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Original Article

Gene expression of cumulus cells in women with poor ovarian response after dehydroepiandrosterone supplementation

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ABSTRACT

Objective: Our previous study showed the potential benefits of dehydroepiandrosterone (DHEA) supplementation in women with a poor ovarian response (POR). Because the connection between cumulus cells (CCs) and oocytes is a key step for oocyte maturation, we supposed that altered gene expression of CCs in women with POR after DHEA supplementation might favor oocyte maturation.
Materials and methods: Women with POR treated with flexible daily gonadotropin-releasing hormone antagonist *in vitro* fertilization (IVF) cycles at The Reproductive Center in Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan between January 2013 and October 2013 were enrolled for this prospective study. CCs were isolated during IVF before and after DHEA (CPH-Formulation, Oakdale, CA, USA) supplementation. Nine genes of isolated CCs, including hyaluronan synthase (HAS2), versican (VCAN), thrombospondin 1 (THBS1), runt-related transcription factor 2 (RUNX2), chromobox homolog 3 (CBX3), tripartite motif-containing 28 (TRIM28), B-cell lymphoma 2 (BCL2), BCL2-associated X protein (BAX), and ankyrin repeat domain 57 (ANKRD57), were compared.
Results: There was a significant difference in the expression of genes in women with POR before and after DHEA supplementation (all $p < 0.05$). All genes related to extracellular matrix (ECM) formation, including HAS2, VCAN, and THBS1, were upregulated. By contrast, all genes involving cell development, differentiation, and apoptosis regulation were downregulated. Unknown function gene ANKRD57 was also downregulated after DHEA supplementation. Although expressions of both BCL2 and BAX were decreased in women with POR after DHEA supplementation compared to those before treatment, the ratio of BCL2 and BAX was significantly increased in women with POR after DHEA supplementation, suggesting that DHEA supplementation might activate the antiapoptosis process of CCs, which might be beneficial to the improvement of ovarian function in women with POR.
Conclusion: The study showed that DHEA therapy positively affected the gene expression of CCs in women with POR, and provided evidence to support the positive effect of DHEA supplementation on women with POR.

Introduction

Diminished ovarian reserve (DOR) and/or poor ovarian response (POR) is a tough and challenging issue encountered during assisted reproductive technology (ART). The Cochrane

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review of interventions for poor responders (women with DOR and/or POR) concluded that there is still insufficient evidence to support the use of any one particular intervention either for pituitary downregulation, ovarian stimulation, or adjuvant therapy to improve treatment outcomes in poor responders during *in vitro* fertilization (IVF) cycles [1]. Because of poor IVF outcomes and very low pregnancy rates (2–4%) [2], it may be suggested to women with POR to receive an oocyte donation after failed IVF cycles. However, this is not always acceptable because of ethical, cultural, and political concerns. Therefore, scientists have tried their best to develop strategies to help women with POR. Among those strategies, dehydroepiandrosterone (DHEA), a precursor of estradiol and testosterone, supplementation has been regarded as a potential intervention to rejuvenate the ovarian condition and improve ART outcomes for these women with POR [3–10]. However, why DHEA supplementation would help these women with POR is still uncertain. Therefore, we thought it would be interesting to know what happens in cumulus cells (CCs) after DHEA supplementation.

CCs, somatic cells that surround the oocyte to form the cumulus-oocyte complex (COC), play a central role in follicular development with oocyte maturation, oocyte meiotic resumption, and ovulation, and are mediated by bidirectional communication between CCs and the oocyte through specialized gap junctions [11–15]. Interruption of the connections between CCs and the oocyte may deteriorate oocyte quality with a resultant poor embryo development and worse pregnancy outcome [15]. Therefore, identifying the quality of oocytes and predicting the IVF outcome is possible by investigating the gene expression of these CCs [16–29]. The potential of CCs to serve as markers of oocyte maturation and competence [16–22], embryo development and quality [17–20,23–25], pregnancy outcome [17,24,26–29], and live birth [26] has been highlighted by gene study of CCs. Furthermore, the procedure used to isolate these CCs does not interrupt IVF cycles, so it is considered as noninvasive [16–29].

However, how many genes of CCs will be involved in oocyte maturation and successful ovulation is yet to be determined. Since interconnection between oocytes and surrounding CCs is a critical step for oocyte maturation and successful ovulation, genes of CCs that are involved in extracellular matrix (ECM) formation, cell development, differentiation, and apoptosis [17–19,23,26,30–41] might be good candidates to explore the possible mechanisms that allow for DHEA supplementation to enhance the ovarian response in women with POR. Therefore, the focus of our current study was the expression of nine genes of CCs in IVF cycles that had been validated as playing important roles in the above-mentioned function, except one [17–19,23,26,30–42]. We supposed that these genes of CCs might be significantly different before and after DHEA supplementation. Three of the nine genes that were involved in ECM formation, namely, hyaluronan synthase (HAS2) [18,23,30], versican (VCAN) [17,19,26,31], and thrombospondin 1 (THBS1) [32,33], were investigated. In addition, three genes regulating cell development and differentiation, namely, runt-related transcription factor 2 (RUNX2) [34,35], chromobox homolog 3 (CBX3) [36,37], and tripartite motif-containing 28 (TRIM28) [26,38] were also explored. Finally, genes controlling apoptosis, such as B-cell lymphoma 2 (BCL2) [39,40] and BCL2-associated X protein (BAX) [39,41] were studied. We also studied one gene whose function is still unknown – ankyrin repeat domain 57 (ANKRD57) [42]. The aim of the current study was to explore the changes in these genes before and after DHEA supplementation and to provide molecular evidence to support the potential benefits of DHEA supplementation in women with POR.

Materials and methods

Patients and design

This prospective study received Institutional Review Board approval from the Committee for Human Research at Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, with the identifier VGHKS13-CT11-17, and was registered at ClinicalTrials.gov with the identifier NCT02150330. All participating women were fully counseled, and their written consent was obtained. Women treated with IVF cycles at The Reproductive Center in Kaohsiung Veterans General Hospital between January 2013 and October 2013 were enrolled into the study. The following clinical situations led to exclusion from the study: (1) ovarian cystectomy; (2) oophorectomy; (3) exposure to cytotoxic or pelvic irradiation for malignancy; or (4) taking herbal drugs or other hormonal agents. Women with POR had to meet Bologna poor responder criteria [43]. In order that the study population might be more homogeneous, enrolled participants had to fulfill the following criteria: (1) serum follicle-stimulating hormone (FSH) > 15 nIU/L, or serum anti-Müllerian hormone (AMH) < 1 ng/mL; (2) abnormally low antral follicle counts (AFC) < 4 on Day 2 of their menstrual cycle; and (3), of importance, an unsuccessful flexible daily gonadotropin releasing hormone (GnRH) antagonist IVF cycle followed by DHEA supplementation (CPH-Formulation, Oakdale, CA, USA) [10]. The detailed IVF protocols can easily be found [10,44].

CCs collection

After oocyte retrieval, CCs were obtained from women with POR before and after DHEA supplementation. The protocol for isolation of CCs has been described in detail [45,46]. In brief, the dispersed cells were transferred to a 15 mL centrifugation tube containing 4 mL of Histopaque 1077 (Sigma Chemical, St. Louis, MO, USA). Human luteinized CCs were separated from red blood cells by centrifugation at 600*g* for 10 minutes. CCs formed a thin layer between the Histopaque and the medium. Cells were removed and placed in a new centrifugation tube and washed using IVF medium, with centrifugation at 600*g* for 10 minutes. The supernatant was discharged and the CCs were placed in at −80°C for RNA extraction.

Gene study

In our original design, a total of 24 genes, involving ECM formation, cell development, cell differentiation, apoptosis, and other uncertain functions in CCs were investigated. We found that 18 of the 24 genes of CCs showed significant changes. Since we could not validate half of these genes (9 genes, data not shown), which were significantly altered after DHEA supplementation, and another six genes (data not shown), which failed to show any significant change before and after DHEA supplementation, we did not further test these 15 genes to explore their potential role in oocyte maturation and/or ovulation (data not shown). However, we investigated the remaining nine genes (Table 1), and the related primers (Table 2). Total RNA was extracted from tissue specimens using the acid-phenol guanidium method [47–50]. Briefly, 1 mL of TRIzol was added to the CCs. The mixture was pipetted to mix and allowed to sit for 5 minutes at room temperature. Chloroform (0.2 mL) was added, mixed, and allowed to incubate at room temperature for 10 minutes. The mixture was centrifuged at 12,000*g* for 20 minutes, and the supernatant was transferred to a fresh tube. Isopropanol (0.5 mL) was added, mixed, and incubated for 10 minutes at room temperature. The solution was centrifuged at 12,000*g* for 30 minutes, and the RNA was purified as above. The pellet was washed