HUMAN FOLLICULAR FLUID IN NORMAL AND POLYCYSTIC OVARIES

H.R. Sadeghipour Roudsari, N. Salsabili, M. Sattarian and F. Ghiafeh Davvodi

Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract - Polycystic ovarian syndrome is a hetrogenous condition with a broad clinical and pathologic spectrum that may reflect the effects of diverse etiologic factors. Polycystic ovarian syndrome is an abnormality in the maturation of the follicles which can result in abnormal composition of follicular fluid. By analysis and comparison of the biochemical composition of human follicular fluid in normal and polycystic ovaries, we undertook to find the etiology of arrested follicular development in this syndrome and to gain an insight into the role of biochemical constituents of follicular fluid on follicular development.

The results of our study indicate higher concentration of potassium, lower concentration of testosterone and higher level of pH in normal ovaries relative to polycystic ovaries. In conclusion, potassium and testosterone concentrations and pH level seem to be some of the most important factors in the oocyte maturation and the acquistion of follicular development; and changes in them may have important role in the etiology of polycystic ovarian syndrome.

Acta Medica Iranica 39 (3): 169-171; 2001

Key Words: Polycystic ovarian syndrome, follicular fluid, biochemical constituents

INTRODUCTION

Follicular fluid (FF) is a complex extracellular fluid that is semiviscous and yellow in colour. It is a derivative of blood plasma and secretions systhesized in the follicle wall (1,2,3).

Ovarian physiology is controlled by many exogenous and endogenous factors including the endocrinological alterations which occur in the follicular fluid throughout the menstrual cycle (4). These changes in follicular fluid may influence steroidogenesis, oocyte maturation, ovulation and transport of the oocyte to the oviduct as well as the preparation of the follicle for subsequent corpus luteum formation and function (5).

Polycystic ovarian syndrome (PCOS) is an abnormality in the maturation of the follicles which can result in abnormal composition of follicular fluid. Recent data suggest that follicular fluid may play an important role in the endocrine balance of PCOS, probably by acting on the theca granulosa cell relationship (6). By analysis of this fluid and comparison of it with normal folicular fluid, we hoped to gain an insight into the role of biochemical constituents of follicular fluid on follicular development and identify any component which might enhance oocyte maturation.

MATERIALS AND METHODS

Study Patients

A total of 40 women with infertility, treated in "IVF Center of Mirzakoochak Khan Hospital", participated in the present study. The age of the patients ranged between 15-40 years. Follicular fluid was collected from the patients who were negative for HIV infection, syphilis and hepatitis B antigen to prevent infection. Patients who had severe endometriosis, immunologic factors or unkown factors were excluded from the follicular fluid collection (7). The patients were divided into two groups: patients with normal ovaries and patients with polycystic ovaries. The diagnosis was made by ultrasound, clinical and hormonal findings according to the proposed diagnostic criteria of the United States National Institute of Health (NIH) (8).

Collection of Follicular Fluid

During the oocyte aspiration in the human IVF programs, follicular fluid was collected from the follicles that were > 18 mm in diameter. Whenever possible non-blood-stained follicular fluid was collected, but the fluid mixed with a small amount of blood was also collected (7).

Preparation of Follicular Fluid

Fluid was centrifuged at 2000 rpm for 20 minutes, and the supernatant was heat-inactivated at 56 °C for 35 minutes. The heated fluid was cooled and sterilized with a filter (0.22 μm pore size filters). All follicular fluid samples were kept at 40 °C until the time of analysis.

Analysis of Follicular Fluid

Follicular fluid samples were subjected to a range of biochemical measurements. Samples were all defrosted at room temperature and analysed after daily calibration of the machines by the analysis of

appropriate controls. A total of 16 tests were carried out on follicular fluid, using Zist Chimi and Pars Azmoon kits.

Statistical Analysis

Results are presented as the mean ± SEM. Comparison of means was done with the unpaired t-test and ANOVA (1-way, 2-way). Significance was set at $P < 0.05$. Statistics were calculated using the Statistical Package for Social Sciences (SPSS) - version 9 program.

RESULTS

Values in the Table 1 represent mean ± SEM concentration of selected molecules, electrolytes, lipids, steroids, pH and osmolarity measured in human follicular fluid (FF) of normal and polycystic ovaries. Also shown are the results of statistical analysis. The ionic composition of the fluid of normal ovaries was characterized by high concentration of K, relative to the fluid of polycystic ovaries ($P<0.05$).

Testosterone concentration in follicular fluid of normal ovaries was significantly lower than follicular fluid of polycystic ovaries ($P<0.01$).

pH studies showed that follicular fluid of normal ovaries is more alkaline than follicular fluid of polycystic ovaries ($P < 0.01$).

There were no significant differences between the concentrations of other components such as Na, P, Cl, Ca, Mg, etc.

Table 1. Mean ± SEM Concentrations of biochemical constituents of human follicular fluid in normal and polycystic ovaries

	Normal ovaries	Polycystic ovaries	P value	
Na (mEq/dl)	148 ± 2.36	145.7 ± 7.61	0.747	NS
K (mEq/dl)	4.03± 0.06	3.71 ± 0.12	0.03	*
P (mg/dl)	3.7 ± 0.1	3.83 ± 0.11	0.38	NS
Ca (mg/dl)	8.47± 0.022	8.58 ± 0.17	0.69	NS
Mg (mg/dl)	1.72± 0.04	1.66 ± 0.05	0.39	NS
Fe (μg/dl)	78.44 ± 1.92	80.5 ± 2.41	0.49	NS
Chol (mg/dl)	22.89 ± 1.04	22.36 ± 1.15	0.73	NS
TG (mg/dl)	115.42 ± 43.1	93.8 ± 29.1	0.43	NS
Tpro (g/dl)	5.61 ± 0.51	5.69 ± 0.2	0.71	NS
Alb (mg/dl)	3.74 ± 0.05	3.81 ± 0.11	0.59	NS
Glu (mg/dl)	62 ± 3.16	58.5 ± 5.17	0.57	NS
Bili (mg/dl)	0.11 ± 0.009	0.11 ± 0.008	0.97	NS
Oestradiol (pg/dl)	1301.9 ± 18.17	1272.7 ± 46.42	0.56	NS
Testosterone (ng/dl)	13.12 ± 1.07	16.9 ± 1.02	0.008	***
Osmolarity (mmol)	282.57 ± 1.02	281.4 ± 1.28	0.49	NS
pH (pH)	7.53 ± 0.05	7.33 ± 0.06	0.019	**

DISCUSSION

Polycystic ovarian syndrome is a hetrogenous condition with a broad clinical and pathologic spectrum that may reflect the effects of diverse etiologic factors (9). Since the follicular fluid is in intimate contact with the oocyte and granulosa cells, changes in the concentration of its different components in PCOS may lead to understand the etiology of the arrested follicular development in this syndrom and to gain an insight in to the role of biochemical constituents of follicular fluid on follicular development. By the time of our study, there was not any other experiment to determine human follicular fluid composition.

The results of our study indicate much higher concentration of K in follicular fluid of normal ovaries than polycystic ovaries. This high concentration may show the effect of K on the maturation of oocyte. This agrees with the findings of Moody and Bosma (10), who showed that potassium level of oocyte increase during meiotic maturation and this increase is closely associated with the migration of the germinal vesicle to the cell periphery. The results of our own study show similar trends to the former studies that reported higher concentration of K in follicular fluid than human serum (11). The mechanism of K effect on the maturation of oocyte is not obvious, but one possibility is its effect on plasma membrane potential (12). Further studies are needed to determine the importance of the ionic composition of the follicular fluid for normal human preimplantation development.

The results obtained in this study indicate that testosterone concentration in follicular fluid of normal

ovaries was significantly lower than follicular fluid of polycystic ovaries. Increased androgen concentrations are thought to be detrimental to oocyte maturation and reproductive potential (13). Depending on the diagnostic data available from patients with polycystic ovaries, various steroidogenic enzyme blocks have been postulated mostly implicating higher-than-normal production of circulation delta 4-androstenedione, testosterone, and in some cases, dehydroisoandrosterone. These high levels of androgens, because of their peripheral conversion to estrogens, lead to inappropriate secretion of gonadotropins in PCOS (9). Arising from these observations was the suggestion that an aromatase enzyme block existed (9). The recent studies suggest a potential role for 5 alpha - reduced androgens in the etiology of PCOS (14).

pH studies showed that follicular fluid of normal ovaries is more alkaline than polycystic ovaries which suggests that a high pH is required for preimplantation embryo development. The mean value of 7.55 in human follicular fluid that we report here is comparable with the value of 7.4 given by Dale et al (15) and is higher than the value of 7.2 given by Edwards (1).

Acknowledgements

The authors thank the staff of "IVF center of Mirzakoochak Khan hospital" and "Laboratory of Mofid hospital" for laboratory facilities and assistance with the recovery of follicular fluid.

REFERENCES

1. Edwards, R.G. Follicular fluid. J. Reprod. Fertil. 37: 189-219; 1974.

2. Kenney, R.M., Condon, W., Ganjam, V.K. and Channing, C. Morphological and biochemical correlates of equine ovarian follicules as a function of their state of viability or atresia. J. Reprod. Fertil., Supp. 27: 163-171; 1979.

3. Yao, J.K., Ryan, R.J. and Dyck P.J. The procine ovarian follicle. VI Comparison of fatty acid composition of serum and follicular fluid at different developmental stages. Biol. Reprod. 22: 141-7; 1980.

4. Eissa, H.M. concentrations of steroids and biochemical constituents in follicular fluid of Buffalo cows during different stages of the oestrous cycle. Br. Vet. J. 152: 573-581; 1996.

5. Peters, H. and McNatty, K.P. The ovary, 1st ed., Granad. Great Britain: Paul Elek; 304-309; 1980.

6. Andreani, C.L., Pierro, E., Lazzarin, N., Lanzone, A., Caruso, A., Mancuso, S. Effect of follicular fluid on granulosa luteal cells from polycystic ovary. Hum. Reprod. 11(10): 2107-13; 1996.

7. Chi, H.J., Kim, D.H., and Chang, S.S. The suitability and efficiency of human follicular fluid as a protein supplement in human in vitro fertilization programs. Fertil. Steril. 70: 871-877; 1998.

8. Legro, R.S. Polycystic ovary syndrome: current and future treatment paradigms. Am. J. Obstet. Gynecol., 179: 101-8; 1998.

9. Mahajan, D.K. Steroidogenesis in human polycystic ovary. Endocrinol. Metab. Clin. North. Am., 17(4): 751-69; 1988.

10. Moody, W.J. and Bosma, M.M. Hormon-induced loss of surface membrane during maturation of star fish oocytes: Differential effects on pottassium and calcium channels. Dev. Biol. 112: 396-404; 1985.

11. Borland, R.M., Biggers, J.D., Lechene, C.P. and Taymar, M.L. Elemental composition of fluid in the human fallopian tube. J. Reprod. Fertil. 58: 479-482; 1980.

12. Wiley, L.M., Yamami, S., Muden, D. Effect of pottassium concentration, type of protein supplement, and embryo density on mouse preimplantation development in vitro. Fertil. Steril. 45 (1): 111-9; 1986.

13. Fridstrom, M., Carlstrom, K., Sjoblom, P., Hillensjo, T. Effect of prednisolone on serum and follicular fluid androgen concentration in women with polycystic ovary syndrome undergoing in-vitro fertilization. Hum. Reprod., 14(6): 1440-4; 1999.

14. Jakimiuk, A.J., Weitsman, S.R., Mogoffin, D.A. 5 alpha-reductase activity in women with polycystic ovary syndrom. J. Clin. Endocrinol. Metab. 84(7): 2414-8; 1999.

15. Dale, B., Menezo, Y., Cohen, J., DiMatteo, L. and Wilding, M. Intracellular pH regulation in the human oocyte. Hum. Reprod. 13 (4): 964-970; 1998.