Gender characteristics of autoimmune hypogonadism*

G. Kh. Safarian[1,2], *D. A. Niauri*[1,2], *Ye. S. Borodina*[1], *L. Kh. Dzhemlikhanova*[1,2], *A. M. Gzgzyan*[1,2]

[1] St. Petersburg State University,
7–9, Universitetskaya nab., St. Petersburg, 199034, Russian Federation

[2] D. O. Ott Research Institute of Obstetrics, Gynecology and Reproductology,
3, Mendeleevskaya liniia, St. Petersburg, 199034, Russian Federation

For citation: Safarian G. Kh., Niauri D. A., Borodina Ye. S., Dzhemlikhanova L. Kh., Gzgzyan A. M. Gender characteristics of autoimmune hypogonadism. *Vestnik of Saint Petersburg University. Medicine*, 2020, vol. 15, issue 1, pp. 9–23. http://doi.org/10.21638/spbu11.2020.102

Autoimmune gonadal lesions represent a highly heterogenous conditions affecting men and women of reproductive age and resulting in loss of endocrine function and, eventually, infertility. Well-known and well-documented risk factors exist, and the presence or suspicion of autoimmune disorder should be regarded as an important one. For the purpose of the present study, ELISA kits according to the proprietary technology for detection of antitesticular and antiovarian antibodies were developed. Among men with autoimmune orchitis an increased level of antibodies to steroid-producing testicular cells, decreased serum total and free testosterone and altered semen parameters were found. Combined autoimmune diseases were present (autoimmune thyroiditis, vitiligo, type 1 diabetes) in 13.5 % of cases. In women with autoimmune oophoritis the main finding was anovulation with preserved serum FSH and LH levels (91.6 %) and luteal insufficiency (8.4 %). In total, 19.4 % of patients had other autoimmune diseases (autoimmune thyroiditis, vitiligo, chronic autoimmune hepatitis, rheumatoid arthritis, systemic lupus erythematosus).

Keywords: antiovarian antibodies, antitesticular antibodies, ovarian insufficiency, hypogonadism.

Introduction

Autoimmune hypogonadism is a condition characterized by the autoimmune process affecting the germinal and steroid-producing gonadal structures and resulting in both altered hormonal and reproductive functions of the gonads.

Nowadays, up to 15 % of couples worldwide suffer from infertility [1]. There is evidence, that up to 31.5 % of patients with normogonadotropic primary ovarian failure have an autoimmune nature of hypogonadism [2; 3]. However, despite the rather high prevalence of the condition, its diagnostic assessment is not carried out routinely in women with infertility.

The immunological basis of male infertility is established by the presence of sperm autoantibodies in the semen or blood. Few clinical studies exist aimed to identify the autoimmune process in the testicular interstitial cells (Leydig cells) that produce the main

* The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists.

androgen, i. e. testosterone. At the same time, in more than 30 % of patients with impaired spermatogenesis and normal levels of serum FSH and LH, the cause of hypogonadism remains unclear [4–6].

According to the world and russian literature data, the autoimmune gonadal lesion is associated with multiple autoimmune conditions: adrenal insufficiency of autoimmune origin, autoimmune thyroiditis, hypoparathyroidism, type 1 diabetes, systemic lupus erythematosus, vitiligo, rheumatoid arthritis, glomerulonephritis, myasthenia, pernicious anemia [3; 7–9]. The presence of concomitant conditions of the same origin points on a common mechanisms in the development of various autoimmune processes.

The aim of the current trial was to evaluate the frequency of autoimmune gonadal lesion in various forms of ovarian failure and normogonadotropic testicular failure, to assess the results of the laboratory and instrumental studies and to identify the association between gonadal lesions of autoimmune origin and systemic autoimmune diseases among women and men.

Materials and methods

In total, 2280 women aged 17–45 y. o. with various forms of ovarian failure and 268 men aged 22–47 y. o. with hypogonadism, resulting in impaired spermatogenesis and testicular insufficiency were examined for the period from 2006 to 2018.

Women who met each of the following criteria were included in the study:

- 17–45 years old;
- Patients with proven ovarian insufficiency according to the results of a hormonal study (serum progesterone ≤ 15 nmol/l on days 19–22 of the menstrual cycle) and/or absence of the corpus luteum on days 19–22 of the menstrual cycle according to pelvic sonogram);
- Day 3–5 serum FSH level more than 1.8 IU/I;
- Day 19–22 serum prolactin level not higher than 700 mIU/l;
- women with serum anti-ovarian autoantibodies level > 350 U/ml;
- BMI < 40.

The study included men who met each of the following criteria:

- 20–47 years old;
- Hypogonadism, characterized by total serum testosterone < 12 nmol/l, free testosterone < 19 pmol/l and/or altered spermatogenesis (semen PR spermatozoa < 20 million/ml in combination with abnormal sperm morphology, i. e. ≤ 14 %);
- Serum prolactin < 700 mIU/l;
- Serum FSH of 1.1–11.8 IU/l and LH of 0.8–8.4 IU/l;
- The presence of serum antitesticular autoantibodies > 400 U/ml;
- BMI < 40.

Exclusion criteria for women with autoimmune oophoritis:

- Endometrial hyperplasia at present;
- Uterine fibroids > 4.0 cm in diameter;
- Ovarian cysts > 4.0 cm in diameter, not regressing on drug therapy or spontaneously during 3 menstrual cycles;

- Active pelvic inflammatory disease and/or identification of pathogenic microorganisms during bacteriological examination;
- History of vascular diseases (coronary insufficiency, stroke);
- History of venous thromboembolism;
- Refractory or untreated hypertension;
- Impaired liver or kidney function;
- Untreated diabetes;
- Present or past history of malignancy of any localization;
- Estrogen and/or progesterone therapy at present or 2 months before the trial;
- Gonadotropin-releasing hormone agonist therapy, if it was not stopped six months before the start of the trial;
- Pregnancy.

Exclusion criteria for men with autoimmune hypogonadism were as follows:

- Prostatic mass > 3.0 cm in diameter and/or PSA values > 10 nmol/l;
- Presence of testicular masses;
- Active genitourinary inflammatory diseases and/or the identification of pathogenic microorganisms on bacteriological examination;
- History of vascular diseases (coronary insufficiency, stroke);
- History of venous thromboembolism;
- Refractory or untreated hypertension;
- Impaired liver or kidney function;
- Untreated diabetes;
- Present or past history of malignancy of any localization;
- Androgen therapy, if it was not stopped six months before the start of the trial.

All the patients of this trial underwent clinical and laboratory examination.

Serum levels of follicle-stimulating (FSH), luteinizing (LH) hormones and estradiol in women on day 3–5, as well as prolactin, progesterone and estradiol on day 19–22 of the spontaneous menstrual cycle were determined using ELISA commercial kits ("Alcorbio", Russia). When indicated, testosterone and its free fraction, dehydroepiandrosterone sulfate (DHEA-S) and 17-hydroxyprogesterone (17-OHP) were measured. In men serum FSH, LH, prolactin, testosterone and free testosterone were examined. Additionally, when indicated, evaluation of thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), antibodies to thyroglobulin (Tg-abs) and thyroid peroxidase (TPO-abs) was performed. For the purpose of antiovarian and antitesticular antibodies determination, ELISA according to the proprietary technology was developed [10].

All examined patients underwent sonography on a Medison SA-8000-Prime ultrasound scanner (Korea) using an abdominal sensor with a variable frequency of 3–7 MHz, an intracavital sensor with a variable frequency of 4–9 MHz and a linear sensor for small organs with a variable frequency of 5–9 MHz. In women, transvaginal sonography (TVS) was performed on days 5–7, 11–14, 19–23 of spontaneous or induced menstrual cycle. The evaluated parameters included: size and echostructure of the uterus and ovaries, as well as dynamic changes in endometrial thickness and follicles size.

Same way, prostate gland and the scrotum were examined in men using ultrasound.

A semen analysis was performed according to the strict morphology criteria to evaluate spermatogenesis [11].

For the purpose of statistical analysis we used EXCEL statistical software packages for WinXP, STATISTICA 6.0, SPSS v 13, Manugistic Statgraphics v. 5.0

Results

Autoimmune orchitis was established by the "two sigma (σ)" rule with the level of circulating antitesticular antibodies > 400 U/ml. Thus, this diagnosis was present in 37 (13.8 ± 2.1 %) of 268 patients with normogonadotropic hypogonadism. The average level of antitesticular antibodies in patients with autoimmune orchitis was 738.7 ± 47.1 U/ml and ranged from 416 to 1571 U/ml. General characteristics of patients are presented in Table 1.

Table 1. **Clinical characteristics of patients with autoimmune orchitis**

Average age (years) 32.1 ± 0.9	32.1 ± 0.9
Duration of the disease (years)	3.9 ± 0.4
Infertility (%)	94.5 ± 3.7
History of varicocele (%)	18.9 ± 6.4
History of unilateral cryptorchism (surgically corrected by age of 3), (%)	5.4 ± 3.7
Erectile dysfunction (%)	5.4 ± 3.7

Majority of the patients have had genitourinary inflammatory disease in the past (Fig. 1) with chronic nonspecific urethritis (Fig. 2) being one of the most frequently diagnosed (45.9 ± 8.2 %).

A history of allergic reactions was present in 6 (16.2 ± 6.1 %) patients: 3 of them had atopic dermatitis with seasonal exacerbations, 2 patients suffered from a combination of allergic rhinitis and conjunctivitis with seasonal exacerbations, and 1 patient was diagnosed with bronchial asthma at the age of 3 y. o. Combined autoimmune diseases were present in 5 (13.5 ± 5.6 %) patients. A diagnosis of puberty onset type I diabetes mellitus was made in one patient, the condition was compensated and no vascular complications were noted. Vitiligo without progression was present in one patient, autoimmune thyroiditis on levothyroxine supplementation at a dose of 50 and 75 mcg/day — in 2 patients.

Scrotal examination revealed testicular hypotrophy in 6 (16.2 ± 6.1 %) patients. Moreover, a decrease in the size of both testicles was noted in four patients; 2 others had isolated left testicle hypotrophy in combination with a history of varicocele. Prostate examination revealed an increase in the size of gland in 8 (21.6 ± 6.8 %) patients with chronic prostatitis and was characterized by a heterogeneous compacted consistency upon palpation.

All patients underwent pelvic and scrotal sonography (Table 2). The sizes of the prostate gland were larger in patients with autoimmune orchitis ($p < 0.001$). Also, the echostructure of the prostate gland in these patients was not uniform. As a rule, a thickened capsule of the gland was seen along with the intermittent contour. There was a significant decrease in the average volume of both testicles in the study group. This difference re-

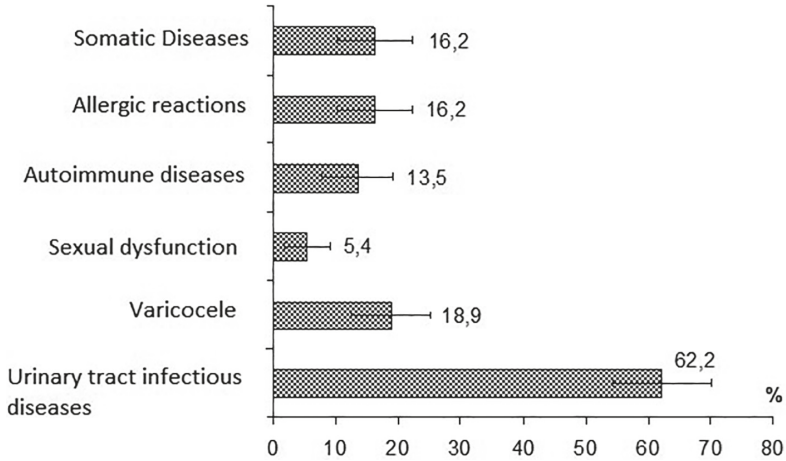

Fig. 1. Concomitant diseases in patients with autoimmune orchitis

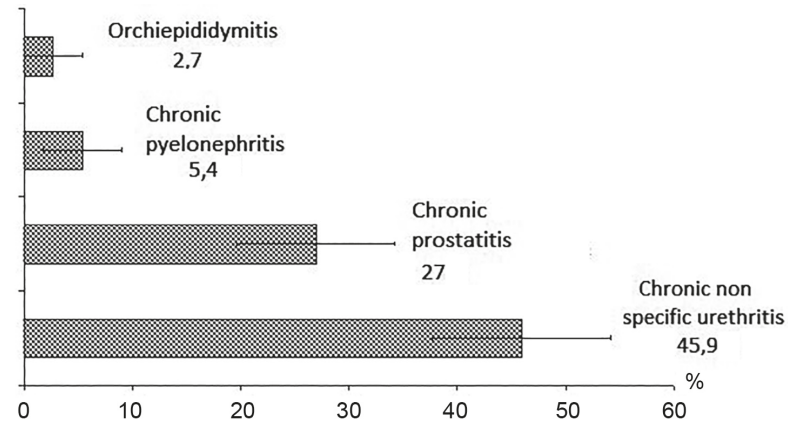

Fig. 2. The prevalence of genitourinary tract inflammatory diseases in patients with autoimmune orchitis

mained significant ($p < 0.001$) even after excluding patients with obvious testicular hypotrophy, when the volume of the testicles did not exceed 15 cm^3. Noteworthy, the total echogenicity of the testes was decreased in cases of testicular volume < 15 cm^3.

The comparative results of hormonal studies in men are shown in Table 3. The average prolactin level in the study group was lower than that of a healthy men ($p < 0.05$). A significant decrease ($p < 0.001$) in the serum testosterone and its free fraction in patients with autoimmune nature of hypogonadism was noted. Spearman analysis revealed a significant positive correlation ($r = 0.3$, $p = 0.04$) between the testosterone and LH levels among patients with autoimmune orchitis.

In the study group, a positive correlation was observed between testicular volume and testosterone level ($r = 0.39$, $p = 0.02$), but not in the controls.

No significant correlation was revealed between the levels of antitesticular autoantibodies and the content of total serum androgens, prolactin, FSH and LH, as well as the size of the testicles.

Table 2. **Pelvic and scrotal sonography results (M ± m, Me) in patients with autoimmune orchitis (1) and healthy men (2)**

Groups / Characteristic	1 (n = 37) (M ± m, Me)	2 (n = 26) (M ± m, Me)	P
Prostate volume (cm^3)	24.2 ± 0.8; 23.0	18.1 ± 0.5; 18.4	< 0.001
Right testicle volume (cm^3)	18.7 ± 0.5; 19.0	22.5 ± 0.5; 22.0	< 0.001
Left testicle volume (cm^3)	17.9 ± 0.7; 18.0	22.7 ± 0.3; 23.0	< 0.001

Table 3. **FSH, LH, prolactin, testosterone and free testosterone levels (M ± m, Me) in patients with autoimmune orchitis (1) and in healthy men (2)**

Groups / Characteristic	1 (n = 37)		2 (n = 26)		P
	M ± m	Me	M ± m	Me	
FSH (IU/L)	4.8 ± 0.5	4.1	4.2 ± 0.3	4.0	> 0.05
LH (IU/L)	3.7 ± 0.3	3.3	4.7 ± 0.4	4.4	> 0.05
Prolactin (mIU/L)	234.2 ± 16.0	220.4	307.5 ± 21.2	313.2	< 0.05
Testosterone (nmol/L)	11.2 ± 0.8	10.2	22.5 ± 1.3	20.0	< 0.001
Free testosterone (pmol/L)	21.7 ± 1.7	18.6	51.1 ± 3.8	48.8	< 0.001

Note: The Mann-Whitney test was used for comparison; differences in the groups were considered significant at $p < 0.05$.

The semen analysis included the following main criteria: volume, sperm concentration, number of progressively motile and morphologically normal spermatozoa (Table 4). According to our results, a significant decrease in the abovementioned parameters was observed in patients with autoimmune orchitis when compared to the controls ($p < 0.001$). The percentage of abnormal spermatozoa was also significantly higher in the study group ($p < 0.001$). According to Spearman, positive correlation was revealed between the number of progressively motile ($r = 0.5$, $p = 0.002$) and morphologically normal spermatozoa. No relationship was observed between the main semen characteristics and the level of serum antitesticular antibodies.

From the data collected, it can be concluded that both hormonal and sperm production functions of the testes are suppressed in patients with autoimmune orchitis, resulting in a significantly lower levels of serum testosterone and free testosterone as well as altered major semen parameters (i. e. ejaculate volume, concentration, the number progressively motile and morphologically normal spermatozoa).

Autoimmune oophoritis was established by the presence of serum antiovarian autoantibodies in titer > 350 U/ml in women with ovarian failure. According to this, the condition was detected in 680 (29.8 ± 1.0 %) of 2280 patients. The mean age of patients was 28.9 ± 0.2 years old (Me — 28.0). 623 (91.6 ± 1.4 %) patients exerted anovulation, and

luteinized unruptured follicle was noted in 57 of them (8.4 ± 1.1 %). Luteal insufficiency was present in 53 patients (7.8 ± 1.1 %), 4 patients (0.6 ± 0.3 %) were diagnosed with corpus luteum cysts (0.6 ± 0.3 %). 518 (76.2 ± 1.6 %) patients complained of menstrual disorders, general characteristics of which is shown in Fig. 3. Despite the preserved menstrual cycle in 162 (23.8 ± 1.6 %) patients, anovulation occurred in 62.3 ± 3.8 % of cases; the remaining women suffered from luteal insufficiency and luteinized unruptured follicle.

Table 4. **Semen analysis (M ± m, Me) in patients with autoimmune orchitis (1) and in healthy men (2)**

Groups / Characteristic	1 (n = 37)		2 (n = 26)		P
	M ± m	Me	M ± m	Me	
Volume (cm^3)	4.3 ± 0.3	4.0	5.2 ± 0.3	5.0	< 0.01
Concentration x 10^6	21.7 ± 0.3	20.0	131.5 ± 11.8	134	< 0.001
Progressive motility (%)	14.7 ± 1.3	16.0	48.9 ± 1.4	46.5	< 0.001
Morphologically normal spermatozoa* (%)	8.2 ± 0.5	8.0	17.5 ± 0.3	18.0	< 0.001

Note: The Mann-Whitney test was used for comparison; differences in the groups were considered significant at $p < 0.05$.

* WHO laboratory manual for the examination and processing of human semen criteria were applied (4^{th} edition).

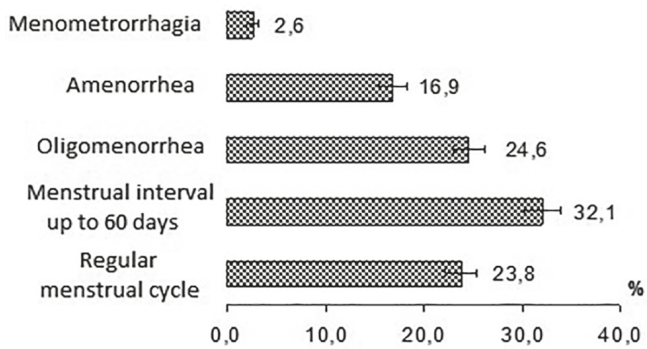

Fig. 3. Menstrual cycle disturbances in patients with autoimmune oophoritis

The mean duration of autoimmune oophoritis was 5.3 ± 0.3 years (Me — 4.0), the mean age of the menarche was 13.2 ± 0.1 years (Me — 13.0) and varied from 12 to 14 years. 401 women (58.9 ± 1.9 %) complained of infertility: 347 (51 %) patients had primary and 54 (7.9 %) patients had secondary infertility. A history of miscarriage was observed in 38 (5.6 ± 0.6 %) women, 15 of which (39.5 %) had secondary infertility. Chronic salpingo-oophoritis was the most common concomitant gynecological disease among the patient from the study group (Fig. 4). The combined incidence of endometriosis in the group of autoimmune oophoritis was 13.7 ± 1.3 %. After the surgical treatment of endometriosis,

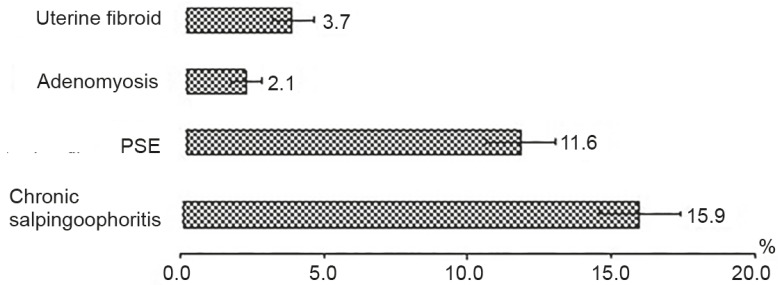

Fig. 4. Concomitant gynecological diseases in patients with autoimmune oophoritis

Note: PSE — peritoneal superficial endometriosis.

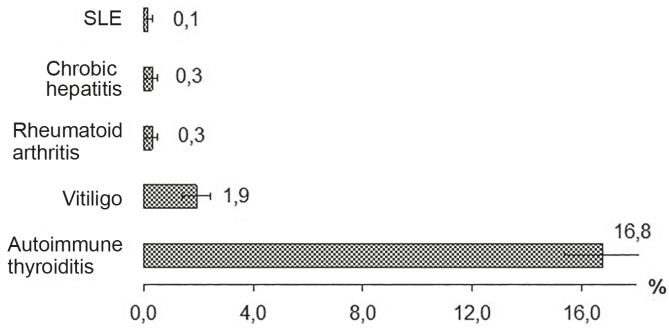

Fig. 5. Combined autoimmune diseases in patients with autoimmune oophoritis

Note: SLE — systemic lupus erythematosus.

46 (58.2 %) patients were treated with Gonadotropin-releasing hormone (GnRH) agonists. The diagnosis of adenomyosis was suspected by sonography and was confirmed by the following hysteroscopy. 1 year hormonal therapy was prescribed to all the women with adenomyosis and was canceled six months before the start of the trial.

Uterine fibroids larger than 4 cm were observed in 25 (3.7 ± 0.7 %) of the examined patients. Seven women (28 %) had previously undergone conservative myomectomy. More than half of patients (64 %) with leiomyoma received estrogen-progesterone hormonal therapy according to the contraceptive regimen, which was canceled six months before the start of the trial. 62 patients had a history of cervical dysplasia combined with chronic salpingitis in up to 74.2 % of cases.

132 (19.4 ± 1.5 %) women with autoimmune oophoritis had at least one more autoimmune condition with autoimmune thyroid disease being the most common (Fig. 5). The age of patients ranged from 16 to 44 y. o., the duration of the disease varied from 1 year to 14 years.

The mean level of antibodies to thyroglobulin (TG-abs) measured in the sera of 81 patients was 199.4 ± 58.3 U/ml, the mean level of antibodies to thyroperoxidase (TPO-abs) obtained from 74 women was 257.3 ± 38.2 U/ml. 72 (63.7 %) patients were on L-thyroxine supplementation (75 mcg/day). Vitiligo was present in 13 patients with autoimmune oo-

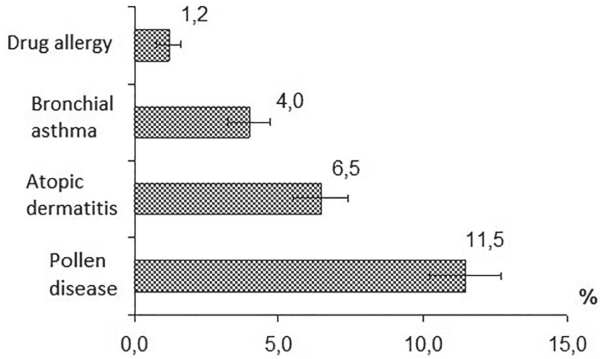

Fig. 6. Allergic conditions in patients with autoimmune oophoritis

phoritis. Two patients had rheumatoid arthritis since puberty. Two patients were diagnosed with chronic autoimmune hepatitis. One patient had systemic lupus erythematosus (in remission). One patient had a history of surgical treatment of thymoma.

A frequent combination of autoimmune oophoritis with hypersensitivity reactions was noted with pollen disease being the most frequent (Fig. 6). 12 (1.8 %) patients had a combination of pollinosis and bronchial asthma. In another 15 (2.2 %) a combination with atopic dermatitis was noted. Two (0.3 %) patients had combination of bronchial asthma, atopic dermatitis and pollinosis. The course of bronchial asthma in all patients was mild.

Autoimmune thyroid disease among the study group was almost 2 times more frequent than diffuse non-toxic goiter and reached 16.8 %. The prevalence of the latter in patients with autoimmune oophoritis was 9.4 ± 1.1 %, which is almost the same as in general population. Nodular non-toxic goiter was found in 9 patients (1.3 ± 0.4 %). History of type 2 diabetes was observed in 4 (0.6 ± 0.3 %) patients with autoimmune oophoritis. Obesity of various degree was observed in 30 (4.4 ± 0.8 %) women.

Upon physical examination, moderate hirsutism was detected in 22 (3.2 ± 0.7 %) patients. Mammary gland exam revealed the presence of fibrocystic disease in 45 (6.6 ± 1.0 %) women from the study group. Enlarged uterus (about 6/7 weeks of gestation) was present upon pelvic palpation in 15 (2.2 %) patients; cervical dysplasia — in 42 (6.2 ± 0.9 %) women. The increase in the size of the ovaries was detected in 66 (9.7 %) patients, 26 of which (39.4 %) had a history of chronic salpingitis, 8 (10.6 %) had peritoneal superficial endometriosis, and the remaining patients had no associated gynecological diseases.

The results of hormonal studies revealed increased serum levels of FSH and LH in 60 (8.8 ± 1.1 %) patients with autoimmune oophoritis ($p < 0.001$) (Fig. 7). Ovarian failure manifested with anovulation in all patients with a hypergonadotropic form of the disease.

The study groups did not differ from the controls when compared by age and body mass index. The mean age of menarche in both groups of patients with autoimmune oophoritis was significantly ($p < 0.001$) higher than in the control group (12.2 ± 0.1 years), and did not differ among patients with hypergonadotropic (13.2 ± 0.04 years) and normogonadotropic forms (13.4 ± 0.2 years) of ovarian failure. The duration of the disease was comparable between the groups (5.3 ± 0.4 and 5.3 ± 0.2 years, respectively). The results of the hormonal studies are shown in Table 5. Significant ($p < 0.001$) difference in levels of serum estradiol and progesterone was noted among patients with the hypergonadotropic

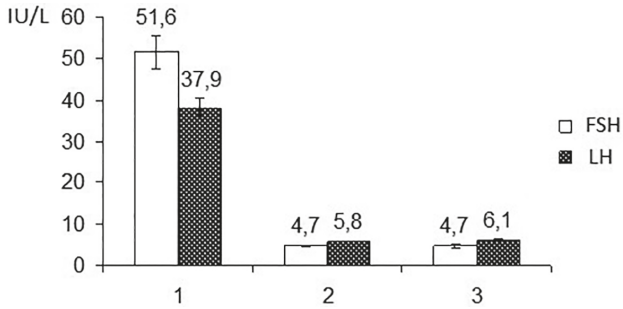

Fig. 7. Serum FSH and LH content in patients with a hypergonadotropic form (1), normogonadotropic form (2) of autoimmune oophoritis and in healthy women (3)

and normogonadotropic forms of autoimmune oophoritis and when comparing each of them with the controls.

Table 5. **Serum prolactin, estradiol and progesterone levels in patients with hypergonadotropic (1), normogonadotropic (2) forms of autoimmune oophoritis and in healthy women (3)**

Groups / Characteristic	1 (n = 60) (M ± m; Me)	2 (n = 620) (M ± m; Me)	3 (n = 28) (M ± m; Me)	P
Prolactin (mIU/L)	205.7 ± 8.3 214.2	284.2 ± 3.8 267.8	376.7 ± 21.9 382.4	P1,2 < 0.001 P1,3 < 0.001 P2,3 < 0.001
Estrodiol (pmol/L)	88.8 ± 7.0 84.4	256.8 ± 4.2 246.2	510.6 ± 22.3 511.3	P1,2 < 0.001 P1,3 < 0.001 P2,3 < 0.001
Progesterone (nmol/L)	0.9 ± 0.1 0.8	7.1 ± 0.4 3.4	27.7 ± 1.3 25.9	P1,2 < 0.001 P1,3 < 0.001 P2,3 < 0.001

Note: when comparing groups, the Kruskal-Wallis one-way analysis of variance was used. The difference was considered significant at p value < 0.015.

To investigate the relationship between the serum estradiol concentration and the menstrual disorders in patients with autoimmune oophoritis, the Kendall's correlation coefficient was used. A significant negative ($\tau = -4.9$, $p < 0.001$) correlation between these signs was revealed; serum estradiol level was lower among women with amenorrhea.

For the purpose of diagnosis autoimmune oophoritis, the determination of serum antiovarian autoantibodies was performed (Fig. 8). The mean level of the latter was 721.5 ± 12.03; Me — 637.0 U/ml. In the group of patients with a hypergonadotropic failure, the level of antiovarian antibodies (612.7 ± 17.9; Me — 579.0 U/ml) was significantly ($p < 0.01$) lower than in patients with a normogonadotropic form (732.0 ± 13.0; Me — 642.0 U/ml). When evaluating the relationship between the level of antiovarian antibodies in the sera with hormonal profile, a positive correlation was found between

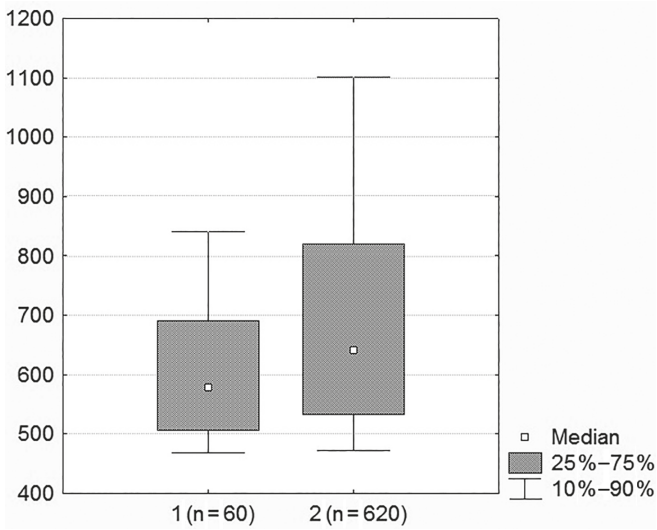

Fig. 8. Serum antiovarian antibodies in patients with a hypergonadotropic (1) and normogonadotropic (2) forms of autoimmune oophoritis

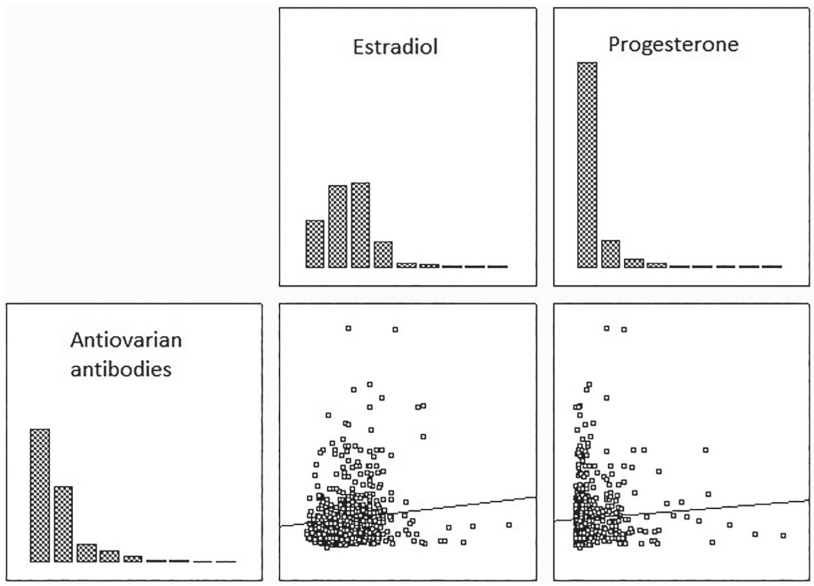

Fig. 9. The relationship of serum estradiol and progesterone levels and antiovarian antibodies titer in patients with autoimmune oophoritis

their level and serum estradiol ($r = 0.1$, $p = 0.007$) and progesterone concentrations ($r = 0.1$, $p = 0.014$) (Fig. 9). Furthermore, a negative correlation existed between the level of antiovarian antibodies and the severity of menstrual disorders ($\tau = -0.1$, $p = 0.002$). According to our results, a higher level of circulating autoantibodies was observed in patients with actively functioning ovaries.

Thus, in the setting of high steroid-producing activity of the ovaries, more autoantibodies are produced. Hormone synthesis by ovarian theca cells decreases with the disease progression due to their partial destruction by the autoimmune process. The amount of antigen progressively decreases leading to lower levels of circulating antiovarian antibodies, which is observed, in particular, in patients with the hypergonadotropic form of autoimmune oophoritis.

Discussion

Establishing the causes of gonadal failure is one of the essential problems of modern reproductive medicine. It is believed, that idiopathic causes play the largest role in the development of male infertility, comprising up to 30 % of cases [4; 5]. Immunological reasons are usually associated with the detection of antisperm antibodies with the frequency up to 15.8 % [12; 13]. It is well known, that maintained paracrine interaction between spermatogenic cells, testicular macrophages, Sertoli and Leydig cells is of fundamental importance for steroid hormone production and in maintaining spermatogenesis, in general. Experimental studies have shown a high importance of local androgen level in the seminiferous tubules on the processes of germ cells division and maturation at certain phases [14; 15]. A large controlled clinical trial including more than 300 patients with spermatogenic failure and infertility, revealed altered functional activity of Leydig cells, resulting in a decreased production of testosterone and free testosterone [16]. In the clinical practice, patients with rheumatoid arthritis have hypogonadism characterized by a decrease in the testosterone production [17]. Androgen deficiency have been common in a significant number of patients with systemic lupus erythematosus [18]. Antibodies to steroid-producing testicular cells were found in all patients with chronic adrenal insufficiency [19]; and autoimmune nature of hypogonadism was suggested in these cases. According to our results, circulating antibodies against the microsomal fraction of Leydig cells were detected by ELISA in 13.8 % of patients with altered spermatogenesis. Significantly lower serum levels of testosterone and free testosterone were found in this patients compared to healthy men, indicating hormonal malfunctioning of the testicles. The obtained clinical and laboratory results point on a significantly impaired hormonal and spermatogenic function of gonads in patients with detected circulating antibodies to Leydig cells. Additionally, the presence of concomitant autoimmune diseases in 13.5 % of these patients, allowed us to suspect an autoimmune nature of the testicular failure. Speaking of concomitant autoimmune diseases, the most frequent was autoimmune thyroiditis (8.0 %), the prevalence of which was higher of that for men in general population — 2.3 % [20]. Clinically, autoimmune orchitis was characterized by decreased volume of testicles when compared to the healthy men of the same age, as well as lower ($p < 0.001$) serum levels of testosterone and free testosterone with maintained level of FSH and LH. Among the study group significantly ($p < 0.001$) lower number of spermatozoa with progressive motility and normal morphology were found when compared to the controls. Infertility comprised the major complaint and the main reason for seeking medical care in most patients with autoimmune orchitis (94.5 %).

Determination of etiological factors causing primary ovarian insufficiency is of another great interest. It is believed, that an important role belongs to the autoimmune damage of the ovaries. Russian researchers [2; 21] came up with a conclusion that autoimmune

oophoritis leads to the development of normogonadotropic primary ovarian failure in 19.2–31.5 % of cases. The present study used ELISA for the detection of antibodies to the microsomal fraction of granulosa cells in order to establish autoimmune oophoritis. According to our own results, ovarian damage due to autoimmunity was detected in 680 (29.8 ± 1.0 %) of 2280 women with normogonadotropic and hypergonadotropic ovarian failure.

Previously, the validity of the method for antiovarian and antitesticular antibodies detection was determined in an additional study: sera from 45 patients with hypergonadotropic and normogonadotropic ovarian failure and the presence of an over-threshold level of antiovarian antibodies detected by ELISA were randomly obtained; all the samples were evaluated later using indirect immunofluorescence analysis. The accuracy of two methods was evaluated using Pearson's chi-squared test (χ^2), which showed a high reliability for both of them ($\chi^2 = 29.7$, $p < 0.0001$). Similarly, the results of antitesticular antibodies determination by ELISA and indirect immunofluorescence were evaluated in 57 patients with normogonadotropic hypogonadism and 26 healthy men. The obtained results coincided with a high degree of reliability ($\chi^2 = 36.2$, $p < 0.0001$). Additionally, all this findings were in agreement with regression analysis carried out.

Anovulation was the most common clinical manifestation of autoimmune oophoritis, comprising 91.6 % of cases, of which luteinization of the unruptured was seen in 8.4 % of women. Luteal insufficiency was noted in 7.8 ± 1.1 % and luteal cysts were diagnosed in 0.6 % of patients with autoimmune oophoritis. Different types of menstrual disorders were present in 518 (76.2 %) women lasting on average for 5.3 ± 0.3 years. In total, 58.9 % of women complained of infertility: 51 % had primary and 7.9 % of patients had secondary infertility. A history of miscarriage was observed in 5.6 % of women. 132 (19.4 %) patients with autoimmune oophoritis had combined autoimmune diseases, with thyroid autoimmunity being the most common (16.8 %). A hormonal check-up revealed a significant ($p < 0.001$) fall in estradiol and progesterone production by the ovaries among patients with autoimmune oophoritis when compared to healthy women of the same age. Such a trend was observed when comparing patients with normogonadotropic and hypergonadotropic forms of ovarian insufficiency ($p < 0.001$). Pelvic sonography revealed pronounced ($p < 0.001$) uterine and ovarian hypotrophy in women with a hypergonadotropic form of autoimmune oophoritis when compared to the corresponding parameters in patients with the normogonadotropic form of the disease and in the control group. It is worth noting that such patients had significantly reduced antral follicle count or their complete absence. The data collected from immunological, hormonal and ultrasound studies indicate almost complete cessation of folliculogenesis in combination with absolute estrogen deficiency, resulting from the autoimmune ovarian damage in women with hypergonadotropic form of the disease. Our results are consistent with the data from experimental studies when histological examination of the ovaries from animals with autoimmune oophoritis [14; 22] was performed. Increased size of the ovaries with large number of small follicles was observed in the cases of normogonadotropic ovarian failure ($p < 0.001$). Dynamic observation revealed a significant ($p < 0.001$) decrease in the dominant follicle diameter. Additionally, in this group, a positive correlation between the level of serum antiovarian antibodies ($p = 0.02$) and the diameter of the dominant follicle and negative correlation ($p = 0.01$) with the antral follicle count were observed, indicating more intense autoimmune process in the actively functioning ovaries. This observation is consistent with the

results of experimental study [23]: a histological examination of the ovaries from animals with autoimmune oophoritis initially revealed a lymphocytic infiltration in the internal theca cells of growing follicles. With the disease progression, it spreads to granulosa cells and gradually forms a dense inflammatory crown around the follicles.

Conclusions

1. Gonadal damage of autoimmune origin is detected in 27.9 % of reproductive-aged women with normogonadotropic primary ovarian insufficiency and in 13.8 % of men with normogonadotropic testicular insufficiency.
2. Autoimmune orchitis is characterized by an increased level of autoantibodies to steroid-producing testicular cells, a significantly decreased serum total and free testosterone and altered spermatogenesis, manifesting by low concentration of spermatozoa, a decreased number of cells with progressive motility and morphologically normal spermatozoa in semen.
3. Autoimmune orchitis is combined with other autoimmune diseases (autoimmune thyroiditis, vitiligo, type 1 diabetes) in 13.5 % of cases.
4. Autoimmune oophoritis is usually characterized by anovulation with preserved serum FSH and LH levels in 91.6 % and luteal insufficiency in 8.4 % of patients, respectively.
5. Autoimmune oophoritis is combined with other autoimmune diseases (autoimmune thyroiditis, vitiligo, chronic autoimmune hepatitis, rheumatoid arthritis, systemic lupus erythematosus) in 19.4 % of patients.

References

1. Kuharić M., Rozić D., Karner I. Thyroid Autoimmunity and Infertility. *SEEMEDJ,* 2017, vol. 1, no. 2, pp. 1–10.
2. Gabelova K. A., Gzgzyan A. M., Potin V. V., Rulev V. V. Autoimmune oophoritis (pathogenesis, clinical presentation, diagnosis, treatment). In: E. K. Ailamazyan (Ed.) *Posobie dlia vrachei*. St. Petersburg, 2010. (In Russian)
3. Lakhotia M., Pahadia H. R., Kumar H., Singh J., Tak S. A Case of Autoimmune Polyglandular Syndrome (APS) Type II with Hypothyroidism, Hypoadrenalism, and Celiac Disease — A Rare Combination. *J. Clin. Diagn. Res.,* 2015, vol. 9, no. 4, pp. OD01–OD3.
4. Bezerra Espinola M. S., Aragona C. New perspectives on male fertility evaluation: Innovative approach for the qualitative analysis of spermatozoa. *Andrologia,* 2019, pp. e13378.
5. Guerri G., Maniscalchi T., Barati S., Busetto G. M., Del Giudice F., De Berardinis E., Cannarella R., Calogero A. E., Bertelli M. Non-syndromic monogenic male infertility. *Acta Bio. Med.*, 2019, vol. 90, no. 10, pp. 62–67.
6. DalPra C., Chen S., Furmaniak J. et al. Autoantibodies to steroidogenic enzymes in patients with premature ovarian failure with and without Addison's disease. *Eur. J. Endocrinol.,* 2003, vol. 148, no. 5, pp. 565–570.
7. Poppe K., Autin C., Veltri F., Kleynen P., Grabczan L., Rosenberg S., Ameye L. Thyroid Autoimmunity and ICSI Pregnancy Outcomes. *J. Clin. Endocrinol. Metab.,* 2018, vol. 103, no. 5, pp. 1755–1766.
8. Chen C. W., Huang Y. L., Tzeng C. R., Huang R. L., Chen C. H. Idiopathic low ovarian reserve is associated with more frequent positive thyroid peroxidase antibodies. *Thyroid*, 2017, vol. 27, no. 9, pp. 1194–1200.
9. Wackenheim C., Hoffman P., Dumestre-Pérard C., Bouillet L., Alfaidy N., Deroux A. Prevalence and characteristics of serum autoantibodies in patients followed for infertility at Grenoble University Hospital. *Presse Med.*, 2019, vol. 48, no. 11, pp. 307–315.

10. Potin V. V., Gzgzyan A. M., Khokhlov N. V., Vorokhobina N. V. Patent no. 2216742 RF. Method for quantitative determination of the concentration of antibodies specific to antigens of steroid-producing human cells in human biological fluids containing specific antibodies. Priority 11.01.2002.
11. *World Health Organization. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. 4th edition, 1999.
12. Yasin A. L., Basha W. S. The Epidemiology of Anti-Sperm Antibodies Among Couples with Unexplained Infertility in North West Bank, Palestine. *J. Clin. Diagn. Res.,* 2016, vol. 10, no. 3, pp. 1–3.
13. Garolla A., Pizzol D., Bertoldo A., De Toni L., Barzon L., Foresta C. Association, prevalence, and clearance of human papillomavirus and antisperm antibodies in infected semen samples from infertile patients. *Fertil. Steril.*, 2013, vol. 99, no. 1, pp. 125–131.
14. Bergh A. Local differences in Leydig cell morphology in the adult rat testis: evidence for a local control of a Leydig cells by adjacent semineferous tubules. *Int. J. Androl.,* 1982, vol. 5, pp. 325–330.
15. Fouquet J. P. Ultrastructural analysis of a local regulation of Leydig cells in adult monkey (Macaca fascicularis) and rat. *J. Reprod. Fertil.*, 1987, vol. 79, pp. 45–96.
16. Andersson A. M., Jørgensen N., Frydelund-Larsen L. et al. Cell Function in Infertile Men: A Study of 357 Idiopathic Infertile Men and 318 Proven Fertile Controls. *J. Clin. Endocrinol. Metab.,* 2004, vol. 89, pp. 3161–3167.
17. Mahmood F., McMurray R. W. *Effect of Treatment of Hypogonadism On Rheumatoid Arthritis In Male Veterans*. Chicago, ACR/ARHP Scientific Meeting, 2011.
18. Kasitanon N., Phrintrakul N., Pattamapaspong N. et al. Hypogonadism and Erectile Dysfunction in Thai Men with Systemic Lupus Erythematosus. *Annals of the Rheumatic Diseases,* 2015, vol. 74, pp. 1100.
19. Petrikova J., Mikulas P., Lazurova I. Antibodies to Gonadal and Adrenal Tissue. *Autoantibodies*, 2014, vol. 3, pp. 391–405.
20. Dedov I. I., Fadeev V. V., Melnichenko G. A. *Endocrinology.* 3rd edition, revised and enlarged. Manual. Moscow, GEOTAR-Media Publ., 2015. (In Russian)
21. Smagina E. E. *The role of autoimmune processes in normogonadotropic ovarian failure development*. Abstract of PhD thesis, 1996, pp. 19.
22. Bagavant H., Sharp C., Kurth B., Tung K. S. K. Induction and Immunohistology of Autoimmune Ovarian Disease in Cynomolgus Macaques (Macaca fascicularis). *Am. J. Pathol.*, 2002, vol. 160, pp. 141–149.
23. Lewis J. Eosinophilic perifolliculitis: a variant of autoimmune oophoritis? *Int. J. Gynecol. Pathol.*, 1993, vol. 12, no. 4, pp. 360–364.

Received: April 17, 2020
Accepted: May 12, 2020

Authors' information:

Galina Kh. Safarian — Postgraduate Student; Galasaf07@gmail.com
Darico A. Niauri — MD, D. Sci. (Medicine), Professor; d.niauri@mail.ru
Yelena S. Borodina — Clinical Resident; lena.borodina9@yandex.ru
Lyailya Kh. Dzhemlikhanova — MD, PhD, Associate Professor; dzhemlikhanova_l@mail.ru
Alexandr M. Gzgzyan — MD, D. Sci. (Medicine), Professor; agzgzyan@hotmail.com