PERMEABILITY OF OVARIAN FOLLICLES TO ELECTRON-DENSE MACROMOLECULES

By

D. G. Cran, R. M. Moor and Mary F. Hay

ABSTRACT

Ferritin (m. w. 500 000) perfused into the ovarian arteries of sheep permeated the theca interna and was also found in the membrana granulosa of both non-atretic and atretic Graafian follicles. Colloidal gold (m. w. 1 000 000) similarly perfused, was found in the theca interna but not in the granulosa. The results are discussed in terms of the blood-follicle barrier.

The concept of a blood-liquor barrier in ovarian follicles, now more commonly called the "blood-follicle" barrier, was introduced by Zachariae (1958). More recently Zamboni (1974) in an ultrastructural study concluded that the follicle barrier has many characteristics of the blood-testis barrier (Fawcett et al. 1970), a barrier which almost completely excludes plasma proteins from the lumen of the seminiferous tubules (Tuck et al. 1970). The conclusions of Zamboni (1974) are, however, at variance with those of Shalgi et al. (1973) who postulated that the blood-follicle barrier behaves as a molecular sieve, allowing passage of plasma proteins into follicular fluid in inverse proportion to their molecular weight.

The purpose of this communication has been to investigate further the permeability of ovarian follicles particularly in relation to their state of atresia and functional activity. Accordingly, we have infused two electron-opaque

* Postal address: 307 Huntingdon Road, Cambridge CB 3 OJQ.
markers, ferritin and colloidal gold, into sheep, a species in which at any time only one or two follicles are steroidogenically active (Moor 1973), and about two-thirds of the other Graafian follicles are undergoing atresia (Brand & de Jong 1973)

MATERIALS AND METHODS

Four Welsh Mountain sheep, on the eighth day of the oestrous cycle, were anaesthetised with sodium pentobarbitone (22.5 mg/kg b. w.) and maintained under light anaesthesia with 1.8 % Fluothane (I.C.I.) in oxygen. A fine steel stilette (0.3 mm external diameter), with attached cannula, was inserted into each ovarian artery with the aid of a stereoscopic microscope (Diploscope, Carl Zeiss (Oberkochen) Ltd) and using surgical techniques designed to reduce to a minimum disruption of the normal ovarian blood flow. Electron-opaque markers were infused over the ensuing 10 min at a rate of 0.5 ml/min using a Harvard infusion pump (Harvard Apparatus Co., Millis, Mass.). A total of 40 mg ferritin (Serva, Heidelberg) suspended in physiological saline was infused into each ovary of three sheep; colloidal gold was similarly infused into the fourth animal. The cannulae were removed after infusion and the ovaries returned to the peritoneal cavity for 30 min to allow time for extra-capillary diffusion of the markers. Thereafter, all the perfused follicles over 2 mm diameter were dissected from the ovaries (for details see Moor 1973), measured and fixed intact in 4 % glutaraldehyde in 0.2 m collidine buffer (pH 7.2). Small portions of the follicle wall were processed and embedded in Epon using standard methods for electron microscopy. A total of 29 follicles was examined in the electron microscope for the presence of ferritin or gold in the capillaries, theca interna and membrana granulosa. The state of atresia of individual follicles was determined using 1 μm Epon sections stained with toluidine blue. In an additional experiment six non-atretic follicles obtained from untreated sheep, were cultured for 24 h in medium containing 3 mg/ml ferritin (for culture procedures see Moor 1973). The follicles were fixed, embedded, and examined in the electron microscope using the methods described above.

RESULTS

In all follicles perfused with ferritin in vivo, many particles were present in the capillaries (Fig. 1). It was clear, however, that the amount reaching them varied considerably both between and within follicles. A count of particle number/unit area indicated that at the time of fixation, the ratio of the number of particles within the capillaries to those in the inter-cellular space in the theca interna immediately outside was of the order of 3:2. It is likely, therefore, that the rate of diffusion from the capillaries was slow. Ferritin particles were present throughout the theca interna (Fig. 2) but with a lower frequency some distance from the capillaries.
Ferritin was also found in the membrana granulosa of both atretic and non-atretic follicles (Fig. 3). In the amount of ferritin reaching the granulosa compartment, no differences were detectable which could be related to either the size of the follicle or its state of atresia. In most cases the ferritin was present between the first three or four layers of cells (probably a reflection of the rate of diffusion) but in a few follicles it had also diffused into the follicular fluid. Ferritin also passed into the granulosa of cultured follicles; in all of them, particles were present in the antral cavity, but in all parts of the follicle the number of particles was considerably less than in perfused follicles.

Colloidal gold was found in the capillaries and throughout the theca interna (Fig. 4). However, in contrast to ferritin, gold was never found within the membrana granulosa even though particles were present along the outer border of the basal lamina.

**DISCUSSION**

The presence of ferritin particles within the membrana granulosa of non-atretic follicles is of particular note. In such follicles the basal lamina separating the granulosa from the theca was well defined but clearly it was not an impenetrable barrier to the passage of the ferritin molecules. The basal 6–8 layers of granulosa cells exhibited a regular well packed arrangement with a cell to cell spacing of approximately 20 nm. This compares with up to 60 nm in the theca interna. The ferritin used in this study had a molecular weight of about 500 000, a Stokes' radius of $65 \times 10^{-1}$ nm (*Harrison*, personal communication) and a measured diameter of 9 nm while the gold had a molecular weight of 1 000 000 and a measured diameter of 23 nm. Thus from a physical viewpoint while both ferritin and gold freely permeated the theca, only ferritin could pass into the granulosa. From our perfusion experiments we can find no support for the suggestion of *Zamboni* (1974) that the blood-follicle barrier is in any way comparable with the structural barrier found in the testicular tubules which is totally impermeable to even very small tracer molecules, for example lanthanum nitrate (*Dym & Fawcett* 1970), which has a molecular weight of 433.

Our results, on the other hand, are in good agreement with the ovarian studies of *Shalgi et al.* (1973) who used a quite different approach. From measurements of the concentrations of proteins on the two sides of the barrier, namely in the follicular fluid and in the blood, they postulated that the barrier was of a molecular sieve type. Their findings and ours both indicate that substances with a molecular weight of the order of 500 000 can enter the follicle, whereas larger macromolecules (mol. wt. 1 000 000) are unable to do so.

From the point of view of permeability however, the charge, size and shape of the molecules are probably more important than their molecular weight.
Fig. 1–4.
Data on the isoelectric point of ferritin (Drysdale & Kirby 1970), LH and FSH (Geschwind 1963) indicated that the net charge on ferritin and FSH are very similar and that on LH is much lower. Furthermore the Stokes' radius of ferritin (65 × 10^{-1} nm) is about twice that of the gonadotrophins (35 × 10^{-1} nm) (Reichert 1972). Therefore it seems unlikely that molecules as small as those of the gonadotrophins are excluded from the follicle by a purely structural barrier.

This is of particular relevance since it has been reported that the levels of LH and FSH in follicular fluid are always much lower than those in serum (McNatty et al. 1975; McNatty & Sawers 1975). If, as the authors suggest, selective exclusion of gonadotrophins forms the basis for the regulation of follicular function, it appears likely that some form of non-mechanical barrier must be operative.

ACKNOWLEDGMENT

We are deeply indebted to Dr. P. Johnson of the Colloid Science Laboratory, University of Cambridge, for a generous gift of colloidal gold.

REFERENCES


---

Fig. 1.
Part of a capillary in the theca interna demonstrating the presence of numerous ferritin particles. E, erythrocyte. × 64 000.

Fig. 2.
Intercellular space (IS) containing ferritin in the theca interna. × 64 000.

Fig. 3.
Intercellular space at the junction between three granulosa cells. Ferritin particles (arrowheads) are present. × 60 000.

Fig. 4.
Theca interna of an ovary perfused with colloidal gold. Gold particles are present within the intercellular spaces. × 34 000.

Received on July 21st, 1975.