Bioelectrochemistry 79 (2010) 193–197

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Dielectric properties of human ovary follicular fluid at 9.2 GHz

Olga A. Gorobchenko [a,*], Anna G. Gerodes [b], Oleg A. Nardid [b], Oleg T. Nikolov [a]

[a] Department of Biological and Medical Physics, School of Radiophysics, V.N. Karazin Kharkov National University, 4 Svobody Sq., 61077 Kharkov, Ukraine
[b] Department of Cryobiophysics, Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, 23 Pereyaslavskaya Str., 61015 Kharkov, Ukraine

ARTICLE INFO

Article history:
Received 25 June 2009
Received in revised form 2 March 2010
Accepted 26 March 2010
Available online 1 April 2010

Keywords:
Follicular fluid
Permittivity
UHF-dielectrometry
Freezing

ABSTRACT

The influence of the follicle size, rapid freezing to −196 °C and cryopreservation in liquid nitrogen within a period of one month of the human ovary follicular fluid (FF) on its dielectric properties is studied by the microwave dielectric method. The FF was obtained from dominant follicles of patients who received treatment for infertility by extracorporal fertilization. We have measured the real part (ε') of the complex permittivity of the native and frozen follicular fluids at the room temperature. A resonator type ultra high frequency (UHF) dielectrometer at the frequency of 9.2 GHz has been used. We have also obtained the values of the total protein, hormones and glucose concentration in the FF. It was found that rapid freezing reduces ε' of the FF. It can result from the bound water increase in the system. It was also found the rise in permittivity and the total protein concentration with the increase of a follicle size, which could be explained by protein dehydration as a result of its clustering and aggregation.

1. Introduction

The ovarian follicular fluid (FF) is a product of granulose cells. It is a compound biological medium that surrounds an oocyte during its maturation. Treatment of human spermatozoa with 50% of the Menezo B-2 medium and 50% of native FF yielded a high fertilizing capacity, progressive motility and lifetime [1]. The observations suggest that exposure to 75% bovine FF at 25 °C for 1 h before freezing may increase the resistance of boar spermatozoa to cryopreservation stress [2]. Thus, the FF protects spermatozoa from cryoinjury. In this case a problem of preservation of FF native bioactive properties for a long period of time arises.

The elaboration of an effective procedure for the long-term storage of FF will extend its usage, in particular, in preparation of spermatozoa for insemination [3], culturing of the oocytes and embryos [1,4], and in other fields of biotechnology and medicine. Usually the biological tissues and fluids are frozen for a long-time storage. However, the deep freeze storage can lead to changes in a number of physicochemical characteristics of biological fluids (FF, blood plasma and serum, etc.) [5]. The properties of proteins in the isolated state change even at +4 °C [6], and the degradation effect of freezing reveals itself in protein denaturation, being sometimes reversible [7]. The basis for freezing damages of proteins, enzymes and hormones is pH lowering, dehydration [8], changing of medium tonic, rise in salt concentration [9], etc. The literature concerning the influence of freezing on isolated or incorporated into tissue proteins suggests that proteins with a quaternary structure are most cryolabile [10]. The changing of pH alone is able to destabilize the proteins with a quaternary structure [11,12]. The concentrated salt and metabolite solution formed in the microphases of the crystallized medium under freezing leads to decomposition of the protein into subunits [8]. Besides, the damage of the peptide-hydrogen bonds produces a change in the alpha helix structure and a native protein changes its structure in the direction of a statistical ball [13]. These changes can damage the structure of macromolecules in biological fluids or affect intermolecular interactions.

It is well known that hydration water, structurally bound with macromolecules, stabilizes its native conformation [14,15]. In this connection, it is of great interest to study the influence of freezing, preservation at −196 °C and consequent melting on physical characteristics of FF, and on hydration of FF macromolecules. We have used a method of estimating hydration based on microwave dielectric measurements [16]. For a polar substance the relative permittivity is a complex value at a microwave frequency: $\varepsilon^* = \varepsilon' - j\varepsilon''$, where the real part ε' is the relative permittivity, which is the ability of the material to be polarized by an external field, and the imaginary part ε'' is the dielectric loss, which proportional to the energy absorbed from the field. In the region of relaxation of the free water molecules (γ-dispersion) the

* Corresponding author. Tel.: +38057 707 55 76; fax: +38057 705 12 61.
E-mail address: olga_bio_f@yahoo.com (O.A. Gorobchenko).

1567-5394/$ – see front matter
doi:10.1016/j.bioelechem.2010.03.010

dielectric properties of an aqueous protein solution are described by the Debye equation [16]:

$$\varepsilon^*(f) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + j(f/f_R)} \tag{1}$$

where ε_s and ε_∞ are the dielectric constants at the low and high-frequency sides of the single relaxation, f_R is the relaxation frequency of water molecules, f is the frequency. The Eq. (1) when expanded into real and imaginary parts gives:

$$\varepsilon'(f) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (f/f_R)^2} \tag{2}$$

$$\varepsilon''(f) = \frac{(\varepsilon_s - \varepsilon_\infty) f / f_R}{1 + (f/f_R)^2}. \tag{3}$$

The dielectric spectroscopy method is widely used for study a water state in biological systems. It has been applied to study aspects of the organization of water in animal tissues [17] and to determine water content and water distribution in ischemic hearts [18,19]. For the most part the dielectric studies have been performed in the low-frequency range or in the range not exceeding 3 GHz. In the present work the complex permittivity measurements were carried out in a dispersion region of free water molecules using a method of ultra high frequency (UHF) dielectrometry at 9.2 GHz. In this region the dielectric properties of tissues are determined by their large water content and dielectric data can be used to compare the relaxation time of tissue water molecules with those of the pure water. The method enables the registration of even slight changes of the water state in biological fluid [20].

The dielectric properties of the FF are not understood. The dielectric parameters of the FF at 9.2 GHz are of obvious interest for study the water state in it. Any structure damages and conformation changes of FF components affect the ratio of free and bound water in the system. Measuring these changes and using water molecules as markers we can obtain the information about both structure changes of macromolecules and their interaction. The aim of this study is to investigate the dielectric properties of the FF after freezing and to obtain the information about the water state in it.

2. Experimental

2.1. Materials

The FF was obtained from 14 patients, who received treatment for infertility at the Human Reproduction Centre "Implant" (Kharkov, Ukraine), by the method of extracorporal fertilization followed by the transfer of embryos to uterine cavity. There were totally 20 follicles. All women were of the reproductive age (25–35 years old), having no endocrine diseases. The stimulation of folliculogenesis was achieved by the drug treatment procedure [21]. The release factor agonist (Suprefact "Hoechst") and human follicle-stimulating hormone (Menogon "Serono") were used. The FF was obtained by the follicular aspiration from the women on the 12–14 days of the menstrual cycle. The human choriogonal gonadotropin (Profasi "Serono") in the amount of 1000 unit was injected 34–36 h prior to the aspiration. The FF was received from dominant follicles 17–22 mm in diameter. The volume of the FF was measured with a volumetric glass and used as an indicator of the follicular size. The oocytes were isolated and their quality was determined by the procedure described [22]. The residual FF was centrifugated at 200 g for 10–15 min to precipitate blood cells so that the supernatant could be used for the study. The obtained FF in the amount of 0.7–1.0 ml was placed in apyrogenic plastic ampoules and then frozen. The freezing was carried out at the rapid rate (300–400 °C/min) that was obtained by the direct immersion of the ampoules into liquid nitrogen. Some of the ampoules were stored at −196 °C for a period of one month. The melting of the FF was carried out in a water bath at 37 °C. The native FF was used as a control and studied for 3–6 h after its obtaining.

2.2. Apparatus

Relative permittivity of the FF was measured by the microwave dielectric method described [23]. We used a resonator type UHF-dielectrometer at 9.2 GHz. The experiment was carried out at room temperature (20 °C). The relative error of the measurements was 0.1% for ε' and 0.5% for ε''.

2.3. Determination of the hormones, protein and glucose concentration

The concentration of estradiol and testosterone in the FF was determined by the method of triphasic immune-enzyme analysis [24]. The total protein content was determined by the Lowry method [25], using human serum albumin as a standard. The glucose content in the FF was determined by the method described [26].

3. Results and discussion

3.1. The effect of freezing on the permittivity of the FF

Fig. 1 shows the measurement of relative permittivity (ε') of the native FF, FF immediately melted after freezing and FF melted after a month storage at −196 °C. Our findings reveal that at the frequency of 9.2 GHz permittivity of the native FF, received from the different follicles, varies from 53.0 to 55.7. Permittivity of the FF, frozen and stored at −196°C for a month, decreases or does not vary within the experimental error. A decrease in ε' suggests that the amount of free water in the system is falling. This can result from an increase in hydration of some FF components, such as proteins [27], which appears to be caused by release of additional sites of binding of the water molecules. These sites appear due to conformation changes in macromolecules during freezing. The rapid freezing to low temperatures, at which the effect of eutectic concentration is short-time, does not lead to the protein cryodenaturation. Due to this, the low temperature preservation of biological media leads to fewer damages

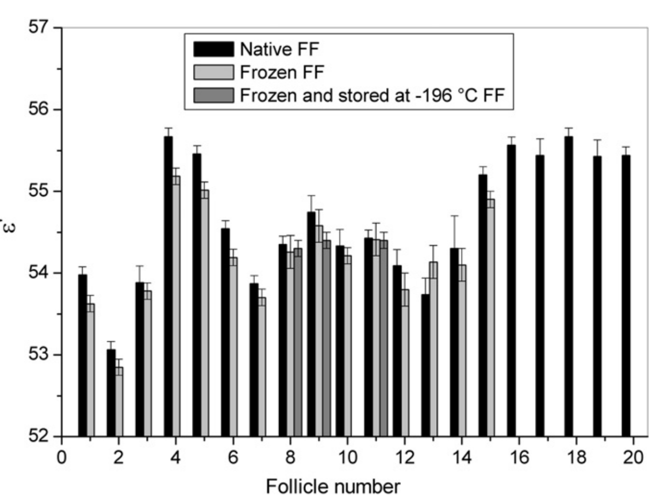

Fig. 1. The values of the real part (ε') of the complex permittivity ($\varepsilon^* = \varepsilon' - j\varepsilon''$) of the native follicular fluid (FF), frozen FF and stored at −196 °C FF.