Follicular development in the sheep after priming with PMSG

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Summary. The administration of PMSG to sheep early in the oestrous cycle (Days 2 or 3) results in the formation of follicular cysts of varied morphology by Days 8 or 9. These may persist for up to 14 days after injection. If PMSG is given on Day 5 or later ~50% of such cysts ovulate. However, when PMSG is given at the beginning of the cycle (Day 2 or 3), the membrana granulosa is lost from the majority of the cysts and the theca interna luteinizes. The major hormone secreted by such luteinized follicles is progesterone. The structure of the steroid-secreting cells of the follicles is similar to that of large luteal cells of granulosa cell origin in cyclic corpora lutea. It is suggested that under suitable luteinizing conditions thecal cells may acquire many of the characteristics of granulosa cells.

Introduction

Once an ovarian follicle begins to grow it is destined to ovulate or to become atretic. Atresia in sheep follicles which grow but do not ovulate is characterized by loss of granulosa and thecal integrity (Hay, Cran & Moor, 1976; O'Shea, Hay & Cran, 1978b). In many other species, however, the thecal cells undergo hypertrophy and have been reported as being involved in the formation of corpora lutea atretica (Brambell, 1956) or glandular interstitial tissue (Mossman & Duke, 1973). In non-ovulating rat antral follicles the theca gives rise to the interstitial tissue. If, however, preovulatory follicles are maintained without the intervention of the LH peak, ovarian cysts are formed accompanied with the loss of both granulosa and theca interna cells (Freeman, Butcher & Fugo, 1970; Peluso, Steger, Huang & Meites, 1979; Peluso, England-Charlesworth, Bolendor & Steger, 1980; Peluso & England-Charlesworth, 1981). This difference between atresia of preovulatory and non-preovulatory follicles has been equated with a loss of LH binding sites (Peluso & England-Charlesworth, 1981).

The present experiments were designed to examine the structural consequences of an extension of the preovulatory phase in sheep by priming with PMSG at a time in the oestrous cycle such that a preovulatory release of LH would not be elicited.

Materials and Methods

Animals and treatment

The onset of oestrus in 38 Welsh Mountain ewes was determined by mating with vasectomized rams. The sheep received 1200 i.u. PMSG on Day 2 (N = 20), Day 3 (N = 11), Day 5 (N = 4), Day 6 (N = 2) or Day 7 (N = 1) of the cycle (onset of oestrus = Day 0). The ovaries were removed at 6, 7, 8, 9, 10, 12, or 14 days after PMSG (see Table 1). PMSG was prepared from plasma from

pregnant mares, essentially according to Goss & Cole (1940). In terms of its ability to induce super-ovulation this preparation was indistinguishable from that obtainable commercially. All follicles with a diameter in excess of 6 mm and all follicles which had undergone apparent luteinization apart from the corpus luteum of the cycle, were dissected from the ovaries using a procedure previously described (Moor, Hay, McIntosh & Caldwell, 1973). Tissue from each sheep was processed for both light and electron microscopy.

Light microscopy

Samples for light microscopy were fixed in Bouin's fluid, wax sections cut at 8 μm and stained with haematoxylin and chromotrope. Alternatively, 4% glutaraldehyde in 0.2 M-collidine buffer, pH 7.2, was used as a fixative with subsequent embedding in glycol methacrylate. When the latter procedure was used, 1 μm sections were cut on a LKB Ultratome and stained with 1% toluidine blue in 1% borax or with phloxine and eosin (Lee, 1977). In addition to routine sections, serial sections were cut through 6 follicles which had undergone apparent luteinization to determine whether an oocyte was present.

Histochemical tests were carried out on fresh tissue frozen in OCT (Raymond A. Lamb, London) over solid CO_2 or frozen rapidly in hexane at -70°C . Alkaline phosphatase activity was examined in 8 μm sections of 20 follicles using the Gomori-Takamatsu procedure (Chayen, Bitensky & Butcher, 1973) with sodium β -glycerophosphate as a substrate. Δ^5 - 3β -hydroxysteroid dehydrogenase (3β -HSD) activity was examined in 16 μm sections of the same follicles by the procedure of Deane, Hay, Moor, Rowson & Short (1966) as modified by Hay & Moor (1975); the substrate used was dehydroepiandrosterone dissolved in acetone. The localization of NAD-diaphorase was also examined in 16 μm sections (Deane *et al.*, 1966).

Electron microscopy

Tissue for electron microscopy was fixed by immersion in 4% glutaraldehyde in 0.2 M-collidine buffer, pH 7.2, followed by post-fixation in 2% OsO_4 and block stained in 2% uranyl acetate in 0.2 M-maleate buffer, pH 5.2. Tissues were embedded in Epon and sections stained with uranyl acetate and lead. Thick sections (1 μm) for light microscopy were stained with 1% toluidine blue in 1% borax.

Measurements of steroids in the culture medium

To examine the steroid output, thin slices of tissue (~ 1 mm thick) were cut with a razor blade, weighed after removing excess moisture and cultured on stainless steel grids with medium 199 (Gibco) containing 10% serum (consisting of 2 parts newborn to 1 part fetal calf serum) at 37°C for 24 h in an atmosphere of 32% oxygen, 5% CO_2 and 63% air. The level of the medium was such that it only touched the inner side of the tissue. Tissue from animals injected on Days 2 (N = 2), 3 (N = 2), 5 (N = 1) and 7 (N = 1) of the cycle and slaughtered 10 or 14, 12 or 14, 10, or 9 days later, respectively, was examined (see Table 2).

The progesterone and total unconjugated oestrogen content of the culture medium were measured using methods and antisera previously described (Moor, Hay, McIntosh & Caldwell, 1973). The cross-reactivity of the progesterone antiserum (Specific Antisera Ltd, South Bank, Davylands, Wilmslow, Cheshire, U.K.) estimated at 50% displacement of labelled progesterone, was with exception of pregnenolone (3.5%) less than 1% for all major steroids secreted by the ovine follicle (Seamark, Moor & McIntosh, 1974). The cross-reactivity of the oestradiol-17 β antiserum (donated by Dr B. J. A. Furr, I.C.I.) was $< 1\%$ for the C_{19} and C_{21} steroids. The cross-reactivity with the C_{18} steroids was 55% for oestrone, 6% for oestradiol-17 α , and 49% for oestrone sulphate. The radioimmunoassay for testosterone was validated by Moor (1977). The cross-reactivity of

testosterone antiserum (Specific Antiserum Ltd) was < 0.01% for C₁₈ and C₂₁ steroids. The cross-reactivity with C₁₉ steroids was 100% for 5 α -dihydrotestosterone, 43% for 5 β -androstane-3 α ,17 β -diol; 41% for 5 α -androstane-3 α ,17 β -diol; 4% for androst-5-en-3 β ,17 β -diol and 0.01% for androstenedione. The limit of sensitivity of the assays was 3 pg/ml for oestrogen, 50 pg/ml for testosterone and 25 pg/ml for progesterone. The within-assay and between-assay coefficients of variation for the assays were, respectively, 6 and 7% for oestrogen, 5 and 7% for testosterone and 6.5 and 11% for progesterone.

Results

Light microscopy

Treatment with PMSG early in the oestrous cycle resulted in marked follicular growth after all injections. Six days after PMSG the follicles were large, about 20 mm in diameter, or occasionally larger, and were well vascularized. Such follicles resembled follicular cysts (Wilson & Zarrow, 1962). Few antral follicles of smaller diameters were observed. The follicular cysts could be assigned to one of five morphological types: (1) those with a thin granulosa and theca interna (Pl. 1, Fig. 1); (2) those with a thick theca interna (~ 10 cell layers thick) and a thin granulosa; (3) those with a thickened theca interna but lacking granulosa (Pl. 1, Fig. 2); (4) those with a thin granulosa and a degenerate theca interna; (5) those lacking both granulosa and theca interna. Type 4 was only seen in 2 follicles. Up to 9 days after PMSG, types 2 and 3, which contained prominent blood vessels, predominated. In about 50% of such cystic follicles the antrum was lined by several layers of elongated cells (Pl. 1, Fig. 2). In addition, fibrous material and blood cells were frequently present within the antrum. From 10 days after PMSG cystic follicles lacking both granulosa and theca interna were more common (type 5), although type 3 follicles were also present. Cystic follicles were present in ovaries up to 14 days after PMSG. The mean ($\pm$ s.e.m.) no. of follicles per sheep given PMSG on Days 2 or 3 and slaughtered 9 days later was 15.4 ± 5.5 . For sheep given PMSG on the same days of the cycle, but slaughtered 10 days later, this fell to 4.8 ± 1.3 (Table 1). The mean number up to and including 9 days after PMSG was 15.1 ± 2.8 per sheep, regardless of the days of injection. The mean from after 10 days was 2.5 ± 0.8 . This later group included ovaries from 3 sheep in which no large follicles were present.

Table 1. Mean number per sheep of cystic follicles and luteinized follicles with or without stigmata after PMSG administration early in the oestrous cycle

Day of cycle PMSG given	Days after PMSG	No. of sheep	No. of cystic follicles > 6 mm diam.	No. of luteinized follicles without stigmata	No. of luteinized follicles with stigmata
2 or 3	6-9	15	15.1 (4-31)*	2.0 (0-7)	0
2 or 3	10	11	4.8 (0-15)	6.6 (1-21)	0
2 or 3	12-14	5	1.6 (0-4)	10.8 (3-27)	0.4 (0-3)
5-7	9-10	7	2.2 (0-6)	3.7 (1-10)	7.3 (0-13)

* The range of follicle numbers is shown in parentheses.

From Day 8 after PMSG, structures with a reddish coloration and an average diameter of 8 mm were observed protruding from the surface of the ovary (Pl. 1, Fig. 3). Due to their probable derivation from follicles they have been termed luteinized follicles. They were generally spherical, but one side was flattened if in close apposition with other follicles. Luteinized follicles were not present 6 or 7 days after PMSG but were present with a frequency of 1.8 ± 1.0 after 8 days, rising to 6.6 ± 1.7 after 10 days for sheep receiving PMSG on Days 2 or 3 of the cycle. In 5 sheep stigmata were present on these structures. Of these sheep, 4 had been injected on Day 5 or later (Table 1).

The remaining animal received PMSG on Day 2 of the cycle and the ovary was removed 12 days later. In a total of 194 luteinized follicles, 43 stigmata were observed in 5 sheep. In addition to the lack of an ovulation point on the majority of luteinized follicles, serial sectioning revealed the presence of degenerate oocytes lying free in the antrum and surrounded by fusiform cells.

The thickness of the wall of the luteinized follicle varied from about 30 cell layers (Pl. 1, Fig. 5) to a form in which the central cavity was obliterated. When an antrum was present it was generally lined by fusiform cells as in type 3 follicles (Pl. 1, Fig. 6). In addition, those luteinized follicles lacking an antrum contained fibroblast-like cells at their centre. Many cells had a round profile with a diameter of $\sim 20 \mu\text{m}$ with a circular nucleus and a high cytoplasmic/nuclear ratio. Interspersed with these cells were elements of the vasculature and an elongate cell type. Occasional luteinized follicles contained radially elongated cells forming cores extending towards the centre of the structure (Pl. 1, Fig. 7). Cells with a structure compatible with that of small luteal cells (O'Shea, Cran & Hay, 1979) could not be detected in any luteinized follicle.

In those follicles containing both theca interna and granulosa, alkaline phosphatase-positive cells were, as has been previously reported for ovarian follicles (O'Shea *et al.*, 1980), only present in the former compartment (Pl. 2, Fig. 9). In luteinized follicles without stigmata, alkaline phosphatase-positive cells were present throughout the structure (Pl. 2, Fig. 8). The reaction product was limited to those cells with a hypertrophied appearance. In luteinized follicles with stigmata, alkaline phosphatase-positive cells were limited to the peripheral areas of the structure and to the cores of the infoldings in about half of those examined (Pl. 2, Fig. 10). In the remainder the reaction was spread throughout the tissue.

As with alkaline phosphatase, a positive reaction for 3β -HSD and NAD-diaphorase was confined to the theca interna of cystic follicles ($n = 5$). A positive reaction was located in luteal cells throughout the tissue of all luteinized follicles ($n = 15$) examined (Pl. 2, Fig. 11). Elements of the blood system and other non-luteal cells were negative.

Oestrogen secretion into the medium during culture for 24 h was very low while that of testosterone was undetectable (Table 2). Progesterone was the major hormone product.

Table 2. Oestrogen and progesterone concentrations in the culture fluid after 24 h culture of slices of luteinized follicles without stigmata*

Day of cycle PMSG given	Days after PMSG	No. of cystic luteinized follicles	Oestrogen (ng mg ⁻¹ 24 h ⁻¹)	Progesterone (ng mg ⁻¹ 24 h ⁻¹)
2	10	3	0.0038 $\pm$ 0.0013	15.1 $\pm$ 1.96
3	12	9	0.012 $\pm$ 0.006	29.3 $\pm$ 1.7
2	14	18	0.0078 $\pm$ 0.003	33.4 $\pm$ 10.5
3	14	4	0.021 $\pm$ 0.009	110 $\pm$ 16
5	10	8	0.003 $\pm$ 0.002	23.7 $\pm$ 4.6
7	9	9	0.006 $\pm$ 0.002	11.6 $\pm$ 1.3

Values are mean $\pm$ s.e.m.

* Testosterone levels were below the limit of sensitivity of the assay.

Electron microscopy

Follicles. The ultrastructure of the theca interna and granulosa of those cystic follicles in which the two tissue compartments were intact and the former unthickened were as described previously (O'Shea, Cran, Hay & Moor, 1978a; Cran, Hay & Moor, 1979).

The theca interna of cystic follicles in which this tissue was thick contained numerous plump cells with abundant tubulo-vesicular endoplasmic reticulum which, while being largely smooth, bore occasional ribosomes (Pl. 3, Fig. 12). Such cells contained occasional lipid droplets and multi-vesicular structures. The mitochondria had predominantly tubular cristae. In addition to this cell form were elongate cells of a less differentiated nature together with endothelial cells and pericytes

of the vasculature. Where present, the granulosa of such follicles had a degenerate appearance (Pl. 3, Fig. 13). In those follicles in which the granulosa was lacking, the antrum was bounded by the remains of the basal lamina (Pl. 3, Fig. 14) or by fusiform cells with a fibroblast-like structure.

Luteal structures. All luteinized follicles examined had similar ultrastructure although as has been described above the gross arrangement of the cells varied. As observed by light microscopy, large luteal-like cells comprised a discrete population. They had an average diameter of about 20 μm with a centrally placed nucleus approximately 8 μm in diameter. A proportion of such cells contained dense granules (see below) and this characteristic has been used to differentiate the population (Pl. 4, Fig. 15). The remaining cells of the tissue were endothelial cells and pericytes of capillaries, fibroblast-like cells which were particularly frequent towards the centre of the luteinized follicles, and occasional leucocytes.

The numbers and proportions of each of the major cell types in a count of 231 cells from 6 luteinized follicles without stigmata and 4 with stigmata are shown in Table 3. Overall, it was observed that 40% of all nuclei present belonged to endothelial cells or pericytes of capillaries, while a further 42% was accounted for by luteal cells of which about 66% had granules. A similar proportion of cells associated with the vasculature was observed for the two types of luteinized follicle. However, although a greater proportion of the follicles with stigmata was comprised of luteal cells and within this cell type there was a higher proportion of cells containing granules, a χ^2 test indicated that there was not a significant difference between the proportions of cells comprising the two forms of luteinized follicle (total $\chi^2 = 6.66$; d.f. = 5; $P = 0.25$).

Table 3. Number and proportions (in parentheses) of different cell types in luteinized follicles with and without stigmata

Type of cell	Luteinized follicles without stigmata	Luteinized follicles with stigmata
Luteal with granules	35 (24)	31 (37)
Luteal lacking granules	21 (14)	8 (10)
Fibroblast	15 (10)	6 (7)
Endothelium and pericyte	63 (43)	30 (36)
Eosinophil	4 (3)	2 (1)
Unidentified	8 (6)	8 (10)
Total	146	85

The luteal cells were regular in outline and were roughly circular or elongate (Pl. 4, Fig. 15). Each cell contained a nucleus which generally had one prominent nucleolus. A small proportion of the cells (< 5%) contained an area of modified cytoplasm bounded by a complete, inverted nuclear envelope (Pl. 4, Fig. 16). The cytoplasm of the luteal cells contained a large amount of endoplasmic reticulum which varied from a predominately vesicular to a predominantly tubular form (Pl. 4, Fig. 17). It generally lacked attached ribosomes. Large numbers of free ribosomes were present.

Mitochondria were numerous and had an average diameter of 0.8 μm . They generally had circular or oval profiles although more elongate forms were observed. Occasional cells had a predominantly elongate form. Both tubular and lamellar cristae were present. Few mitochondria contained inclusions.

One or more small Golgi complexes with associated vesicles were present. About two thirds of luteal cells contained dense granules in various amounts (Pl. 4, Fig. 18). These had a diameter of $0.27 \pm 0.006 \mu\text{m}$ ($n = 50$). A single membrane enclosed the electron-dense contents which were uniformly opaque at high magnification. The dense core was generally separated by less dense material from the bounding membrane. Some pleomorphy of the core was observed. Occasional 'dumb bell' configurations and profiles suggestive of two or more cores were present. In addition, cores of widely different sizes were seen. The granules were distributed evenly throughout the cytoplasm and were often found close to the plasma membrane. Electron-dense granules were also

found outside the cell in which situation they had lost their bounding membrane (Pl. 5, Fig. 19).

Granular endoplasmic reticulum occurred as small patches scattered throughout the cytoplasm (Pl. 5, Fig. 20). Microtubules, microfilaments and lipid droplets were also observed but were not prominent features of the luteal cells.

Sites of apposition between luteal cells were frequent (Pl. 5, Fig. 21). These zones were characterized by the presence of numerous cytoplasmic interdigitations between the neighbours. These processes generally appeared as circular or elongate of profiles. No close surface relationship was seen between the plasma membranes of luteal cells and fibroblasts or with endothelial cells or pericytes of capillaries with which they often bore a close spatial relationship.

Discussion

The circumvention of an LH surge by priming of sheep early in the oestrous cycle with PMSG resulted in the formation of follicular cysts and of a structure similar to that which has been described as a corpus atreticum (Bloom & Fawcett, 1968; Mossman & Duke, 1973) or a luteinized follicle. During growth many follicles lost their membrana granulosa as evidenced by the presence of degenerate cells in the antrum and of a lack of cells internal to the basal lamina. Further, the widespread presence of alkaline phosphatase-positive cells in the wall of the luteinized follicles argues strongly for predominantly thecal origin (O'Shea *et al.*, 1980). This form of atresia contrasts markedly with that observed in non-stimulated follicles (Hay, Cran & Moor, 1976) in which, although necrotic changes are most evident in the granulosa, both it and the theca interna degenerate without luteinization taking place in the latter tissue. After PMSG priming, a population of stimulated follicles was produced, a proportion of which had stigmata after 10 days or more. In some cystic follicles without stigmata at this time, both the granulosa and theca interna were lacking.

In many mammals the thecal tissue of atretic non-preovulatory follicles gives rise to interstitial gland tissue (Mossman, Koering & Ferry, 1964). However, in some hystricomorph rodents this tissue may be derived from the granulosa (Weir, 1971). The cells of this tissue have a structure highly suggestive of a steroidogenic function (Gondos & Hobel, 1973). However, to date no clear intraovarian function has been assigned to it. In rabbits it is believed to be a source of progestagens (Hilliard, Archibald & Sawyer, 1963; Simmer, Hilliard & Archibald, 1963), while in the human it is considered to have an androgen-secreting capacity (Hammerstein, Rice & Savard, 1964; Rice & Savard, 1966; Savard, 1969). It has been stated that atresia of preovulatory follicles does not give

PLATE 1

Fig. 1. Cystic follicle with thin theca interna (T) and granulosa (G). $\times 720$.

Fig. 2. Cystic follicle with markedly thickened theca interna (T) and no granulosa. Note the presence of fusiform cells (F) bounding the antrum. $\times 480$.

Fig. 3. Ovary from a sheep given PMSG on Day 3 of the cycle and slaughtered 10 days later. Numerous luteinized follicles protrude from the surface (arrows). $\times 1.9$.

Fig. 4. Oocyte from a luteinized follicle. It is bounded by fusiform cells (arrows). $\times 200$.

Fig. 5. Luteinized follicle demonstrating the markedly thickened wall. $\times 30$.

Fig. 6. Detail of a luteinized follicle demonstrating the presence of fusiform cells (F) lining the antrum. $\times 120$.

Fig. 7. A luteinized follicle in which the cells are elongated to form radial cords. $\times 120$.

PLATE I

Fig. 8. Distribution of alkaline phosphatase activity in a non-ovulated luteinized follicle. All luteal cells are positive. $\times 170$.

Fig. 9. Localization of alkaline phosphatase activity in a cystic follicle containing both theca interna (T) and granulosa (G). Activity is confined to the theca interna. $\times 90$.

Fig. 10. Alkaline phosphatase activity is localized in the septae (arrows) of luteinized follicles with stigmata. $\times 140$.

Fig. 11. Localization of 3β -HSD in a luteinized follicle. Activity is spread throughout the tissue. $\times 360$.

rise to interstitial tissue (man: Mossman *et al.*, 1964; rat: Peluso, Steger & Hafez, 1977). Notwithstanding this, the structure of luteal tissue resulting from PMSG priming in sheep is similar to that of interstitial tissue found in, for example, rabbits and women. However, although the ontogeny of this tissue and many of its ultrastructural features resembles that of interstitial tissue, the detailed structure of the cells and their steroidogenic capacity are more comparable with that of large luteal cells of cyclic corpora lutea and thus the distinction between the two may be somewhat artificial.

When primed on Day 5 of the cycle or later, an ovulation response could be elicited. In such follicles alkaline phosphatase-positive cells were confined to the septae. For normally cyclic sheep, O'Shea *et al.* (1980) have demonstrated that such a localization is indicative of ovulation having taken place within the previous 24 h. At such a time the follicles were already cystic but were presumably capable of secreting sufficient oestrogen to elicit an LH surge at the time of luteal regression which occurs between Day 13 and 15 (Bjersing *et al.*, 1972).

The predominant steroid secreted by the luteinized follicle was progesterone, with very low or undetectable levels of oestrogen and testosterone. Since the cells which showed a positive reaction for β -HSD had the structural characteristics of steroid-secreting cells (Christensen & Gillim, 1969) it is clear that both functional and morphological luteinization had taken place. During atresia of non-stimulated follicles, although testosterone secretion by the theca interna is reduced from non-atretic levels, it is still maintained at an appreciable level (Moor, Hay, Dott & Cran, 1978). PMSG has therefore had the effect of enhancing and altering the steroidogenic capacity of the theca interna. A similar observation has been made in the rabbit in which intrafollicular injection of LH and FSH resulted in luteinization and concomitant progesterone synthesis (Jones & Nalbanov, 1972).

PLATE 3

Fig. 12. Thecal cells from a cystic follicle in which this tissue is thickened. They contain abundant smooth endoplasmic reticulum (S), lipid droplets (L) and vesicular structures (V). They are interconnected by a gap junction (GJ). $\times 13\ 000$.

Fig. 13. Part of a cystic follicle demonstrating degenerating granulosa cells (G); basal lamina (arrows). $\times 7000$.

Fig. 14. Cystic follicle in which the granulosa has degenerated leaving the basal lamina (arrows) exposed T, theca interna. $\times 10\ 800$.

PLATE 4

Fig. 15. Typical luteal cells from a luteinized follicle showing profiles of vesicular endoplasmic reticulum (ER) and dense granules (arrows). $\times 7500$.

Fig. 16. Nucleus from a luteal cell from a luteinized follicle. The nuclear inclusions (I) are filled with cytoplasmic material. $\times 5600$.

Fig. 17. Detail of a luteal cell from a luteinized follicle demonstrating the tubular nature of the endoplasmic reticulum (arrows). $\times 12\ 600$.

Fig. 18. Dense membrane-bounded granules present in luteal cells from a luteinized follicle. One (arrow) contains three dense inclusions. $\times 9600$.

PLATE 5

Fig. 19. Detail of membrane-bounded granules (arrowheads.) One, lacking a bounding membrane (arrow) is lying in an extracellular position. $\times 30\ 000$.

Fig. 20. Strands of rough endoplasmic reticulum (ER) present in luteal cells from a luteinized follicle. $\times 11\ 300$.

Fig. 21. The plasma membranes of adjacent luteal cells (L) from a luteinized follicle show marked interdigitation (arrows). No such relationship exists between fibroblasts (F) or other cell types; capillary (C). $\times 5000$.

At neither the light nor electron microscope level was there any indication of two populations of luteal cells as has been reported for the cyclic corpus luteum of the pig (Corner, 1919) and sheep (Mossman & Duke, 1973; O'Shea *et al.*, 1979). In addition, the proportion of the tissue occupied by luteal cells was similar to that occupied by both large and small luteal cells of the sheep corpus luteum (O'Shea *et al.*, 1979).

At an ultrastructural level the luteal cells resembled the large luteal cells of the corpus luteum more than they did the small. Features they had in common with the large luteal form included predominantly smooth endoplasmic reticulum, scattered stacks of rough endoplasmic reticulum, regularly shaped mitochondria of smaller dimensions than those found in small luteal cells, and numerous dense membrane bound granules. The cells were larger than small luteal cells ($\sim 20 \mu\text{m}$ compared with $15 \mu\text{m}$) and were of a more even outline, but were smaller than large luteal cells ($30 \mu\text{m}$). The only feature observed which is characteristic of small luteal cells was the presence of occasional nuclei with regions of inverted nuclear envelope.

The granules, which were secreted into the extracellular space, were less numerous, more pleomorphic and slightly larger ($0.26 \mu\text{m}$ compared to $0.2 \mu\text{m}$) than those of the cyclic CL (Gemmell, Stacy & Thorburn, 1974; Parry, Willcox & Thorburn, 1980). However, their overall structure was similar and some variation in structure is also present within the cyclic CL (unpublished observation).

After ovulation in the sheep, cells of the theca interna give rise to small luteal cells which have an intimate relationship with luteal cells of granulosa origin (O'Shea *et al.*, 1980). It is now clear that as a result of priming with PMSG theca cells may also acquire structural and secretory characteristics similar to those derived from the granulosa. Apart from the ability to secrete progesterone and respond to LH (Lemon & Loir, 1977; Hunter, 1981) little is known regarding the function of small luteal cells. The apparent developmental plasticity of thecal cells may indicate that they potentially have a function similar to those of granulosa origin and that further differentiation of thecal luteal cells is inhibited by those originating from the granulosa.

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