Oestradiol feedback stimulation of androgen biosynthesis by human theca cells

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This study analysed the effect of oestradiol on basal and LH-stimulated production of androstenedione and progesterone by human theca cells in monolayer culture. Incubations were carried out for either 2 days (seven experiments) or 4 days (four experiments), in the presence or absence of luteinizing hormone (LH), oestradiol (10⁻⁹ – 10⁻⁶ M) or inhibin. Medium collected at 48 and 96 h was stored until radioimmunoassay for steroid content. Theca pooled from small follicles (<10 mm) was used in all but two experiments; in these, ovaries were obtained from ovulatory women in the mid-follicular phase of their cycle and theca from small and large follicles was pooled. Oestradiol inhibited progesterone production in a dose-dependent manner in all experiments, irrespective of follicle size, ovulatory status and ovarian morphology, with maximum effect at 10⁻⁶ M. At this dose, oestradiol had no effect on androstenedione production by theca from four anovulatory women with polycystic ovaries but produced a significant augmentation of both basal and LH-stimulated androstenedione production in theca from five of the seven ovulatory women, with maximal response in theca from the two pre-ovulatory subjects. During the 48–96 h period of incubation, oestradiol augmented androstenedione production in all four experiments and had a greater stimulatory effect than the physiological dose of inhibin (10 ng/ml). This is the first report of oestradiol regulating human theca cell steroidogenesis in a dose-dependent manner.

Key words: androgen biosynthesis/human theca/monolayer cultures/oestradiol

Introduction

The growth and development of a healthy Graafian follicle depends on the close cooperation of theca and granulosa cell layers. Structurally, theca interna and mural granulosa cells lie side by side, separated by a thin, basement membrane. The primary role of mural granulosa cells is to synthesize 17β-oestradiol but, as these cells lack 17α-hydroxylase, 17,20-lyase, androgen substrate supply is provided exclusively by the theca layer. This two-cell, two gonadotrophin model of steroidogenesis (Armstrong and Dorrington, 1979; Hillier et al., 1980) has, in recent years, been considerably refined as various intra-ovarian modulators have been identified (Hillier et al., 1994). While data on theca from human ovaries are limited, LH-dependent androgen biosynthesis has been shown to be augmented by inhibin (Hillier et al., 1991a), insulin, insulin-like growth factor-I (IGF-I) (Bergh et al., 1993; Gilling-Smith et al., 1993; Nahum et al., 1995) and IGF-II (Gilling-Smith et al., 1993; Nahum et al., 1995) and inhibited by activin (Hillier et al., 1991b). By fine-tuning androgen substrate availability, these modulators may play a role in the regulation of granulosa cell oestrogen production.

A modulatory role for oestradiol has not been explored in the human ovary. Oestradiol has been shown to inhibit basal and LH-stimulated androgen and progesterone production in both rat thecal tissue (Leung et al., 1978; Leung and Armstrong, 1979; Magoffin and Erickson, 1982) and porcine theca cell cultures (Hunter and Armstrong, 1987), while in bovine theca it stimulates androgen but inhibits progesterone production (Fortune, 1986; Roberts and Skinner, 1990a, b). In contrast to the rat and pig, the bovine ovary is, like the human ovary, mono-ovulatory and a useful animal model for studying the role of various endocrine and paracrine modulators of folliculogenesis. In this species, oestradiol regulates follicular growth through both an autocrine effect on granulosa cell oestrogen production and a paracrine action on theca cell androgen production. If such a local feedback were to exist in the human ovary, it is plausible that this effect might be defective or absent in anovulatory women such as those with polycystic ovary syndrome (PCOS).

We have developed a model for studying steroid production by isolated human theca cells in vitro which retains its responsiveness to luteinizing hormone (LH). Theca cells, obtained from unstimulated human ovaries, are dispersed and cultured as monolayers in serum-free medium. During the first 48 h of culture, they respond to LH in a dose-dependent manner but thereafter, both androgen production and LH-responsiveness progressively decline (Gilling-Smith et al., 1994). The primary aims of this study were to use this monolayer culture system, firstly, to determine the effect of oestradiol on basal and LH-stimulated androstenedione and progesterone accumulation by human theca and, secondly, to define whether any observed effects could be related to the patient’s ovulatory status or stage of the menstrual cycle. In order to quantify the relative role of oestradiol as a modulator of theca cell steroidogenesis, a further aim was to compare oestradiol action on theca with that of inhibin.

Materials and methods

Patients and tissue collection

The study received approval from the Kensington, Chelsea and Westminster Health Authority Ethics Committee. Ovaries were
Ovarian morphology was defined at the time of dissection according to medium in the dose range (10^-9 – 10^-6 M), which is the range found obtained from 11 women undergoing uni- or bilateral oophorectomy for benign, non-ovarian gynaecological disease. Medication to either stimulate or suppress ovariectomy had been stopped at least 3 months before surgery. Note was made of the patient’s age, menstrual cycle history, day of cycle at the time of oophorectomy, pre-operative ultrasound findings and ovulatory status, if previously determined. Ovarian morphology was defined at the time of dissection according to previously published criteria (Gilling-Smith et al., 1994). Seven women had a history of regular ovulatory cycles and a corpus luteum was present in the ovaries at the time of dissection. Within this group of ovulatory women, one subject had normal ovaries, two had polycystic ovaries (PCO), and four had normal ovaries but with cystic changes associated with chronic pelvic pain. We have previously described these ovaries as being found exclusively in women with a history of pelvic venous congestion and refer to them as ‘pelvic pain cystic’ (PPC) ovaries (Gilling-Smith et al., 1995; Mason et al., 1995). The remaining four women had irregular cycles, PCO and a clinical history of anovulation. Patient details are summarized in Table I.

### Table I. Patient profiles

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ovary type</th>
<th>Age (years)</th>
<th>Cycle</th>
<th>Day of cycle</th>
<th>Cells/well × 10,000</th>
<th>Follicles &lt;10 mm</th>
<th>Follicles ≥10 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>OV</td>
<td>33</td>
<td>11</td>
<td>15</td>
<td>0</td>
<td>1 (24 mm)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>PPC</td>
<td>35</td>
<td>OV</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>PPC</td>
<td>36</td>
<td>OV</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>PPC</td>
<td>31</td>
<td>OV</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>PPC</td>
<td>38</td>
<td>OV</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>PCO</td>
<td>32</td>
<td>OV</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>PCO</td>
<td>34</td>
<td>ANOV</td>
<td>NK</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>PCO</td>
<td>28</td>
<td>ANOV</td>
<td>10</td>
<td>11</td>
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<td></td>
</tr>
<tr>
<td>IX</td>
<td>PCO</td>
<td>44</td>
<td>ANOV</td>
<td>6</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>PCO</td>
<td>40</td>
<td>ANOV</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>PCO</td>
<td>31</td>
<td>ANOV</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = normal ovary, PPC = normal ovary with cystic changes associated with pelvic venous congestion, PCO = polycystic ovary, OV = regular ovulatory cycle, ANOV = irregular anovulatory cycle, NK = not known.

Women, West Midlands, UK: LH activity 12 700 IU/mg; follicle-stimulating hormone (FSH) 7.0 IU/mg; thyroid-stimulating hormone (TSH) 6.5 mIU/ml was added to half the wells at a dose of 2.5 ng/ml which we have previously shown to be the maximum effective dose required for androgen production (Gilling-Smith et al., 1994).

Oestradiol (Sigma, Poole, Dorset, UK) was added to the incubation medium in the dose range (10^-6 – 10^-6 M), which is the range found in human follicular fluid throughout the menstrual cycle, and all experiments were performed in the presence and absence of LH 2.5 ng/ml. In a single experiment, the effects of oestradiol were compared with those of recombinant inhibin-A (kindly donated by Genentech, San Francisco, CA, USA). Cultures were maintained for either 2 days (seven experiments) or 4 days (four experiments) at 37°C under a humidified atmosphere of 5% CO2 and 95% air. The overlying culture medium was collected on days 2 and 4 of culture and stored at –20°C until radioimmunoassay for androstenedione and progesterone. For each experiment, a series of cell-free wells containing incubation medium and each dose of added hormone served as blanks to exclude cross-reactivity with oestradiol for both the androstenedione and progesterone assays. All experiments were performed using either duplicate or triplicate wells.

In order to determine whether the effect of oestradiol could be maintained in long-term cultures, in a single experiment, following the initial 48 h period of culture in serum-free medium, the cells were washed with 500 µl HBSS and incubated in culture medium enriched with 2% UltraSero G (Sigma). The medium was changed on alternate days. After 10 days, the cells were washed with 500 µl HBSS and the initial experiment repeated, i.e. the cells incubated for 48 h in 1 ml serum-free culture medium with increasing doses of oestradiol in the presence or absence of LH (2.5 ng/ml).

Previous studies have identified the main variables affecting the magnitude of steroid accumulation to be dose of LH, density of cell plating, duration of incubation and follicle size (Gilling-Smith et al., 1994). When the effects of oestradiol on theca from ovulatory and anovulatory subjects were compared, the first three variables were kept constant. With respect to follicle size, theca from small follicles (<10 mm) was used in all experiments except in two cases, where the theca was collected during the mid-follicular stage of the cycle and a dominant follicle was present. In these two cases, theca from both small and large follicles were pooled and the results analysed separately.

Contamination of the culture system by granulosa cells was excluded functionally by the observation that FSH added to the incubation medium, at doses known to produce maximal stimulation...
Oestradiol stimulation of theca cell androgen synthesis

Figure 1. Dose-dependent effects of oestradiol during the first 48 h of culture on (a) androstenedione and (b) progesterone accumulation by theca cells in the absence (−LH) and presence (+LH) of luteinizing hormone (2.5 ng/ml). Data taken from subject II on day 10 of an ovulatory cycle. Theca from small and large follicles were pooled. Data are mean ± SEM (n = 3). Analysis of variance of logarithmic transformed data: (a) androstenedione: in absence of LH, F value 9.419, P < 0.001; in presence of LH (2.5 ng/ml), F value 4.682, P = 0.005. (b) Progesterone: in absence of LH, F value 35.37, P < 0.0001; in presence of LH (2.5 ng/ml), F value 71.615, P = 0.0001.

of granulosa cell steroidogenesis, had no effect on oestradiol accumulation. Hence all observed effects in the above experiments could be assumed to be due exclusively to added, as opposed to granulosa cell-derived, oestradiol.

Radioimmunoassay
Both androstenedione and progesterone in the stored incubation medium were measured by direct radioimmunoassay of the unextracted culture medium using antibodies and antiserum as previously described (Gilling-Smith et al., 1994). For each cell culture experiment, duplicate samples from each well were assayed in a single batch, and if replicates had a coefficient of variation >3%, the assay was repeated. Cross-reactivity with the added oestradiol was excluded in both assays by running the cell-free incubates in parallel with the experimental samples.

Statistical analysis
Steroid accumulation is expressed in pmol steroid/1000 viable cells/48 h of culture ± SEM unless otherwise stated. Since the data were not normally distributed, they were subjected to logarithmic transformation before performing analysis of variance.

Results
Dose-dependent effects of oestradiol on androstenedione and progesterone accumulation
Full dose-response curves for oestradiol (10⁻⁹–10⁻⁶ M) were obtained in four experiments. Figure 1 shows representative curves taken from a single experiment on theca cells from subject II and shows androstenedione and progesterone accumulation during the first 48 h of culture.

Table II. Effect of oestradiol (10⁻⁶ M) on androstenedione and progesterone accumulation by theca cells under basal and luteinizing hormone (LH)-stimulated conditions between 48 and 96 h of culture

<table>
<thead>
<tr>
<th>Oestradiol</th>
<th>Androstenedione (pmol/1000 cells/48 h)</th>
<th>Progesterone (pmol/1000 cells/48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0 (0–1.9)</td>
<td>11.7 (0–101)</td>
</tr>
<tr>
<td>LH</td>
<td>0 (0–1.0)</td>
<td>27.5 (0–140)</td>
</tr>
<tr>
<td>Oestradiol 10⁻⁶ M</td>
<td>3.1 (3.0–5.2)</td>
<td>0 (0–23.0)</td>
</tr>
<tr>
<td>LH + oestradiol 10⁻⁶ M</td>
<td>3.2 (2.5–4.8)</td>
<td>10.6 (0–25.5)</td>
</tr>
</tbody>
</table>

Data are pooled from four experiments (subjects V, VI, VIII and XI). Incubations were carried out either in duplicate or triplicate wells in the absence or presence of LH (2.5 ng/ml). Data are expressed as median steroid accumulation/1000 viable cells/48 h of culture with the range given in parentheses. Plating density range 5–15×10⁴ cells/well.

Progesterone production was inhibited by oestradiol in a dose-dependent manner in all four subjects, with maximum inhibition occurring at a dose of 10⁻⁶ M. The effect of oestradiol on androstenedione production was variable. In two experiments, theca cells were obtained from ovulatory women in the mid-follicular phase of their cycle (subjects I and II) and theca from small and large follicles were pooled. Here, a dose-dependent augmentation of androstenedione production was observed, both in the absence and presence of LH. The maximum effective dose of oestradiol was 10⁻⁶ M and LH had a synergistic, rather than an additive, effect on androgen production. However, in the two other experiments (subjects VII and X), in which theca was pooled from small follicles (<10 mm) only, there was no significant androstenedione production.
Figure 2. Dose-dependent effects of oestradiol following a 10 day period of culture in serum-rich culture medium showing (a) androstenedione and (b) progesterone accumulation by theca cells in the absence (–LH) and presence (+LH) of luteinizing hormone (2.5 ng/ml). Data are taken from subject II on day 10 of an ovulatory cycle. Theca from small and large follicles were pooled. Data are mean ± SEM (n = 3).

response to oestradiol during the first 48 h of culture. These preliminary studies defined the maximum effective dose of oestradiol to be 10^(-6) M and this dose was therefore used in all subsequent experiments where a single dose of oestradiol was used.

**Time-dependent effects of oestradiol on androstenedione and progesterone accumulation**

Table II shows the accumulation of androstenedione and progesterone in pooled data from four experiments in which incubations were carried out for four days. In all cases, there was a significant fall in basal androstenedione accumulation (to undetectable levels in three cases) and some loss of LH responsiveness during the 48–96 h period of culture. However, irrespective of oestradiol effects during the first 48 h, oestradiol (10^(-6) M) produced a significant augmentation of androstenedione accumulation and inhibition of progesterone accumulation in all cases.

In subject II, theca cells were cultured for 10 days in serum-rich medium and the experiment repeated. The effect of oestradiol on androstenedione and progesterone accumulation in this case is shown in Figure 2. Although LH responsiveness was lost, oestradiol effects on both androstenedione and progesterone were maintained.

**Oestradiol effects in ovulatory and anovulatory subjects**

In order to establish whether oestradiol is an important modulator of androgen biosynthesis in the developing follicle, the results of 11 experiments were analysed according to each patient's ovulatory status. Three different groups of theca were compared:

- **Group I** (n = 4): anovulatory women, theca from small follicles (<10 mm)
- **Group II** (n = 5): ovulatory women, theca from small follicles (<10 mm)
- **Group III** (n = 2): ovulatory women, theca pooled from small and large follicles (<10 mm and ≥10 mm).

Median and range androstenedione and progesterone production during the first 48 h of culture under basal and LH-stimulated conditions are shown in Tables III and IV. The number of experiments in each group was too small to allow formal statistical comparison using the Mann–Whitney U test. In qualitative terms, oestradiol inhibited basal and LH-stimulated progesterone accumulation in all three groups, with no obvious differences in the magnitude of effect between groups. Among the ovulatory patients (n = 7), basal and LH-stimulated androstenedione production was augmented in five out of seven cases [median (range) basal: 252% (84–663%); LH: 237% (71–577%), n = 7]. Overall, oestradiol had no effect on androstenedione production in theca from the four anovulatory patients [basal: 101% (50–180%); LH: 79% (47–104%), n = 4]. In theca from the two preovulatory subjects (Group III), the androstenedione response to oestradiol was much greater [basal: 361% (252–470%); LH: 298% (248–348%), n = 2]. These data are shown graphically in Figure 3.

**Comparative effects of oestradiol and inhibin**

The comparative effects of inhibin and oestradiol on androstenedione accumulation are shown in Figure 4. This experiment was performed on an anovulatory subject (XI) with
polycystic ovaries. During the first 48 h of culture, inhibin stimulated a dose-dependent augmentation of androstenedione accumulation, in contrast to oestradiol which had no effect (Figure 4a). However, during the subsequent period of culture (48–96 h), when basal and LH-stimulated androstenedione accumulation were low, oestradiol produced a greater augmentation of androstenedione than the physiological dose of inhibin, 10 ng/ml, although not so great as the supraphysiological dose of 100 ng/ml (Figure 4b).

**Discussion**

This is the first report of oestradiol modulating steroidogenesis by human theca cells in monolayer culture. Of the 11 experiments performed, oestradiol produced a significant augmentation of androstenedione accumulation, in contrast to oestradiol which had no effect during the first 48 h of culture in six. In a further two cases, despite there being no clear-cut effect during the first 48 h of culture, androstenedione augmentation was noted between 48 and 96 h. In the experiments where oestradiol stimulated androstenedione accumulation, the effect was dose-dependent with maximum effect at 10⁻⁶ M, which corresponds to oestradiol concentrations found in pre-ovulatory follicles (McNatty et al., 1980; Seibel et al., 1989). These findings suggest that oestradiol, derived from either follicular fluid or granulosa cells, and acting through a short-loop feedback paracrine action, may be an important modulator of theca cell androgen production.

Since the initial period of culture is characterized by a marked loss of accumulated stores of androstenedione (Gilling-Smith et al., 1994), it is possible that in some experiments, this outpouring of steroids during the first 48 h of culture masked any stimulatory effect of oestradiol. In keeping with this explanation is the observation that the sensitivity of our theca cell monolayer system to the effects of insulin, IGF-I and IGF-II is significantly increased following a 12 h period of preincubation in serum-free medium (Gilling-Smith et al., 1993). In bovine theca, a similar time-dependent effect of oestradiol on androstenedione accumulation has been reported, with oestradiol producing only a slight increase in androstenedione production after 24 h in culture but a much greater rise after 3 days (Roberts and Skinner, 1990b). An alternative explanation, proposed by these workers, is that oestradiol induces theca cell differentiation which in turn affects steroidogenesis.

Inhibin, like oestradiol, rises significantly in the preovulatory follicle and has been shown to produce a twofold augmentation of basal and LH-stimulated theca cell androstenedione accumulation (Hillier et al., 1991a). This response is equivalent to that obtained with LH alone, but significantly less than the effect of IGF-I and LH, which together act in a synergistic fashion. These data come from studies in which experiments were carried out after a 24 h period of preincubation in serum-rich medium. In our study, the relative roles of inhibin and oestradiol on theca cell androstenedione production were compared in a single experiment following immediate incubation. Neither a physiological dose of inhibin (10 ng/ml) nor oestradiol (10⁻⁶ M) had a significant effect on androstenedione accumulation during the first 48 h of culture, in contrast to the supraphysiological dose of inhibin (100 ng/ml), which produced a twofold rise in both basal and LH-stimulated

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**Table III. Effect of oestradiol (10⁻⁶ M) on androstenedione accumulation by theca cells grouped according to the subject's ovulatory status**

<table>
<thead>
<tr>
<th>Group</th>
<th>Anovulatory (f &lt; 10 mm)</th>
<th>Ovulatory (f &lt; 10 mm)</th>
<th>Ovulatory (f &lt; and ≥10 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>26.6 (6.8–32.1)</td>
<td>3.7 (1.9–86.9)</td>
<td>25.6 (1.3–49.9)</td>
</tr>
<tr>
<td>LH</td>
<td>48.2 (14.3–109)</td>
<td>9.1 (3.0–72.6)</td>
<td>39.8 (2.8–76.8)</td>
</tr>
<tr>
<td>Oestradiol 10⁻⁶ M</td>
<td>23.9 (12.3–35.2)</td>
<td>12.9 (3.0–72.6)</td>
<td>66.1 (6.3–126)</td>
</tr>
<tr>
<td>LH + oestradiol 10⁻⁶ M</td>
<td>32.1 (14.8–69.6)</td>
<td>21.7 (4.5–104)</td>
<td>137 (7.0–267)</td>
</tr>
</tbody>
</table>

Incubations were carried out either in duplicate or triplicate wells in the absence or presence of luteinizing hormone (LH) (2.5 ng/ml). Data are expressed as median steroid accumulation/1000 viable cells/48 h of culture with the range given in parentheses. Plating density range 5–15 ×10⁴ cells/well. f = follicles. See legend for Figure 3 for patient details.

**Table IV. Effect of oestradiol (10⁻⁶ M) on progesterone accumulation by theca cells grouped according to the subject's ovulatory status**

<table>
<thead>
<tr>
<th>Group</th>
<th>Anovulatory (f &lt; 10 mm)</th>
<th>Ovulatory (f &lt; 10 mm)</th>
<th>Ovulatory (f &lt; and ≥10 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>46.2 (16.9–215)</td>
<td>13.4 (5.8–147)</td>
<td>329 (41.0–617)</td>
</tr>
<tr>
<td>LH</td>
<td>80.2 (26.1–359)</td>
<td>17.3 (3.2–85.5)</td>
<td>713 (94.9–1332)</td>
</tr>
<tr>
<td>Oestradiol 10⁻⁶ M</td>
<td>15.4 (0–48.6)</td>
<td>7.2 (6.9–106)</td>
<td>89.1 (10.6–168)</td>
</tr>
<tr>
<td>LH + oestradiol 10⁻⁶ M</td>
<td>24.4 (0–81.7)</td>
<td>10.9 (6.9–106)</td>
<td>209 (26.3–391)</td>
</tr>
</tbody>
</table>

Incubations were carried out either in duplicate or triplicate wells in the absence or presence of luteinizing hormone (LH) (2.5 ng/ml). Data are expressed as median steroid accumulation/1000 viable cells/48 h of culture with the range given in parentheses. Plating density range 5–15 ×10⁴ cells/well. f = follicles. See legend for Figure 3 for patient details.
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controlling androgen substrate supply, particularly in the pre-ovulatory follicle.

A dose-dependent fall in progesterone accumulation was observed in all experiments with maximum effect, once again, at $10^{-6}$ M. This paradoxical augmentation of androstenedione and inhibition of progesterone induced by oestradiol is identical to that observed in the bovine ovary and difficult to interpret from steroid accumulation experiments alone. The precise mechanism of action of oestradiol on human theca can only be clarified by either pulse-chase experiments, using labelled precursors, or enzyme kinetic studies. Since we did not add steroid precursors to the incubation medium, the fall in progesterone accumulation could have been due to substrate depletion following stimulation of 17α-hydroxylase, 17,20-lyase by oestradiol. This explanation would be in keeping with the fact that oestradiol augmented androstenedione production in all the longer (48–96 h) incubations. Since the relative concentrations of progesterone produced by theca cells in vitro can be up to 100-fold greater than those of androstenedione (Gilling-Smith et al., 1994), an alternative explanation is that oestradiol has a direct and selective inhibitory action on progesterone biosynthesis by competitive inhibition of the 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme, as has been reported in a number of steroid secreting tissues. These include cultured swine (Veldhuis et al., 1985), chicken (Lee and Bahr, 1990) and porcine (Tonetta et al., 1987) granulosa cells, human fetal adrenal cells, human (Williams et al., 1979) and rhesus monkey (Butler et al., 1975) luteal cells and Leydig tumour cells (Freeman, 1985). The degree of 3β-HSD suppression by oestradiol has been shown to be substrate sensitive, being significantly less for DHEA than for pregnenolone (Fortune, 1986), which would enable oestradiol selectively to augment androstenedione production while inhibiting progesterone production. Selective suppression of pregnenolone conversion to progesterone by oestradiol at this stage of the cycle could be a mechanism for maximizing the synthesis of androstenedione from DHEA and limiting the premature secretion of progesterone prior to ovulation.

Oestradiol effects on theca cell steroidogenesis did not appear to be affected by ovarian morphology, although there were insufficient data on theca from normal ovaries to make a statistical comparison. By contrast, ovulatory status did appear to be an important factor in the androstenedione, but not the progesterone, response to oestradiol. In theca from five of the seven ovulatory women, oestradiol produced a significant augmentation of androstenedione accumulation under both basal and LH-stimulated conditions. Furthermore, in the follicles from two pre-ovulatory women, oestradiol and LH acted synergistically to augment androstenedione production, although significant effects were only noted at higher concentrations of oestradiol. In the only comparable work on human ovaries, McNatty et al. (1980) co-cultured human theca cell explants with granulosa cells in vitro and demonstrated that granulosa-derived products enhanced theca cell androgen production in a synergistic manner, which depended on the size of follicle from which the cells were isolated, and the duration of incubation. A greater and more rapid augmentation of androstenedione production was observed in cells from larger

Figure 3. Effect of oestradiol ($10^{-6}$ M) in the absence and presence of luteinizing hormone (LH) (2.5 ng/ml) on androstenedione accumulation by theca cells grouped according to follicle size and patient’s ovulatory status. To account for the variation in androstenedione accumulation between experiments, data are shown as percentage androstenedione accumulation during the first 48 h of culture with reference to the basal values of 100% for each group. Pooled data for each group are shown as median and ranges. f = follicles. Each experiment was performed in duplicate or triplicate wells and plating density range was between 5–15 × 10^4 cells/well. Group I: theca from small follicles (<10 mm) from four anovulatory subjects with polycystic ovaries (subjects VIII, IX, X and XI). Group II: theca from small follicles (<10 mm) from five ovulatory subjects, four with normal ovaries but with cystic changes and chronic pelvic pain (PPC) (subjects III, IV, V and VI) and one with polycystic ovaries (subject VII). Group III: pooled theca from small (<10 mm) and large (≥10 mm) follicles from two ovulatory subjects, one with normal ovaries (subject I) and one with PPC ovaries (subject II).

androstenedione production. More importantly, during the 48–96 h period of culture, oestradiol produced a significant rise in androstenedione accumulation whereas physiological doses of inhibin had no discernible effect. These data suggest that oestradiol, derived from granulosa cells, follicular fluid and/or blood has as important a role as follicular fluid inhibin in
Oestradiol stimulation of theca cell androgen synthesis

Figure 4. Comparative effects of inhibin (INH) and oestradiol on androstenedione and progesterone accumulation by human theca cells. Results are from a single experiment on theca from subject XI. Theca cells from small follicles only (<10 mm) were incubated for 4 days in serum-free culture medium with inhibin 10 ng/ml (INH 10), inhibin 100 ng/ml (INH 100) or oestradiol (10⁻⁶ M) in the absence (−LH) and presence (+LH) of luteinizing hormone (2.5 ng/ml). The medium was changed after 48 h. Data are expressed as mean ± SEM of triplicate wells showing androstenedione accumulation between (a) 0–48 h of culture and (b) 48–96 h of culture and progesterone accumulation between (c) 0-48 h of culture and (d) 48–96 h of culture.

Figure 5. Proposed feedback action of granulosa cell derived oestradiol (E₂) on androstenedione (A) and progesterone (P) production by human theca cells. LH = luteinizing hormone, FSH = follicle-stimulating hormone.

In summary, oestradiol, within the concentration range found in human follicular fluid, appears to have an important action on androstenedione and progesterone production by human theca cells in vitro. The concomitant stimulatory and inhibitory effect of oestradiol on theca cell androstenedione and progesterone accumulation respectively has only previously been reported in the bovine ovary which, like the human ovary, is mono-ovulatory. These findings would support the concept of an intra-ovarian short-loop feedback mechanism, whereby granulosa cell-derived oestradiol controls its own substrate supply, which in turn promotes unifollicular development and follicular fluid and the ovarian circulation prior to ovulation, theca cells respond by providing the increased androgen substrate supply required for the final stages of follicular growth. Absence of this effect in small follicles, at low doses of oestradiol, could be a mechanism for ensuring atresia of all but the dominant follicle.

Follicles (≥8 mm diameter). Our findings are consistent with these and would suggest that the ability of theca cells to respond to oestradiol may be crucial to successful Graafian follicle development. As oestradiol concentrations rise in both
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successful ovulation (Figure 5). This hypothesis is further supported by the observation that oestradiol effects onandrosterone production were significantly amplified in theca from pre-ovulatory follicles.

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References


