Improvement in quality of oocytes in polycystic ovarian syndrome in programs of in vitro fertilization

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ABSTRACT
Inositol therapy is aimed at improving the quality of oocytes during preconception care in patients with polycystic ovarian syndrome (PCOS), a cause of infertility and reproductive dysfunction. The objectives of this observational comparative multicentre study were to evaluate the effectiveness of inositol in improving the quality of oocytes/embryos and IVF cycle outcome. Group 1 patients (N = 133) received inositol 1000 mg (Inofert or Nutrilinea) + folic acid 0.1 mg. Group 2 consisted of patients with preserved ovarian reserve without PCOS (N = 137), not administered inositol prior to pregnancy. Effectiveness criteria were numbers of mature oocytes and good quality embryos, pregnancy rates per ET, ‘take home baby’ index and miscarriage rates. Pregnancy rates per ET (87.0% vs. 87.4%), ‘take home baby’ index (79.6% vs. 89.4%) and miscarriage rates (14.3% vs. 10.6%) were comparable. Use of inositol in patients with PCOS during preconception care is an effective method allowing improvement of oocytes quality and positively affecting IVF cycle prognosis. High pregnancy rates per ET and ‘take home baby’ index after treatment are justifying inositol usage in patients with PCOS and infertility.

Introduction
Polycystic ovarian syndrome (PCOS) is a widespread endocrine disease that occurs in 20% of women of reproductive age [1]. PCOS is characterized not only by hyperandrogenism and menstrual disorders, but also by infertility associated with the absence of ovulation, as well as metabolic syndrome. Obesity is one of the most important factors in the development of PCOS. On the one hand, from 30% to 70% of women with PCOS are overweight, and on the other hand, PCOS often occurs with an increase in body mass index of more than 28.0 kg/m² [1]. At the epigenetic level, during the period of embryofoetal development, infancy and adolescence, the excess of androgens leads to the formation of abdominal and visceral obesity and at a later age to the insulin resistance and hyperinsulinemia [2], which subsequently closes the vicious circle of insulin resistance: ‘hyperinsulinism – hyperandrogenism – PCOS.’ According to various authors [3], the insulin resistance can be found in women with PCOS regardless of body mass index.

There are two ways of hyperproduction of androgens at PCOS. The first is related to a high level of luteinizing hormone (LH) and can be found in patients without obesity and insulin resistance, the second – on the contrary – is typical for patients with obesity, insulin resistance and normal level of LH [4]. Insulin is synergized with LH, stimulating testosterone synthesis by theca-cells of the ovary. Thus, it has a cagonadotropic effect on the ovary, increasing the LH-induced synthesis and the secretion of androgens by the ovaries [5]. In addition, hyperinsulineemia can stimulate the development of antral follicles, increasing the sensitivity of granulosa cells to follicle-stimulating hormone (FSH), thereby increasing the number of simultaneously growing antral follicles. Even at the end of the twentieth century it was shown that this process is accompanied by an increase in the ovary volume with the formation of small-cystic lesions in it [6]. The importance of insulin resistance at PCOS is indirectly evidenced by the fact that insulin sensitizers are suggested as an alternative for the treatment of hyperinsulinemia-induced ovarian dysfunction [7].

In order to diagnose PCOS the Rotterdam criteria of the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) are the most frequently used: (1) oligo- or non-ovulation; (2) clinical and/or biochemical signs of hyperandrogenism; (3) polycystic ovary morphology according to sonography [8]. The diagnosis is established if two of the three criteria listed above are present.

Inositol (vitamin B8) is involved in the insulin-signaling cascade and has a specific effect on many organs and systems. More than half of the inositol-dependent proteins are involved in supporting the vital functions of the cardiovascular, central nervous and immune systems. Inositol-dependent peptides also participate in the reproductive system. Studies have shown [9] that the use of this micronutrient reduces the risk of metabolic disturbances in patients with overweight at PCOS, having a beneficial effect on metabolic processes and hormonal regulation of ovarian function. According to randomized controlled trials, inositol is a highly effective pathogenetic agent in the combined therapy of infertility in patients with PCOS [10].

In 2013, the consensus of the world’s leading specialists in obstetrics and gynecology [11] found that the use of inositol is...
necessary for the compensation of deficiency of this micronutrient at PCOS, which is clinically oriented toward normalizing the levels of androgens, FSH, LH and restoring the ovulatory menstrual cycle. In addition, use of inositol helps to normalize the lipemic index. The targeted effect of inositol on such manifestations of PCOS as insulin resistance, overweight, hyperandrogenia, oligo- and menolipsis, high levels of LH and antioxidant effects made it the drug of choice included in the PCOS treatment program (achievement of ovulation and maturation of a single qualitative oocyte) and preparation for infertility therapy by IVF in Europe, USA, Japan, South Korea, etc.

Use of inositol at the stage of preparation for infertility treatment by IVF is a promising method aimed at improvement in quality of oocytes, which allows accelerating the onset of ovulation and improving the results of IVF cycles in patients with PCOS [9].

The objectives of the study were as follows: (1) to evaluate the effectiveness of inositol in patients with PCOS for improving the quality of oocytes; (2) to evaluate the quality of embryos after application of inositol at PCOS; (3) to evaluate the outcomes of IVF cycles after inositol therapy at PCOS.

Methods

The study was conducted with the participation of clinics for assisted reproductive technologies (ART) included in the self-regulating organization ‘Association of Clinics for ART’ (President – MD E.V. Vartanyan) [12], including in the Clinic for Assisted Reproductive Technologies «Test-Tube Babies» (Director – MD E.V. Vartanyan) – at the clinical base of the Department of Obstetrics, Gynecology and Reproductive Medicine of the Faculty of Continuing Medical Education of the Peoples’ Friendship University of Russia (Head of the Department - corresponding member, Prof. V.E. Radzinsky). For the observational comparative multicentre study the patients with primary and secondary combined infertility (N = 270) who applied to the clinics of assisted reproductive technologies of the Russian Federation in the period from July 1, 2015 to December 31, 2016 were selected. The first group consisted of women with PCOS (n = 133), the second group (n = 137) consisted of patients with preserved ovarian reserve without PCOS.

Inclusion criteria: age of <45 years, the absence of severe extragenital pathology, ET in the cycle of ovarian stimulation. Exclusion criteria: age of 45 years and older, severe extragenital pathology, X-linked genetic syndromes, causing oocyte-derived factor of infertility, ET in the IVF cryoprotocol. Criteria for the effectiveness of therapy: outcomes of IVF cycles: the number of mature oocytes and embryos of good quality, the pregnancy rates per ET, the ‘take home baby’ index, the miscarriage rates.

Before entering the IVF protocol on days 2 – 3 of the menstrual cycle, the transvaginal ultrasound (TVUS) was performed. Images of the uterus and ovaries in the sagittal and transverse plane were sequentially obtained. Monitoring of follicular growth in the cycle of controlled multifollicular ovarian stimulation was carried out by folliculometry (using TVUS), also concentrations of FSH, LH, estradiol in the blood serum were determined by microwhitenonfluence method. The normative values for the first phase of the menstrual cycle were 3.5–13.1 mIU/ml for FSH, 1.7–13.3 mIU/ml for LH, 23–179 pg/ml for estradiol, respectively.

During the preconception period, all the patients of the first group received inositol 1000 mg + folic acid 0.1 mg, 1 sachet/day (Inofert, Humana Pharma International SpA or Nutrilinea Srl for Italfarmaco SpA, Italy) orally for 3 months and during the ovarian stimulation. Patients in the second group received only folic acid 400 µg + cyanocobalamin 2 µg, 1 tablet/day (Foliber, Italfarmaco S.p.A., Italy) orally for 3 months, during the ovarian stimulation and further up to 12 weeks of gestation.

Controlled induction of ovarian stimulation was carried out according to two protocols:

1. using FSH (follitropin alfa 150 IU/day, subcutaneously, according to the schedule), ordering it from the 3–5 day of the menstrual cycle. The duration of administration of the FSH was from 9 to 12 days and depended on the size of the leading follicle according to folliculometry. From 5 to 12 days, at the continued ovarian stimulation, gonadotropin releasing hormone antagonist (ant-GnRH) (cetrorelix 0.25 mg, 1 syringe/day, subcutaneously, according to the schedule);

2. in the protocol with desensitization of the hypothalamic–pituitary–ovarian system, this was done using gonadotropin releasing hormone agonists (aGnRH) (tryptorelin acetate 0.1 mg/day subcutaneously from 21 day of the previous cycle and for 18 days) with direct proceeding to the controlled induction of ovarian stimulation with FSH (follitropin alfa 150 IU/day, subcutaneously, according to the schedule).

As an ovulation trigger the recombinant human chorionic gonadotropin (hCG) choriogonadotropine alfa 250 µg (6500 IU) was administered subcutaneously, given as a single dose.

In order to support the luteal phase of the cycle, micronized progesterone of 200–600 mg/day was used intravaginally (Iprozine, Catalent France Beinheim SA or Capsugel Ploermel, France for Laboratoires EFFIK, France – Italfarmaco SpA, Italy) during the entire preconception period from day 15–25 of each menstrual cycle, including the IVF protocol, until determining the level of β-hCG and further up to 12 weeks of gestation [13].

All the patients were assessed for β-hCG in peripheral blood 14 days after ET. At a level of β-hCG of more than 50 IU/L the pregnancy test was considered as positive.

Mathematical assessment of the results was carried out using descriptive statistics methods on a personal computer using IBM SPSS Statistics v 20 and Microsoft Excel 2007 software. The number of women in the group is indicated by N, and the number of cases in the group is n (n/N). Descriptive statistics of quantitative variables is presented by medians (Me), interquartile range (L–H), where L is 25 (lower) quartile, H is 75 (upper) quartile. The confidence interval (CI) is indicated as Me ± m, where Me is the median, and m = 2σ (2 standard deviations). To compare the quantitative variables, the nonparametric tests (the Mann–Whitney test) were used in two independent samples. A comparative analysis of categorical variables was carried out using the χ² criterion. When analyzing the contingency tables 2 × 2 the Yates correction or Fisher exact test were used. Differences were considered significant at p < 0.05. The ‘take home baby’ index was calculated as the number of children who lived more than 27 postpartum days per the number of embryo transfer procedures ratio.

Results

The mean age and the body mass index in the groups were comparable; the mean age was 30.2 ± 6.3 years, the mean body mass index was 26.5 ± 11.4 kg/m².

The key factors of infertility in the groups were as follows: the endocrine factor prevailed in the first group (104/133 (78.2%) vs. 8/137 (5.8%), p = .001), whereas the second group was dominated by tubal and/or uterine factor (15/133 (11.3%) vs. 67/137
(48.9%), \( p < .001 \) and the male factor (9/133 (6.8%) vs. 48/137 (35.0%), \( p = .002 \)).

In the first group the protocol of controlled ovarian stimulation with an ant-GnRH (93.2% vs. 81.8%, \( p = .002 \)) was more often used. Primary outcomes for assessing the effectiveness of inositol use in women with PCOS were analyzed by the indicators of IVF cycles (Table 1).

The total number of mature oocytes in the first group was significantly higher than in the second group (\( p < .001 \)).

Data on the outcome of the IVF cycle was obtained from 108/133 (81.2%) patients from the first group and from 119/137 (86.9%) from the second group. Data on the outcome of pregnancies after IVF and ET were obtained in 49/133 (36.8%) of women from the first group and in 47/137 (34.3%) from the second group. The outcomes of IVF cycles, estimated according to the pregnancy rates per ET (94/108 (87.0%) vs. 104/119 (87.4%), the ‘take home baby’ index (39/49 (79.6%) vs. 42/47 (89.4%)) and the miscarriage rates (7/49 (14.3%) vs. 5/47 (10.6%)) did not differ significantly in the groups.

**Table 1.** Indicators of IVF cycles in the observed women.

| Measured outcome                      | Group 1       | Group 2       | \( P \) |
|---------------------------------------|--------------|--------------|------|
| Number of mature oocytes, IU, Me (L–H)| 12 (1–37)    | 8 (0–24)     | <.001|
| Number of good quality embryos, IU, Me (L–H) | 4 (0–11)    | 3 (0–10)     | .336 |

**Discussion**

There is no doubt that the endocrine factor is the key factor of infertility among women with PCOS. In our study, based on the results of examination of the hormonal status, the prevalence of the ratio of LH/FSH was defined, along with the high values of AMH. These features of the hormonal panel of women with infertility among women with PCOS are encouraging: such criteria for efficacy as the pregnancy rates per ET, the ‘take home baby’ rates and the miscarriage rates are comparable with those in patients without PCOS. However, there is no doubt that further large-scale randomized trials are needed in this direction.

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**Disclosure statement**

The authors declare no financial or any other conflict of interest that could be affecting the results and conclusions of this study.

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