Formation of the Rupture Site in Preovulatory Hamster Follicles: Morphological and Morphometric Analysis of Thinning of the Granulosa and Thecal Layers

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The wall of hamster ovarian follicles is composed of the following cell layers: granulosa, theca interna, theca externa, and the surface epithelium. To determine the morphological changes occurring in the follicle during formation of the rupture site, we measured: the thickness of each layer, the number of cells in each layer, and size of cells in each layer, at the apex and base of follicles at specific times during the final 13 hr before ovulation. Changes in the wall occur in 3 stages. During stage 1 (T0–T8), which includes the first 8 hr following the surge in luteinizing hormone, the apical and basal walls thin at the same rate and the antrum increases in size. During stage 2 (T9–T12), there is no change in the thickness of the apical and basal follicle wall nor in the size of the antrum. During the third stage (T12–T13), the size of the antrum decreases slightly and there is an increase in the thickness of the basal wall, which is correlated with its constriction caused by the contraction of smooth muscle cells. The apical wall rapidly thins to the point of rupture. The morphology of cells from each layer is described. Theca interna cells form the final tissue preventing escape of the oocyte-cumulus complex. The roles of cell migration, stretching, and death in thinning of the apical wall are described, and the mechanisms involved in follicle rupture are discussed.

Key words: ovulation, morphology, mammals

INTRODUCTION

Complete knowledge of the structural changes occurring in the preovulatory follicle is necessary to understand the complex events that lead to ovulation in the mammalian ovary. Although there have been numerous morphological studies on mammalian follicles, it is apparent that each species must be examined in detail because considerable variation among commonly tested animals has been noted [referenced in Talbot et al, 1987]. In addition, the entire follicle, not just the apical wall, must be studied as a unit. This has not been done previously for any species.

We have analyzed morphological changes that occur in the surface epithelium (SE) of preovulatory hamster and mouse follicles [Talbot et al, 1987]. In both species,
the SE cells stretch, degenerate, and slough from the follicle apex prior to the loss of any other layer of the follicle wall.

The purpose of this report is to present morphometric and morphological data on changes in the remainder of the wall in preovulatory hamster follicles. We have measured the size of the antrum, thickness of the apical and basal follicle wall, number of cell layers in the granulosa and theca, and the size of individual cells in each layer of the follicle wall at various preovulatory times. We have also examined changes in the ultrastructure of the thecal and granulosa cells as ovulation approaches. These observations provide a framework for understanding the mechanism of ovulation in hamsters.

MATERIALS AND METHODS

Animal Care

Mature female golden hamsters (Mesocricetus auratus), 8–20 weeks old, were maintained on a 12:12 light:dark photoperiod and allowed free access to Purina laboratory chow and water. Animals were induced to superovulate by an intraperitoneal injection of pregnant mare's serum gonadotropin (PMSG, 25 IU, Sigma) on the first day of their 4-day estrous cycle. At 11 p.m. on the evening of day 3, hamsters were given an intraperitoneal injection of human chorionic gonadotropin (hCG, 25 IU, Sigma) to simulate the natural surge of luteinizing hormone (LH). In our laboratories, hamsters routinely begin to ovulate 12.5–13 hr after the hCG injection.

Tissue Preparation

Follicles were prepared for examination by light microscopy (LM) and transmission electron microscopy (TEM) at 0, 4, 8, 9, 10, 11, 12, and 13 hr after the hCG injection. Ovaries were removed from hamsters, rinsed in 0.85% saline, and immersed in 3% glutaraldehyde in 0.1 M sodium cacodylate pH 7.4 at room temperature. After 1 hr, individual follicles were dissected from the ovary. Isolated follicles were fixed for an additional 2 hr, then washed 5 min in 0.1 sodium cacodylate buffer. Follicles to be examined by LM were dehydrated in an ethanol series and embedded in plastic [Spurr, 1969]. Sagittal sections, 1 μm thick, were cut, placed on glass slides, and stained with methylene blue. To obtain sections through the thinnest part of the apical follicle wall, proper orientation and trimming of the follicle are essential. Any deviation in the angle or position of the section will accentuate the thickness of the apical wall. Therefore, follicles to be sectioned for measurements were not postfixed with osmium tetroxide because it blackens the tissue and makes it difficult to obtain the proper orientation. Serial sections were cut through follicles, and measurements were used only from sections that included both the thinnest part of the apical wall and the greatest area of the antrum.

For examination of the morphology of the follicle wall using TEM, follicles were prepared as described for LM except the follicles were postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate for 1 hr prior to dehydration in ethanol. Thin sections were cut on a Porter-Blum MT2-B ultramicrotome, picked up on copper grids, stained 1 hr with uranyl acetate and 5 min with lead citrate, and viewed in a Hitachi HU 11A or H 500 TEM. To enhance the contrast of the basal laminae in some follicles, tannic acid (1%) was added to the glutaraldehyde fixative for 1 hr, and then follicles were processed as described above.
The follicle apex was also prepared for examination by scanning electron microscopy (SEM) as described previously [Talbot et al., 1986].

Biometrical Procedures

The pointer in one of the eyepieces of the light microscope was used as a line and was rotated so that it ran perpendicular to the surface epithelium at the thinnest part of the follicle wall. The thickness of the granulosa, theca interna and externa, the thickness of individual cells, and the number of cells in each layer were measured (using an ocular micrometer) or counted along this axis. Measurements were made at the follicle apex and on the theca interna and granulosa layers in the basal follicle wall at a point 180° from thinnest part of apical follicle wall. The area of follicle was measured by projecting images of thick (1 μm) sections onto a Hipad digitizing tablet and tracing the circumference of the antrum, using Bioquant software interfaced with an IBM PC microcomputer. Descriptive statistics (mean standard deviations and 95% confidence intervals) were computed using the statistical package for the Social Sciences [SPSS; Nie et al., 1975].

A single classification analysis of variance (ANOVA, SPSS) was used to examine heterogeneity among the sample means across the different time periods. Variance in measurements of the apical wall in follicles 13 hr after the hCG injection was not equal to variance at other time periods (Bartlett's-Box F = 3.814). This can be explained because not all follicles rupture at the same time; T13 includes some follicles with extremely thin apical walls (less than 2 cell layers) that will most likely rupture within minutes and other follicles with relatively thick apical walls that will not rupture for longer periods of time (but within 60 min because all follicles have typically ruptured by 14 hr after the hCG injection; unpublished observations). We separated these two populations on morphological grounds; follicles with hemispherical outlines to their basal follicle wall remained in T13, whereas follicles with constricted basal follicle walls were placed in T13* [see Martin and Talbot, 1981a, for discussion of basal wall constriction in the hamster follicle]. When T13* was omitted from the ANOVA test, all criteria for this analysis were fulfilled (Bartlett-Box F = 0.642). The rationale for elimination of this time period from analysis of the entire wall is that only the theca interna layer remains at this time; the surface epithelium, theca externa, and granulosa layers are absent from the thinnest part of the apical follicle wall. Separate ANOVA tests were made for individual layers of the apical and basal follicle wall and analysis of statistical differences between successive time periods were analyzed using the multiple range test, Scheff's procedure.

Trends in wall thinning were analyzed by nonparametric correlations (Kendall's rank order correlation coefficients) because the time intervals are not a continuous variable.

All hypothesis testing was evaluated at the 0.05 significance level and all computations were performed on the Prime 9955 computer at Occidental College.

RESULTS

Measurements on Preovulatory Follicle Walls

The overall thickness of the wall, the thickness of individual cell layers (granulosa and theca interna and surface epithelium plus theca interna), and the thickness of individual cells (granulosa, theca interna) were measured at the apex and base of
folicles at specific times during the final 13 hr before ovulation (Figs. 1–7). Based on these measurements, changes in the thickness of the apical follicle wall occur in 3 stages (Fig. 1). Stage 1 lasts from T0–T8 and is characterized by a gradual thinning of the wall. During stage 2, which lasts from T9–T12, there is no significant change in the overall thickness of the apical wall. Stage 3 includes the final hour before rupture, T12–T13*, and is characterized by a rapid decrease in the thickness of the apical follicle wall.

The decrease in wall thickness that occurs during stages 1 and 3 results mainly from a decrease in the relative thickness of the granulosa layer (Fig. 2). During stage

![Graph showing thickness changes](image1)

**Fig. 1.** Thickness (in μm) of the apical follicle wall at various times after the hCG injection. Values are the means of 49 observations. Standard deviations are not shown as they usually did not exceed the diameter of the point indicator. □ = thickness of the total apical wall. △ = thickness of the granulosa layer, ○ = thickness of the theca interna. The total thickness differed significantly (0.05 level at T0 and T4, T8 and T12, and T13, and T13 and T13*). The thickness of the granulosa layer differed significantly at T0 and T4, T8 and T12, and T13 and T13*. The thickness of the theca interna differed significantly at T0 and T4, and T13 and T13*. * = T13*.

![Graph showing percentage changes](image2)

**Fig. 2.** The relative thickness of the surface epithelium and theca externa (□), theca interna (●), and granulosa layer (□) at various times after the hCG injection. The percentage of the wall comprised of each of these layers is shown at 0, 4, 8, 9, 11, 12, 13, and 13* hours after the hCG injection. Data on the left are for the apical wall; data on the right are for the basal wall. * = T13*. 
Fig. 3. The number of cell layers in the granulosa (Δ) and theca interna (0) in the apical follicle wall at various times after the hCG injection. Each point is the mean ± SD of 40 or more observations. The number of cell layers in the granulosa decreases significantly between T₀ and T₄ and again between T₁₂ and T₁₃*. The number of cell layers in the theca interna is not significantly changed until T₁₂. * = T₁₃*.

Fig. 4. The thickness (in μm) of cells in the granulosa (Δ) and theca interna (0) at the apex at various times after the hCG injection. Each point is the mean ± SD of 40 or more observations. There is no significant (0.05 level) change in the size of granulosa cells whereas the thickness of these interna cells decreases from T₁₁ to T₁₃. * = T₁₃*.

1, this decrease is due solely to a decrease in the number of granulosa cell layers (Fig. 3); there is no change in the size of individual granulosa cells during this time (Fig. 4). The thinning of the apical wall in the area of the rupture site during stage 3 is due to both a decrease in the number of cell layers in the granulosa and theca interna (Fig. 3) and a decrease in the thickness of individual cells in these layers (Fig. 4). By T₁₃*, almost all cells in the area of the rupture site are theca interna cells; most epithelial, granulosa, and theca externa cells are lost from this region by T₁₂ (Fig. 2).

Changes in the basal follicle wall also occur in 3 stages (Fig. 5). Stage 1 (T₀ to T₈) is characterized by gradual thinning of the wall. The rate of thinning of the basal follicle wall is similar to that occurring at the apex and is due to a decrease in the relative number of granulosa cells (Fig. 6). The number of layers of theca interna
Fig. 5. Thickness (in $\mu$m) of the basal follicle wall at various times after the hCG injection. Each point is the mean of 40 or more observations. Standard deviations are not shown as they seldom exceed the diameter of the point indicator. $\square$ = thickness of the entire basal wall. $\triangle$ = thickness of the granulosa. $\bigcirc$ = thickness of the theca externa. Values for total wall thickness were significantly (0.05 level) different for the following pairs: $T_0$ and $T_4$, $T_4$ and $T_8$, $T_{11}$ and $T_{12}$, $T_{12}$ and $T_{13}$. Values for the granulosa layer were significantly different at $T_0$ and $T_4$, $T_4$ and $T_8$, and $T_{11}$ and $T_{12}$. Values for the theca interna were significantly different only at $T_{12}$ and $T_{13}$. $* = T_{13}$*

Fig. 6. Number of cell layers in the basal granulosa ($\triangle$) and basal theca interna ($\bigcirc$) at various times after the hCG injection. Each point is the mean $\pm$ SD of 40 or more observations. Values for the number of cell layers in the granulosa were significantly (0.05 level) different for the following pairs: $T_0$ and $T_4$, $T_4$ and $T_8$, $T_{11}$ and $T_{12}$, $T_{12}$ and $T_{13}$. No pairs of values for the number of granulosa layers were significantly different. $* = T_{13}$*

cells remains the same (Fig. 6) as does the size of individual granulosa and theca interna cells (Fig. 7). During stage 2 ($T_9$–$T_{12}$), there is no significant change in the overall thickness of the basal wall, whereas stage 3 is characterized by a rapid increase in the thickness of the basal follicle wall (Fig. 5). This change is brought about by constriction of the basal follicle wall.

To quantify swelling of the follicle, the area of the antrum seen in sagittal sections was measured during the final 13 hr before rupture (Fig. 8). During stage 1, the area of the antrum more than doubled, whereas during stage 2, $T_9$–$T_{13}$, there
Fig. 7. Thickness (in \(\mu m\)) of cells in the basal granulosa (\(\triangle\)) and basal theca interna (\(0\)) at various times after the hCG injection. Each point is the mean \(\pm\) SD for 40 or more observations. There is no significant change in the thickness of granulosa cells, whereas the thickness of theca interna cells differs significantly from \(T_5\) to \(T_8\) and \(T_9\) to \(T_9^*\).

Fig. 8. Area of the antrum (in \(\mu m^2\)) at various times after hCG injection. Significant differences (0.05 level) occurred between \(T_0\) and \(T_4\), \(T_4\) and \(T_8\), and \(T_{13}\) and \(T_{13}^*\). Each point is the mean \(\pm\) SD for 40 or more observations. \(^* = T_{13}^*\).

was no significant change in the size of the antrum. Between \(T_{13}\) and \(T_{13}^*\), the antral area actually decreased slightly, and this is correlated with constriction of the basal follicle wall.

**Shape of the Follicle and Morphology of the Apical Follicle Wall**

T0: At T0, follicles have spherical antra, and the layers of the wall are clearly distinguished in sections (Fig. 9). Granulosa cells are spherical to polyhedral in shape, possess filipodia, and contain typical cell organelles (Figs. 9–12). Gap junctions up to 4.5 \(\mu m\) long and annular nexuses are common (not shown).

Cells of the theca interna are ovoid (Fig. 11). Plasma membranes of adjacent cells are joined by a few short gap junctions and are highly interdigitated (Fig. 13). These interdigitations are still present at T4 but not at T8. In addition to typical organelles, these cells contain distinctive ring-shaped mitochondria that are not found
Fig. 9. Light micrograph of a hamster follicle at $T_0$. The antrum (A) is spherical and the granulosa cells (G) are clearly distinguished from the remaining layers of the wall. ×100.

Fig. 10. Scanning electron micrograph of granulosa cells from a $T_0$ hamster follicle. The cells are spherical to polyhedral in shape and have filopodia (arrows). ×3,900.

Fig. 11. Light micrograph through the apex of a hamster follicle at $T_0$; 3-5 layers of theca externa cells (TE) are present beneath a single layer of flat surface epithelial cells (arrow). Cells of the theca interna (TI) and granulosa (G) are large and at the light microscope level similar in appearance. ×880.
in any other type of cell in the follicle (Fig. 13 and Fig. 14 from T8). The plasma membrane has numerous coated pits (not shown).

A network of capillaries, previously named the inner vascular wreath, lies in the theca interna along the basal lamina, which separates the thecal and granulosa cells (not shown) [see Lipner, 1973]. Other capillaries are scattered throughout the theca interna.

The theca externa is composed of 3–5 layers of cells, which are thinner, longer, and less densely packed than the interna cells (Fig. 11). Theca externa cells are similar at T0 and T8, contain typical organelles, and on occasion possess a basal body and cilium (Fig. 14). A thin layer of microfilaments (60 Å diameter) is often seen near the plasma membrane. Desmosomes are occasionally observed between cells. Collagen fibrils, which are more abundant in this layer than in the theca interna, are oriented in small clusters parallel to the circumference of the follicle.

Epithelial cells cover the follicle and have been described previously [Talbot et al., 1987].

T8: In T8 follicles, the antrum is ovoid (Fig. 15); the width of the antrum exceeds its height. In some follicles, only a single or double layer of granulosa cells separates the antrum from the thecal layers (Fig. 16). Granulosa cells attached to the basal lamina are often elongate, but otherwise the morphology of these cells shows no change from the T0 description. The basal lamina lying between the granulosa and theca interna is intact.

The ultrastructure of the theca interna cells has not changed by T8, but the cells are more elongate, less densely packed, and the interdigitations of the plasma membrane are absent (compare Figs. 13, 14). Fewer capillaries appear to be present in the inner vascular wreath and in the outer part of the theca interna.

No changes are observed in the theca externa.

T10: The antrum is ovoid; its width exceeds its height, and it is common for the apex of these follicles to partially collapse and become inverted during fixation (Fig. 17). Granulosa cells are unchanged except for the appearance of blebs on the cell surface (Fig. 18). In some follicles, the basal lamina separating theca interna and granulosa cells is continuous at the apex, but in most follicles it is patchy or has disappeared completely. Without the basal lamina, the distinction between the granulosa and theca interna layers is not always clear at the LM level. However, cells of these layers can be identified using TEM by the presence of ring-shaped mitochondria (Fig. 19), which are found only in theca interna cells.

Occasionally dying interna cells are observed (Fig. 20); they stain lightly and often contain only mitochondria, ribosomes, and dilated RER. The inner vascular wreath is no longer recognizable, but capillaries are still common throughout the theca interna. The basal lamina around capillaries is often discontinuous or missing. When present, it is often penetrated by blebs from the endothelial cells (Fig. 23). Some capillaries are intact, whereas others are torn and/or clogged by platelets (Figs.

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Fig. 12. Transmission electron micrograph of granulosa cells from a T0 follicle. The cells contain RER, mitochondria (arrow), small vesicles (V), and lipid droplets (L). ×11,900.

Fig. 13. Transmission electron micrograph of a theca interna cell from a T4 follicle. Cells in this layer are similar at T0 and T4. The plasma membranes are highly interdigitated (arrow), and the cells contain a distinctive type of mitochondria (M). ×34,200.
Fig. 14. Transmission electron micrograph through the apical wall of a follicle at $T_8$. The fibroblasts (*) of the theca externa are similar to those observed at $T_0$; they are separated by small intercellular spaces containing collagen (arrow). The cells of the theca interna (TI) now have smooth plasma membranes that do not interdigitate with membranes of adjacent cells. The theca interna cells are readily identifiable by their distinctive mitochondria. $\times 8,900$.

Fig. 15. Light micrograph through a follicle at $T_6$. The antrum is ovoid and the apical wall is thinner. $\times 56$.

Fig. 16. Higher magnification of the apical follicle wall at $T_8$. The thickness of the granulosa layer is greatly reduced. A distinct boundary formed by an intact basal lamina exists between the theca interna (*) and granulosa layer (G). $\times 760$.

Fig. 17. Light micrograph of a follicle at $T_{10}$. The antrum is still ovoid at this time point and the apical wall often collapses during fixation. $\times 56$.

Fig. 18. Scanning electron micrograph of granulosa cells from a $T_{10}$ follicle. It is common to see blebs (arrow) on these cells at this time. $\times 5,100$. 

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21–22). Erythrocytes are commonly seen free in the follicle wall (Figs. 19, 21). Capillaries at the base of follicles show none of these changes.

The theca externa is composed of 2–3 layers of cells separated by relatively large extracellular spaces (Fig. 19). Collagen fibrils are less abundant than at T8. The surface epithelial cells begin to detach from the follicle apex at this time, thereby allowing views of the theca externa by SEM. In these views, the cells are flat with irregular outlines (Fig. 24). Branched microvilli are common around the periphery of the cell. When examined by TEM, some externa cells show the same signs of cell death as the interna cells; swollen nuclear envelopes and cisternae of RER. Other cells have breaks in the plasma membrane and their cytoplasm is leached. Organelles such as mitochondria have been seen in the extracellular space of the theca externa.

T11–T13: The antra of follicles at these times are ovoid to irregular in shape. Granulosa cells are either absent from the apical follicle wall or are present singly or in small clusters. Many granulosa cells are spherical with numerous blebs, but some are elongate and not easily distinguished from interna cells. The walls of many capillaries are torn, and free erythrocytes are common in the follicle wall. Cells of the theca interna make up most of the apical wall (Fig. 25). They are elongate and contain long parallel cisternae of RER and ring-shaped mitochondria. The plasma membranes of adjacent cells run parallel to each other for long distances, but there are no specialized junctions between them. A few collagen fibrils are closely bound to the cell surface. Leached interna cells, which are presumed to be dead, are common. Basal laminae are now completely absent as the apex.

The theca externa is absent from the thinnest part of the apex. Along the sides of the follicle, the externa reappears as a region of 1–3 layers of elongate, widely separated cells enmeshed in an extracellular matrix.

T13*: The T13*-hr follicles have a constricted basal follicle wall (Fig. 26) The basal part of the antrum is reduced to a narrow lobe(s). This constriction forms in follicles within a few minutes of rupture [Martin and Talbot, 1981; Talbot and Chacon, 1982]. In T13* follicles, only theca interna cells and remnants of the capillaries are present at the thinnest part of the wall (Figs. 27, 28). In some follicles judged to be a within a few seconds of rupture, even the interna cells and vessels do not form a continuous layer. Spaces up to 15 μm wide separate interna cells and/or remnants of the capillaries that form the final wall (Figs. 27, 28).

DISCUSSION

Based on morphometric and morphological data, the final 13 hr before rupture of the hamster follicle can be divided into 3 stages. Stage 1 (T0–T8) is characterized by gradual swelling of the follicle and thinning of the entire apical and basal follicle wall. During stage 2 (T8–T12), there is no significant change in the thickness of the follicle wall, but degeneration of the apical follicle wall begins. Stage 3 includes the final hour (T12–T13*) before follicle rupture when the size of the antrum decreases slightly, the basal wall increases in thickness, and the apical wall thins to the point of rupture.

During stage 1, the area of the antrum (measured in thick sections) doubles. This is probably due to the movement of plasma into the antrum [Lipner, 1973; Parr, 1975; McNatty, 1978] as ovarian vessels are highly permeable [Payer, 1975; Ellinwood et al, 1978], follicular fluid is similar in composition to plasma [Lipner, 1973;
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McNatty, 1978], and blood flow to the ovary increases after the LH surge [Wurtman, 1964; Lee and Novy, 1978]. Ovarian hyperemia may be due directly to LH or to compounds released in response to LH such as histamine [Szego and Gitin, 1964; Wurtman, 1964] and PGE [Lee and Novy, 1978; see Espey, 1980]. Actual movement of plasma into the antrum and its retention there is probably influenced by deposition of extracellular hyaluronic acid [Eppig, 1981a,b] during expansion of the cumulus oophorous. Hyaluronic acid is a large polyanion that absorbs water to neutralize its negative charges [Toole and Gross, 1971]. Its secretion would tend to draw and hold plasma in the antrum, leading to gradual swelling of the follicle. This suggestion is consistent with the observations that cumulus expansion in the rat [Dekel and Kraicer, 1978] and the hamster [unpublished data] begins 4 hr after the LH surge and is complete by T8 [Liebfried and First, 1982; unpublished data] when follicle swelling is also complete [this study].

During stage 1, the basal and apical walls of hamster follicles thin to 67% and 44% of their T0 thickness. Most of the thinning is caused by a loss of cells from the granulosa layer and is probably due either to: 1) detachment of cells from the wall and their inclusion into the expanding cumulus oophorus, or 2) a redistribution of the granulosa cells along the expanding follicle wall. The size and shape of the granulosa cells do not change during this time, and cell death has not been observed.

The theca interna also thins during stage 1, and by T8, it is only 74% of its T0 thickness. This thinning appears to be due to unfolding of the cell’s plasma membranes as the follicle expands. A similar loss of lateral plasma membrane interdigitations and a reduction in the number of microvilli is observed in SE cells during stage 1 [Talbot et al., 1987].

Swelling of the follicle and thinning of its walls are probably necessary events in the normal ovulatory sequence. However, there is evidence that these events are not sufficient by themselves to cause rupture. When intrafollicular pressure is artificially raised in follicles, only those follicles that show degenerative changes in the apical wall and are close to natural rupture will extrude the oocyte cumulus complex [Espey and Lipner, 1963; Bronson et al., 1979; Talbot and Schroeder, 1982]. No evidence of cell death or breakdown of collagen fibrils and the basal laminae have been observed during stage 1 of hamsters. In the absence of these events, ovulation would not be expected.

Fig. 19. Transmission electron micrograph through the wall of a T0 follicle. The spaces (*) between theca externa cells are much larger than at previous times. Free erythrocytes (E) are often seen in the wall. Cells of the theca interna (T) and granulosa (G) are no longer separated by a basal lamina. ×3,900.

Fig. 20. Transmission electron micrograph showing a dying theca interna cell from a T10 follicle. The cytoplasm is leached and the nuclear envelope (arrow) swollen. ×9,300.

Fig. 21. Light micrograph through the apex of a follicle fixed a T10. Numerous free erythrocytes (E) are present in the wall, and a capillary clogged with platelets (arrow) is shown. ×600.

Fig. 22. Transmission electron micrograph of a T10 follicle showing a capillary wall clogged with platelets. ×20,000.

Fig. 23. Transmission electron micrograph of a capillary from a T10 follicle. Blebs (arrows) from the endothelium have penetrated the basal lamina around the capillary. ×28,500.

Fig. 24. Scanning electron micrograph of the apical surface of a follicle at T10. The surface epithelium has detached and the basal lamina is absent. Cells of the theca externa (*) are flat and irregular in shape. ×630.
During stage 2 (T8–T12), there is no major change in the size of the hamster follicle or in the thickness of the follicle wall. In contrast, rabbit follicles apparently enlarge and the apical wall thins at a constant rate throughout the entire period between the LH surge and follicle rupture [Walton and Hammond, 1928; see Lipner, 1973].

The collagenous support (collagen fibrils and basal laminae) of follicles must be weakened for ovulation to occur [Espey and Rondell, 1968; Espey, 1978], and collagenase activity has been detected in preovulatory mammalian follicles [Curry et al. 1985]. In hamsters, we observed significant morphological changes in the collagenous components of follicles during stage 2, suggesting that collagenase is active during this time. The specific changes observed include loss of: 1) some of the collagen fibrils in the theca externa at the apex, 2) the basal lamina separating the granulosa and theca interna in all regions of the follicle, 3) the basal lamina surrounding capillaries at the apex, and 4) the basal lamina beneath the surface epithelium at the apex. A similar decrease in abundance of collagen fibrils has been noted in the rabbit at a time equivalent to stage 2 [see Lipner, 1973]. The basal lamina around vessels in the base of follicles remains intact throughout ovulation, whereas loss of the basal lamina around apical capillaries results in their tearing as the follicle swells. Erythrocytes are commonly seen outside vessels in the apex as early as T10, but free erythrocytes are not seen in other parts of the follicle even at later times. Our observations on degradation of collagenous components in hamsters follicles are in good agreement with the studies by Ichikawa et al. [1983 a,b] showing the collagenase inhibitors block ovulation if applied to follicles during stage 2.

Stage 3 includes the final hour before follicle rupture. During this time there is a slight decrease in the size of the antrum [Talbot, 1983a; present study], the basal wall becomes thicker, and apical wall thins to the point of rupture. Epithelial, granulosa, and theca externa cells are lost from the apical follicle wall either by detaching and/or cell death, leaving only remnants of capillaries and cells of the theca interna to form the final barrier preventing escape of the oocyte-cumulus complex. Thinning of the interna layer involves stretching of the cells and cell death. Stretching is apparent in TEM micrographs where the cell diameters thin from 5.2 μm at T12 to 3.0 μm just before rupture. We assume that cell stretching is a passive process, yet we cannot rule out the possibility of active cell elongation. Degenerating cells are first identified by swollen organelles and later by breaks in the plasma membrane and leaching of the cytoplasm [Wyllie, 1981]. It is difficult to obtain images of the follicle wall when it is composed only of interna cells, presumably because this condition lasts for a brief time. Adjacent interna cells closely parallel one another, but no specialized junctions, membrane densities, or supporting filaments that would help hold these cells together are apparent. Several micrographs have been published showing gaps in the apical wall prior to release of the oocyte [Parr, 1974; Schroeder and Talbot, 1982; present study]. The cohesiveness of the oocyte-cumulus complex prevents escape of the oocyte until the gaps have enlarged to form the rupture site.

In sections through hamster follicles, the circumference of the follicle wall increases due to cell stretching, which occurs throughout the final 13 hr before follicle rupture. However, the area of the antrum does not increase significantly during stage 2 and, in fact, there is a slight decrease in antrum size just before rupture, stage 3 [Talbot, 1983a; present study]. These two conditions can occur simultaneously because the basal hemisphere of hamster follicles constricts prior to rupture [Martin and
Fig. 25. Transmission electron micrograph of a follicle at T_{12}. Most of the cell layers of the wall have disappeared except for the theca interna (*). Small bundles of collagen (arrows) adhere to the surfaces of the interna cells. ×10,000.

Fig. 26. Light micrograph of a follicle at T_{13}*. The base of the follicle is constricted and V-shaped. The apex of the follicle has thinned almost to the point of rupture. In some regions only a single layer of cells maintains the integrity of the follicle. ×64.

Fig. 27. Higher magnification of the apical wall from a T_{13}* follicle. The apical wall is comprised mainly of theca interna cells (*) at this time, although granulosa cells (G) are also observed. There are small areas completely lacking cells (arrows). ×840.

Fig. 28. Transmission electron micrograph of the apex of a T_{13}* follicle showing cells of the theca interna (*) and granulosa (G). A small space is present between adjacent interna cells (arrow). ×2,500.
Talbot, 1981a]. The formation of this constriction is correlated with: 1) contraction of smooth muscle cells in the basal theca externa, and 2) a change in the profile of the follicle from a low dome to a tall dome. Drugs that block contraction of smooth muscle cells block constriction of the basal follicle wall and inhibit ovulation [Martin and Talbot, 1981b]. We suggest that the constriction of the basal follicle wall allows the antrum to maintain a relatively constant pressure while the circumference of the follicle wall is increasing due to cell stretching. As the wall is stretched farther, holes are formed in the apex, which is the area most weakened by proteinases. This accounts for the observation that intrafollicular pressure remains constant or decreases slightly at the time the smooth muscle cells contract [Schroeder and Talbot, 1982]. In this model, the function of smooth muscle cells is not to increase the pressure within the follicle, a notion popular until 1963 [see Rouget, 1858; Espey and Lipner, 1963; Blandau and Rumery, 1963], but to maintain it against an increasingly leaky apical wall. Constant intrafollicular pressure is necessary for: 1) continued thinning of the apical follicle wall, and 2) evacuation of the oocyte-cumulus complex from the ruptured follicle [Espey and Lipner, 1963; Parr, 1975; Talbot, 1983b]. Similar changes in the shape of follicles in other species [Bolling et al, 1941; Goethe, 1967; Blandau, 1967] suggest that this mechanism is not restricted to hamsters. However, differences in the structure of preovulatory follicles among mammals suggests that variations in the relative importance of particular mechanisms involved in ovulation are to be expected.

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