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Ovarian follicular fluid contains immunoreactive estriol: lack of correlation with estradiol concentrations

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Key words: ESTRADIOL, ESTRIOL, ESTROGEN, FOLLICLE, OVARY

ABSTRACT

Estradiol and estrone concentrations in ovarian follicular fluid change according to the ovulatory cycle, but no studies on the possible presence and/or changes of estriol are available. The aim of the present study was to evaluate whether estriol is measurable in follicular fluid and how its concentration changes according to the volume of ovarian follicles and to the maturational stage of oocytes.

A group of women (n = 39) undergoing a program of induction of ovulation was included in this study and divided into three groups according to the causes of infertility: those with unexplained infertility (n = 11); those with endocrine disturbances (n = 5); and normal ovulatory women (n = 23) (controls). The follicles recruited (n = 116) on the basis of morphology and the appearance of the oocyte cumulus–corona complex were divided into: mature (n = 22); intermediate (n = 75); immature (n = 11); and atretic (n = 8). Ovarian follicles were also divided according to the diameter of each: < 1.5 cm (n = 38); 1.6–2.4 cm (n = 66); and > 2.5 cm (n = 12). Ovarian follicular fluids were aspirated under ultrasound guidance and a blood specimen was collected from each subject. Estriol and estradiol concentrations were evaluated by radioimmunoassay in serum and follicular fluid following an ether extraction.

Estriol was found in high concentration in each sample of follicular fluid, significantly higher than in the respective serum sample ($p < 0.01$). Although the estradiol concentration was significantly lower in follicles containing immature and atretic oocytes than in intermediate or mature follicles ($p < 0.01$), the estriol concentration did not depend upon the maturational stage. In addition, the follicular fluid estriol concentration did not differ according to the causes of infertility. Follicular fluid and serum estradiol concentrations showed significant correlation ($p < 0.01$), whereas no significant correlation was observed between serum and follicular estriol concentrations.

The present data show that follicular fluid contains a high concentration of estriol and that its changes are independent of the ovulatory cycle and estradiol concentrations, supporting an independent origin and suggesting a different function for estriol.

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INTRODUCTION

It is well known that the main estrogen produced by the ovary is estradiol, while estrone is secreted in limited amounts. Granulosa cells of Graafian follicles are the major source of estrogen secretion, estradiol and estrone being found in both follicular fluid and the systemic circulation. Another estrogen, estriol, is detectable in the circulation as free (unconjugated). The liver, peripheral adipose tissue and the placenta are considered the sources of estriol, most probably through the metabolic conversion of both estradiol and estrone. It is considered that the plasma estriol concentration is the result of 16α-hydroxylation of estrone or estradiol in the peripheral tissues, but the placenta and peripheral tissues may also be sources by an autonomous pathway[1].

Antral follicular fluid concentrations of estrogens, progesterone and androgens have been measured in samples collected from ovarian follicles of fertile and treated women[2]. A role of estradiol in the intrafollicular environment has been clearly demonstrated by the association between high follicular estradiol concentrations and successful *in vitro* fertilization (IVF) and pregnancy rates[3–9]. The correlation between follicle concentrations of estradiol and stage of ovarian follicle maturation of the oocytes showed that the concentrations of estradiol are significantly lower in the follicles carrying immature oocytes when compared to intermediate and mature oocytes[10–12].

The aim of the present study was to evaluate the possible presence of immunoreactive estriol in follicular fluid and whether a relationship exists between follicular fluid estriol concentration and the stage of follicular maturation in women treated by controlled ovarian hyperstimulation.

MATERIALS AND METHODS

Subjects

A group of women ($n = 39$) undergoing a program of ovarian hyperstimulation was recruited after they had given their informed consent. Three causes of infertility were identified:

(1) Unexplained infertility ($n = 11$);

(2) Endocrine disturbances ($n = 5$) distinct in women with hypogonadotropic hypogonadism ($n = 3$) or hyperprolactinemia ($n = 2$);

(3) Male factor infertility or mechanical stenosis in normal ovulatory women ($n = 23$) (controls).

All women received a treatment of ovarian hyperstimulation: purified menofollitropin (pFSH Metrodin®; Serono, Rome, Italy) (150 IU) from the 3rd to the 5th day of the menstrual cycle plus human menopausal gonadotropin (hMG) (Pergonal®; Serono, Rome, Italy) (2 ampules) from the 6th day of the menstrual cycle.

Ovarian follicular fluid ($n = 116$) was sampled under ultrasound guidance within 12 h from the onset of the endogenous luteinizing hormone (LH) peak. Follicles were divided on the basis of morphology and the appearance of the oocyte cumulus–corona complex, according to the criteria of Acosta and colleagues[13], into: mature ($n = 22$); intermediate ($n = 75$); immature ($n = 11$); and atretic ($n = 8$). Ovarian follicles were also divided according to the diameter of each: < 1.5 cm ($n = 38$); 1.6–2.4 cm ($n = 66$); and > 2.5 cm · ($n = 12$). In each follicular fluid sample, estriol and estradiol concentrations were evaluated. A blood specimen was drawn from each patient at the time of oocyte recruitment, and estriol and estradiol concentrations were measured in serum.

Assays

Before assay, each serum and follicular fluid sample was subjected to an ether extraction procedure. The extracts were then subjected to chromatographic purification using Sep-Pak® C_{18} cartridges previously activated with 10 ml of aqueous methanol (50 : 50 v/v). The cartridges were sequentially washed with 50% aqueous methanol containing 1% acetic acid and the unconjugated steroid fraction was eluted with absolute methanol and taken to dryness under nitrogen. Analytical grade solvents were purchased from Merck (Darmstadt, Germany); C_{18} Sep-Pak cartridges were obtained from Waters Corporation (Milford, USA).

Follicular fluid and serum estradiol concentrations were measured by a specific radioimmunoassay (RIA) by using a commercially available kit (Radim®; Pomezia-Rome, Italy).

A specific buffer dilution for each follicular fluid sample was performed, as previously described[14]. The RIA for estradiol determination had a high degree of specificity (Table 1). The percentage of cross-reaction was calculated by using the Abraham formula ($X/Y \times 100$), X and Y being the weight of the substance to be assayed and the weight of the interfering substance, both able to reduce the binding capacity by 50%. The assay sensitivity for estradiol evaluation was 10 pg/ml; the intra- and interassay coefficient of variation was 2.1% and 3.5%, respectively.

Follicular fluid and serum estriol concentrations were measured by using a specific RIA. High concentrations of estriol in human follicular fluid made it necessary to dilute the samples, as well as the first and second antibodies, prior to assay. A rabbit antiserum raised against estriol coupled to bovine serum albumin and sheep IgG anti-rabbit antibody were the first and the second antibodies, respectively. The antibodies were diluted 1 : 8 and 1 : 3, respectively. The tracer was diluted 1 : 2. The sensitivity of the method was 50 pg/ml. Inter- and intra-assay coefficients of variation ($n = 10$) were 7.9% and 4.8%, respectively. The recovery test (with unlabelled steroid) was 93.5 ± 11.8%. The percentage of cross-reaction was calculated using the Abraham formula. The method had a high degree of specificity for estriol (Table 2).

Statistics

Data obtained are expressed as mean ± standard error of the mean (SEM). Hormonal concentrations were statistically analyzed and expressed by using the multiple analysis of variance and Duncan test for multiple comparisons. A linear regression was also performed between the different parameters evaluated.

Table 1 Cross-reaction between estradiol antiserum and various steroid hormones

Steroids	*Cross-reactivity* (%)
Estradiol	100
Estriol	1.05
Estrone	0.93
Dehydroepiandrosterone	< 0.015
Danazol	< 0.015
Cortisol	< 0.015
Progesterone	< 0.015
17β-progesterone	< 0.015
Androstenedione	< 0.015
Testosterone	< 0.015

RESULTS

High concentrations of estriol were detectable in each follicle (Figure 1), and were higher than in the respective serum specimen (Table 3). Dilution curves confirmed that follicular fluid and serum immunoreactive estriol is identical to the original steroid. No correlation was found for estriol levels between follicular fluid and serum. The estriol concentration did not show significant variation according to the follicular diameter (Figure 2) or to the morphology of the oocytes (Figure 1). No significant differences were observed in follicular fluid estriol levels in the three different groups of women according to the causes of infertility.

Table 2 Cross-reaction between estriol antiserum and various steroid hormones

Steroids	*Cross-reactivity* (%)
Estriol	100
Estrone	< 0.12
17β-estradiol	< 0.6
11-OH-progesterone	< 0.12
Testosterone	< 0.06
Progesterone	< 0.06
5α-androsten-3β-17-diol	< 0.06
Dehydroepiandrosterone	< 0.06
Dihydrotestosterone	< 0.06
Cortisol	< 0.06
Cholesterol	< 0.06

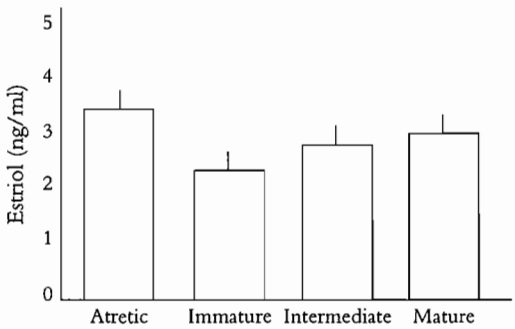

Figure 1 Mean ± SEM estriol concentration in follicular fluid at different maturational stages of follicle development

Table 3 Serum and follicular fluid estradiol and estriol concentrations in women at different maturational stages of follicles (mean ± SEM)

	Estradiol		*Estriol*	
	Serum (ng/ml)	*Follicular fluid* (ng/ml)	*Serum* (pg/ml)	*Follicular fluid* (ng/ml)
Atretic	0.62 ± 0.08	351 ± 40	11.2 ± 0.4	3.32 ± 0.4
Immature	0.53 ± 0.1	295 ± 20	11.8 ± 0.6	2.08 ± 0.2
Intermediate	0.81 ± 0.07	451 ± 31	15.3 ± 0.2	2.6 ± 0.4
Mature	1.12 ± 0.09	480 ± 35	14.8 ± 0.8	2.8 ± 0.2

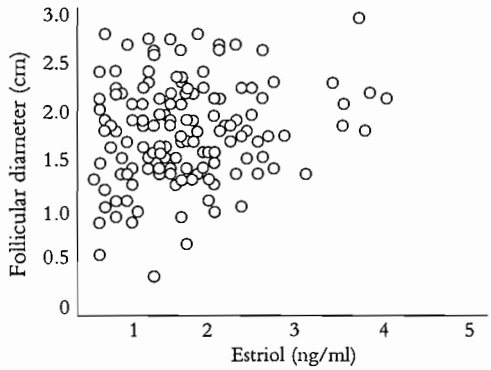

Figure 2 Correlation between ovarian follicular size and estriol concentration

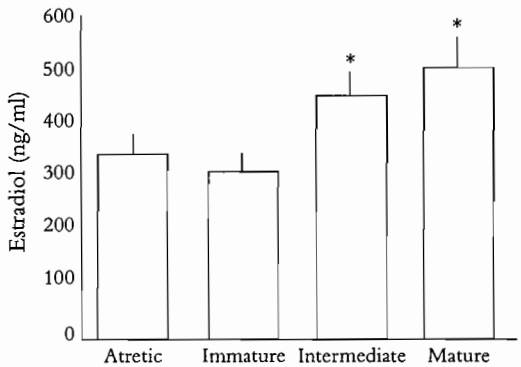

Figure 3 Mean ± SEM estradiol concentration in follicular fluid at different maturational stages of follicle development (*$p < 0.05$ intermediate and mature vs. immature and atretic)

Taking into account the different morphology and follicular diameter, serum estradiol concentration correlated with follicular fluid estradiol concentration ($p < 0.01$), and levels in mature and intermediate follicles were significantly higher ($p < 0.05$) than in immature and atretic follicles (Figure 3).

DISCUSSION

For the first time, the present study has shown that estriol is detectable in the human ovarian follicular fluid and that its concentration does not correlate with follicular maturation or with estradiol concentration. In agreement with previous studies, a correlation between follicular fluid estradiol concentrations and the maturational stage of the follicles was confirmed. While estradiol concentration was significantly lower in follicles containing immature and atretic oocytes than in intermediate or mature follicles, the estriol concentration did not depend upon the maturational stage. The presence of estriol in follicular fluid and the absence of correlation with estradiol concentration at the follicular level suggests that estriol does not seem to be an end-product of the metabolism of estradiol[15], but it could have an autonomous pathway of production from granulosa and theca interna cells. Moreover, the follicular fluid estriol concentration did not correlate with follicular volume or causes of infertility, suggesting a possible involvement in other functions, but not in follicular development.

Estriol produced or metabolized in the follicles may affect ovarian functions. *In vitro* studies have demonstrated that estriol has only 20–30% affinity for the estrogen receptor compared to estradiol; therefore, it is rapidly cleared from a cell. If the effective concentration of estriol is kept equivalent to that of estradiol, however, the biological effects on target tissues are similar[16]. In fact, during pregnancy, the serum concentration of estriol increases and it is an important hormone for the monitoring of fetal–maternal well-being. The biological relevance of estriol is also confirmed by the exogenous administration in hypoestrogenic women:

estriol is considered the hormone of choice for the treatment of climacteric and postmenopausal disturbances and for preventing osteoporosis, cardiovascular disease and vaginal atrophy[17–19].

Further studies are required to elucidate the possible paracrine or autocrine effect of estriol in ovarian function and to investigate the specific enzymatic pathway involved in its synthesis.

In conclusion, our data indicate that estriol is detectable in human ovarian follicular fluid and that its production does not depend upon estrogen or the functional status of the follicles.

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