Structure of the corpus luteum in the ovulatory polycystic ovary

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BACKGROUND: Women with polycystic ovaries (PCO) have a wide spectrum of presentation from anovulation and amenorrhoea to apparently regular, ovulatory menstrual cycles. We have recently reported a subtle defect in steroidogenic function in the luteal phase in the latter and an increase in the number of degenerating corpora lutea (CL) were observed in ovulatory PCO (ovPCO) during dissection. The possibility was therefore investigated of differences in structure or degeneration in CL formed during ovulatory cycles in women with PCO. METHODS: This study compared the histology of the CL in ovPCO with that in the normal ovary. Corpora lutea were collected from nine normal ovaries (days 1–27 of the cycle) and from 13 women with ovPCO (days 5–38). RESULTS: Variations in the degree of regression, both in relation to onset of menses and between different areas within individual CL, were recorded in both groups. During development and regression no obvious differences were observed between either group apart from an apparent increase in luteal haemorrhage, which was more common and more extensive in CL from PCO. CONCLUSIONS: The findings suggest that possible luteal phase abnormalities of steroid secretion in women with ovulatory PCO are not associated with obvious morphological defects in the CL, however it is possible that the persistence of luteal structures seen in PCO was a consequence of increased luteal haemorrhage.

Key words: corpus luteum structure/ovulatory polycystic ovaries

Introduction

Polycystic ovarian syndrome (PCOS) is the most common cause of anovulatory infertility (Hull, 1987) and is often associated with obesity, hirsutism, insulin resistance and excess androgen production (Yen, 1980; Conway et al., 1989; Franks, 1989). With the advent of routine use of pelvic ultrasound scanning, it is now clear that around 20% of women of reproductive age in the ‘normal’ population have polycystic ovaries (PCO) (Polson et al., 1988; Clayton et al., 1992; Farquhar et al., 1994). This suggests that many women have no obvious clinical manifestation of PCOS, despite the presence of PCO, and that these women may have regular ovulatory menstrual cycles. There are very few data regarding the frequency of ovulation in the latter, but there is some evidence to suggest that the cycles in women with ovulatory PCO (ovPCO) may not be entirely normal. In one study (Polson et al., 1988), 76% of women found to have PCO on ultrasound reported irregular cycles on close questioning. This indicates that the follicular phase may be prolonged in many cases but, as with other aspects of PCO, it is likely that there is a spectrum between the ovulatory and the anovulatory conditions.

Morphologically, PCO have been shown to have increased numbers of follicles developing from the primordial stage (Hughesdon, 1982). Although the reason for this is not known it may be the result of a relative decrease in follicular atresia, with failure of apoptosis (Homburg and Amsterdam, 1998). In our ovarian studies we have collected a large number of ovaries from women undergoing total abdominal hysterectomy and bilateral salpingo-oophorectomy for benign gynaecological disease. By gross appearance and microscopic dissection a group of ovaries which showed the morphological features of PCO, but which contained a dominant follicle or a corpus luteum (CL) were identified and collected from women who reported regular cycles (Mason et al., 1994). Within these ovaries an apparent increase in the number of degenerating CL and corpora albicantia was noticed compared with normal ovaries (Mason, 2000, unpublished observations). This may be due to a disturbance in the normal process of luteal regression which proceeds, at least in part, by apoptosis. This would provide an additional indicator of a failure of this process in PCO.

In a recent study to investigate luteal phase progesterone secretion in women with ovPCO, early luteal phase urinary pregnanediol-3-glucuronide/creatinine ratios were lower than in women of proven fertility with normal ovaries. Interestingly,
several of the PCO group showed sudden alterations in production suggestive of abnormal luteal function (Joseph-Horne et al., 2000). The possibility of morphological aberrations in the CL formed during an ovulatory cycle in PCO has not been examined and it was the purpose of the present study to undertake such an investigation.

Materials and methods

Subjects

Normal and PCO were obtained from women undergoing oophorectomy for non-ovarian gynaecological pathology. Approval was granted by the local ethics committees of the hospitals concerned. The tissue was obtained as a result of procedures which the gynaecologist, independently from this study, had deemed necessary for successful patient management. Informed consent was given by each subject prior to surgery and none of the women had received any medication for stimulation or suppression of ovarian function for at least 3 months prior to surgery. Details of menstrual cycle history (cycle length, duration of menses) were obtained prior to surgery, which was performed at random stages of the cycle. Although the date of the last menstrual period prior to surgery was determined the precise day of ovulation was not ascertained.

Post-surgical examination and criteria for inclusion in the study

Following surgery and examination of tissue by the pathologist, ovaries were transported on ice to the laboratory. Ovarian morphology was assessed in relation to menstrual cycle history and macroscopic features at the time of dissection. A polycystic ovary was defined as one in which >10 follicles of 2.0–8.0 mm diameter were contained, plus one or more of the following criteria: increased ovarian volume (>9.0 ml), an increase in the amount and density of stroma and thickening of the tunica (Adams et al., 1985; Mason et al., 1994). Ovaries meeting the above morphological criteria collected from patients with a history of regular cycles and containing a dominant follicle and/or a recent CL, were designated ovPCO. Normal morphologies were assigned to women with regular menstrual cycles whose ovaries were of normal size with small amounts of spongy stroma and contained ≤5 follicles >2.0 mm in diameter. Corpora lutea were dissected intact from the ovary where possible, with a small portion of adherent stroma to allow for orientation of the sections after processing. When more than one CL was identified the most recent was collected as judged visually with respect to size, degree of vascularity and structural integrity.

The material collected is listed in Table I and represents tissue from the period 1990 to 2000. These CL were present incidentally in ovaries collected for previously reported studies (Mason et al., 1994, 2001).

Tissue processing

Following dissection of the intact CL from the ovary, tissue to be examined using conventional haematoxylin and eosin staining was fixed either in Bouin’s fixative, or 4% buffered formalin for conventional thick section processing. The tissue was transferred from fixative to 70% ethanol to await processing to paraffin blocks. After sectioning, tissue was stained with haematoxylin and eosin. Some tissue was also fixed in glutaraldehyde and processed to araldite-embedded blocks, from which semi-thin sections were prepared and stained with Toluidine Blue. All sections were examined using an Olympus BH2 microscope with conventional light-field objectives. Photomicrographs were prepared from selected regions using conventional 35 mm photography, and the 35 mm slides subsequently scanned to produce a digitized image in Adobe Photoshop, from which prints were prepared using a Kodak dye sublimation printer.

Cycle staging

The presumptive age of the CL was based on time from onset of the last menstrual period. Corpora lutea were allocated to early-, mid- or late luteal, assuming that these stages occurred from days 15–19, 20–24 and 25–28 from the onset of the last menstrual period (LMP), respectively. Additionally, CL in ovaries collected from days 1–14 since the onset of menses, i.e. during the following follicular phase, were also classified as late luteal.

Microscopic examination

Although the tissue selected was primarily luteal, when sections were initially assessed for gross microscopic appearance, the additional presence or absence of stroma, follicles, and corpora albicans was recorded. Within the CL, the presence of a luteal cavity, the occurrence, distribution and degree of bleeding and the thickness of the luteal wall were noted. Sections were then examined at higher magnifications to assess the stage of development or regression. Parameters assessed included differences between lutein cells derived from the follicular granulosa and theca compartments, the presence of mitotic figures, degenerative changes in cell nuclei and changes in cell cytoplasm, in terms of vacuolation and lipid retention.

Results

General

Fourteen CL were identified in ovaries designated as polycystic. These were compared with 10 CL collected from normal ovaries. Patient details are given in Table I. In both groups

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*Not recorded in notes, but stated as being ‘regular’.
**Not recorded in notes.

PCO = polycystic ovary.
the CL were distributed from the time of the last menstrual period up to 28 days later with the exception of one in the ovPCO group which was collected 38 days after the LMP.

For the control group, paraffin-embedded, haematoxylin and eosin-stained sections were prepared for CL from all 10 subjects, covering the range 1–27 days from the onset of menses. Additionally, araldite-embedded, Toluidine Blue-stained sections were processed for five of these, covering days 1–17 from the LMP. For the ovPCO group, haematoxylin sections were prepared for 13/14, and Toluidine Blue for 4/14, covering the periods 5–38 and 5–23 from onset of the last menses, respectively.

A typical example of a semi-dissected ovPCO is shown in Figure 1a. This was an enlarged ovary in which the copious amount of dense central stroma had been microscopically dissected to reveal an 18 mm pre-ovulatory follicle. This follicle contained an immense number of granulosa cells, a thick and highly vascular theca and high concentrations of estradiol in the follicular fluid. In contrast to normal ovaries, at least 10 other follicles of 3–8 mm were identified in this ovPCO. Another visible characteristic feature is the thickened tunica albuginea and both this and the large amount of dense stroma are consistently seen in ovaries of this morphology. A degenerating CL can also be seen in the ovary illustrated, clearly identifiable by the surrounding yellow lutein and central degenerating blood clot. A complete mature CL, dissected intact from an ovPCO and then divided, is shown in Figure 1b. This shows the nature of the infolded wall of cells which constitutes the CL, surrounding the central luteal cavity which contains a quantity of blood.

**Early corpora lutea (days 15–19 from onset of LMP)**

**Control group (CL#7 and #8)**

In the early luteal phase, CL comprised a convoluted, infolded wall of variable thickness with lutein cells bounded by the ovarian stroma and containing a central luteal cavity. The cavity contained residual fluid, large numbers of erythrocytes and some fibroblasts, but few leukocytes were present. Both cases were typified by granulosa cells which were elongated, not yet fully enlarged, and contained less cytoplasm than those in the mature CL. The differentiating granulosa lutein cells were loosely arranged radially and showed evidence of residual streaming toward the ovulatory stigmata (Figure 2a). The luteal wall was highly vascular, with larger vessels from the theca lutein compartment entering the infoldings which were bounded by differentiating granulosa lutein cells on the side proximal to the cavity. Haemorrhage was evident to a variable degree between CL from the early luteal phase, the free erythrocytes lying both within the luteal cavity and between cells of the developing granulosa lutein layer. In general, the morphological differences between the theca and granulosa lutein cells were not clearly pronounced at this stage: however, the development of the lutein cells was variable between regions within the same section. This regional micro-heterogeneity was particularly evident in differentiating granulosal lutein cells, which varied in both shape and cytoplasmic:nuclear ratio. This absence of homogeneity throughout individual CL was a recurring theme.

**Figure 1.** (a) An ovulatory polycystic ovary (ovPCO) with the central stroma dissected away to reveal a large preovulatory follicle (POF) (18 mm) and at least 10 other follicles of 3–7 mm in diameter (black arrowheads). Also visible is a regressing corpora lutea (CL), identifiable by the surrounding yellow lutein (white arrowhead). (b) A mature CL dissected intact from an ovPCO and opened to reveal the luteal cavity and thickness of the wall. (c) The characteristic mitotic cells (arrowed) in the early CL from a normal ovary. Original magnification ×100, scale bar = 50µm.

Although mitotic figures were rare throughout the lifespan of the CL they were most common in the early luteal phase, as exemplified in Figure 1c.
Figure 2. The microscopic appearance of corpora lutea (CL) from normal ovaries compared with CL from ovulatory polycystic ovary (ovPCO). For each paired comparison, photomicrographs were prepared at the same magnification. (a) normal and (b) ovPCO: Representative CL from the early luteal phase. Note the 'streaming' appearance and the occurrence of the erythrocytes (e) in the luteal cavity (c) with little difference between the granulosa and theca lutein cells. Original magnification ×10. Scale bar = 1000 µm. (c) normal and (d) ovPCO: Representative CL from the mid-luteal phase. Note the well-formed and clearly distinguishable granulosa (G) and theca (T) lutein cells and the highly vascular nature of the tissue, with luminal microvessels (arrowed) and numerous endothelial cells. Original magnification ×50, scale bar = 200 µm. (e) normal and (f) ovPCO: Representative CL from the late luteal/early follicular phase. Note the disorganized appearance of the regressing CL during luteolysis with poorly identifiable region of regressing granulosa lutein cells (G). Original magnification ×50, scale bar = 200 µm.

ovPCO group (corpora lutea #16–19)

All of the features recorded for the early CL in the control group were also observed in the tissue collected from ovPCO. The luteal wall was characteristically convoluted, with blood vessels at the base of the infoldings clearly evident. The theca-derived lutein cells in the luteal wall proximal to the stroma showed little difference from those granulosa lutein cells proximal to the cavity, and the regional variations in the degree of differentiation observed in the control material were also evident in the ovPCO group. A representative section from an
early CL in an ovPCO is shown in Figure 2b. Overall, there were few obvious morphological differences in the presumptive early CL between the normal and ovPCO groups, however there appeared to have been more extensive haemorrhage within the early CL from ovPCO.

**Mature corpus luteum (days 20–24 from onset of LMP)**

*Control group (CL#9 and #10)*

In contrast to the appearance of the early CL, the typical features of the mature CL are illustrated in Figure 2c. The luteal tissue was characteristically folded, and the granulosa lutein cells proximal to the luteal cavity were bordered by fibrous connective tissue, where the fibrillar nature of the material was clear. The theca and granulosa lutein cell layers were now clearly distinguishable. As the granulosa lutein cells developed with time, they showed gradual changes in shape from being slightly elongate, with incomplete differentiation and expansion of the cytoplasm, through to fully developed, mature cells, with abundant eosinophilic cytoplasm and a polyhedral to spherical morphology. The theca lutein cells were characterized by their position, smaller size and the presence of cytoplasmic lipid droplets which result in grey rather than lilac staining in Toluidine Blue sections. The CL were heavily vascularized, with innumerable endothelial cells evident.

*ovPCO group (CL#20 and #21)*

As can be seen in the representative section of a CL from the mid-luteal ovPCO group (Figure 2d), the appearance of the CL is similar to that recorded in the control group for this stage of the cycle.

Importantly it was not possible to see any differences in gross microscopic or higher magnification appearance between mature CL from normal or ovPCO other than the fact that haemorrhage was more common, heavier and more extensive in the latter.

**Regressing corpus luteum (days 1–14 and days 25–28 from onset of LMP)**

*Control group (CL#1–6)*

Luteal regression, as shown in Figure 2e, was evident by day 27. Early degenerative changes included a reduction in the nuclear to cytoplasmic ratio of granulosa lutein cells and consequently in the thickness of the luteal wall due to the preponderance of cells of this type. As degeneration progressed it became more difficult to distinguish between granulosa and theca lutein cells, due in part to the increase in lipid droplets in the cytoplasm in both cell types and the reduction in size of the former. This increase in cytoplasmic lipid was evident in Toluidine Blue sections where abundant lipid droplets were present in the cytoplasm. Regressive change appeared to be initiated in cells proximal to the central cavity and gradually extended towards the periphery of the CL. Throughout the luteolysis process the number of cells underwent a rapid reduction, but it was not possible to determine the underlying mechanism for this, since cells showing nuclear fragmentation were rarely observed and, using the light microscope, it is not possible to state with certainty that these cells are undergoing apoptosis. In the most regressed CL almost all of the lutein cells had disappeared and had been replaced by an amorphous ground substance, presumably the forerunner of the collagenous material forming the corpus albicans. Also noted was extensive deposition of a brown pigment, which is likely to be haemosiderin. This was most evident in the degenerating luteal wall.

*ovPCO group (CL#11–15 and #22–24)*

A representative example of a late luteal phase CL is shown in Figure 2f. This closely resembles the morphological characteristics seen in control tissue as depicted in Figure 2e. Indeed, the regressive changes noted in CL from the ovPCO group were as already recorded for the control ovaries. As observed for the mature CL, the indications were that haemorrhage had been more extensive in the ovPCO group.

In comparing the two groups, it was apparent that, in both normal and ovPCO, there was a clear progression of degenerative change. Consequently, not all of the features characteristic of regression were necessarily evident in a single degenerating CL. Despite the trend of progressive luteolysis, the degree to which this had occurred was not always consistent with the stage of the cycle at which the tissue had been collected. This was evident even within the group of CL collected from the normal ovaries, indicating that it was not simply a characteristic of the regressing CL in the ovPCO patient. Particularly during the follicular phase, when chronological staging is more precise, wide variations in regressive change were seen between CL of the same age, relative to day of onset of the LMP. Additionally, within any single CL from either group, it was noted that the degree to which degeneration was occurring was not uniform throughout, with clear regional variations both between and within sections from the same CL.

Overall, no clear differences were seen in the structure or speed of onset of degeneration between CL from normal or ovPCO, however the occurrence and degree of bleeding was again higher in the ovPCO group.

**Discussion**

It appears that this is the first report in the literature of the structure of the CL formed during ovulatory cycles in women with PCO. The principal conclusion is that it was not possible to detect any gross morphological or degenerative differences between CL from normal or PCO, suggesting that, once ovulation has occurred in the ovPCO, development and subsequent regression of the CL proceeds normally.

Ideally, it would have been preferable to have had a larger number of CL for comparison, but the specimens examined had already taken 10 years to collect. Another limitation of this study was the inability to be able to state with certainty the age of the CL collected during the luteal phase. The use of the onset of the LMP as a marker for luteal age is confounded by potential differences in cycle length, even when these are supposedly regular, such differences in length normally resulting from variations in the duration of the follicular rather than of the luteal phase. Although one of the most striking findings was the wide variation in the degree of regression both between and within ovPCO and control CL at the same
presumptive stage of the cycle, similar findings have been reported previously (Corner, 1956) for the normal CL. Indeed, the disparity in the expected degree of breakdown relative to the stage of the cycle was given as the reason for over half of the specimens being rejected from this study. Variations have also been reported in the morphology of the CL prior to and during menses and the occurrence of microheterogeneity within individual CL (Brewer, 1942; Brewer and Jones, 1947). Corner (1956) stated that ‘the human corpus luteum during menstruation shows great variability in degenerative changes’ (Corner, 1956). As changes in the CL proceed relatively rapidly it is expected that some variations in the stage of the processes within individual tissues will occur. However, this makes the process of identifying differences between specimens inherently difficult. This absence of homogeneity within CL also suggests that conclusions based on reverse transcription–polymerase chain reaction of biopsies or even full depth strips of luteal tissue might be misleading because of these intracorporeal regional variations.

The occurrence of luteal tissue has been noted previously in sections of PCO. In the classic study by Hughesdon (1982) 11 out of 17 of the ovaries studied contained CL and only one did not contain at least a corpus albicans (Hughesdon, 1982). Although the vast majority of patients in this study by Hughesdon reported irregular cycles or amenorrhoea it is clear that even these women must ovulate spontaneously on occasion. No comment was made on the morphology of these structures.

The findings of the current study indicate that, although the follicular phase may be prolonged, once a dominant follicle is formed and ovulation occurs, the process of subsequent CL formation and degeneration proceeds relatively normally in the ovPCO. In a previous study a greater variability was found in luteal phase urinary pregnenediol-3-glucuronide in women with ovPCO compared with women with normal ovaries and proven fertility (Joseph-Horne et al., 2000). In particular, as a group they had significantly reduced levels in the first half of the luteal phase. A failure to detect structural changes in the CL from the ovPCO does not conflict with the functional abnormalities suggested by the study of progesterone excretion but may infer that the latter are not the result of microscopically observable changes in lutein cells. It is probable that for ovulation to occur, the dominant follicle is structurally and functionally similar to that in a normal ovary and therefore the resultant CL is also structurally normal. That the dominant follicle in PCO may be indistinguishable from that in the normal ovary was also the conclusion of a study by Magoffin et al., (1995), who reported that the concentrations of insulin-like growth factors and their binding proteins were similar in preovulatory follicles from polycystic and normal ovaries (Magoffin et al., 1995).

In the normal ovary, developing follicles are lost by apoptosis and it has been postulated that the increase in the number of follicles in the PCO is the result of a reduction or failure of follicular atresia (Homburg and Amsterdam, 1998). Although the process by which cells are lost during luteal regression has not yet been completely resolved, apoptosis has also been proposed as a mechanism that results in deletion of cells during luteolysis (Rodger et al., 1995). If this is the case, then changes in the numbers of cells undergoing apoptosis could influence the rate at which luteal regression progresses and result in an increase in the number of regressing structures present. The current study was not able to provide any morphological evidence for this; however apoptosis in a single cell is rapid, and may easily be missed, especially if it should occur in a narrow time-window during luteal regression, as has been suggested by the studies in the non-human primate (Young et al., 1997) and in the human (Morales et al., 2000). In the present study nuclear fragmentation was also rarely seen and even when it was recorded, it was not possible to state unequivocally that such nuclear change was representative of apoptosis, or denoted nuclear breakdown by an alternative mechanism. The studies of Fraser et al. (1999), using the electron microscope, suggested that not only were apoptotic cells rare in the regressing primate CL, but that other methods for cell deletion may predominate during luteal regression (Fraser et al., 1999). It may be that in PCO a failure of apoptosis occurs only in the follicles in anovulatory ovaries, but this remains to be demonstrated.

Another aspect of ovarian physiology having the potential to influence both follicular and luteal development and regression is the process of angiogenesis, the development of the blood vascular system from pre-existing vessels (Fraser and Lunn, 2000, 2001). Vascular endothelial growth factor (VEGF), one of the major factors associated with angiogenesis and change in vascular permeability and the important role of VEGF in the primate CL has been described (Fraser et al., 2000). In women with PCOS the levels of VEGF are high, and the ovaries show increased vascularity (Agrawal et al., 1998). Interestingly, Gaytan and his associates (Morales et al., 2000), have introduced the concept of interaction between the occurrence of blood in the luteal cavity with the nature of the final demise of the CL, suggesting that only CL in which a blood clot and a large luteal cavity are present, progress to formation of true corpora albicantia. In the absence of such a blood clot, they believe that the CL does not undergo extensive hyalinization resulting in the formation of a corpus albicans but, instead, involutes to form small clusters or scars of pigment-filled granulosa-lutein cells. If the enhanced vascularity in the PCO is associated with increased vascular permeability, then this may account for the finding in the present study that the occurrence of haemorrhage in and around the CL of PCO is greater than that observed in luteal tissue from morphologically normal ovaries. Additionally, it would support the suggestion of Morales et al. (2000) that it is the occurrence of a large blood-filled luteal cavity which results in regression of the CL to the corpus albicans (Morales et al., 2000) and might explain the increased numbers of corpora albicantia found in PCO.

In conclusion it was not possible to demonstrate any obvious morphological differences in CL structure between normal and ovPCO which may explain the apparent increase in the numbers of degenerating luteal structures present in the latter. It remains to be determined whether there are differences in the occurrence of apoptosis and regression or changes in the vasculature of the ovPCO CL that could result in the persistence of regressing luteal structures or affect luteal steroidogenesis.
References

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