THE HUMAN OVARY IN EARLY PREGNANCY

A. D. T. GOVAN

Research Department, Glasgow Royal Maternity Hospital, Rottenrow, Glasgow, C 4

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SUMMARY

A histological and histochemical study has been made of ovaries obtained from patients in early pregnancy, the duration of pregnancy varying from 6 to 20 weeks. During the first 10 weeks of pregnancy ovarian structure is virtually unchanged, compared with the post-ovulatory state. From 10 weeks onwards new Graafian follicles appear. These are limited in size, most achieving a maximum diameter of 4 mm. They are characterized by an early and excessive thecal development. Atresia overtakes these follicles and this occurs at all stages of development, suggesting a sudden and possibly recurrent change in gonadotrophic stimulation. Despite atresia the theca persists but histochemical tests suggest that its functional activity is limited. This loss of activity is associated with a recognizable histological change in the thecal cell. It is suggested that most of the alterations in ovarian structure are due to changes in the quality and quantity of gonadotrophins produced at this period of pregnancy.

INTRODUCTION

Although considerable attention has been paid to the structure of the corpus luteum at all stages of human pregnancy (Gillman & Stein, 1941; Brewer, 1942; Hertig & Rock, 1941, 1945, 1949; Papanicolau, Traut & Marchetti, 1948; White, Hertig, Rock & Adams, 1951) little is known about changes in the remainder of the ovarian tissue in early pregnancy. Some mention is made by Nelson & Greene (1953, 1958) and Sauramo (1954), but the number of human ovaries investigated is very small. The reason for this is that satisfactory material is extremely difficult to obtain. It is important, however, that as much as possible should be known of ovarian function during the first weeks of pregnancy in view of the importance of proper nidation of the blastocyst and its relationship to abortion and other abnormalities of pregnancy. The following account describes the histological appearance of ovaries from 28 subjects covering the period from the 6th week to the 20th week of pregnancy.

MATERIAL AND METHODS

In twelve instances both ovaries were obtained from the 28 subjects. The duration of pregnancy in these twelve subjects was as follows: 6–7 weeks in one woman, 11 weeks in one woman, 12 weeks in five women, 16 weeks in four women, 20 weeks in one woman. Removal of the ovaries was carried out as a therapeutic measure in four patients suffering from carcinoma of the breast. The other subjects died
as a result of intercurrent disease. Clinical details of these 12 patients are given in Table 1. The remaining 16 subjects had tubal pregnancies and only one ovary was available from each. Operation for removal of the tubal pregnancy and the associated ovary took place at the seventh week in three instances, at 8 weeks in five instances, at 10 weeks in five instances and at 12 weeks in three instances. In all the ectopic pregnancies trophoblastic villi were still healthy and changes in the ovary were therefore not likely due to degeneration.

In three cases ovaries were obtained immediately after the operation, quickly frozen and were sectioned on a cryostat for enzymatic studies. Incubation with suitable substrates was carried out for the detection of alkaline phosphatase (Pearse, 1961) and 3β-ol dehydrogenase (Levy, Deane & Rubin, 1959). The substrate for the latter reaction was dehydroepiandrosterone. Control sections were incubated without the specific substrate. The duration of pregnancy in these three women was 7, 11 and 16 weeks, respectively. Material from the other patients was received already fixed in 10% formalin. Frozen sections were stained for neutral fat and the remainder of the material was embedded in paraffin and stained with haematoxylin and eosin.

Table 1. Clinical details of twelve patients with intra-uterine pregnancies from whom both ovaries became available

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yr.)</th>
<th>Parity</th>
<th>Duration of pregnancy (weeks)</th>
<th>Associated disease</th>
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<td>3</td>
<td>6–7</td>
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<tr>
<td>2</td>
<td>37</td>
<td>2</td>
<td>11</td>
<td>Carcinoma of breast</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>3</td>
<td>16</td>
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</tr>
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<td>4</td>
<td>39</td>
<td>2</td>
<td>20</td>
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</tr>
<tr>
<td>5</td>
<td>29</td>
<td>2</td>
<td>12</td>
<td>Influenzal myocarditis</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
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<td>16</td>
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<td>3</td>
<td>12</td>
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<td>16</td>
<td>Rheumatic heart disease</td>
</tr>
</tbody>
</table>

RESULTS

A preliminary survey shows some individual variation, but at about 10 weeks the ovary shows changes which seem characteristic of pregnancy. In some instances these changes are delayed beyond 10 weeks and in one woman they were present at 8 weeks.

Seven to ten weeks of pregnancy

Up to 10 weeks the most striking feature was the presence of large mature Graafian follicles with an average diameter of 8 mm. The granulosa in all of these, apart from the cumulus, was thin, being no more than three cells thick. The nuclei were dark and crowded together, roughly round but with a tendency to irregularity. An occasional nucleus shows karyorrhexis and an odd polymorph was seen here and there. The cytoplasm of these cells was very scanty and the cell outlines were indeterminate. In most of these follicles the ovum was degenerate and the nucleus was no longer visible,
but some retained an ovum of reasonably normal histological appearance. Similarly, the zona pellucida was absent in most. Irregular spaces were present in the cumulus. They were not Call-Exner bodies but appeared to be the result of degenerative processes. The theca surrounding these follicles was inconspicuous, being a thin layer no more than three or four cells thick consisting of spindle cells with narrow spindle nuclei. A few cells were quite plump with larger nuclei and showed a rather basophil cytoplasm. No evidence of so-called luteinization was seen. An occasional polymorph was present among the thecal cells.

Primordial follicles were present, but the only new development seen was the presence of occasional solid, pre-antral follicles with four or five layers of granulosa cells and a central ovum. At this stage no evidence of thecal development can be seen.

**Eleven to twenty weeks of pregnancy**

A new phase has been reached at this stage. At 12 weeks the large mature follicles noted in the earlier weeks were still present. Further degenerative changes had taken place and in most follicles the granulosa was ragged and individual cells show karyolysis. The changes tended to be focal so that in parts the wall of the follicle was denuded of granulosa cells. At a later stage, at 16–20 weeks, these follicles were atretic and completely devoid of granulosa cells; they had shrunk and were no more than half their previous size. The theca which had not been noticeable up to the time became more distinct. Further examination showed, however, that this was not due to proliferation, but to shrinkage of the follicle resulting in crowding of the cells, the nuclei of which were reverting to the stromal type.

Meanwhile many new follicles were seen to develop. Preantral follicles at a more advanced stage than those found at 10 weeks were present. The granulosa cell layers had increased in number to 6 or 8 and mitoses were present. A pale zone, about four cells deep around the follicle, indicated the developing theca. These were large cells with plump, pale spindle-shaped nuclei. The whole structure measured 40 μ in diameter.

Small antral follicles, measuring 40 μ in diameter exclusive of theca, and 60 μ including the theca interna, were characterized by a marked increase in the amount of thecal tissue and differentiation of its cells (Pl. 1, fig. 1). The granulosa cells apart from the appearance of the antral space and development of the cumulus had not altered greatly. They were small cells with dark nuclei, pallisaded in the outer layer, but more or less polygonal and arranged in concentric rows in the inner zones. One noticeable feature was the appearance of one or two rosettes usually at the periphery of the cumulus. Not only was the internal theca prominent but the cells showed a remarkable degree of differentiation. They were large and the majority were irregularly polygonal with abundant cytoplasm. The latter was pale and slightly eosinophilic but finely granular, a feature more obvious in frozen sections than in paraffin preparations. The nuclei were rounded. Some of these cells were round and, in paraffin sections, apparently colourless with a slightly eccentric nucleus. A faint skein of cytoplasm gave the impression of vacuolation but this may be an artifact (Pl. 1, fig. 2).

Larger follicles, 0.5 mm. in diameter, showed further extension of the changes described. The granulosa layer, apart from the site of the cumulus, was approximately
four cells thick and the arrangement and character of the cells was as before. Thecal development had increased in quantity, the larger being about eight cells in depth. The nuclei were large, pale and round and the cytoplasm was abundant and slightly eosinophilic (Pl. 1, figs. 3, 4). Thecal cones had developed and while some were directed towards the surface of the ovary others were parallel to, or even directed towards, the medulla. Mitoses were present in both granulosa and theca.

Further growth of follicles was apparent usually up to a maximum of 4 mm. Very occasionally a much larger follicle, 6 mm. in diameter, was found. Many of the follicles, measuring 2 mm. in diameter, showed an extraordinary number of rosettes, some without colloid, in the granulosa at all points of the circumference (Pl. 2, fig. 5). In larger follicles they were almost completely absent. Pyknosis of nuclei was present in many of the granulosa cells in the 2 mm. follicles and degenerative changes were advanced in those measuring 4 mm. Karyorrhexis was prominent and polymorphs were common among the granulosa cells at this stage. Thecal development was even more pronounced as the follicle enlarged. At the same time the thecal cells changed once more in character. The nuclei were now uniformly round and rather dark; the cytoplasm was more opaque and markedly eosinophilic and the cells were arranged compactly, frequently in radial columns.

With the exception of the odd follicle measuring 5–6 mm. no further development of the follicle as such occurs beyond this point. There were, however, considerable changes. The degenerative changes in the granulosa continued and ultimately the whole lining disappeared leaving an atretic follicle lined by a layer of spindle cells. No degeneration of the thecal layer was observed and it may even seem to increase in thickness although no mitoses were seen. The thecal cells were now cuboidal with small dark nuclei and showed a rather hyaline and very eosinophilic cytoplasm. Although the cavity of the follicle may persist for a while the fluid is gradually absorbed and the thecal cells come to form a solid mass, ovoid in shape with a small central connective tissue core (Pl. 2, figs. 6–8). Later, a very narrow wavy line of corpus albicans material appeared; the thecal cells were reduced in size, their nuclei became crowded and irregular and ultimately they disappeared leaving a fine corpus albicans.

Although this description suggests an orderly progression with a regular growth of follicles up to a maximum of 4 mm. in diameter before any serious degeneration occurs, this is not an inevitable sequence of events. Degeneration and disappearance of the granulosa was seen in follicles of all sizes in the same ovary, from the early antral type measuring 40 μ in diameter upwards. The degeneration of the granulosa was always associated with excessive development of the theca and a change in the thecal cells to a cuboidal eosinophilic type. One other feature noted in these ovaries was the appearance of developing follicles in the medulla and near to the hilum.

Staining for fat showed fine droplets in the cytoplasm of the thecal cells. These droplets appeared at the stage of antral formation. No fat was seen in the granulosa layer until atretic degenerative changes occurred. At this stage coarse droplets appeared in both the granulosa and theca. At a later stage when the thecal cells formed a solid body almost no fat was seen.

In growing follicles a strong positive reaction for alkaline phosphatase was present in the thecal layer, the granulosa gave a negative reaction. The reaction in the thecal
The ovary in early pregnancy

cells became progressively fainter as atresia developed. However, a slight reaction was still present in the solid thecal masses.

A positive reaction for 3β-ol dehydrogenase was present in the theca of growing follicles. In the early stages of maturation the granulosa is negative, but when atretic changes appear an apparently positive reaction is observed in this layer. The granules, however, are irregular and in close relation to lipid globules and the possibility of artifact is obvious. Little or no reaction for 3β-ol dehydrogenase was seen in the solid thecal masses.

DISCUSSION

The most striking feature found is the change in ovarian structure at about the 10th week of pregnancy. Up to that time the cyclical formation of follicles seems to be suspended and the ovary virtually remains in the same structural state as it was just around the time of ovulation. Despite the rising concentration of gonadotrophin no new follicles appear, the mature follicles which have not ovulated persist but show gradual deterioration and no luteinization apart from the corpus luteum itself is apparent. After 10 weeks many new Graafian follicles develop. Since this follicular development is usually less marked in the ovary containing the corpus luteum it might be thought that the corpus luteum itself was responsible for depressing follicular activity. The corpus luteum is said to reach full development between 7 and 10 weeks but this is maintained until mid-pregnancy and obviously does not influence follicular development during this period. The most reasonable suggestion seems that the character of the gonadotrophic influence differs before and after 10 weeks. In 1953, Lyon, Simpson & Evans investigated the influence of human pregnancy urine on the ovaries of hypophysectomized rats. Urine from patients up to 3 weeks pregnant showed a slow increase in gonadotrophin content. Thereafter there was a rapid increase up to 7–8 weeks of pregnancy. More important was the finding that the gonadotrophin during the first 2–3 weeks of pregnancy was only capable of producing repair of interstitial cells in the rats, in other words it was of luteinizing nature. After 5 weeks of pregnancy the urinary gonadotrophin showed an increasing power to stimulate follicular growth as well as interstitial cell repair. In addition, the urine also showed thyroid-stimulating activity.

Even with the appearance of new follicles several departures from the usual sequence of events found in non-pregnant individuals can be shown. Perhaps the most important departure is the absence of ovulation. This is apparently true for all species studied (van der Horst & Gillman, 1945). It may be due to the activity of the corpus luteum as claimed by Loeb (1911) who found that removal of corpora lutea in the guinea-pig accelerated the onset of ovulation. It is unlikely, however, that it is due to the direct action of luteal steroids on the ovary. Recent studies with progestational compounds in the form of contraceptive pills would tend to suggest that their main action is to alter gonadotrophic activity and thus to produce an indirect effect on ovarian function (Vorys, Ullery & Stevens, 1965; Greenwald, 1965b; Ryan, Goss & Reid, 1966).

Closely associated with the lack of ovulation is the limitation of follicular growth, no follicle exceeding 6 mm. and most of them being no more than 4 mm. in diameter. The limitation in the growth of the follicles at this stage of pregnancy is difficult to
understand in view of the large amounts of gonadotrophin being produced. As long ago as 1935, Selye, Collip & Thomson demonstrated that the rat ovary was more sensitive to gonadotrophins during pregnancy than in the non-pregnant state. This is the basis for one method of estimating follicle-stimulating hormone (Steelman & Pohley, 1953). It is very doubtful whether the presence of the corpus luteum is of any significance in this respect since progesterone does not influence the maturation of healthy Graafian follicles (Greenwald 1965a). Moreover, in pregnancies associated with an abnormal amount of circulating gonadotrophic hormones as in twins (Weigle & Thatcher, 1955; Horner & Young, 1955), hydrops foetalis (Lerner, Villaneuva & Tannhauser, 1958) and also in some cases of hydatid mole and chorion-epithelioma (Dubreuil, 1953) the ovaries contain large cystic follicles. Up to the present, however, there has been a tendency to assume that in pregnancy only chorionic gonadotrophin is present. This is almost certainly not true and this would mean that many conclusions drawn from experimental work and based on this assumption are unjustified. There is a need for more detailed examination of this problem particularly in relation to the interaction of the various types of gonadotrophic principles. In rats, according to Greenwald (1966), there is a marked variation in the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), during pregnancy. This has been little investigated.

Whereas in normal non-pregnant females thecal differentiation and luteinization becomes obvious in the final stages of follicle maturation prior to ovulation, in early pregnancy these changes are apparent at a very early stage in follicular development. Seitz (1906) was probably the first to report on this change and has stated that it was marked by the third month of pregnancy. There is some evidence to link thecal proliferation and differentiation with the corpus luteum at least in a negative sense. According to Papanicolaou, et al. (1948) these changes only become prominent in pregnancy when the corpus luteum function begins to decline. In the non-pregnant female similar changes are only found in conditions such as the Stein-Leventhal syndrome which are characterized by absence of ovulation. A further indication of the inverse relationship between corpus luteum function and thecal activity may possibly lie in the work of several observers (Ludwig, 1965; Mall-Haefeli, Ludwig, Keller & Cloeren, 1965) who had found that progestational compounds prevent thecal development in relation to follicles. As stated previously, however, these substances appear to act indirectly by altering gonadotrophic activity. It may be that the corpus luteum acts in a similar manner to control thecal proliferation in the early stages. The subsequent excessive thecal proliferation could then be related to a further change in gonadotrophins. Histochemical tests indicate that the theca around these developing follicles is chemically active, possessing enzymes necessary for steroidogenesis. On the other hand, the solid thecal masses which persist after atresia has overtaken the follicle show diminished enzymatic activity. For the histologist this is important. The term 'theca luteinization' is used in a very loose fashion, and included under the heading are cells with pale translucent cytoplasm, cells with colourless cytoplasm, cells with pale hyaline cytoplasm, and cells with deeply coloured opaque cytoplasm in haematoxylin and eosin preparations. There is a need for a more precise definition. In the present series diminution in alkaline phosphatase and 3β-hol dehydrogenase was associated with a change from a larger irregular polygonal cell
The ovary in early pregnancy

with pale translucent cytoplasm and a large pale nucleus to a smaller compact cuboidal cell with small dark nucleus and opaque eosinophilic cytoplasm.

Although thecal proliferation continues the granulosa degenerates and the follicle becomes atretic. The important point is that this atresia may overcome the Graafian follicle at any stage of development and can be seen in follicles of all sizes in the same ovary. This, together with the presence of solid thecal masses of varying size, can only be explained by some marked change in environment which is recurrent. Little is known about the process of atresia. Experimentally it can be produced by oestrogens or testosterone (Greenwald, 1965b).

A possible explanation for the main changes in these ovaries might be that as the placenta begins to manufacture chorionic gonadotrophin the production of pituitary luteinizing factors falls off. At the same time pituitary FSH secretion begins and is not suppressed by the chorionic LH. Nevertheless, there appears to be a limitation to the action of the FSH and this may be a permissive activity of the chorionic hormone.

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REFERENCES


**DESCRIPTION OF PLATES**

**PLATE 1**

Staining: haematoxylin and eosin.

Fig. 1. Early human Graafian follicle demonstrating the marked development of the surrounding theca interna. (× 80.)

Fig. 2. Grossly vacuolated thecal cells. Frequently these are in clusters within the thecal band surrounding Graafian follicles. (× 800.)

Fig. 3. Hyperplastic theca interna surrounding a 4 ml. Graafian follicle. (× 200.)

Fig. 4. A high-power view of the thecal cells in fig. 3. Note the large nuclei with prominent nucleoli and fine chromatin set in abundant granular cytoplasm. (× 800.)

**PLATE 2**

Fig. 5. Graafian follicle showing extraordinary number of rosettes in the granulosa. (× 80.)

Fig. 6. Low-power view of thecal body showing the ovoid structure with thin central connective tissue core. (× 80.)

Fig. 7. Higher power view of thecal body showing the almost columnar arrangement of the cells. (× 200.)

Fig. 8. Details of the cells in fig. 7. The nuclei of the thecal cells are now small and dark with coarse chromatin knots. The cytoplasm is now opaque. (× 800.)