CHANGES IN THE CONCENTRATION OF PITUITARY AND STEROID HORMONES IN THE FOLLICULAR FLUID OF HUMAN GRAAFIAN FOLLICLES THROUGHOUT THE MENSTRUAL CYCLE

K. P. McNATTY,1 W. M. HUNTER,2 A. S. McNEILLY3 AND R. S. SAWERS4

1MRC Unit of Reproductive Biology, 39 Chalmers Street, Edinburgh, EH3 9ER; 2MRC Radioimmunoassay Team, 2 Forrest Road, Edinburgh, EH1 2QW; 3Department of Chemical Pathology Research, St Bartholomew's Hospital, West Smithfield, London, EC1A 7BE and 4Department of Obstetrics and Gynaecology, Royal Infirmary, Edinburgh, EH3 9YW

(Received 25 July 1974)

SUMMARY

The concentrations of FSH, LH, prolactin, oestradiol and progesterone were measured in peripheral plasma and follicular fluid of women throughout the menstrual cycle. With the exception of prolactin, concentrations of pituitary and steroid hormones in follicular fluid correlated with those in peripheral plasma.

Follicle-stimulating hormone was present in a greater number of small follicles (< 8 mm) during or just after the peaks of FSH in peripheral plasma. During the mid-follicular phase the concentration of both FSH and oestradiol in fluid from large follicles (≥ 8 mm) was high. During the late follicular phase the large follicles (≥ 8 mm) contained high amounts of progesterone in addition to oestradiol, low physiological levels of prolactin, and concentrations of LH and FSH about 30 and 60% respectively of those found in plasma. By contrast no large ‘active’ follicles (≥ 8 mm) were found during the luteal phase although many contained both LH and FSH. Luteinizing hormone was present in a proportion of small follicles (< 8 mm) during the late follicular and early luteal but not at other stages of the menstrual cycle.

It is suggested that a precise sequence of hormonal changes occur within the microenvironment of the developing Graafian follicle; the order in which they occur may be of considerable importance for the growth of that follicle and secretory activity of the granulosa cells both before and after ovulation.

INTRODUCTION

There is considerable evidence to suggest that pituitary gonadotrophins are responsible for the later stages of follicular growth, maturation of the oocyte and ovulation (see reviews of Baker, 1972; Biggers & Scheutz, 1972; Greep, 1973). Although it is possible to correlate changes in gonadotrophins in peripheral plasma
with the production of steroids from the ovary, the actions of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) on the follicle remain obscure. It has been suggested that gonadotrophins within the follicular fluid itself may have important consequences for follicular steroidogenesis (Channing, 1970, 1972; Edwards, 1974; McNatty, Sawers & McNeill, 1974). growth of the follicle, oocyte maturation and evolution (Edwards, 1974).

The changing concentration of peripheral plasma oestradiol during the follicular phase of the human menstrual cycle provides an index of follicle growth (Baird, 1971). Oestradiol in the peripheral plasma during this phase of the cycle is derived almost solely from the actively growing follicles (Baird & Fraser, 1974) which also contain high concentrations of oestradiol (Smith, 1960; Short & London, 1961; Giorgi, 1965; Edwards, Steptoe, Abraham, Walters, Purdy & Fotherby, 1972; Baird & Fraser, 1975; Sanyal, Berger, Thompson, Taymor & Horne, 1974). Furthermore, during the immediate preovulatory phase there is an increase in the concentration of progesterone in follicular fluid (Edwards et al. 1972; McNatty et al. 1974; Sanyal et al. 1974) which is also reflected in peripheral plasma (Johansson & Wide, 1969; Yussman & Taymor, 1970).

In the present study the concentrations of LH, FSH, prolactin, oestradiol and progesterone were measured in samples of peripheral plasma and follicular fluid collected from women at varying stages of the menstrual cycle. It was hoped in this way to investigate the relationships between the concentrations of pituitary and steroid hormones in follicular fluid and to relate them to concentrations in plasma, follicle size, and the stage of the menstrual cycle.

MATERIALS AND METHODS

Subjects

Ninety-seven subjects (aged 21–48 years) who were at varying stages of the menstrual cycle were undergoing hysterectomy for various gynaecological conditions. The indications for surgery were stage 0 carcinoma of the cervix (17), menorrhagia due to fibroids (35), dysfunctional uterine bleeding (10), endometriosis (9), or chronic pelvic pains or dysmenorrhoea (26). Those with stage 0 carcinoma of the cervix had regular menstrual cycles (21–32 days) and were considered to be endocrinologically normal (Baird & Fraser, 1974). The previous menstrual cycles of the group with menorrhagia varied in length from 21–34 days and about 50% of these subjects had ovulated in the cycle under study, as indicated by the presence of a secretory endometrium and of at least one corpus luteum at the time of the operation. The remaining subjects were in the proliferative phase as assessed by their endometrial histology and date of the last menstrual period.

Dating the menstrual cycle

An endometrial biopsy and a peripheral blood sample (collected before oophorectomy) were obtained during the operation. The endometrium was examined histologically and dated according to the criteria of Noyes, Hertig & Rock (1950). The stage of the menstrual cycle was assessed in all subjects from the date of the last menstrual period, the concentrations in plasma of LH, FSH, oestradiol and pro-
gesterone, endometrial histology and the presence or absence of a corpus luteum. The menstrual cycle was divided into six phases: early follicular, still menstruating (EF); mid-follicular (MF); late follicular (LF); early luteal (EL); mid-luteal (ML) and late luteal (LL).

**Ovarian morphology**

Ovaries were examined *in situ* to assess gross morphology and to record the presence or absence of a corpus luteum. In some cases excised ovarian specimens were examined by light microscopy after removal of the Graafian follicles to ascertain that no major ovarian pathology existed.

**Collection of follicular fluid**

The follicular fluid analysed in this study was obtained from ovaries of the 97 subjects described above. Two different methods for the collection of the fluid were used.

In 23 subjects antral fluid was aspirated from 36 Graafian follicles through a 23G needle into a syringe during surgery. It was technically not possible to measure these follicles accurately, consequently they were classified as large (≥ 8 mm) or small (< 8 mm).

In 74 subjects ovarian specimens (whole ovaries or wedge biopsies) were collected into chilled Medium 199 containing Hanks' salts and HEPES buffer (20 mM) (Flow Laboratories) and all follicles which were ≥ 4 mm diameter (153) were dissected out within 2 h of surgery. The diameter of each isolated follicle was measured and the antral fluid aspirated through a 27G needle into a 500 μl Hamilton syringe.

Fluid collected by either method was frozen at −20 °C until assayed. No attempt was made to recover the oocyte.

Follicles without a lining of granulosa cells, irrespective of size, were defined as 'cystic'. Subsequent data reported for the cystic follicles are considered separately from those obtained for normal follicles.

**Radioimmunoassay of pituitary and steroid hormones**

**Luteinizing hormone and follicle-stimulating hormones**

The radioimmunoassays for LH and FSH in plasma and follicular fluid were based upon those previously described (Hunter, Edmond, Watson & McLean, 1974) with modifications for the antral fluid in order to accommodate the small volumes. The original concentrations of standards, tracer and antisem were retained but the incubation volume was reduced to 150 μl of which 50 μl were neat (or diluted) follicular fluid or standard. The counting time was increased proportionately so that in this respect assay precision was undiminished. The following standards were used: LH, MRC 68/40 assumed 77 units/ampoule; FSH, MRC 68/39 assumed 32·8 units/ampoule (MRC National Institute for Biological Standards and Control). The concentrations of LH and FSH are expressed as mu./ml, where 1 mu. LH = 11·6 ng LER 907 and 1 mu. FSH = 44·6 ng LER 907. The assays would normally measure LH and FSH respectively over the ranges 0·8–12·8 and 0·4–6·4 mu./ml in undiluted follicular fluid. With large follicles, ≥ 8 mm diameter, assays were in general carried
out in duplicate, whilst those for follicles < 8 mm diameter were single determinations. A number of LH assays were also carried out on larger volumes of pooled fluid from small follicles.

**Prolactin**

Prolactin in peripheral plasma and follicular fluid was measured using a specific double antibody radioimmunoassay (RIA) previously described (McNeilly, 1973; McNeilly & Hagen, 1974). Purified human prolactin for standards and labelling with $^{125}$I was generously supplied by Dr H. G. Friesen, University of Manitoba, Winnipeg, Canada. The concentration of prolactin is expressed as ng Friesen prolactin/ml of which 1 ng = 20 μu. MRC 71/222. The minimum detectable level of prolactin was 1·5 ng/ml for both follicular fluid and plasma. Assays were carried out in duplicate in both specimens of plasma and fluid.

**Progesterone**

Progesterone was measured in peripheral plasma using a radioimmunoassay similar to that described by Thorneycroft & Stone (1972), and in follicular fluid by the method of Neal, Baker, McNatty & Scaramuzzi (1975). Follicular fluid (2–10 μl) was diluted 100- to 1000-fold in phosphate-buffered saline (0·1 mol/l, pH 7·0) and 0·1 ml samples were assayed directly without extraction. The precision and accuracy of the assay was similar to that described by Neal et al. (1974). The progesterone antiserum (RI-4) was raised in a rabbit against progesterone-11α-hemisuccinate conjugated to bovine serum albumin and the specificity was similar to that previously reported (Dighe & Hunter, 1974). The assays were conducted in duplicate and the minimum detectable level of progesterone was 300 pg/ml in plasma and 10 ng/ml in follicular fluid.

**Oestradiol**

Antiserum for the assay of oestradiol was raised in a rabbit to a conjugate of oestradiol 6-carboxymethyl–oxime and bovine serum albumin. Cross-reactions of other steroids using the routine assay conditions were: oestrone, 3 %; oestriol, 0·4 %; 6-oxo-oestradiol, 100 %; testosterone, 0·003 %; progesterone, 0·0002 %.

For estimations of oestradiol in plasma an ether extract was evaporated to dryness, equal volumes of 0·05 m-NaOH and carbon tetrachloride were added and shaken together with the residue. Samples of the aqueous phase were then neutralized in a slightly acid diluent for incubation in the RIA system as described by A. Bolton and F. Rutherford (personal communication). Oestradiol in follicular fluid was assayed without prior extraction. The assays were carried out in duplicate and the minimum detectable level of oestradiol was 8 ng/ml in follicular fluid and 20 pg/ml in plasma.

**RESULTS**

Concentrations of hormones in plasma and follicular fluid in subjects with stage 0 carcinoma of the cervix were compared with those of patients with menorrhagia and were not found to be significantly different. The results from all patients have therefore been pooled.

Concentrations of FSH, LH, oestradiol and progesterone in the antral fluid of
follicles aspirated in situ were not significantly different from those aspirated in vitro \((P > 0.2)\). The results obtained for each hormone from the two methods of collection have therefore been pooled. Concentrations of prolactin in the antral fluid of follicles aspirated in situ are not reported. These samples were stored at \(-20^\circ\text{C}\) for at least 12 months before being assayed. The immunological and biological activity of prolactin in physiological fluids declines with prolonged storage (H. G. Friesen, personal communication).

**Concentration of luteinizing hormone, follicle-stimulating hormone, prolactin, progesterone and oestradiol in peripheral plasma**

Mean values \((\pm \text{s.e.m.})\) obtained for the concentrations of LH, FSH, prolactin, progesterone and oestradiol in peripheral plasma with respect to the stage of the menstrual cycle are shown in Fig. 1.
Distribution of the size of excised follicles in relation to the stage of the menstrual cycle

A scatter plot of follicle size in relation to the stage of the menstrual cycle is shown in Fig. 2. The greatest range of follicle sizes (4–20 mm) was found in the late follicular and late luteal phases. The greatest number of 'cystic' follicles (45%) was found in the early follicular phase whereas none was found during the late follicular or early luteal phase.

The concentrations of pituitary and steroid hormones in the follicular fluid of individual Graafian follicles

Follicle-stimulating hormone

There were no significant differences in the concentration of FSH in follicular fluid from follicles aspirated in situ when compared with those aspirated in vitro (P > 0.2). The mean concentration of FSH in all antral fluids in relation to size and the stage of the menstrual cycle is shown in Fig. 3a. The minimum detectable level of FSH in the
smallest follicles (4 mm) was 1.3 μU/ml, which was therefore chosen as the limit of detection for all follicles. The percentage of follicles with detectable FSH at each stage of the cycle is shown in Table 1. In general, the greatest proportion of small follicles with detectable FSH are found either during or immediately after the increase in levels in the plasma (Fig. 1 and Table 1). In contrast, the concentration of FSH in some large follicles was high when the plasma concentrations were low (Fig. 1 and Fig. 3). Follicle-stimulating hormone was detectable in a proportion of the
smallest follicles examined (see Tables 1 and 2). However, at no time during the cycle was the concentration of FSH in follicular fluid more than 60% of the levels found in plasma.

Table 1. Percentage of follicles with detectable levels of LH, FSH or LH + FSH in relation to size at each phase of the human menstrual cycle

<table>
<thead>
<tr>
<th>Pituitary hormone (limit of sensitivity)</th>
<th>Follicle size (mm)</th>
<th>Follicular phase</th>
<th>Luteal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (1·3 mu./ml)</td>
<td>≥ 8</td>
<td>EF: 38·0</td>
<td>EL: 45·5</td>
</tr>
<tr>
<td></td>
<td>&lt; 8</td>
<td>MF: 15·5</td>
<td>MF: 20·0</td>
</tr>
<tr>
<td>LH (2·8 mu./ml)</td>
<td>≥ 8</td>
<td>LF: 82·5</td>
<td>LF: 28·0</td>
</tr>
<tr>
<td></td>
<td>&lt; 8</td>
<td></td>
<td>EL: 46·5</td>
</tr>
<tr>
<td>LH + FSH</td>
<td>≥ 8</td>
<td></td>
<td>MF: 0</td>
</tr>
<tr>
<td></td>
<td>&lt; 8</td>
<td></td>
<td>EF: 3·5</td>
</tr>
</tbody>
</table>

EF, MF and LF refer to early (still menstruating), mid- and late follicular phase, respectively, while EL, ML and LL refer to early, mid- and late luteal phase respectively.

Table 2. Concentration of FSH in fluid from different sized follicles at each phase of the human menstrual cycle

(Values are means ± s.e.m. in mu./ml. Samples less than the detection limit were assumed to have a concentration of 1·3 mu./ml for the purposes of the group mean.)

<table>
<thead>
<tr>
<th>Stage of menstrual cycle*</th>
<th>Follicle diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-8</td>
</tr>
<tr>
<td>EF</td>
<td>1·50±0·09</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
</tr>
<tr>
<td>MF</td>
<td>1·30±0·05</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>LF</td>
<td>1·76±0·26</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
</tr>
<tr>
<td>EL</td>
<td>1·66±0·23</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
</tr>
<tr>
<td>ML</td>
<td>1·30</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
</tr>
<tr>
<td>LL</td>
<td>1·46±0·13</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
</tr>
</tbody>
</table>

* For abbreviations see Table 1. Number of observations in parentheses.

Luteinizing hormone

The concentration of LH in the antral fluid of follicles aspirated in situ was not significantly different from those aspirated in vitro (P > 0·2). The mean concentration of LH in all antral fluids examined with respect to follicle size and the stage of the menstrual cycle is shown in Fig. 3b. The percentage of follicles containing detectable concentrations of LH at various stages of the cycle is shown in Table 1; the lowest detectable level in the smallest follicles examined (4 mm) was 2·8 mu./ml, which was therefore chosen as the limit of sensitivity. There was a greater proportion of large follicles with detectable LH during the late follicular phase (Table 1), when the
concentrations were higher than at any other stage of the cycle (Fig. 3b). Luteinizing hormone was not detectable in any follicle of <8 mm during the early and mid-follicular and mid- and late luteal phases. Fluids from 20 early and mid-follicular phase follicles were pooled to give a total volume of 50 µl, and the concentration of

![Graphs showing concentrations of (a) prolactin, (b) oestradiol and (c) progesterone in follicular fluid in relation to follicle size and stage of the cycle. Vertical lines represent ±1 S.E.M. White bars, mean concentration in follicles ≥ 8 mm; black bars, mean concentration in follicles < 8 mm. Minimum detectable level of prolactin (1.5 ng/ml) is indicated by the broken line. See Fig. 1 for abbreviations. Numbers of observations in parentheses.](https://example.com/graphs.png)
LH was 0.96 mu./ml (detection limit 0.8 mu./ml). Throughout all stages of the cycle the fluid concentration of LH was < 30% of that found in peripheral plasma. Furthermore, LH was only found in those follicles that also contained FSH (Table 1).

**Prolactin**

Unlike LH and FSH, prolactin was detectable in almost all the follicles examined. The minimum detectable concentration was 1.5 ng/ml. The concentration of prolactin was significantly lower in the large follicles during the late follicular phase than in any other stage of the menstrual cycle (P < 0.05) (Fig. 4a), with the exception of the large follicles during the early luteal phase. The general pattern of prolactin in antral fluid indicates a progressive fall in concentration during the follicular phase followed by a rise in concentration during the luteal phase.

**Oestradiol**

The concentration of oestradiol in the antral fluid of follicles aspirated *in situ* was not significantly different from those aspirated *in vitro* (P > 0.2). This finding is similar to that reported by Sanyal et al. (1974). The concentration of oestradiol with respect to size of follicle and stage of the cycle is shown in Fig. 4b. The levels of oestradiol in follicles at all stages of the cycle are between 40 and 40,000 times higher than those in peripheral plasma. During the mid- and late follicular phases the large follicles contained a significantly higher concentration than that in small follicles at the same phase (P < 0.001). During the luteal phase there were no significant differences in the concentration of oestradiol between small and large follicles (P > 0.05) and the levels did not exceed those found in follicles during the early follicular phase.

**Progesterone**

The concentration of progesterone in the antral fluid of follicles aspirated *in situ* was not significantly different from those aspirated *in vitro* (P > 0.2). The concentration of progesterone with respect to size of follicle and stage of menstrual cycle is shown in Fig. 4c. Levels of progesterone in large follicles during the proliferative phase were significantly higher than those found in the corresponding small follicles (P < 0.001). The most dramatic increase was found in the large follicles during the late follicular phase, where the levels were up to 20 times higher than in any other follicle throughout the cycle. By contrast, the small follicles (< 8 mm) during the early luteal phase had a significantly higher concentration of progesterone than any other follicle during the luteal phase (P < 0.001).

**Hormones in the fluid of recently ruptured and ‘cystic’ follicles**

Haemorrhagic fluid was aspirated *in vitro* from two recently ruptured follicles in subjects whose endometria still showed very late proliferative changes. The concentrations of hormones in the fluids were: progesterone, 8600–10,800 ng/ml; oestradiol, 335–210 ng/ml; LH, 9.6–7.6 mu./ml; FSH 7.9–6.8 mu./ml; prolactin, 14.6–12.1 ng/ml. The levels of LH and FSH were similar to those in peripheral plasma whereas the concentrations of prolactin in follicular fluid were about half the peripheral plasma concentrations. The steroid concentrations were 100- to 1000-fold
higher than the concentrations of oestradiol and progesterone in peripheral plasma. The concentration of progesterone in the fluid was also four to eight times higher than in late follicular phase fluid, but the concentration of oestradiol was at least ten times lower.

Table 3. Hormone levels in human cystic follicles

(Samples less than the detection limit were assumed to have a concentration of 1.3 µg/ml, 2.8 µg/ml, 1.5 ng/ml for FSH, LH and prolactin respectively for the purposes of the group means.)

<table>
<thead>
<tr>
<th>Stage of cycle*</th>
<th>Follicle size (mm)</th>
<th>LH (µg/ml)</th>
<th>FSH (µg/ml)</th>
<th>Prolactin (ng/ml)</th>
<th>Oestradiol (ng/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>20</td>
<td>2.8</td>
<td>2.9</td>
<td>13.8</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>6.0</td>
<td>1.7</td>
<td>39.0</td>
<td>205</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.8</td>
<td>1.4</td>
<td>25.2</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.8</td>
<td>1.2</td>
<td>47.0</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>MF</td>
<td>20</td>
<td>2.8</td>
<td>6.6</td>
<td>19.9</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>12.0</td>
<td>1.3</td>
<td>23.0</td>
<td>230</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.8</td>
<td>2.2</td>
<td>28.2</td>
<td>36</td>
<td>58</td>
</tr>
<tr>
<td>ML</td>
<td>10</td>
<td>2.8</td>
<td>1.3</td>
<td>23.0</td>
<td>150</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>10.0</td>
<td>1.3</td>
<td>21.0</td>
<td>104</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.8</td>
<td>1.3</td>
<td>21.0</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>LL</td>
<td>10</td>
<td>14.0</td>
<td>2.4</td>
<td>3.0</td>
<td>19</td>
<td>48</td>
</tr>
</tbody>
</table>

* For abbreviations see Table 1.

Table 4. Concentrations (a) of oestradiol in the follicular fluid with or without FSH and (b) of progesterone in follicular fluid with or without LH (values are means ± S.E.M. in ng/ml)

<table>
<thead>
<tr>
<th>Stage of menstrual cycle*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>(a) Oestradiol concentrations</td>
</tr>
<tr>
<td>FSH (≤ 1.3 µg/ml)</td>
</tr>
<tr>
<td>(b) Progesterone concentrations</td>
</tr>
<tr>
<td>LH (&gt; 2.8 µg/ml)</td>
</tr>
</tbody>
</table>

* For abbreviations see Table 1. Number of observations in parentheses. Limit of detection for FSH is 1.3 µg/ml and for LH is 2.8 µg/ml.
† No follicles containing LH.

Data relating to the hormone levels in cystic follicles are shown in Table 3. All these follicles contained low levels of oestradiol and progesterone irrespective of the stage of the cycle, suggesting that they were 'inactive' (Baird & Fraser, 1975).

Relationship between follicular concentrations of hormones

Those follicles which had detectable levels of FSH during the follicular phase without exception contained significantly higher concentrations of oestradiol than those with undetectable FSH (Table 4a) (EF, MF, LF; P < 0.01, P < 0.001,
levels low these peripheral follicles physiological and lar prolactin hormones production concentration two late Large previously at occurring (Table 2) (MF, LF, EL, ML, LL; \( P < 0.001, P < 0.001, P < 0.001, P < 0.05, P < 0.01 \) respectively). All the follicles containing LH and a high concentration of progesterone were \( \geq 8 \) mm diameter and also contained FSH.

During the mid-follicular, late follicular and late luteal phase of the cycle the concentration of oestradiol in follicular fluid was correlated with that of progesterone \( (r = 0.44, n = 43, P < 0.01; r = 0.88, n = 33, P < 0.001; \) and \( r = 0.49, n = 37, P < 0.01, \) respectively).

**DISCUSSION**

There was no difference in the concentration of hormones in fluid collected from follicles *in vitro* from those collected *in vivo* suggesting that there was little if any production or metabolism of hormones in the time between ovariectomy and aspiration of fluid (cf. Giorgi, Addis & Colombo, 1969; Sanyal *et al.* 1974). Although patients were undergoing surgery for a variety of reasons, the general pattern of hormones in peripheral plasma (Fig. 1) was similar to that described in normal women during the menstrual cycle (e.g. Robyn, Delvoye, Nokin, Vekemans, Badawi, Perez-Lopez & L'Hermite, 1973). However, the concentration of prolactin in peripheral plasma was up to sixfold higher than that in normal women (McNeilly, Evans & Chard, 1973; McNeilly & Chard, 1974). This is probably because the samples were collected under the stress of surgery which is known to stimulate the release of prolactin (Robyn *et al.* 1973). The overall mean concentration of prolactin in follicular fluid \( (20 \pm 5 \text{ ng/ml}, n = 189) \) was much lower than that in peripheral plasma. In the absence of information about the rate of exchange of prolactin between plasma and follicular fluid *in vivo* it is impossible to determine whether this difference is of physiological importance or whether it is an artifact due to the elevated prolactin concentration in peripheral plasma as a result of stress. Since all the samples were collected under similar conditions, the striking change in follicular fluid concentration at different stages of the cycle (Fig. 4a) is probably not an artifact.

The distribution of follicle size in relation to the stage of the cycle is similar to that previously described in women (Block, 1951) and rhesus monkeys (Koering, 1969). Large follicles \( (\geq 8 \text{ mm}) \) were much commoner during the mid- and late follicular and late luteal phases than at any other stage of the cycle. Although it is difficult to draw conclusions about the dynamics of follicle growth from these cross-sectional observations, it is tempting to speculate that these large follicles represent the end result of two waves of follicular development initiated by the peaks of secretion of FSH occurring at the onset of menses and again at mid-cycle. The minimum time necessary to develop a follicle from the antral to the mature preovulatory stage in women is probably 6–10 days (Gemzell & Johansson, 1971; Bertrand, Coleman, Crooke, Macnaughton & Mills, 1972). The concentration of FSH in small follicles reaches a maximum during the early follicular, late follicular or early luteal phases of the cycle (Table 2) which is either during or just after the peak FSH concentration in peri-
Hormones in follicular fluid

pheral blood. Furthermore, the concentration of FSH in these follicles apparently increases as they develop into mature preovulatory follicles in spite of the falling levels of FSH in peripheral blood (cf. Table 2 and Fig. 1). Some factor may increase the affinity of these follicles for FSH. Oestradiol, the concentration of which is extremely high in the mid- and late proliferative phase follicles, is known to increase the sensitivity of the ovary to FSH (Goldenberg, Vaitukaitis & Ross, 1972). The high levels of oestradiol in the large follicles during the mid- and late follicular phase are similar to those previously reported by other workers in normal (Smith, 1960; Short & London, 1961; Sanyal et al. 1974; Baird & Fraser, 1975) and gonadotrophin-stimulated ovaries (Short, 1964a; Edwards et al. 1972). Follicles which have detectable levels of FSH during the follicular phase also have a high concentration of oestradiol whereas those follicles with undetectable levels of FSH have significantly lower levels of oestradiol (see Fig. 3a and Table 4a).

When the concentration of FSH in peripheral plasma was high (early follicular phase and at mid-cycle) FSH was detectable in only a minority of small follicles (diameter < 8 mm) (Tables 1 and 2). Presumably follicles in which the concentration of FSH is high are those which are stimulated to further development (Table 4a). It is not known how this minority of small antral follicles are selected although it has been suggested that oocytes are ‘programmed’ for development in the order in which they are formed during foetal life (Henderson & Edwards, 1968).

About 17% of the small follicles (diameter < 8 mm) in the early luteal phase of the cycle had measurable amounts of FSH and LH (Table 1), presumably as a result of the preovulatory peaks of the gonadotrophins. In contrast to the follicular phase of the cycle, none of the follicles in the luteal phase were functionally ‘active’, as indicated by the persistently low concentrations of oestradiol. From the time of the LH ‘surge’ there is an abrupt fall in the mitotic activity of granulosa cells (Delforge, Thomas, Roux, Caneiro de Siqueiro & Ferin, 1972). Thus the presence of LH in some of these small follicles (Table 1) may interfere with their normal orderly development and consequently their steroidogenic potential (Tables 4a and b). The marked increase in cystic follicles (Fig. 2) from the mid-luteal phase is probably a consequence of this. It may be that an ordered sequence of gonadotrophins, e.g. FSH alone followed by LH and FSH, is necessary for normal follicular development.

Luteinization of the granulosa cells of the preovulatory follicle begins some 24–36 h before ovulation in response to the mid-cycle LH surge (Hertig, 1967; Delforge et al. 1972). After exposure to LH, the preovulatory follicle secretes increasing amounts of progesterone as indicated by the rise in concentration in ovarian (Mikhail, 1970; Lloyd, Lobotsky, Baird, McCracken, Weisz, Pupkin, Zanartu & Puga, 1971) and peripheral plasma (Johansson & Wide, 1969; Yussman & Taymor, 1970). The relatively high concentration of progesterone in follicular fluid of preovulatory follicles is similar to that found by other workers in normal (Sanyal et al. 1974) and gonadotrophin-stimulated ovaries (Short, 1964a; Edwards et al. 1972), and it is likely that the luteinizing granulosa cells are the source of this steroid (Channing, 1969, 1970; Edwards et al. 1972).

The large preovulatory follicles were characterized by a highly vascular appearance (Short, 1964b). The granulosa cells were very loosely attached to one another; the walls were slimy and mucoid, and the follicular fluid was bright yellow and
viscous. When the granulosa cells from large follicles collected during the pre-ovulatory phase were cultured in vitro they secreted maximal amounts of progesterone in response to minimal physiological concentrations of gonadotrophins in the medium (Channing, 1970; K. P. McNatty & R. S. Sawers, unpublished observations). The granulosa cells are stimulated in vivo by the relatively high concentrations of LH in blood and follicular fluid. Presumably the metabolic requirement of these actively secreting cells is high and may account for the relatively low oxygen tension within the follicle (Fraser, Baird & Cockburn, 1973). The relatively low concentration of prolactin may also reflect utilization or metabolism of the hormone by the follicle. It may also play a key role in controlling steroid synthesis, for the production of progesterone by human granulosa cells in vitro is inhibited when the concentration of prolactin in the medium exceeds 30 ng/ml (McNatty et al. 1974).

It is apparent that despite the presence of FSH in antral fluid the granulosa cells do not secrete progesterone in the absence of LH. Furthermore, the concentration of progesterone in follicular fluid of small follicles during the luteal phase is significantly lower than in preovulatory follicles, even though they contain both FSH and LH. The failure of the granulosa cells in small follicles to secrete substantial amounts of progesterone when exposed to LH suggests that these cells require time before they are capable of responding fully. The latter finding is consistent with the observation that granulosa cells harvested from preovulatory follicles in the pig have a significantly greater number of receptors for LH when compared with the cells harvested at other stages of the oestrous cycle (Channing & Kammerman, 1973; C. P. Channing, personal communication).

The concentration of oestradiol in the haemorrhagic fluid of recently ruptured follicles was very much lower than that found in the large preovulatory follicles. Although most of the fluid is lost at ovulation the low concentration of oestradiol in the haemorrhagic fluid is consistent with the low plasma levels during the immediate post-ovulatory period (Fig. 1) (Moor, 1974). In contrast, however, the levels of progesterone were higher than those found in the preovulatory follicle and this is consistent with the increased plasma levels indicating the growing secretory capacity of the luteinizing granulosa cells.

Steroid levels in the cystic follicles were low irrespective of the stage of the cycle and in many the pituitary gonadotrophin concentrations were high and similar to those found in plasma. These findings suggest that cystic follicles are functionally inactive and are incapable of secreting oestradiol or progesterone even in the presence of gonadotrophins.

These data suggest that a precise sequence of hormonal changes occurs within the microenvironment of the developing Graafian follicle; the order in which the changes occur may well be of considerable importance for the growth of that follicle and the secretory activity of the granulosa cells both before and after ovulation. Figure 5 shows the possible inter-relationships between the concentrations of pituitary hormones in plasma and follicular fluid with respect to the steroidogenic activity and growth of a follicle during the follicular and luteal phase of the menstrual cycle. The changes in the concentrations of pituitary hormones in follicular fluid throughout the menstrual cycle are related to those which occur in peripheral plasma. The presence of FSH in the smallest follicles appears to be important for their development. The
presence of FSH and oestradiol in the follicular phase follicles prepares them for the preovulatory production of progesterone under the influence of LH. The very low concentrations of prolactin in these follicles may be related to the upsurge in metabolic activity within the follicle during this time. The antral fluid of the preovulatory follicle contains relatively large amounts of oestradiol and progesterone, low physiological levels of prolactin, and concentrations of LH and FSH approaching 30 and 60% respectively of those found in plasma. These changes in the growth of a follicle destined to ovulate are different from those proposed for the pig (Channing, 1972). Such differences that exist will possibly depend on the permeability of a Graafian follicle in different species to protein hormones (see Edwards, 1974, for review).

Furthermore, it is suggested that the large inactive follicles found during the luteal phase are a consequence of LH interference during the growth of these follicles under the influence of FSH. The consequence of these findings may prove to be important in the treatment of amenorrhoeic or anovulatory patients with exogenous gonadotrophins.

We acknowledge the assistance of Dr A. Bolton for the plasma oestradiol assays, Mr D. Love for the follicular fluid oestradiol assays, Mr L. Mackenzie for assistance in dating the endometrium, Mrs E. Hunter for the radioimmunoassays of LH and FSH. A. McN. acknowledges the Wellcome Foundation for financial support and K. McN. is a recipient of a New Zealand N.R.A.C. fellowship. We wish to acknowledge the
gynaecological consultants at the Royal Infirmary, Edinburgh for their valuable assistance in obtaining the ovarian specimens and Drs R. V. Short and D. T. Baird for their assistance in arranging this collaborative project and their advice in the preparation of this manuscript.

REFERENCES


D. A morphometric analysis. Fertility and Sterility 23, 1–11.


Hormones in follicular fluid


