Polycystic ovarian patient's serum decreases in vitro development of mouse embryo

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders which cause anovulatory infertility and hyperandrogenism in young women. The common feature in PCOS women is increased ovarian androgen secretion which can effect on the prevalence of miscarriage rate.

Objective: The aim of this study was to investigate the effect of PCOS patient's serum on in vitro developmental stages of mouse embryo from two cells to hatching blastocyst.

Materials and Methods: After superovulating and fertilizing Balb/c mice, 219 two cells embryos were retrieved, 109 embryos were cultured in 10% PCOS patient's serum and 90% medium and 110 embryos were cultured in 10% normal serum and 90% medium to hatching blastocyst stage. The PCOS patient's serum which added to medium had higher hormonal concentrations than normal serum. The early developmental stages of embryos were studied in 2, 4, 8 cells, morula, early, late and hatching blastocyst stages.

Results: The statistical analysis confirmed the decreasing rate in the number of embryos in all developmental stages from 2 cells to hatching blastocyst in PCOS group in comparison with the normal group ($p < 0.05$).

Conclusion: The PCOS patient's serum causes the decreasing rate of in vitro development of the early stage in mouse embryos.

Introduction

The most widely accepted definition of polycystic ovarian syndrome (PCOS) is related to chronic anovulation and hyperandrogenism in the absence of specific disorders of ovaries, adrenals and pituitary (1). PCOS is the most common endocrine disorders affecting 4% to 12% of women of reproductive age (2) and infertility is one of the problems in PCOS women (3). PCOS is closely associated with decreased fertilization rate (4) and when pregnancy happened the miscarriage rate is as high as 30% - 50% (5). Homburg stated the prevalence of spontaneous miscarriage in women who have PCOS is not known (6). Therefore more studies are necessary to determine the effect of additional infertility factors on women with PCOS.

One of the common features in PCOS is high concentration of androgen and luteinizing hormone (LH) level (7).

An excess androgen promotes higher rate of implantation failure and miscarriage rates (8-9). Also high LH level decreases oocyte quality (10), and causes low pregnancy rate of in vitro fertilization (IVF) in PCOS women (11).

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Numerous experiments in animal models of PCOS have been developed to show the features of PCOS. In rat model both testosterone and LH levels are significantly induced and increased (12-13). Some of evidences emphasized about the other factors such as obesity (14), leptine (15) and resistin (16) which affect on infertility of PCOS women. It has been indicated that PCOS women suffer from poor oocyte quality, fertilization and implantation failure, which cause higher rates of miscarriage. However it proposed that the variety changes of hormone have dominate influence in these processes (17).

Since, the effect of high concentration hormone on oocyte maturation, fertilization, implantation and high miscarriage rate in PCOS women have been confirmed (8-10), so we decided to evaluate the effect of hormonal concentrations in PCOS women on developmental stage of mouse embryo from 2 cells to hatching blastocyst. We used mouse embryos to eliminate the other infertility factors such as poor oocyte, failure fertilization and inequality embryos to study the direct effects of PCOS patient's hormones on development stage of mouse embryo.

Materials and methods

2 cell embryo

6–8 week old Balb/C female mice were received an i.p. injection of 7/5 IU Pregnant Maress` Serum Gonadotropin (PMSG; Organon OSS Netherlands) in order to increase the number of oocytes. 48 hours later the mice were injected 7/5 IU Human Chronic Gonadotropin (HCG; Organon OSS Netherlands) to induce the simultaneously ovulation of oocytes. The female mice were transferred to cage with male mice for copulation, next day when the vaginal plague was observed. It is possible to retrieve the 2 cells embryo 24-38 hours after fertilization (24-57 hours after ovulation). 30 -32 hours after fertilization the animal were killed by cervical dislocation. The 2 cells embryos were collected by flushing technique, transferred in 35 mm dishes and washed 2 times in 50 µl drops of medium. The high quality embryos finely were gathered with Pasteur pipette. Total embryos of each mouse randomly were divided in two groups. Finally 109 embryos in 2 cells stage were cultured in medium (contain PCOS serum as case group A) and 110 in medium (contain normal serum as control group B).

Serum

PCOS patient's serum was obtained from 31 years old woman and the PCOS diagnosis in this patient was based on Rotterdam criteria 2003 for PCOS (18). The biochemical lab results of this patient indicated free testosterone 3.1 pg/ml, DHEA-S 253 µg/dl, FSH 6.1 mIU/ml, LH 14.7 mIU/ml, prolactine 2.8 ng/ml, progesterone 0.5 ng/ml, estradiol 10.0 pg/ml. While, the other biochemical serum factors were in normal range according to lab references.

Normal serum was obtained from 31 years old woman signified no disease in endocrine glands and deficiency in ovaries. The biochemical lab results in this woman indicated free testosterone 0.8 pg/ml, DHEA-S 176 µg/dl, FSH 4.3 mIU/ml, LH 2.1 mIU/ml, prolactine 160 ng/ml, progesterone 0.8 ng/ml, estradiol 74.0 pg/ml while the other biochemical serum factors were in normal range based on the same lab references like PCOS. 10 cc of each serum were purified by centrifuged at 4000 rpm for 10 min and the supernatant stored in -4° C.

It was an effort about effective factors e.g. age, BMI, marriage status and health history in PCOS woman and normal woman to be similar.

Embryo culture

10% of PCOS serum was added to 90% of medium (HTF; Irvine Scientific) for group A and 10% of normal serum was added to 90% of medium for group B. After washing each embryo, they were cultured in 100 µl drops of medium, covered by light paraffin oil and incubated in 37° C in 5% CO_2.

The developmental stages were evaluated under the microscope every 12 hours. The embryos in group A were transferred to fresh medium (10% of PCOS serum) and group B to fresh medium (10% of normal serum) per 24 hours.

The major criteria for embryo survival was based on continuing and arriving to the next developmental stage after the expected time according to Theiler developmental stages of mouse embryo (19). Base on Theiler developmental stages in murine, the embryos were cultured for over 6 days.

The rate of developmental stages was recorded in 2 cells (0 times of our study or 30 – 32 hrs after fertilization), 4 cells 24-26 hrs later, 8 cells 36-38 hrs, morula 70-72 hrs, early blastocyst (EB) 96-100 hrs, late blastocyst (LB) 120-124 hrs and hatching blastocyst (HB) after 140-144 hrs.

Statistical analysis

Statistical analysis was Chi-square and Fisher's exact test; the $p < 0.05$ was considered for significant differences.

Results

The recorded data showed the decreasing rate in the number of embryos in all developmental stages from 2 cells to hatching blastocyst in PCOS group in comparison with the normal group has significant differences.

The data showed in group A, 100 (91.7%) embryos out of 109 total embryos and in group B, 109 (99.1%) embryos out of 110 total embryos in 2 cell stage developed to 4 cells, the significant differences $p<0/01$. In this stage only 1 embryo died in normal group meanwhile in PCOS group 9 embryos died. In the next stage from the total cell in group A, 93 (85.3%) and in group B 104 (94.5%) arrived in 8 cell stage, $p<0/05$. In morula stage, in group A, 77 (70.6%) and in group B, 99 (90%) embryos developed to early blastocyst, the significant differences $p<0/001$. In the early, late and hatching blastocyst stages the recorded data were confirmed, from the total cell in group A, 50 (45.9%) arrived in EB, 16 (14.7%) in LB and finally 6 (5.5%) embryos were able hatched from zona pellucida (ZP). Meanwhile in group B, 84 (76.4%) embryos arrived in Eb, 53 (48.2%) in LB and 27 (24.5%) hatched from ZP successfully. The statistical analysis in all blastocyst stages presented significant differences $p<0/001$. The number of embryos which can develop to next stage and the expect time for each stage are shown in table I.

Table I. The results of in vitro developmental stages of mouse embryo in PCOS and normal serum and the expect time for each stage.

Embryos Time	2 cells --0 hrs†	4 cells*24 hrs	8 cells**38 hrs	Morula*** 72hrs	EB***100 hrs	LB***124 hrs	HB***144 hrs
Group A embryos (in PCOS serum)							
Alive	109 (100)‡	100 (91.7)	93 (85.3)	77 (70.6)	50 (45.9)	16 (14.7)	6 (5.5)
Dead		9 (8.2)	16 (14.7)	32 (29.4)	32 (54.1)	93 (85.3)	103 (94.5)
Group B embryos (in normal serum)							
Alive	110 (100)	109 (99.1)	104 (94.5)	99 (90)	84 (76.4)	53 (48.2)	27 (24.5)
Dead		1 (0.9)	6 (5.5)	11 (10)	26 (23.6)	57 (51.8)	83 (75.5)

†The 0 time of our study (30-32 hours after fertilization),   ‡No. (%)
(Early blastocyst) EB, (late blastocyst) LB, (hatching blastocyst) HB.
*$p<0/01$ **$p<0.05$ ***$p<0.001$

Discussion

The data of our study showed the hormonal factors of PCOS patient's serum decreased the rate of early developmental stages of mouse embryo. It means except anovulation in PCOS women, there are other factors which can decrease the rate of fertility. In our research the PCOS patient's serum which was used as a supplement to culture medium has high androgen concentrations.

In several reports, it has been claimed, exposing of immature murine oocytes to testosterone reduce the ability of maturation (19). In PCOS women, testosterone level is 2 times higher than normal women (20). High LH concentration (> 10 IU/L) have a causal factor in early pregnancy loss (21-23), higher rate of implantation failure (8) and decrease fertilization rate of mature oocytes (11). In current study we found the testosterone level in PCOS serum was 3 times higher than normal serum, and LH level was also high. Therefore we expected the significant lower rate in all developmental stage of mouse embryo in PCOS group to happen. In the other hand, the high concentration of testosterone has been appeared to induced animal's model of PCOS and the high androgen in PCOS women could be as potential source for her fetus (12-13). The former evidences indicated the detrimental effect of testosterone and LH in different aspect of pregnancy in PCOS women. In our research the PCOS serum contains high testosterone and LH which have harmful effect on the rate of embryo development.

The research in rat model of PCOS, declared that apoptotic rate of granulose cells was higher (24). Study in animal model of PCOS determined, changing in expression of cytoskeletal proteins and cellular adhesion molecule have been happened. They stated it could be related to hormonal changes in animal model of PCOS. It has indicated that cell survival and proliferation depend on signal mediate and adhesion molecules (12).

Therefore these factors influence on proliferation of embryonic cell in different stages. It has been emphasized that in PCOS patients, immature oocytes and low cleavage rates lead to decrease of implantation and pregnancy rates (17-25). But, it hasn't stated whether the PCOS hormonal factors influence on oocytes and causes the lower rate of pregnancy or the hormonal factors directly has an influence on embryo development. In current study, for eliminating the hyperandrogen effects, which have influence on quality of oocytes, fertilization and implantation, we used the

qualified mouse embryos. Therefore we can postulate that the high hormonal concentrations in PCOS directly affect the developmental stage of embryos. The data of our research showed the increase exposure time of embryo from 2 cells to HB in PCOS group decrease the embryo survival significantly (Table I). We used the PCOS serum as one parameter to evaluate the effect of high hormonal concentrations on the rate of embryo development in different stages.

According to different evidences about the negative effects of high testosterone (19) and LH (21) level separately on reproductive process, we proposed that effect of excess multiple hormones simultaneously in PCO women could increase the harmful effects of hormones. However in spite of using high quality embryos, and controlling the detrimental effect of oocyte fertilization and embryo implantation, there are other factors which should be considered in treatment of infertility in PCOS women. Besides the new procedures e.g. in vitro maturation and IVF to eliminate the side effect of gonadotrophins stimulation and infertility in PCOS women (26), control of hyperandrogen in infertility PCOS women should be managed. Large-scale studies are needed to determine contributors of additional factors in infertility women with PCOS.

Conclusion

The hormonal factor in PCOS woman's serum causes the decreasing rate of in vitro development of early stage in mouse embryos.

Therefore the lower pregnancy rate and prevalence of miscarriage in PCOS women could be due to lower developmental rate of embryo in preimplantation stage.

Acknowledgment

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