to functional disorders of various organs and body systems. Evidently, cryoglobulinemia may have a negative influence on the reproductive system's condition, thus, this process requires detailed study.

**Material and methods** Clinical examples, indicating connection between cryoglobulinemia and spermatogenesis impairment are presented in the article. The proof is improvement of spermogram due to inhibition of cryoglobulin synthesis by the method of intradermal immunization with native autoleukocytes. Leukocytes are isolated by precipitation of a patient's heparinized peripheral venous blood in thermostat at 37°C for 90–140 minutes. After precipitation, blood plasma is aspirated, leukocytes are precipitated by centrifugation. Autoleukocytes are injected in the dose 0.1 ml into 8–12 points of the skin in the back.

**Results** In the spermogram indices considerably improved due to the inhibition of cryoglobulin synthesis in patients with idiopathic oligoasthenoteratozoospermia. However, we have determined that in men with other forms of infertility, including severe ones, presence of an increased level of cryoglobulins significantly deteriorates prognosis considering successful correction of spermatogenesis impairment. Their elimination, in most cases, has a positive influence on spermatogenesis and makes additional conditions for efficacy improvement in the treatment of infertility.

**Conclusions** Examination for cryoglobulinemia should be included to methodical recommendations/instructions for examination of patients with impaired fertility, since the reduction of cryoglobulin level by means of intradermal immunization with autoleukocytes has a positive influence on spermogram indices.

**Key Words:** male infertility <> spermogram <> cryoglobulinemia <> native autoleukocyte immunization

## INTRODUCTION

A problem of male infertility is current for all countries and requires detailed study and elaboration of new effective methods for diagnostics and therapy. Peculiar difficulties, according to literature data, occur because in 40% of cases it is impossible to determine the causes of spermatogenesis impairment (idiopathic infertility) [1].

The aim of our earlier described investigation [2, 3, 4], was a determination of the etiology and pathogen-

esis of oligozoospermia (OZ) in patients, who after examination, according to the WHO recommendations, were considered as 'actually healthy people'. In these investigations, it was established that more than the third of patients with idiopathic OZ have an increased level of cold-shock precipitating proteins – cryoglobulins in blood. It is clear that in patients referred to actually healthy individuals, doctors did not detect signs of cryoglobulinemia syndrome. However, in some of them moderate cold extremities, acrocyanosis and/or worsening of cold tolerance

were observed. Unfortunately, most doctors do not pay attention to these symptoms of cryoglobulinemia. Besides, as it is known, level of cryoglobulins in blood serum may be determined in generally healthy people.

Since cryoglobulins often cause vasculitis of small (rarer medium, rare large) vessels, which may lead to impairment of functions of various organs and body systems [5–9], a tendency to study a possible connection between cryoglobulinemia and oligoasthenoteratozoospermia appeared. Thus, it was studied whether inhibition of cryoglobulin synthesis influenced spermogram indices.

## MATERIAL AND METHODS

Method of intradermal immunization with native autoleukocytes was used for inhibition of cryoglobulin synthesis. Thus, leukocytes were isolated by precipitation of a patient's heparinized peripheral venous blood in thermostat at 37°C for 90–140 minutes. After precipitation, blood plasma was carefully aspirated, avoiding mixing; leukocytes were precipitated by centrifugation at 200 g for 7–8 minutes. Autoleukocytes were injected with syringe intradermally in the dose 0.1 ml into 8–12 points of the skin in the back. This method was described earlier [2–5, 10, 11]. In our investigations [2, 3, 4], it was established that inhibition of cryoglobulin synthesis has a positive impact on production of spermatozoa, their motility and morphology. Thus, in most patients with idiopathic oligozoospermia, when autoleukocyte immunization resulted in a considerable decrease in the concentration of cryoglobulins, the amount of spermatozoa increased to 20 mln/ml and more (85.71%). Simultaneously, their progressive motility and percentage of normal forms improved [2].

However, despite high efficacy of this method, its implementation into clinical practice occurs slowly and incompletely. For this reason, in addition to previously published materials, we describe clinical examples, which show the efficacy of this method.

## RESULTS

A clinical case, which is analogical to those, which were observed in most treated patients with idiopathic oligozoospermia.

Patient K., 41-year old, first marriage (8 years). The couple did not use contraceptives, but there were no children. On thorough examinations, according to the adopted algorithm and WHO recommendations, the patient was considered actually healthy: besides spermatogenesis impairment, pathologic conditions were not found in the body. However,

in our examination, cryoglobulins of the 3<sup>rd</sup> type were detected in the blood serum: 42 conditional units (norm below 10 conditional units). Clinical manifestations, specific for cryoglobulinemia syndrome, were not detected in the patient.

Before immunization, concentration of spermatozoa was 1 mln/ml, total of 2.4 mln in ejaculate; non-motility – 100% (analysis dated 18.12.2010 year). However, already in 12 days after intradermal autoleukocyte immunization, the concentration of spermatozoa increased to 111 mln/ml, totally in ejaculate – 307.8 mln; active spermatozoa – 50% (46 and 4% of A and B categories, respectively).

Previously described results of the investigation, which we already mentioned, concerned only patients with idiopathic oligozoospermia. However, afterwards when taking into consideration the fact that cryoglobulinemia may result in different clinical signs, we expanded the range of treatment significantly. At the same time, the reduction of cryoglobulin level leads to improvement of spermatogenesis in patients with numerous, even the severest pathological conditions.

A clinical case: Patient M., 37 years old, was examined and treated for infertility in a clinic abroad. Diagnosis Kallmann-Syndrome (azoospermia, anosmia, divergent strabismus) was made.

Karyotype 46XY; microdeletion of Y-chromosome in AZF region was not detected, mutations of CFTR gene were not found.

Replacement hormone therapy was administered: chorionic gonadotropin and follicle-stimulating hormone with gradual dose increase. Thus, from November 2013 to July 2014 the patient was taking chorionic gonadotropin in the dose 1500 units three times per week and follicle-stimulating hormone – 150 units three times per week. In July 2014, FSH dose was increased to 300 units three times per week, and the dose of chorionic gonadotropin increased from

**Table 1.** Amount of cryoglobulins and concentration of spermatozoa before and after autoleukocyte immunization in patients with idiopathic oligoasthenoteratozoospermia

	Before immunization	After immunization	P
Amount of cryoglobulins (CU)	70 CU	34 CU	<0.001
Median (min. – max.)	(18–165)	(0–73)	
Number of patients			
Concentration of spermatozoa, mln/ml	Below 5	11 (64.71%)	1 (5.88%)
	5–10	6 (35.29%)	1 (5.88%)
	10–20		2 (11.76%)
	> 20		13 (76.47%)

**Table 2.** Concentration of spermatozoa before and after autoleukocyte immunization of patients with various infertility types

Type of infertility	Patients	Cryoglobulin (CU)		Total concentration of spermatozoa (mln)		% motile (A+B)+C*		% normospermia	
		Before immunization	After immunization	Before immunization	After immunization	Before immunization	After immunization	Before immunization	After immunization
Primary secretory infertility (n 2)	1.	30	<10	0	4.5	0	single motile	–	–
	2.	20	<10	0.5	5	0	single motile	–	–
Secondary secretory infertility (n 3)	3.	29	<10	1.5	8.5	0	36+9	0	11
	4.	24	<10	0.8	46	0	31+5	0	4
	5.	33	<10	1.24	98.6	0	7+3	0	6
	6.	86	<10	14.4	57.2	3+3	34+10	3	4
	7.	22	<10	0.126	56	0	7+16	0	4
	8.	22	<10	6.1	16.2	20+7	21+10	0	11
Varicocele (n 7)	9.	35	<10	76.2	94.2	18+19	44+21	2	4
	10.	19	<10	11.5	91.4	10+7	15+7	2	14
	11.	27	<10	27.9	32.4	29+12	41+8	1	4**
	12.	35	<10	12.2	30.2	12+10	27+18	2	4**
	13.	61	<10	40.1	41.2	6+8	33+10	3	4**
Excretory-toxic infertility (n 7)	14.	25	<10	9	16.3	2+5	21+40	3	11**
	15.	33	<10	6	60	25+25	38+10	0	4**
	16.	19	<10	15	75	12+13	29+13	0	5**
	17.	34	<10	22.6	25.5	15+5	20+22	3	4
	18.	34	22	5.5	6	9+9	10+9	2	3
	19.	30	32	75	74	10+7	11+11	0	2
All patients (n 19)		P <0.001		P <0.001		P <0.001		P <0.001	

\*A – fast progressive motility; B – slow progressive motility; C – non-progressive motility; \*\* – a wife became pregnant in a natural cycle

March 2015 to 3000 units three times per week. Replacement hormone therapy lasted from November 2013 to May 2015. However, it was not successful.

In January 2015, the patient came to our clinic and was diagnosed with cryoglobulinemia of the 2<sup>nd</sup> type: level of cryoglobulins in blood serum was 30 conditional units (norm to 10 conditional units).

For inhibition of cryoglobulinemia synthesis, we performed intradermal immunization with autoleukocytes on April 2, 2015. In two weeks (April 16, 2015) after control of cryoglobulin level, which decreased to six conditional units (norm to 10 conditional units), analysis of ejaculate was done – at last, spermatozoa appeared in ejaculate: concentration – 600000/ml, total of 2.5 mln, sperm cells of A+B category – 1.5 mln.

From April to July 2015, several collections of ejaculate were done for sperm cryoconservation and its use in cycle Intracytoplasmic Sperm Injection (ICSI).

At this time, indices of ejaculate remained approximately within 200000 – 300000 /ml with the presence of sperm cells of A+B category about 25%.

Thus, elimination of cryoglobulinemia resulted in spermatogenesis improvement and enabled conduction of ICSI procedure for the couple. Six embryos were obtained. Two embryos at the stage of early blastocyst were implemented.

Nevertheless, the pregnancy did not occur, and the remaining embryos degenerated and ceased development. However, this example shows that cryoglobulinemia is a considerable negative factor of influence on spermatogenesis, since reduction of cryoglobulin level caused improvement, even in case of the most severe disorder of male fertility.

Patient S., 29 years old, first marriage (5 years). The couple did not use contraceptives; however, he did not have any children with his wife (or other sexual partners). On anamnesis record, it was established that the patient had been examined in several medical establishments in Lviv city, and he had been diagnosed with severe oligoasthenoteratozoospermia. In ejaculate analysis (from October 2013 to September 2014), concentration of spermatozoa was below 1 mln/ml and not more 1.24 mln totally; motility – from single active to 100% inactive.

Level of sex hormones on 01.11.13:

Luteinizing hormone (LH) – 2 mIU/ml (norm 1.7–8.6 mIU/ml), FSH – 1.3 mIU/ml (1.3–12.4 mIU/ml), total testosterone – 11.34 nmol/ml (8.6–29.0 nmol/l), estradiol – 41.73 ng/ml (7.63–42 ng/l).

The patient was diagnosed with autoimmune thyroiditis of unknown etiology. In addition, the patient complained of frequent arthralgia, which intensified in cold seasons.

Indices of the thyroid at the time of specific therapy with L-thyroxin 15.04.14: TSH – 92.52 mcIU/ml (norm to 4.2), Prolactin – 16.22 ng/ml (norm 4.04–15.2 ng/ml); Thyroid peroxidase (TPO) Antibodies – 276.2 IU/ml (norm to 34.0), antibodies to thyroglobulin – 293.80 IU/ml (norm – to 115 IU/ml). It should be noted that due to the levothyroxine intake (100 mg/day), a tendency to the worsening of the thyroid indices was observed: in June 2014 TSH level became >100 mcIU/ml, TPO Antibodies – increased to 384.7 IU/ml; level of prolactin somewhat increased as well (16.59 ng/ml).

Cryoglobulinemia was diagnosed in September 2014: level of cryoglobulins in blood serum was 33 conditional units (norm to 10 conditional units). As a result, 16.10.14 intradermal immunization with autoleukocytes was performed. In 30 days following intradermal autoleukocyte immunization (17.11.14) cryoglobulins were not detected anymore, level of TPO Antibodies and antibodies to thyroglobulin decreased, and the concentration of spermatozoa significantly increased – to 17 mln/ml, total in ejaculate 98.6 mln; 7% active spermatozoa, categories A+B and C – 4 %, (before immunization concentration of spermatozoa constituted less than 1 mln/ml, less than 1.24 mln totally; motility – from single active to 100% inactive).

Further, the patient was offered selection and cryoconservation of sperm for use in the cycle of assisted reproductive technologies.

Thus, intradermal autoleukocyte immunization had a positive impact on the autoimmune process: synthesis of cold-shock pathological proteins – cryoglobulins ceased; production of antibodies to the thyroid antigens partially decreased in the patient. Spermatogenesis improvement occurred due to the positive influence on the autoimmune process (usually on cryoglobulin synthesis) (Tables 1, 2).

## DISCUSSION

From the suggested examples, it is seen that in the case of oligozoospermia, caused by cryoglobuline-

mia, intradermal immunization with native autoleukocytes is a highly effective method of treatment in spermatogenesis impairment. Even in severe pathological conditions, when spermatogenesis normalization does not occur, immunization results in concentration increases and improvement of spermatozoa motility. It promotes improvement of results in IVF methods.

Synthesis of cryoglobulins is known to be an autoimmune process, resulting in the deposition of precipitating cold-shock proteins, mainly in vessels with small diameter. Depending on intensity of vascular damages and their localization, various pathological processes may occur, particularly those, which, according to our data, lead to (or promote) formation of male infertility. Cryoglobulinemia may have indirect negative influence on spermogram indices; however, direct influence on the condition of hematotesticular barrier, as well as morphological peculiarities and functional activity of spermatozoa is theoretically possible. Moreover, in our opinion, physiologically lower temperature in the testicles (34.2–34.8°C), which is necessary for complete spermatogenesis, but at the same time results in reactions of cryoglobulin precipitations, is an additional factor, which promotes damage to testicular vessels.

Oligozoospermia, caused by cryoglobulinemia, has been mistakenly suggested to be idiopathic, though it may be referred to the group of immunologic infertility.

In other severe forms of spermatogenesis impairment, cryoglobulinemia is an additional complicating factor, which plays a significant role in the pathogenesis of the male infertility.

## CONCLUSIONS

Thus, patients with spermatogenesis impairment, especially those with atypical or severe signs of concomitant diseases / or conditions, should be examined for the presence of cryoglobulins in the blood serum. Such approaches should be included to methodical guide / instructions for examination of patients with fertility disorders, since the reduction of cryoglobulin level by means of intradermal immunization with autoleukocytes has a positive impact on the synthesis of spermatozoa.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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