

Impact of antiovarian antibodies (AOA) on ovarian responsiveness in vitro fertilization and embryo transfer

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Abstract

OBJECTIVE: This study was to investigate the effects of antiovarian antibodies (AOA) on ovarian responsiveness in vitro fertilization and embryo transfer (IVF/ET).

METHODS: 233 infertility women younger than 36 years undergone IVF/ET because of single salpingemphraxis were attended in the study, whose fast blood was taken to detect serum AOA by ELSA. Among them there were 35 women with serum AOA positive composed the study group and the other with serum AOA negative composed the control. Ovarian volume, antral follicle number, basal FSH, gonadotropin dosage and recollected oocytes were compared between the 2 groups.

RESULT(S): There's no difference in ovarian volume between serum AOA positive group with negative group, $p > 0.05$. The number of antral follicles was less and basal FSH was higher in AOA positive group than in control, $p < 0.05$; what's more, the ampoules of gonadotrophin consumed in AOA positive group exceed significantly in control, and less recollected oocytes in AOA positive group than in control, $p < 0.01$. Of the 233 infertility women, the serum AOA positive rate was 73.08% in the women who got less than 5 recollected oocytes, significantly higher than the other women in the study, $p < 0.01$.

CONCLUSION(S): The existing of anti-ovarian antibodies had suppressive effects on the ovarian responsiveness in ovarian stimulation in IVF/ET cycle and on ovarian function. AOA should be detected before IVF/ET to evaluate ovarian responsiveness and further treatment seems necessary.

Abbreviation

AOA	antiovarian antibodies
SLE	systemic lupus erythematosus
FSH	follicle-stimulating hormone
pSs	Sjogren's syndrome
IVF/ET	in vitro fertilization-embryo transfer

INTRODUCTION

Antiovarian antibodies (AOA) target many kinds of components in ovary such as oocyte, zona pellucida, membrana granulosa, theca folliculi interna and lutein cells [1], even target components such as steroidogenic enzymes, gonadotrophins and their receptors out ovary. The mechanism of serum AOA generation remains obscure. According to literature, serum AOA always presents in premature ovarian failure caused by autoimmune oophoritis, in which AOA acts as partial humoral immunity taking part in ovary local immunity. There comes to elevated corpora atretica, even to the extinction of the follicular apparatus and fibrotization of the ovarian cortex [2]. Other diseases such as systemic lupus erythematosus (SLE) [3], Sjogren's syndrome (pSs) [4], Myasthenia gravis [5] and autoimmune thyroid disease [6] sometimes also demonstrate serum AOA positive. The common pathological change is impaired ovarian function in the above diseases, which is the etiology or the results needs further study. However, in polycystic ovarian syndrome, no consensus reaches on the existence and function of AOA [7, 8].

In vitro fertilization, it has been proved in some study that AOA could produce by the microtrauma induced by repeated puncture of ovarian follicles [9]. Animal experiment also verified ovary autotransplantation could produce AOA [10]. But it showed in other study that the ovarian trauma like laparoscopic excision of ovarian cysts does not result in AOA production [11]. In clinical manifestation, the existing of AOA could disturb ovarian function to demonstrate menstrual cycle disorders or infertility [1]. The presence of antiovarian antibodies always corresponds to reproductive failure in IVF/ET program. [12,13] Appropriate treatments with corticosteroids [14] or hormonal replacement therapy [15] are useful for minimizing ovarian destruction and improving the success rate in previous IVF failure correlated with serum AOA.

The relationship of antiovarian antibodies with ovarian diseases has been verified in many studies. However, the effects of serum AOA existence to normal ovarian function were rare reported before. In vitro fertilization and embryo transfer procedure, controlled ovarian stimulation was the first step. The factors affected ovarian responsiveness needs to be considered in ovarian stimulation. There was no reported on the effects of antiovarian antibodies till now. In the present study, we analyzed the effects of serum AOA to ovarian function in its stimulating station to demonstrate the influence of AOA to ovarian responsiveness, which may be benefit for guiding further treatment.

MATERIALS AND METHODS

Patients sample

233 infertility women younger than 36 years undergone IVF/ET because of single salpingemphraxis who visited Reproductive Center in women and children medical center, Qingdao, from May 2003 to October 2007 were included in this study All patients undergoing fresh IVF/ET cycles were treated with a long protocol for controlled ovarian stimulation. Basal endocrine were detected at the menstrual 2nd before IVF/ET and ovarian volume (length×width×height/2)and follicle number (Diameter 2–12mm) were also detected at that day by vaginal ultrasound (GE, LOGIQ a200, USA). Ethics approval for this study was obtained from the institutional ethics committee and each participant signed an informed consent for her participation in the study.

Venous blood was taken from the participants in the morning after an overnight fast. The blood was taken at menstrual 2nd and hCG injection day in IVF/ET therapy cycle and menstrual 2nd before the cycle respectively. It is be decided as serum AOA antibody positive for twice serum positive in the above blood-taken times. Thromboplastic tubes with blood were centrifuged at 1500 rpm for 5 min to separate the serum, which was stored at -20° until it could be assayed for AOA levels.

Among the 233 women, 35 of them with serum AOA positive composed the study group, and the other with serum AOA negative composed the control.

AOA antisera detect

For detection of AOA we used commercial kit AOA-ELISA (Biological, Anqun, Shenzhen, China). The microtiter strips are coated by oxidized AOA as the antigen. Prediluted serum samples, positive control and negative control were added into each well and incubated for 30 min at 37°. After washing, the anti-human IgG antibody conjugated with peroxidase was added into each well and incubated for 30 min at 37°. The wells were washed, the substrate was added and the microplate was incubated for 15 min at 37°. The enzymatic reaction was stopped by addition of sulfuric acid. 2.1 times of the optical density levels of AOA higher than negative control were considered as positive.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows, version 10.0 (SPSS Inc, Chicago, IL, USA.), and statistical significance was taken at the two-tailed 0.05 *P* level. Tthe Friedman M and Newman-Keuls test or Pearson probabilities using a 2×2 table.

Table 1. Comparison of ovarian volume, antral follicle number, basal FSH, gonadotropin dosage and recollected oocytes between AOA positive and negative groups. * $p < 0.05$; ** $p < 0.01$, AOA positive group vs. AOA negative group

Group	n	Ovarian volume (ml)	antral follicles	Basal FSH (mIU/mL)	Gonadotropin (ampoule)	Recollected oocytes
AOA positive	35	11.5±0.8	6.5±2.1*	12.3±5.2*	34.4±15.0**	9.1±5.0**
AOA negative	198	12.0±0.6	10.3±4.6	5.7±1.6	27.9±8.4	16.4±6.8

Table 2. Serum AOA positive rate in different recollected oocytes. $\Delta p < 0.01$, other groups vs. recollected oocytes less than 5.

Recollected oocytes number	patients	Patients with AOA positive	AOA positive rate (%)
≤5	26	19	73.08 Δ
6~10	57	10	17.54
11~15	57	6	10.52
16~20	54	0	0.00
≥21	39	0	0.00

RESULTS

There's no difference in ovarian volume between serum AOA positive group with negative group, $p > 0.05$. The number of antral follicles was less and basal FSH was higher in AOA positive group than in control, $p < 0.05$; what's more, the ampoules of gonadotrophin consumed in AOA positive group exceed significantly in control, and less recollected oocytes in AOA positive group than in control, $p < 0.01$. (Details in table 1.)

Of the 233 infertility women, the serum AOA positive rate was 73.08% in the women who got less than 5 recollected oocytes, significantly higher than the others, $p < 0.01$. (Details in table 2.)

DISCUSSION

On the condition of trauma to ovary such as oophoritis, follicles puncture and enucleation of ovarian cyst, lots of ovarian antigens were released into blood to stimulate antigen-antibody reactions to produce antiovarian antibodies. The common serum AOA positive rate in infertility women is 1–3% [16], however, the AOA positive rate was relatively higher in women undergone IVF/ET maybe partly because of follicles puncture [9]. Of the 35 serum AOA positive women in the study, 22 of them who had twice or more IVF/ET therapy, which verified repeated ovarian puncture might be one of the reasons to induce AOA production.

The common evaluations of ovarian storage are ovarian volume, basal antral follicles and basal FSH level. From table 1 in the study, the AOA positive group had higher basal FSH level and more gonadotrophin ampoules consume with less basal antral follicles and recollected oocytes, which was reflected the decreased ovarian storage in these cohorts of women. From the results, we could see the women undergone IVF/ET

with serum AOA had poor ovarian storage. Poor ovarian storage always means to poor ovarian responsiveness when stimulating with gonadotrophin, that's why in the women with serum AOA though more gonadotrophin used in controlled ovarian stimulation, less oocytes were recollected. From table 2, the 73.08% serum AOA positive rate of the women who got less than 5 recollected oocytes also verified this. The antigen-antibody reaction arose by AOA in ovarian could induce granular cells apoptosis and disturb steroid metabolism, follicular development and oocyte mature was interfered with in the end [17].

In all, repeated follicles puncture in IVF/ET increased chances to expose ovarian antigen, which stimulates antiovarian antibodies expression. The existing of antiovarian antibodies had suppressive effects on the ovarian responsiveness in ovarian stimulation in IVF/ET cycle. Therefore, AOA should be detected before IVF/ET to evaluate ovarian responsiveness and further treatment seems necessary.

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