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Ovarian Steroid Production

POLYCYSTIC OVARY SYNDROME: INTERACTION OF FOLLICLE STIMULATING HORMONE AND POLYPEPTIDE GROWTH FACTORS IN OESTRADIOL PRODUCTION BY HUMAN GRANULOSA CELLS

STEPHEN FRANKS* and HELEN D. MASON
Department of Obstetrics and Gynaecology, St Mary's Hospital Medical School, Imperial College of Science, Technology & Medicine, London W2 1PG, England

Summary—The mechanism of the ovarian dysfunction in polycystic ovary syndrome, the most common cause of anovulatory infertility, remains obscure. Clinical data suggest that follicle stimulating hormone (FSH) action may be inhibited at the ovarian level by paracrine factors derived, presumably, from interstitial cells. The greater responsiveness to FSH of granulosa cells isolated from polycystic ovaries (PCO) compared with that seen in cells derived from normal ovaries, provides some support for this hypothesis and we present data which suggests that epidermal growth factor, or more likely transforming growth factor α, could be a candidate for this inhibitor. It should be emphasized, however, that the cardinal biochemical feature of the PCO is hypersecretion of androgens by interstitial cells. Stromal tissue from the PCO will secrete significant quantities of androstenedione in response to LH, whereas there is a negligible response in stroma from normal ovaries. It remains to be determined whether androgens have a direct inhibitory effect on FSH-induced oestradiol production in the human follicle, or whether they might exert an indirect effect by activating inhibitory polypeptide growth factors.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility. In a series of 175 anovulatory women, 97 were found to have polycystic ovaries (PCO) [1, 2] and although this diagnosis was based primarily on ultrasound scanning of the ovaries, similar data have been produced in a study by Hull [3] who demonstrated that, using more conventional clinical and biochemical criteria to make the diagnosis, 75% of women with anovulatory infertility had PCOS. Despite the high prevalence of this disorder, PCOS remains an enigma; little is known about the mechanism of anovulation and this is of considerable practical significance since the necessarily empirical treatment of anovulation in women with PCOS is not uniformly successful. In subjects who are resistant to anti-oestrogen treatment, conception rates during treatment with exogenous gonadotrophins fall somewhat short of those obtained during similar therapy in women with gonadotrophin deficiency. In the following, the possible mechanism of anovulation in women with PCO is discussed and, in this context, results are presented from studies of endocrine–paracrine interactions in the control of oestradiol (E_2) production by human granulosa cells of both normal ovaries and PCO.

MECHANISM OF ANOVULATION IN WOMEN WITH PCOS

The characteristic feature of anovulation in women with PCOS is a failure of follicle stimulating hormone (FSH)-dependent follicular maturation [2, 4]. Although LH and androgen concentrations are typically elevated in women with PCOS (and either or both may have a part to play in modulating FSH action on the ovary [5]—see below), administration of FSH alone, even in relatively small doses, can induce development and ovulation of a dominant follicle [6, 7]. Anovulation in PCOS could therefore be due to one (or more) of three possible mechanisms: (a) inadequate serum concentrations of FSH; (b) decreased biological activity of circulating FSH; or (c) inhibition of

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*To whom correspondence should be addressed.

FSH action at the ovarian level by paracrine or other endocrine factors.

Serum concentrations of immunoreactive FSH are similar in anovulatory women with PCOS to those in the early to mid follicular phase of the menstrual cycle in ovulatory women with normal ovaries [2, 8]. Furthermore, when comparing the biochemical features of women who ovulate during treatment with the anti-oestrogen, clomiphene, with those who do not (and who do not develop a dominant follicle) no significant differences were found between the groups in the peak serum FSH concentrations induced by clomiphene treatment [9]. These findings raise the question as to whether the biological activity of circulating FSH in women with PCOS is decreased compared with normal. However, serum concentrations of bioactive FSH (as measured by a rat granulosa cell bioassay) were similar in women with both clomiphene-responsive and -resistant PCOS to those in the mid follicular phase of the normal cycle [10] (Fig. 1). Could anovulation in PCOS therefore be related to inhibition of FSH action at the ovarian level?

PARACRINE REGULATION OF FSH ACTION

Both ovarian steroids and polypeptide growth factors have been shown, in animal studies, to modulate the effects of FSH on E_2 production by granulosa cells *in vitro* [2]. Clearly, in the context of PCOS, a potentially inhibitory effect of androgen must be considered. Work on the primate follicle suggests

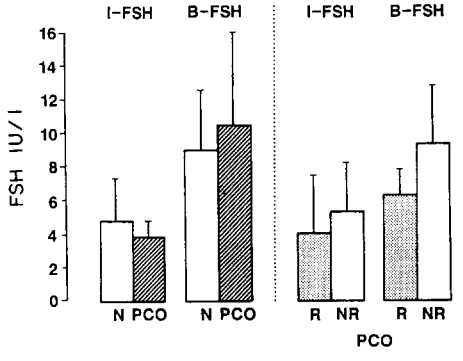

Fig. 1. Serum concentrations (mean + SD) of bioactive (B-FSH) and immunoactive (I-FSH) FSH in women with either normal ovaries (□, $n = 5$) or PCO (▨, $n = 11$) are shown in the left-hand panel. The right-hand panel shows I-FSH or B-FSH levels in two groups of women with anovulatory PCOS: those who had ovulated in response to clomiphene (■) and those who had not (□). There were no significant differences between groups of patients (i.e. N vs PCO or R-PCO vs NR-PCO) in either I-FSH or B-FSH.

that androgens have a biphasic effect on FSH-induced E_2 production, i.e. augmentation of FSH action in small antral follicles but inhibition in the preovulatory follicle [11]. But it remains to be determined whether or not androgens have a similar inhibitory effect on FSH action in the large follicles of the human ovary. There is a host of polypeptide growth factors which have been shown to affect granulosa function in laboratory animals. Of these, insulin-like growth factor-1 (IGF-1) and transforming growth factor α (TGFα) have been shown to be produced by human theca cells [12, 13]. TGFα and the homologous epidermal growth factor (EGF) are of particular interest because they have been shown to be potent inhibitors of E_2 production and might therefore subserve such a role in the mechanism of anovulation in women with PCOS.

CULTURE OF HUMAN GRANULOSA CELLS

Normal ovaries were obtained from patients with a history of spontaneous ovulatory cycles who were undergoing oophorectomy for non-ovarian disease. Each had normal ovarian morphology on ultrasound and on histological examination. PCO tissue was obtained from patients undergoing ovarian wedge resection for chronic anovulation and infertility. None of the patients had received any medication for stimulation or suppression of ovulation for at least 3 months prior to surgery.

Follicles were identified and dissected intact from the ovaries as described previously [14]. They were examined under the microscope for signs of atresia and only those which appeared well-vascularized and contained no blood-clots were used. The follicles were gently incised and the granulosa cells removed by flushing with a stream of medium. The tissue was then gently scraped to remove any remaining cells. Cells from follicles of similar sizes were pooled (except in occasional experiments where cells from a single follicle were used) centrifuged, washed and counted. The cells were then distributed in multiwell plates at a density of approx. 50,000 viable cells in a 200 μl volume of Medium 199 (Gibco BRL Ltd Uxbridge, Middx., England) with the addition of antibiotics and 200 mM glutamine.

All incubations were carried out in serum-free medium with the addition of 10^{-7} M testosterone (Sigma Chemical Co., Poole, Dorset,

England). Medium was collected after incubation of the cells for 48 h in the presence of highly-purified human FSH (kindly supplied by Dr S. Lynch, Birmingham and Midland Hospital for Women, W. Midlands, England) with or without the addition of purified growth factors, i.e. murine EGF (Peninsula Labs, St Helen's, Merseyside, England) or IGF-1 (Novo-Nordisk, Gentofte, Denmark). Samples of medium were frozen and stored for measurement of oestradiol by RIA using an antibody supplied by Steranti (St Albans, Herts., England).

COMPARISON OF FSH-STIMULATED E_2 PRODUCTION BY GRANULOSA CELLS OF NORMAL OVARIES AND PCO

The results of 9 experiments in normal ovaries and a similar number in PCO are summarized in Fig. 2. There was a dose-dependent increase in E_2 production in the presence of FSH in cells of both normal and polycystic ovaries, but the responsiveness and sensitivity of granulosa cells from PCO was significantly greater than that observed in those derived from normal ovaries. These results indicate that not only are granulosa cells from PCO capable of responding to FSH *in vitro*, but, perhaps surprisingly, they are also more responsive than cells from non-PCO. One interpretation of these data is that there is, *in vivo*, an endogenous inhibitor of FSH action (possibly derived from the interstitial cells) in women with PCO and that once granulosa cells have been removed from the influence of this putative inhibitor they become hyper-responsive to FSH.

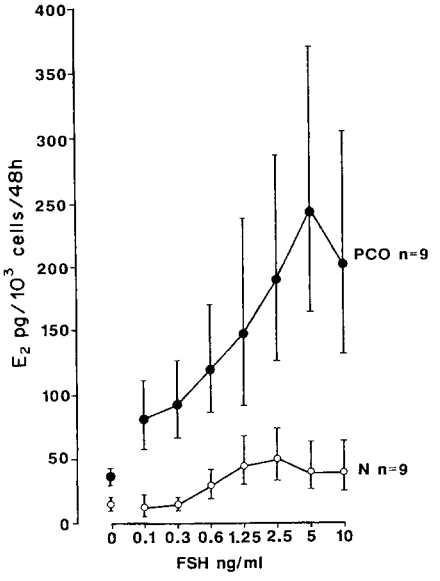

Fig. 2. Mean ± SE E_2 response to FSH in granulosa cells from either 9 normal ovaries (○) or 9 PCO (●). Note the increased sensitivity and responsiveness of cells to FSH in cells from PCO. The dose of FSH is shown on a log scale.

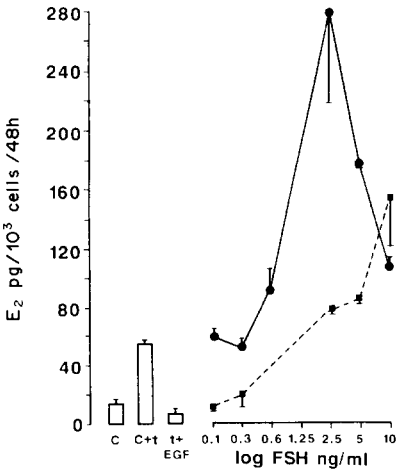

Fig. 3. E_2 responses to the addition of testosterone (t) alone (□) and t + FSH (●), in granulosa cells from a normal ovary. The addition of EGF at 50 pg/ml caused a marked inhibition of E_2 production (■). Values represent mean ± SE of triplicate experiments. Data from Ref. [14].

These data were obtained from analysis of the results of experiments using pooled granulosa cells from follicles of different sizes (3–8 mm dia) and remain to be confirmed by comparison of cells from individual follicles of similar size in normal ovaries and PCO. Nevertheless, the results are very striking and it seems likely that these findings represent a true difference in responsiveness between cells from the two types of ovary.

EFFECT OF EGF ON FSH-STIMULATED E_2 PRODUCTION *IN VITRO*

When EGF (50 pg/ml) was added to the culture medium it resulted in marked inhibition of FSH-induced E_2 production (Fig. 3). The effect was similar in cells from both normal ovaries and PCO. The overall data from 10 experiments are summarized in Table 1. EGF

Table 1. Effect of EGF (50 pg/ml) on peak response of E_2 to FSH

	Mean (range) peak[a] E_2 response to FSH (pg $E_2/10^3$ cells/48 h)			
	Follicle size (mm)	−EGF	+EGF	Mean % inhibition
PCO	⩽5 (2)	468 (74, 863)	289 (0, 578)	42
	>5 (4)	596 (39–1669)	373 (46–1119)	66
Normal ovaries	⩽5 (3)	76 (30–121)	38 (0–110)	53
	>5 (3)	150 (37–237)	65 (8–167)	68

[a]Peak calculated as maximal increase in E_2 above control (testosterone alone).

Results are divided according to follicle size and to type of ovary. The number of experiments is shown in parentheses. From Ref. [14].

(50 pg/ml) caused a mean inhibition of the peak E_2 response to FSH of 57% (range 42–68%). This inhibitory effect was similar in cells obtained from both types of ovary. There was also a dose-related inhibitory effect of EGF in the presence of a fixed concentration of FSH (i.e. 0.5 or 1.0 ng/ml) (Fig. 4).

The inhibitory effects of EGF on human granulosa cells from unstimulated normal ovaries and PCO are consistent with the findings in experiments using rat granulosa cells [15] and from those of Steinkampf *et al.* [16], who demonstrated that EGF had an inhibitory effect on aromatase activity in human granulosa cells obtained after superovulation therapy. Although there is no evidence that EGF is synthesized in the ovary, TGFα, which is homologous to EGF and which binds to the EGF receptor, is secreted by cells from human theca and stroma [13]. An enhanced inhibitory effect of TGFα in the granulosa cells of women with PCO could be explained by either increased responsiveness to TGFα or increased production of TGFα by theca or stroma. The responsiveness of granulosa cells from PCO was similar to that observed in normal ovaries but it remains to be determined whether production of TGFα by interstitial cells is increased in follicles from PCO.

EFFECT OF IGF-1 ON E_2 PRODUCTION

Erickson *et al.* [17, 18] have demonstrated that E_2 production by cells from both normal ovaries and PCO is greatly enhanced by the addition of IGF-1 to the culture medium. Our preliminary studies, illustrated in Fig. 5, confirm that IGF-1, in the absence of FSH, is able to stimulate aromatase activity and that there is a marked synergistic effect of IGF-1 and FSH. Like Erickson *et al.*, we observed these effects in tissue from both normal ovaries and PCO. The results of these studies are, however, difficult to interpret either in physiological terms or in the context of anovulation in women with PCO. This is because the action of IGF-1 in the ovary is likely to depend on its interaction with specific binding proteins which are synthesized locally within the ovary. As yet, there are no data to indicate whether such binding proteins inhibit or augment the effects of IGF-1 in human granulosa cells. Thus, whilst it is tempting to suggest that IGF-1 or insulin may have a role in the hypersecretion of *androgen* by theca cells of the PCO [19, 20], it would not be wise, at this stage, to speculate on its role in normal or disordered *granulosa cell* physiology in the human follicle. One reason for a cautious approach is the observation that whilst human growth hormone (hGH) was found to stimulate E_2 secretion by human granulosa cells, IGF-1 could not be detected in the medium either before or after hGH stimulation [21].

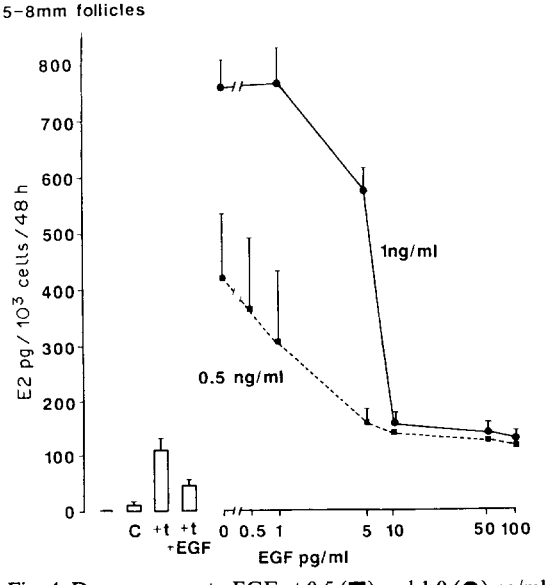

Fig. 4. Dose response to EGF at 0.5 (■) and 1.0 (●) ng/ml FSH in granulosa cells from small follicles from a normal ovary. Values represent mean ± SE of triplicate estimations. From Ref. [14].

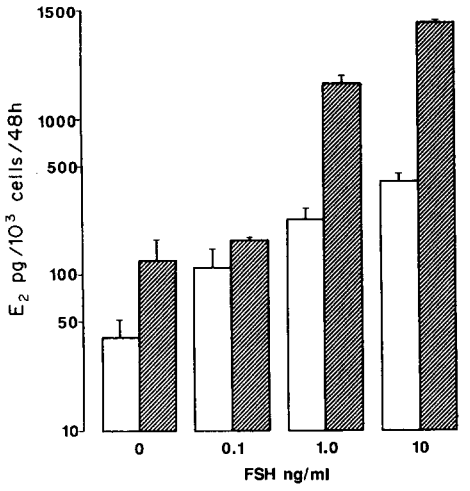

Fig. 5. E_2 response in granulosa cells from a PCO to FSH alone (□) or FSH + IGF-1 at 50 ng/ml (▨). Values represent mean + SE of triplicate experiments; t at 10^{-7} M was added as substrate. Note E_2 production is on a log scale.

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