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Molecular Phenotyping of Reproductive Aging in Women with Subfertility and Infertility

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Abstract

There is no scientific data on the molecular mechanisms of reproductive aging in women. The purpose of the study is the development of molecular phenotypes of the aging in women with gynecologic pathology of reproductive and post-reproductive periods. The present study included 90 women with subfertility of varying degrees of severity and infertility. All the patients were divided into three groups: I group (n=30) included patients with a weak degree of subfertility; II group (n=30) with middle and high degree of subfertility; III group (n=30) with clinical infertility. All the patients underwent the routine diagnosis of the sub- and infertility. Molecular phenotyping of blood serum and cervicovaginal fluid processed with methods in proteomics: the prefractionation, the separation of proteins with standard sets, MALDI-TOF-MS/MS. Bioinformatics analysis of the molecules in the biosamples was based on the integrated database «Bioinformatic Harvester». Statistical analysis of the survey data was performed using the software «Statistica 7.0». Proteomic analysis helps in the detection of differences in the component composition of the serum proteins and CVF in women with subfertility of varying degrees of severity and infertility compared with the control group of fertile women. We have data on the universal molecular pathways of the development of reproductive aging in women with subfertility and infertility. IJBM 2012; 2(3):174-178.

Key words: *proteomics, bioinformatics, molecular interactions.*

Introduction

The purpose of this study is the development of molecular phenotypes of aging in women with a gynecologic pathology of the reproductive and post-reproductive periods.

The concept of «the aging of a person» is closely connected with concepts of «reproductive health» and «reproductive aging». The main clinical phenotypes of reproductive aging in the female organism are connected with the development of gynecological diseases and conditions, including uterine fibroid tumors, ovarian cysts, polycystic ovary syndrome, endometriosis in women of reproductive age, inflammatory diseases of the female genitals and menstrual disorders among young girls, miscarriage and other complications of pregnancy and childbirth, subfertility and infertility, oncological pathology in women, diseases pertinent to women during pre- and post-menopause, including several other female reproductive system abnormalities [1].

Reproductive aging of the female organism in demographic researches is presented by indicators such as birth rate, mortality, the birth of a child in middle age, and the time prior to pregnancy (TTP). In the 21st century, two

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tendencies became obvious in Russia, with respect to the birth rate: the reduction of the number of children per family and the displacement of the period of childbirth in women to a later age. The success of the birth of the second child by a woman until 40 years, decreases as a rule, correlating with the progress of the processes of subfertility and infertility, as clinical phenotypes of reproductive aging.

Today, some molecular interactions in the mechanism of human aging have been identified. The fields of female reproductive biology best studied by applying proteomics, include gynecological cancers, endometriosis and infertility [2, 3]. There is no scientific data about the molecular mechanisms of reproductive aging in women. The creation of the database of new intermolecular interactions and molecular targets for subsequent development of new drugs for the prevention of reproductive aging in women is becoming highly relevant.

Material and Methods

The present study included 90 women with subfertility of varying degrees of severity and infertility, according to the WHO criteria (1996), data of Habbema J. et al., and the value of TTP. The control group consisted of 30 women based on the next criteria, which included women with two or/and more safe pregnancies within a year of sexual activity without contraception. The women of the control group, included those with three or less pregnancies with no spontaneous abortions, although with four and more pregnancies they could have had spontaneous abortion. The average age of the women with subfertility and infertility was 36±1.2 years of age, whereas the women with fertility in the control group were 35±1.1 years of age. All the patients were divided into three groups: I group (n=30) included patients with a weak degree of subfertility, TTP=6 unsuccessful cycles; II group (n=30) with middle and high degree of subfertility, TTP=12 unsuccessful cycles; III group (n=30), TTP=48 months, with clinical infertility. All the patients underwent the routine diagnosis of infertility, including, total and special gynecological examination; ultrasound examination of the female pelvic organs; determination of blood group and Rh-factor; complete blood count; blood tests for syphilis, HIV, hepatitis B and C; the study of biocenosis of the urethra, vagina and cervix; hysterosalpingoscopy and laparoscopy on indications; endometrial biopsy; bacteriological examination and polymerase chain reaction of the urethral and cervicovaginal secretions; cervical smear tests; the definition of concentrations of follicle-stimulating hormone, luteinizing hormone, estradiol, prolactin, testosterone, cortisol, progesterone, triiodothyronine, thyroxine, thyrotropin, somatotropin (ADVIA CENTAUR, Bayer Diagnostics, Germany); the detection of antisperm antibodies and antiphospholipid antibodies; screening for infections (Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma genitalium, Herpes simplex virus, Cytomegalovirus, Toxoplasma gondii, Rubella virus); the typing of HLA-A, -B, -C, -DR-antigens of lymphocytes of the pair; the study of genetic factors of habitual miscarriage and risks (G20210A prothrombin gene mutation, factor V G1691A gene mutations, MTHFR C677T gene mutation; ICycler IQ-5, BioRad, USA). Molecular phenotyping of biosamples (blood serum, CVF) processed with methods in proteomics: the prefractionation, the separation of proteins with standard sets (MB-HIC C8 Kit, MB-IMAC Cu, MB-Wax Kit, «Bruker», USA), matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS, Ultraflex II, «Bruker», USA). The partially identified sequences were then submitted to «BLAST protein-protein» and screened against the Homo sapiens Swissprot database to check if this identification matched the MASCOT-identification (Matrix Science). Bioinformatics analysis of the molecules in the biosamples was based on the integrated database "Bioinformatic Harvester" (Karlsruhe Institute of Technology, Germany). The data of the molecular interactions and functional features of proteins were received with STRING 8.1 and STITCH databases. Statistical analysis of the survey data was performed using the software «Statistica 7.0».

Results

The study revealed several forms of female subfertility and infertility, significantly, endocrine subfertility and infertility, associated with the violation of ovulation (32%); Fallopian tube subfertility and infertility (28%); gynecological diseases as the causes of the subfertility and infertility (25%); immune subfertility and infertility (1%); and unexplained subfertility and infertility (14%).

The compatibility was for one antigen HLA-DQ in 14 out of 90 pairs (15.5%) with subfertility (4 pairs; 4.4%) and clinical infertility (10 pairs; 11.1%), for HLA-B - in 21 out of 90 pairs (23.3%) with subfertility (9 pairs; 10%) and clinical infertility (12 pairs; 13.3%). Combinations for HLA in infertility were found in 35 out of 90 pairs (38.9%).

Factor V G1691A gene mutations were heterozygous in two women with subfertility and four women with clinical infertility, and in one woman of the control group. G20210A prothrombin gene mutation has not been identified. The analysis of MTHFR C677T gene mutation rare allele 677T was found in 21 women of I group (70%): in homozygous - in 4 women (13.3%), in the heterozygous – in 17 women (56.7%); in 30 women of II group (100%): in homozygous - in 5 women (16.7%), in heterozygous – in 25 women (83.3%); in 30 women of III group (100%): in homozygous - in 7 women (23.3%), in heterozygous – in 23 women (76.7%); in 7 women of the control group (23.3%): in homozygous - in 2 women (66.7%), in heterozygous – in 5 women (16.7%).

Proteomic analysis helps in the detection of differences in the component composition of the serum proteins and cervicovaginal fluid (CVF) in women with subfertility of varying degrees of severity and infertility compared with the control group of fertile women (Table 1, 2).

Bioinformatics analysis revealed the presence of molecules, which are the participants of the universal pathways of reproductive aging in women and the molecular interactions involved.

Table 1
Qualitative profile of serum proteins in women with the subfertility, infertility and in control group of fertility women

	Protein name	n (the number of women with the expression of the serum protein)					Functional process (source: Bioinformatic Harvester, KIT, Germany)
		Control group (n=30)	I group (n=30)	II group (n=30)	III group (n=30)	[1]MW, [2]Da	
1	Beta-defensin 2	30	12	10	2	7820	Immunity and defense, nucleoside, nucleotide and nucleic acid metabolism
2	Metalloproteinase inhibitor 1	0	7	24	30	23794	Developmental processes, protein metabolism and modification
3	Cystatin-C	2	15	18	28	13300	Protein metabolism and modification
4	Apolipoprotein A-II	0	6	23	30	11175	Lipid, fatty acid and steroid metabolism, transport
5	Apolipoprotein B-100	1	5	14	30	515605	Lipid, fatty acid and steroid metabolism, transport
6	Glucose-6-phosphate isomerase	30	21	14	3	59991	Carbohydrate metabolism
7	Glycodelin	1	15	18	30	16361	Developmental processes
8	Macrophage migration inhibitory factor	0	22	26	30	12476	Immunity and defense
9	Metalloproteinase inhibitor 2	1	25	29	30	72000	Protein metabolism and modification
10	Carcinoembryonic antigen-related cell adhesion molecule 8 precursor	0	0	14	30	38154	Cell adhesion, signal transduction
11	Repetin	0	20	22	27	90731	Cell proliferation and differentiation, developmental processes
12	Kallikrein-14	2	17	22	26	29122	Protein metabolism and modification

Notes: *[1]MW - molecular weight; [2]Da - Dalton.*

Discussion

Proteomic analysis has revealed an increase in the absolute number of women with protein expression performing certain biological functions and having various localizations in the intra - and extracellular spaces (Table 1, 2).

Molecules was seen to interact among themselves and with other molecules as participants of universal pathways in reproductive aging in women, which is the chief cause for subfertility and infertility: the hormonal signaling pathway (insulin and insulin-like signaling, growth hormone signaling, steroid signaling, Klotho signaling, AC5, TGFβ), nutrient sensing and signaling pathway (sirtuin deacetylases, AMP-activated protein kinase, TOR and translation signaling, FOXA/PHA-4 transcription factor signaling, NRF1/SKN-1 signaling), mitochondria and ROS signaling (electron-transport chain signaling, stress-induced protein kinases: JNK and MST-1), genome surveillance pathways (tumor suppressors and antagonistic pleiotropy).

Each protein molecule in the functional group interacts with other protein molecules. For example, the

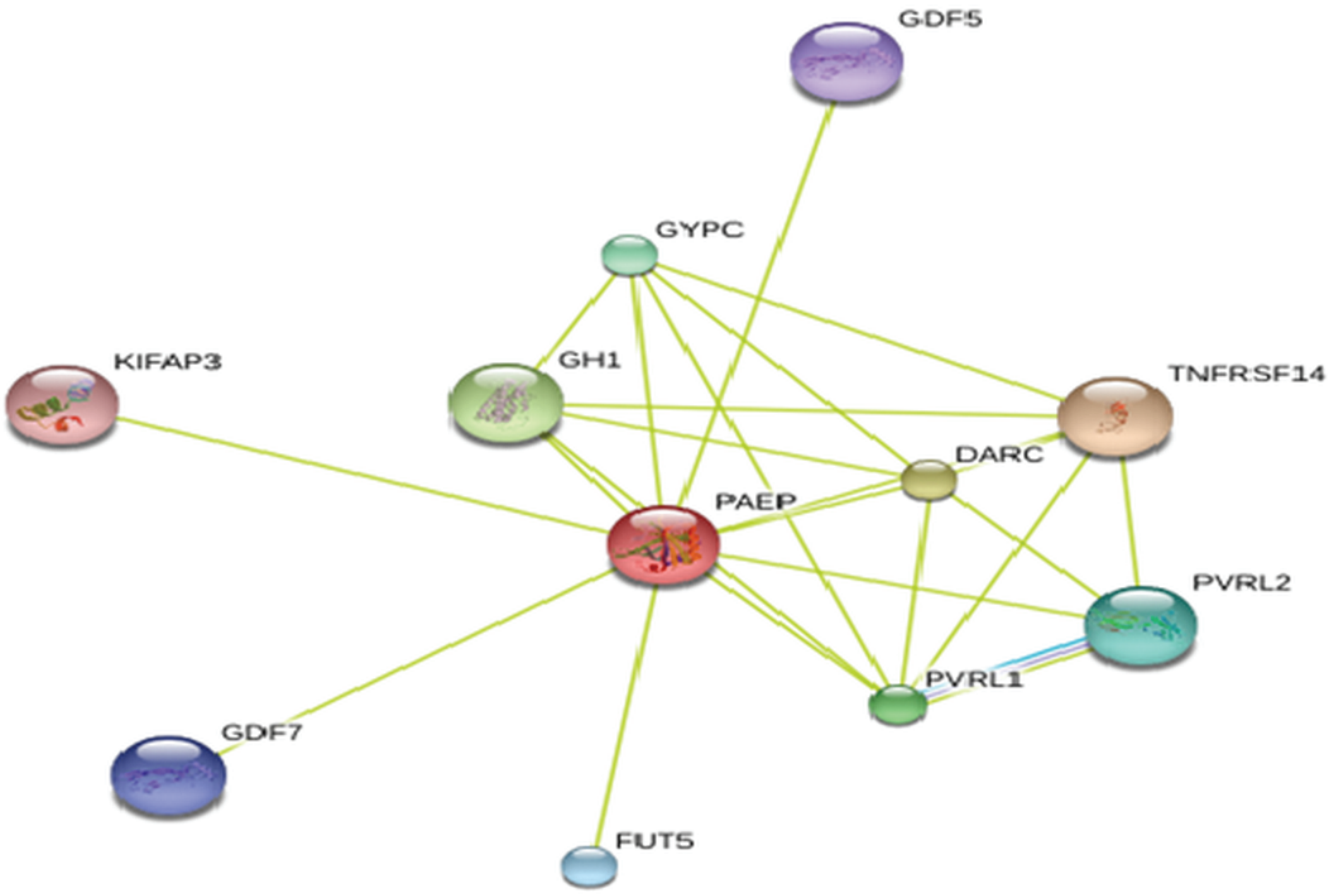
Table 2

Qualitative profile of proteins of cervicovaginal fluid in women with the subfertility, infertility and in control group

	Protein name	n (the number of women with the expression of the protein in CVF[1])					Functional process (source: Bioinformatic Harvester, KIT, Germany)	Cellular localization
		Control group (n=30)	I group (n=30)	II group (n=30)	III group (n=30)	[2]MW, [3]Da		
1	Putative tropomyosin alpha-3 chain-like protein	15	27	28	24	26269	Cell structure and mobility	Cytoplasm
2	Voltage-dependent P/Q-type calcium channel subunit alpha-1A	12	16	27	26	282365	Muscle contraction, neuronal activities, transport	Nucleus
3	Actin-related protein 2/3 complex subunit 2	30	22	6	4	40950	Protein metabolism and modification, cell structure and mobility	Cytoskeleton
4	Histone H2B type 1-K	30	10	8	2	13890	Immunity and defense, nucleoside, nucleotide and nucleic acid metabolism	Nucleus
5	Probable phospholipid-transporting ATPase VB	20	25	25	27	165391	Lipid, fatty acid and steroid metabolism, transport	Membrane
6	Hemoglobin subunit delta	8	12	14	18	16055	Blood circulation and gas exchange, transport	Cytoplasm
7	Tropomyosin beta chain	30	27	25	23	32851	Cell structure and mobility, developmental processes, muscle contraction	Cytoskeleton
8	Clathrin light chain B	5	8	21	30	35000	Intracellular protein traffic	Golgi Apparatus
9	Carboxypeptidase M	0	2	4	30	36520	Protein metabolism and modification	Membrane
10	B-Raf proto-oncogene serine/threonine-protein kinase	0	4	9	30	84437	Apoptosis, cell proliferation and differentiation, oncogenesis, signal transduction	Membrane
11	Small proline-rich protein 2E	12	16	17	21	7855	Developmental processes, cell proliferation and differentiation	Cytoskeleton
12	Peroxiredoxin-6	3	23	28	28	27100	Immunity and defense	Cytoplasm
13	Carnitine O- palmitoyl-transferase 2, mitochondrial	6	15	27	24	73777	Amino acid metabolism, lipid, fatty acid and steroid metabolism	Envelope
14	Nesprin-2	1	18	26	26	796442	Cell structure and mobility	Nucleus
15	Toll-like receptor 7 precursor	5	12	17	28	120922	Developmental processes, signal transduction	Membrane

Notes: *[1]CVF - cervicovaginal fluid, [2]MW - molecular weight; [3]Da - Dalton.*

Figure 1
Molecular interactions of progestagen-associated endometrial protein (glycodelin) (STRING 8.1 database).



Notes: *PAEP - progestagen-associated endometrial protein (glycodelin); TNFRSF14 - tumor necrosis factor receptor superfamily, member 14; DARC - Duffy blood group, chemokine receptor; GH1 - growth hormone 1; PVRL1 - poliovirus receptor related 1; GYPC glycophorin C; PVRL2 - poliovirus receptor-related 2; FUT5 - fucosyltransferase 5; GDF7 - growth differentiation factor 7; GDF5 - growth differentiation factor 5; KIFAP3 - kinesin-associated protein 3.*

molecular interactions of glycodelin or progestagen-associated endometrial protein, are presented (Fig. 1). The concentration of glycodelin rises in the serum of infertile women with abnormal tubes compared with fertile controls.

Conclusion

We identified potentially new biomarkers that differ among women with subfertility and infertility and that could aid in developing a noninvasive, serum-based diagnostic test. This study is the first step in the identification of potentially new biomarkers of reproductive aging in women. Future identification of the proteins and further validation in a second population is needed before these findings can be applied in clinical practice.

References

1. Seeber B, Sammel MD, Fan X, Gerton GL, Shaunik A, Chittams J, Barnhart KT. Proteomic analysis of serum yields six candidate proteins that are differentially regulated in a subset of women with endometriosis. Fertil Steril 2010; 93(7):2137-44.
2. Anderson NL. The clinical plasma proteome: a survey of clinical assays for proteins in plasma and serum. Clinical Chemistry 2010; 56 (2):177-185.
3. Hong Zhang, Yidong Niu, Jie Feng, Huifang Guo, Xue Ye, Heng Cui. Use of proteomic analysis of endometriosis to identify different protein expression in patients with endometriosis versus normal controls. Fertil Steril 2006; 86(2):274-282.