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LUTEOLYSIS INDUCED BY PROSTAGLANDIN $F_{2\alpha}$ COMPARED WITH NATURAL LUTEOLYSIS IN THE EWE

AN ULTRASTRUCTURAL STUDY

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SUMMARY

Normal ovine luteolysis shows a series of transformations of luteal cell ultrastructures and ultimate disappearance of organnelles. In order to know if infusion of $PGF_{2\alpha}$ into the uterine vein at mid cycle induces the same luteolysis, an ultrastructural study was performed. It showed rapid disappearance of dense bodies and later appearance of numerous lipid droplets. In these experimental conditions, the blood supply seemed to be altered as shown among other observations by scattered dead cells in the corpus luteum. With previous observations and others it is thought that $PGF_{2\alpha}$ induces the release of dense bodies and inhibits the progesterone synthesis chain but does not mimic natural luteolysis in this experiment.

INTRODUCTION

Arguments in favour of an induction of natural luteolysis by $PGF_{2\alpha}$ have accumulated since 1970. The ultrastructural transformations occurring in the corpus luteum of the ewe at the end of the oestrous cycle have been described (BJERSING *et al.*, 1970 ; CORTEEL, 1975).

By infusing pharmacological doses of prostaglandin in the uterine vein, luteolysis may be obtained at mid-cycle. The purpose of this work is an attempt to answer the following question : « does luteolysis induced by exogenous prostaglandin mimic natural luteolysis? » To our knowledge, such a comparison has been made only once in the pseudopregnant rabbit (KOERING and KIRTON, 1973). The first ultrastructural study of the effect of $PGF_{2\alpha}$ was performed in the pseudo-pregnant

rat but without reference to normal luteolysis (OKAMURA *et al.*, 1972). In a study in the Rhesus-Monkey (KIRTON and KOERING, 1973) $PGF_{2\alpha}$ was administered too late in the cycle to allow a conclusive demonstration of induced luteolysis.

MATERIALS AND METHODS

On the 8th day of the cycle, $PGF_{2\alpha}$ (1) diluted in a M/15 phosphate buffer was infused in the uterine vein (C. L. side) at the rate of 50 µg per hour for two hours (THORBURN and NICOL, 1971).

When there was a corpus luteum in each ovary, hemicastration was carried out before perfusing. A sham operation was performed in a control ewe by infusing the same quantity of vehicle.

Ovaries were fixed by perfusing the ovarian artery with 4 p. 100 glutaraldehyde in a 0.1M phosphate buffer containing dextran (CORTEEL, 1973). They were dissected, post-fixed with osmium tetroxide, washed, dehydrated and embedded in epon. Sections were stained first with uranyl acetate, then with lead citrate solutions.

Observations will be dated in hours from the beginning of the infusion referred to as H_0.

RESULTS

A. — *Characteristics of most luteal cells*

1) Dense bodies all disapeared at the beginning of the infusion (Pl. 1 (2)). They were expelled rapidly from the cell as shown in a corpus luteum fixed within 30 minutes from H_0 and in which only a few dense bodies may be seen in the cytoplasm while they appear in great numbers in the intercellular spaces (Pl. 2 (2)). In some cells, they may be seen again at H_{18}. In natural luteolysis they disappear progressively.

2) The « elements » of the Golgi appartus (CHRISTENSEN and GILLIM, 1969) which produce whole or part of the dense bodies, remained dense to the electrons while in natural luteolysis, they seem to become enlarged and empty (Pl. 1 (1)).

3) Lipid droplets appeared in great numbers at H_{18} and their number was much larger at H_{24} and H_{48} (Pl. 2 (2) and 3 (1)) than that observed in natural luteolysis. Under induced luteolysis, there were also some exceptionally large lipid droplets (Pl. 2 (2)).

4) The rough endoplasmic reticulum did not develop after $PGF_{2\alpha}$ infusion while it became much more abundant in the cells at the very beginning of naturally occurring luteolysis (Pl. 1).

5) The smooth endoplasmic reticulum did not migrate to peripheral areas as in natural luteolysis.

6) Mitochondria were not modified following $PGF_{2\alpha}$ while they aggregated and seemed to fuse at times during natural luteolysis.

B. — *General characteristics of corpora lutea*

At H_{24} or H_{48}, there were dead cells scattered among the previously described cells (Pl. 3 (1)). In natural luteolysis there was no heterogeneity or mosaïc-like distribution.

(1) $PGF_{2\alpha}$ manufactured by Fuji Yakuhin Kogyo Cie, gracefully given by Dr WISHART, Searle lab. High Wycombe, England, to which we express our thanks.

The only corpus luteum studied at H_{72}, observed macroscopically in a longitudinal section, showed two distinct areas : the deep part was white (devoid of blood supply) and the upper part was still pale pink. Upon observation with the electron microscope, the cells of the samples belonging to the deeper area were regressed and shrunk, the intercellular spaces were large and filled with collagen. This part of the corpus luteum looked like the old corpus luteum on the first or second day of the following cycle (Pl. 3 (2)), but its cells had less myelin-like structures. The cells of the apical area had the appearance of luteal cells on the 11th day of the cycle with dense bodies persisting in the cells as well as being expelled (Pl. 3 (3)).

DISCUSSION

In this study we have used only one dose of $PGF_{2\alpha}$, only two hours of infusion and only one route of administration *i. e.* through the uterine vein. The differences observed suggest some hypotheses about the luteolytic action of exogenous $PGF_{2\alpha}$ when infused in the uterine vein.

1) It is known that high doses of this chemical compound have a vasoconstricting effect. Some authors have shown that in the ovary, the blood flow is reduced by lower pharmacological doses of infused $PGF_{2\alpha}$ (Review, PHARRIS and SHAW, 1974).

It is thought that a reduced blood supply may lead either to anoxia of a few cells or to an irregular distribution of prostaglandin in the tissue, resulting in the mosaïc-like luteal cell distribution or in the bipartition of the corpus luteum at H_{72}.

Another reason for thinking that $PGF_{2\alpha}$ infusion has altered the blood supply is the ineffective fixation of the smooth endoplasmic reticulum. As a general rule, the fixation used in this study as in previous ones preserves the tubular structure of the smooth endoplasmic reticulum while in the present case most of the samples have a vesicularised smooth endoplasmic reticulum, which would indicate a poor penetration of the fixative when administered by the arterial route.

2) The infusion of $PGF_{2\alpha}$ provokes almost immediately the expulsion of the dense bodies. Among several possible explanations two may be advanced : Pharmacological doses of $PGF_{2\alpha}$ lead to overall contraction of the smooth muscle cells of the ovary (COUTINHO and MAIA, 1971 ; VIRUTAMASEN *et al.*, 1972 ; DIAZ-INFANTE, 1974) which might provoke the release of the dense bodies. Myofibrils presumably present at the periphery of the luteal cells might react in the same manner as the smooth muscle cells to $PGF_{2\alpha}$ and favour release of dense bodies.

The parallelism previously described between peripheral plasma progesterone levels and numbers of cellular dense bodies (CORTEEL, 1973 ; GEMMELL *et al.*, 1974) is confirmed again since frequent enough progesterone assays after the beginning of PG infusion show an increase in the concentration of this hormone preceeding its decrease. The increase would correspond to release of dense bodies.

3) The ultrastructural transformations undergone under natural or provoked luteolysis do differ : under natural conditions the appearance of rough endoplasmic reticulum as early as the 12th day of the cycle leads to the suggestion of a modification of cell metabolism as observed during corpus luteum formation (CORTEEL, 1973). In the conditions prevailing here for provoked luteolysis, there is no increase

of the rough endoplasmic reticulum and there is an accumulation of lipid droplets in numbers equal to or larger than the accumulation occurring at the last day in the natural cycle. This suggests an inhibition of the progesterone synthesis chain by $PGF_{2\alpha}$. It is probably a reflection of the inhibition of cholesterol ester synthetase or cholesterol esterase studied by BEHRMAN *et al.*, (1971). In the normally functionning corpus luteum the accumulation of cholesterol precursors is avoided by the fast turn-over of progesterone synthesis and secretion. Therefore lipid droplets do not accumulate. The progesterone synthesis inhibition provoked by $PGF_{2\alpha}$ seems to be incomplete and/or reversible below a given threshold. This would explain the ineffectiveness of very low doses of $PGF_{2\alpha}$, the reappearance of dense bodies in some cells and even the resumption of activity of the apical part of the H_{72} corpus luteum without any apparent abnormality of the cell in this region. A low dose of exogenous or endogenous PG as in the D_{12} corpus luteum (GODING, 1973) would provoke a beginning of luteolysis *i. e.* a « functional » luteolysis (BAIRD, 1975) eventually reversible ; a high or cumulated doses as in $D_{12} + D_{15} + D_{16}$ of the cycle would provoke a « morphological » luteolysis that is irreversible with a large accumulation of lipid droplets, a transformation of all the ultrastructures and cell degeneration.

The observations reported here give rise to a discussion of the comparison between natural luteolysis and luteolysis provoked by $PGF_{2\alpha}$ but with the dose used and the route of administration chosen, we have not been able to mimic natural luteolysis.

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RÉSUMÉ

COMPARAISON DE LA LUTÉOLYSE NATURELLE ET DE LA LUTÉOLYSE INDUITE PAR LA PROSTAGLANDINE $F_{2\alpha}$ CHEZ LA BREBIS. ÉTUDE ULTRASTRUCTURALE

Une étude a été entreprise pour comparer les transformations des ultrastructures des corps jaunes de brebis dont la lutéolyse est induite par l'infusion de $PGF_{2\alpha}$ dans la veine utérine, à celles des corps jaunes en lutéolyse naturelle. Elle montre, dans le cas de la lutéolyse provoquée, la disparition brutale des corps denses et vingt-quatre heures plus tard l'apparition d'un nombre excessif de gouttelettes lipidiques. Il y a également une perturbation de la vascularisation traduite entre autres signes par des cellules mortes dispersées dans le corps jaune. Ces observations ainsi que d'autres décrites, font penser que la prostaglandine $F_{2\alpha}$ entraîne la libération des corps denses et bloque la chaîne de synthèse de progestérone mais elle ne mime pas la lutéolyse naturelle dans ces conditions expérimentales.

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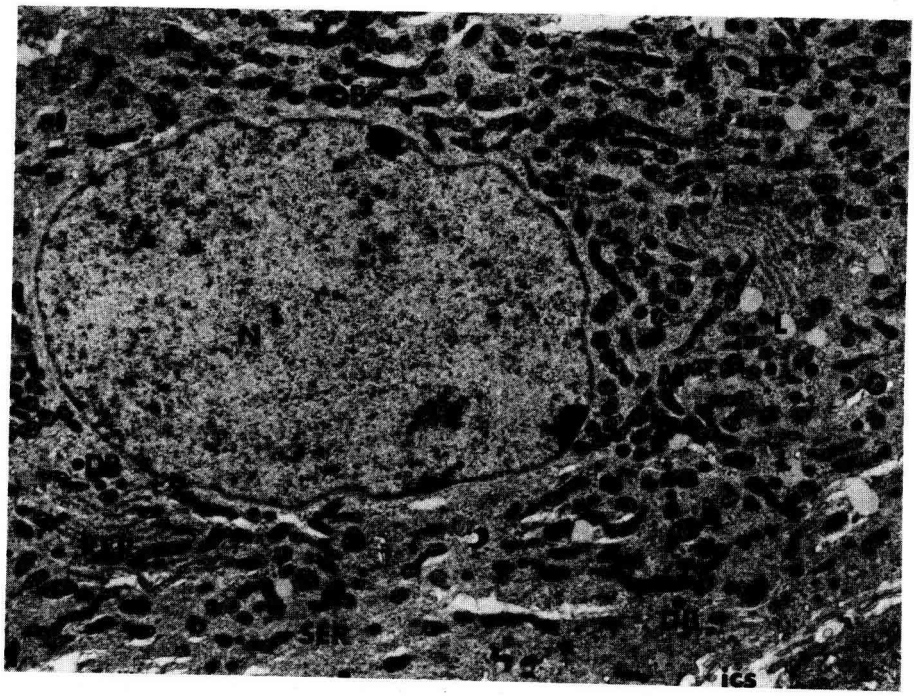
PLATE I

1. — *A luteal cell at the 12th day of the cycle*

At this time in natural conditions, stacks of Rough Endoplasmic Reticulum (RER) are more numerous, Mitochondria (M) more elongated, cisternae of the Golgi elements (G) more dilated than earlier. Dense Bodies (DB) are always present. This micrograph does not show peripheral area of Smooth Endoplasmic Reticulum (SER) which are segregating at this time, it shows Nucleus (N) and Lipid droplets (L) (× 7,050).

2. — *A luteal cell at* H_2

At the beginning of induced luteolysis, the luteal cells have a lack of dense bodies. They have collapsed Golgi elements (G) and the same round Mitochondria (M) as before infusion. The micrograph shows a nucleolus (n) in the Nucleus (N), vesicularised Smooth Endoplasmic Reticulum (SER), and normal Lipid droplets (L) (× 9,000).



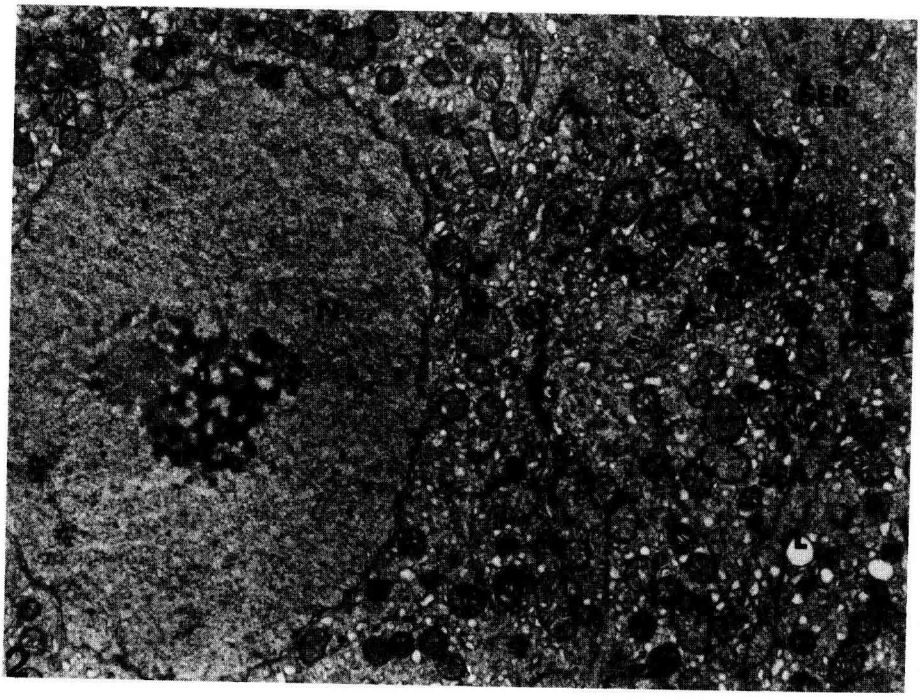


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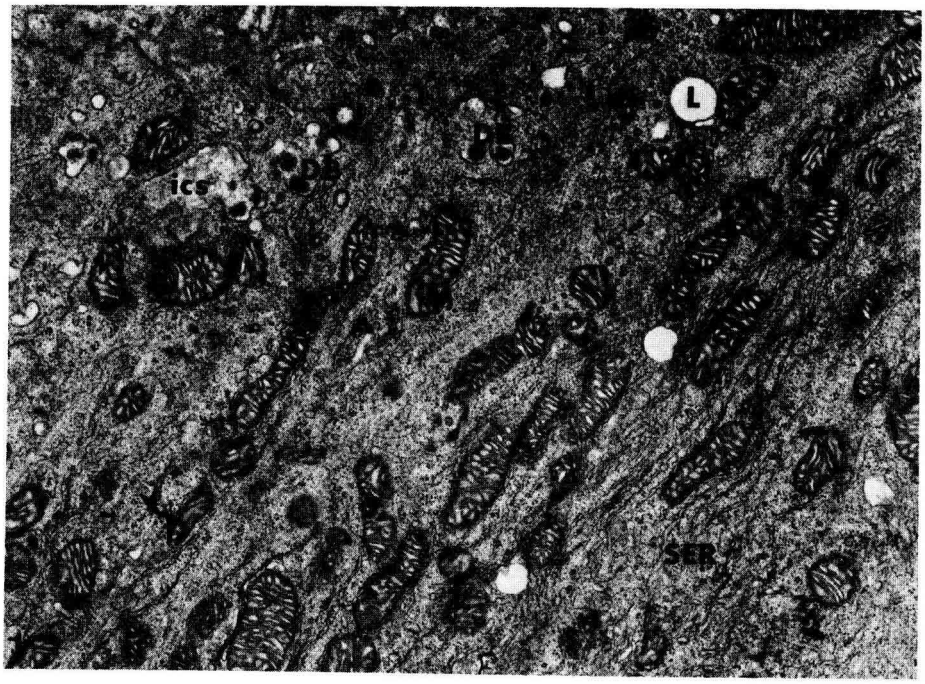
PLATE 2

1. — *A luteal cell in the* 30 *minutes after* H_0 : In intercellular spaces (i. c. s.) there are Dense Bodies (DB) while there are few in cytoplasm. In this cell Mitochondria (M) are a little elongated and Smooth Endoplasmic Reticulum (SER) is tubular. There are few Lipid droplets (L) (× 13,750).

2. — *Luteal cells at* H_{48} : this micrograph shows the great numbers of Lipid droplets (L) in the luteal cells and some exceptionally large or « giant » lipid droplets (gld). Dense Bodies (DB) appear again in a few cells, Golgi elements (G) are dilated in this micrograph (× 4,500).



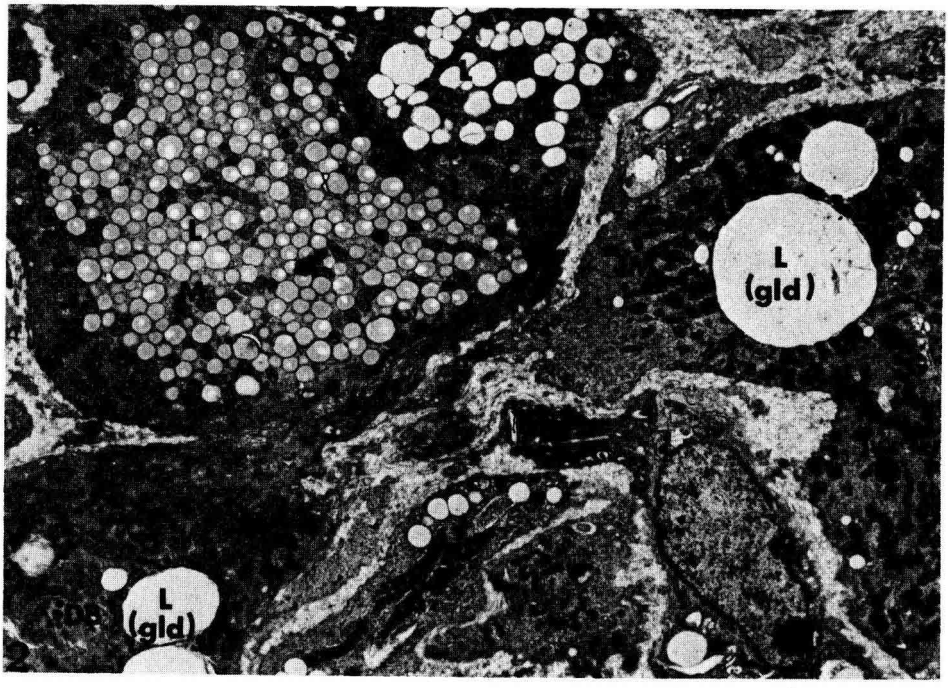


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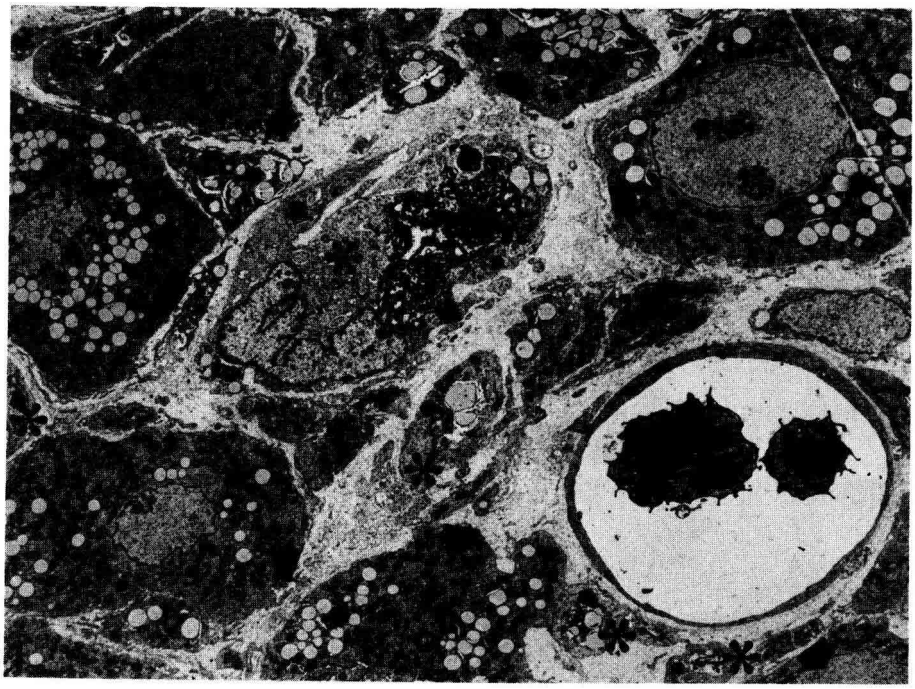
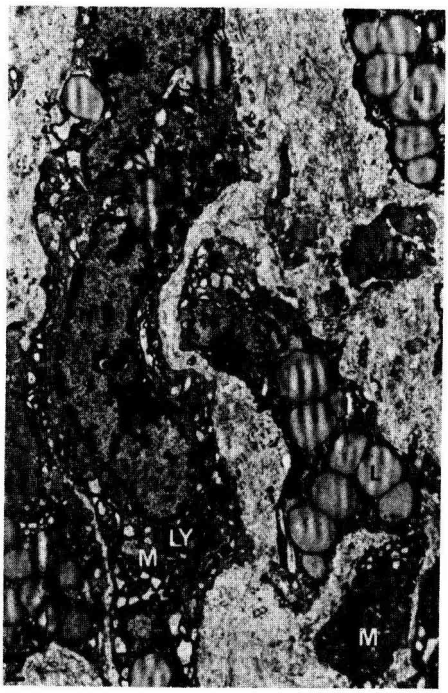
PLATE 3

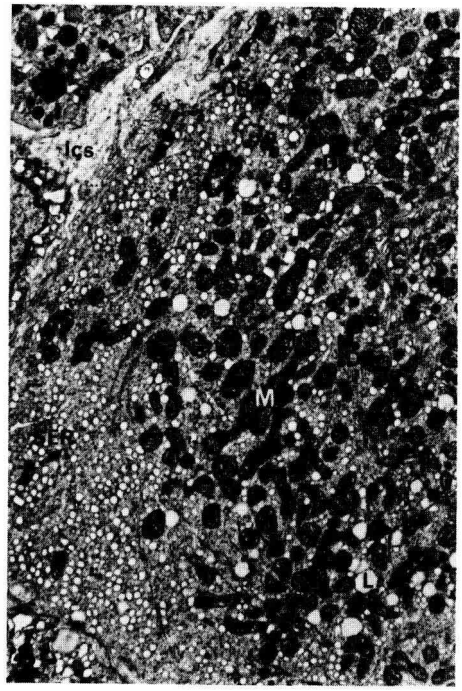
1. — *General view of a portion from a corpus luteum at* H_{24} : Dead cells (*) are scattered among cells modified by $PGF_{2\alpha}$ (→). A vessel is seen at the right lower (× 2,500).

2. — *Some cells from the deeper part of the corpus luteum at* H_{72} : These cells like the cells of old corpora lutea are shrunk, their Nucleus (N) is pycnotic they have numerous and large Lipid droplets (L) as well as Lysosomes (LY), and poor Mitochondria (M) (× 4,000).

3. — *Part of a cell from apical area of the corpus luteum at* H_{72} : This cell is like luteal cells at the 11th day of the cycle. It has Smooth Endoplasmic Reticulum (SER) in peripheral area, scattered Dense Bodies (DB), Lipids (L) and Mitochondria (M), and dense inner cisternae of Golgi elements (G). In the upper part, a Dense Body (DB) is seen in the intercellular space (ics) close to the cell : it may indicate cellular activity (× 5,450).







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