Cytomorphological changes in the rabbit oviductal epithelium after human chorionic gonadotropin treatment

A.M. Bondi, M.G. Gabrielli, L. Marchetti, G. Materazzi and G. Menghi
Department of Molecular, Cellular and Animal Biology, University of Camerino, Camerino, Italy

Summary. An electron microscopic investigation was performed to examine the ultrastructural changes occurring in the rabbit oviductal epithelium after human chorionic gonadotropin (HCG) administration. Mainly, the non-ciliated secretory cells proved to be affected by the hormonal treatment which resulted in qualitative and quantitative modifications of the secretory patterns differently expressed in the ampulla and isthmus. Thus, morphological evidence of intense secretion was observed in both the oviduct regions at preovulatory stages. Following ovulation, timing of expression of active secretory patterns in the ampulla and isthmus correlated well with the rate of gamete transport and relative functional roles of the oviductal regions in the reproductive process. At present, HCG-induced changes concerning the ciliated cells seem to consist of the occurrence of secretory granules responsible for the appearance of "mixed cells".

Key words: Oviduct, Rabbit, HCG, Ultrastructure

Introduction

Based on its multiple involvements in the reproductive function, the mammalian oviduct has an obvious appeal for morphologists, physiologists and biochemists. Recently, increasing research interest has been addressed to identification and characterization of secretory products released by the oviductal epithelium. Their presence acquires special relevance in relation to physiological events occurring in the oviduct, such as gamete transport, capacitation and fertilization, and early embryonic cleavages (Menezo, 1979; Oliphant, 1986; Harper, 1988; Boice et al., 1990; Gerena and Killian, 1990; McDowell et al., 1993; Gandolfi, 1995; Jansen, 1995; Abe, 1996).

In view of the importance of carbohydrates as constituents of the oviducal secretory products (Kapur and Johnson, 1988; Robitaille et al., 1988; Kan et al., 1990), the characterization and distribution of glycoconjugates along the mammalian oviduct have been under our investigation for several years (Menghi et al., 1984, 1985a,b, 1986, 1988a,b, 1989a,b). We also investigated the effect of human chorionic gonadotropin (HCG) treatment on the glucidic components of the rabbit oviductal epithelium. The occurrence of bacterial hyaluronidase labile material on secretory cells of both ampulla and isthmus as well as the presence of strongly acidic glycoconponents, susceptible to chondroitinase and testicular hyaluronidase digestion, within the isthmus secretory cells were pointed out (Menghi et al., 1992). Additional research, concerned with lectin histochemistry, revealed that ampulla and isthmus undergo affinity changes to lectins at different stages of the hormonal treatment (Menghi et al., 1995).

Also structural changes in both muscle coat and lining epithelium of the oviductal wall during estrous cycle and after hormone treatment have been widely documented (Verhage and Brenner, 1975, 1976; West et al., 1976; Bajpai et al., 1977; Pathak et al., 1979a,b; Verhage et al., 1979; Odor et al., 1980, 1983; Fuentealba et al., 1988; Abe and Oikawa, 1993; Abe, 1996). More recently, the structural relationship of cumulus-oocyte complex and oviductal wall during transport of unfertilized cumulus-oocyte complexes in mice was investigated (Sato et al., 1995).

In order to get further insights into the morphological features of the oviduct function, we aimed here to examine, at electron microscope level, the fine structure of the oviductal epithelium of rabbits induced to ovulate by HCG treatment. Indeed, it is well established that, in rabbits, administration of gonadotropin (HCG, LH), as well as mating, has a rapid and pronounced effect on sex steroid secretion (Hilliard and Eaton, 1971; Spilman and Wilks, 1976; Weinberg and Pauerstein, 1980). On the other hand, the coexistence of estrogen and progesterone receptors has been demonstrated in both the secretory and ciliated cells and smooth muscle cells of the rabbit oviduct (Karbowskii et al., 1992), thus providing further evidence...
of their biological significance.

Finally, the ultrastructural investigation approach provides a means of visualizing in situ cytological events which, as they depend on macromolecular events, may contribute functional information. Comparison of corresponding cell types undergoing changes consequent to HCG administration can be easily made between the polarized secretory cells as well as the highly structured ciliated cells.

Materials and methods

Animals

Sexually mature, New Zealand strain, female rabbits weighing on average 3kg were maintained in individual cages at 22 °C, with a photoperiod of L:D = 14h:10h; water and feed were provided ad libitum. Tissues were obtained from 15 estrous rabbits injected with 100 IU of human chorionic gonadotropin (USB, Cleveland, Ohio), divided into five groups and sacrificed 9, 13, 18, 24, and 72 h after administration; 3 rabbits in estrus served as controls. The estrous condition was established by preliminary examination of vulva and vaginal smear and, subsequently, by direct inspection of ovaries. Animals were stunned and killed by cervical dislocation.

Tissue preparation

Oviducts were excised and placed in an ice-cold petri dish, lined with saline-saturated gauze, and rapidly dissected free of adipose and connective tissue. The oviduct was divided into ampulla and isthmus. Fimbriae, preampulla and ampullary-isthmic junction were eliminated.

Small fragments of ampulla and isthmus were treated as follows: a) fixation in 3% glutaraldehyde in phosphate buffer 0.1M, pH 7.4, for 3h at 4 °C, postfixation in 1% veronal acetate-buffered osmium tetroxide, ethanol dehydration and embedding in Durcupan ACM (Fluka, Buchs, Switzerland); b) fixation in half-strength Karnovsky fluid (2.5% glutaraldehyde-2% paraformaldehyde) for 3h at 4 °C, postfixation in 1% osmium tetroxide, dehydration and embedding as above. Thin sections were counterstained with uranyl acetate and lead citrate by an LKB Ultrostainer and observed with a 201C Philips transmission electron microscope.

Results

The best morphological results originated from samples fixed in half-strength Karnovsky fluid; accordingly, only these findings were documented by

Fig. 1. Ampulla. Estrous stage. Survey of the epithelial lining consisting of secretory (SC) and ciliated (CC) cells. Note the heterogeneous electron-density of granules within SC and the numerous mitochondria within CC. x 8,800
Rabbit oviduct after HCG injection

Both in the ampulla and isthmus of the rabbit oviduct, our observations on the control samples at estrous stage (Figs. 1, 2) confirmed the ultrastructural peculiarities of ciliated and non-ciliated cells, as previously described (Nilsson and Reinius, 1969; Jansen and Bajpal, 1982). Ciliated cells were found to be more abundant in the ampulla than in the isthmus. Their specific features were the high content in mitochondria, showing narrower diameter and denser matrix than those of the non-ciliated cells, as well as lack of secretory granules. At the luminal cell surfaces, cilia and scattered microvilli were observed. Non-ciliated secretory cells occurred at a higher proportion in the isthmus. They showed short microvilli at their luminal membranes and contained a lot of secretory granules, mainly restricted to the apical cell portions. The oviductal granules at estrous stage were typically heterogeneous both in size and degree of electron-density, and often exhibited an unhomogeneous matrix. This was more evident in the isthmic secretory cells whose granules, less dense than those in the ampulla, showed pale contents with peripheral dark spots (Fig. 2).

HCG administration produced appreciable effects at all the stages here examined.

Nine hours after HCG injection, a large occurrence of granules, similar to those of estrous samples, was observed in the ampulla secretory cells. Their compartmentalization to the apical cell portions appeared to be more pronounced than in controls and often resulted in apical protrusions, largely projecting into the lumen. Ciliated cells were characterized by high occurrence both of vacuoles, scattered in the supranuclear cytoplasm, and nuclei lined by nuclear envelopes with deep indentations (Fig. 3). In the isthmus, a more heterogeneous pattern of secretory cells was observed. In a few samples, the secretory cells were rich in electron-lucent granules located in apical stumpy protrusions. In other findings, secretory cells, with less or no luminal blebbing, contained few or no granules but were sometimes found to be connected finely to luminal fragments of cytoplasm filled with secretory granules. Ciliated cells could occasionally be detected in the isthmic epithelium at this stage. Cytoplasmic vacuoles and distinct nuclear aspect were common features of the isthmic epithelium, too (Fig. 4).

In the samples examined 13h after HCG treatment, marked changes were observed, more pronounced in the ampullary than in the isthmic epithelium. Morphological evidence of secretory process could be rarely found in the ampulla. Indeed, the non-ciliated cells, though largely represented, were lower and wider than previously, almost flat apically or with only low apical protrusions, often devoid of secretory granules. When present, the granules were located in the supranuclear cytoplasm. A higher number of granules was found in the isthmus epithelium. In both the regions, the granules appeared strongly electron-dense, as compared with

---

Fig. 2. Isthmus. Estrous stage. The secretory cells (SC) are filled with secretory granules which appear more or less electron-dense, with (arrows) or without peripheral dark spots. Ciliated cell (CC) cytoplasm contains numerous mitochondria. x 10,500

Fig. 3. Ampulla. 9h after HCG administration. Montage of the epithelial lining. Note the predominance of ciliated cells containing narrow mitochondria to which large cytoplasmic vacuoles are associated. The secretory cells exhibit apical blebs packed with moderately electron-dense granules. Nuclei, irregular in shape, are present, lined by nuclear envelopes with deep indentations. x 7,400

Fig. 4. Isthmus. 9h after HCG treatment. Some secretory cells show very irregular luminal profiles. Stumpy cytoplasmic protrusions, with heterogeneous secretory granules, differently project into the lumen and often appear to be broken off from the cell. Many swollen mitochondria (arrows), containing membrane residues, can be observed. x 7,800
Fig. 5. Ampulla. 13h after HCG administration. A secretory cell, intercalated between ciliated cells, contains only a few secretory granules which appear more electron-dense than previously. x 9,500

Fig. 6. Isthmus. 13h after HCG injection. More numerous secretory granules were found in the isthmus than in the ampulla non-ciliated cells. Intensification of granule density appear to be characteristic of this stage. The secretory granules maintain, however, a heterogeneous feature with and without peripheral spots inside them. Junctional complexes (arrows) and deep interdigitations of lateral membranes (paired arrows) can be observed. x 11,000

Fig. 7. Ampulla 13h after HCG treatment. Ultrastructural peculiarities relative to one of the oviductal samples examined. a. Nuclear degenerative process is visualized by the presence of large nuclear fragments clearly illustrating the peripheral clumping of the chromatin. b. Features indicative of nuclear and cytoplasmic degeneration, including a myelin-like body. x 12,000
Rabbit oviduct after HCG injection

Fig. 8. Ampulla. 18h after HCG administration. A montage of the epithelial lining with alternating secretory and ciliated cells. Ciliated cells usually have a less dense cytoplasmic matrix than secretory cells; however, it should be noted that, at this stage, secretory cells can also appear with a light or dark cytoplasm. Both secretory cell types contain strongly electron-dense granules. An interesting observation is the presence of secretory granules near the nucleus (arrows) or near the basal bodies (paired arrows) within the ciliated cell cytoplasm. x 7,600
Rabbit oviduct after HCG injection

those of the previous stage, though maintaining a higher density in the ampulla than in the isthmus. Ciliated cells of both the oviduct portions proved to be unmodified (Figs 5, 6). One of the samples examined at 13h after HCG treatment showed a quite unusual morphological pattern: in both the oviduct regions, undifferentiated cells were found, lacking in both secretory granules and well structured cilia. In addition, features indicative of degenerative changes could be largely observed. They included nuclei with deep membrane folds within the nucleoplasm and large patches of condensed chromatin along the nuclear envelope as well as fragmentation of nuclei (Fig. 7a,b).

Eighteen hours after HCG administration, the strongly electron-dense granules were found in high number in the ampullary secretory cells, mainly concentrated in the apical cell protrusions projecting into the lumen. In the ampulla, a specific feature at this stage of treatment was the occurrence of “dark” and “light” secretory cells. In some cases, “dark” cells displayed morphological peculiarities of both secretory and ciliated cells. Also, typical ciliated cells were seen which contained secretory granules (Fig. 8). In the isthmus, the secretory cells were similarly filled with a lot of granules located at the supranuclear cell portions. The granules contained less electron-dense matrix than those of the ampulla, with peripheral dark patches. Marked evidence of secretion derived from finding some secretory cells which were discharging into the lumen a lot of heterogeneous granules, dipped into fragments of apical cytoplasm (Fig. 9). At the isthmus level, we were unable to identify different secretory cell types.

Examination of samples 24h after HCG injection showed no appreciable structural modifications of the oviductal ciliated and non-ciliated cells, compared to the previous stage, except for a more reduced occurrence of granules in the secretory cells of the ampulla.

Seventy-two hours after HCG administration, the non-ciliated cells of the ampulla displayed sparse secretory granules which could also be occasionally detected in ciliated cells (Fig. 10a,b). In the isthmic region, heterogeneous secretory cells were identified, showing markedly different types of secretory granules (Fig. 11a). Golgi complexes were very well developed both in secretory and ciliated cells. Some ciliated cells showed secretory granules containing lens-shaped spots (Fig. 11b).

Discussion

Marked ultrastructural changes have been here documented to occur in the oviductal epithelium of rabbits induced to ovulate by HCG treatment.

Our investigation was carried out at different stages between 9 and 72h after the hormone injection; indeed, Harper (1965) reported no appreciable ovum transport-

Fig. 9. Isthmus. 18h after HCG injection. Intense secretory activity is illustrated by massive discharging into the lumen of large cytoplasmic portions filled with secretory granules. The picture is highly suggestive of an apocrine mode of secretion. Note the heterogeneous electron-density of the secretory granules, often containing denser areas. x 7,600
associated modifications in the oviduct epithelium between 60h and 15d of pseudopregnancy.

The most relevant effects, discussed below, refer to morphological patterns of the secretory cells since, at the moment, only slight modifications can be convincingly demonstrated in the ciliated cells. Thus, 9h after the hormonal treatment, morphological evidence has been found in favour of an active secretory process occurring

Fig. 10. Ampulla. 72h after HCG treatment. 

a. The secretory cells show little evidence of any secretory activity, as indicated by the presence of a few remnant secretory granules in the cell apical tips. x 6,800.

b. The extensive profiles of rough endoplasmic reticulum and Golgi complexes (G) suggest, however, some synthetic activity in the non-ciliated cells. Junctional complexes (arrows). x 18,000

Fig. 11. Isthmus. 72h after HCG administration.

a. Note the large occurrence of different types of secretory cells packed with heterogeneous, moderately electron-dense granules. x 5,800.

b. A ciliated cell exhibits unhomogeneous secretory granules in the supranuclear cytoplasm. x 20,000
Rabbit oviduct after HCG injection

in the ampulla and, more evidently, in the isthmus. The package of the numerous secretory granules into pedunculate portions of the ampullary non-ciliated cells, which strongly extruded into the lumen, is a specific feature of the ampulla at this stage, fully consistent with previous results of immunocytochemical studies (Oliphant et al., 1984). In the isthmus, the finding of granule groups, surrounded only by a thin rim of cytoplasm, which lie free into the lumen or are still connected by a narrow stalk to the apical surface of the non-ciliated cells, raises the possibility of an apocrine mode of secretion, as also supported by findings at following stages. A similar mode of release of secretory products in the oviduct epithelium has been elsewhere suggested (Odor et al., 1983; Jansen, 1995; Odor and Augustine, 1995).

On the basis of similarities in cell and granule features of the morphological patterns, the secretory process at 9h after HCG administration seems likely to represent an enhanced expression of similar secretory activity already occurring in the oviduct epithelium during estrus. The increased levels of estrogen, which reach peak concentration approximately 6h after HCG injection (Weinberg and Pauerstein, 1980), may be responsible for modulating such a secretory process. Accordingly, steroid-modulated and cycle-specific changes in rabbit oviductal secretory proteins, characterized as sulphated oviductal glycoproteins, have been described (Erickson-Lawrence et al., 1989a,b).

A relevant ultrastructural aspect of the induced secretion is represented by the morphological differences between the secretory granules of the ampulla and isthmus, also previously observed during estrus (Jansen and Bajpai, 1982; Jansen, 1995) and recently reviewed (Abe, 1996). When considering that additional differences between the oviductal regions have been pointed out by differential patterns both of glucidic component (Menghi et al., 1992, 1995) and specific glycoprotein (Oliphant et al., 1984) distribution, regional differences in the composition of oviductal secretions may be proposed. Furthermore, morphological evidence of more pronounced secretion in the isthmus than in the ampulla after HCG treatment has been here provided. The above findings, taken together, support the hypothesis of a differential distribution and/or regulation of steroid receptors along the rabbit oviduct, probably related to differential involvements of the oviductal regions in the earliest events of the reproductive process. At this time, indeed, while the ampulla has still to receive oocytes, the isthmus would be more directly involved in participating in sperm migration.

A somewhat astonishing finding in some of the samples examined at 9h after HCG treatment was the large occurrence of cytoplasmic vacuoles. Although they were detected at both the oviductal regions, a coupling seemed to exist, in the isthmus, between morphological expression of secretory activity and number of vacuoles in the non-ciliated cells. In the ampullary epithelium, conversely, the cytoplasmic vacuoles were almost always restricted to the ciliated cells. On the basis both of their intracellular localization, closely associated to mitochondria, and identification of matrix residues and membrane fragments inside them, we think that the cytoplasmic vacuoles may represent mitochondria undergoing large-amplitude swelling. We exclude, for two reasons, that the presumptive swelling of mitochondria in our study is due to artifactual effects of bad fixation: the coexistence of both normal and swollen mitochondria in the same cell and the failure to detect similar ultrastructural alterations in morphological patterns other than those relative to 9h after HCG administration. Similar alterations of mitochondria have been recently described in rat embryos exposed to diabetic environment both in vivo and in vitro during organogenesis (Yang et al., 1995). A relation has been proposed between oxidative stress, deriving from a relative overload of substrates, and peroxidative destruction of the mitochondrial membrane integrity resulting in the swelling of mitochondria (Vladimirov et al., 1980; Bindoli, 1988). On the other hand, mitochondria may also act as a source of reactive oxygen species to oxidatively impair other cellular components, such as DNA and cellular membranes (Hruszkewycz, 1988). It is of interest that several investigators have observed mitochondrial swelling in association with cell injury and cell death (Laitho and Trump, 1975; Verhage et al., 1984).

Some other results, occasionally found at 9h after HCG injection, are difficult to explain. They concern the finding, mainly in the ampulla epithelium, of regressive changes which displayed more pronounced expression at 13h after hormonal treatment, though in only one animal examined. Among the ultrastructural features indicative of degenerative processes here observed, fragmentation of nuclei has also been described in the baboon and human oviductal epithelium as early stages of apoptosis (Verhage et al., 1979, 1990). Cell death by apoptosis was reported to be a characteristic feature of the steroid-withdrawn and progesterone-treated oviduct (Verhage et al., 1984; Brenner and Maslar, 1988). In our study, both the above hormonal conditions pertained. Indeed, a sharp decrease of sex steroids up to undetectable levels does occur at ovulation time; in addition, because of the earlier withdrawal of estrogen, progesterone remains for some time the only hormonal factor acting on the oviductal epithelium. On the other hand, progesterone treatment, in the presence or absence of estradiol, is well known to cause atrophy, regression and loss of secretory activity in the oviductal epithelium (Odor et al., 1983; Verhage et al., 1990; O'Day-Bowman et al., 1995). Nevertheless, because of both the very short time in which such modifications would occur and the uniqueness of the morphological evidence of regression which has emerged here, it is reasonable to be cautious when generalizing the present results until additional, more aimed investigations are made. Finally, we cannot rule out, at the moment, that apparent discrepancies in our findings may depend on the morphological patterns...
belonging either to basal or apical regions of the epithelial folds which might display simultaneously different morphofunctional behaviour.

Apart from the presently unconvincing findings on regressive modulations, it is however noticeable that, in all the other samples examined at ovulation time, the most striking feature was a deeply modified morphology of the oviductal epithelium. Indeed, probably correlating with the presence of the newly released oocytes which reach the ampullary-isthmus junction within minutes after ovulation, the structural patterns of the ampullary non-ciliated cells documented a severe decline of secretory activity at 13h after HCG treatment. When present, the granules proved, moreover, to differ markedly from the preovulatory ones: in all the samples examined, they appeared homogeneous and strongly electron-dense, thus fairly resembling serous-type granules (Jansen, 1995). By comparing the above findings with those relative to the previous stages, we might conclude that ovulation not only coincides with a lowering in the secretion rate of the ampulla epithelium but also modulates the transition from a prevalently mucous to a serous type of secretion. Similar modifications from lucent to dense appearance were observed in the secretory granules of the isthmus, though to a lesser degree than in the ampulla.

Because of the nearly undetectable levels of serum steroids at ovulation and, at least, in the next 30h (Spilman and Wilks, 1976), it is possible that the distinct secretory patterns at this time are due to modulators other than estrogen and progesterone or, more likely, represent the expression of a constitutive secretory pathway. Possible participation of the cumulus-oocyte complex in modulating directly or indirectly the rabbit oviduct secretory status may, at present, only be speculated.

Quantitative modulations of the non-ciliated cell activity were observed at the successive stages examined. At 18h after HCG treatment, important morphological correlates of increased secretory activity in the ampulla epithelium consisted of larger occurrence of the homogeneously electron-dense granules in different, unusual types of secretory cells, such as “dark” and “light” non-ciliated cells. Sometimes, granules were also present in otherwise typical ciliated cells, a finding which does not seem unique to our work. Similar observations have indeed been reported for estrous and estradiol-treated rabbits (Merchant, 1969; Odor and Blandau, 1988; Odor et al., 1989) and, recently, for baboon (Odor and Augustine, 1995), while secretory cells exhibiting ciliogenesis have been described in the oviductal epithelium of newborn hamsters treated with estradiol (Abe and Oikawa, 1993). In the quail oviduct, the appearance of “mixed cells” was induced by the administration of estradiol and progesterone (Sandoz and Boisvieux-Ulrich, 1976; Sandoz et al., 1976). The possible conversion of ciliated cells into secretory cells and vice versa in the oviductal epithelium was suggested (Sandoz et al., 1976).

Significant changes in the ampulla morphological patterns were not found until 72h after HCG treatment, when most of the secretory cells contained no or only sparse granules at their apical portions, and little or no evidence of secretory activity could be recognized. At the same time, ultrastructural features suggestive of granule synthesis and secretion were markedly present in isthmus non-ciliated cells. We suggest that the increased levels of serum progesterone, following corpora lutea development, may impair the expression of secretory activity in the ampulla epithelium. In accordance with the proposal of a steroid-responsive gradient throughout the oviduct, the isthmus epithelium should be unaffected by progesterone and, indeed, it did continue to express its constitutive secretory pathway, in fair agreement with the rate of ovum transport.

In summary, some interesting points emerge from our ultrastructural investigation. In particular, qualitative and quantitative modulations of the oviduct secretory activity were visualized at different times after HCG treatment, fairly consistent with both the relative changes in the serum sex steroids and their known effects on the oviductal epithelium. Studies on the composition of the rabbit oviductal fluid contribute additional data for the interpretation of the present morphological findings. Indeed, several secretory products have been identified which vary during the estrous cycle and after hormone treatment (Kay and Feigelson, 1972; Oliphant and Ross, 1982; Oliphant et al., 1984; Hyde and Black, 1986; Abe, 1996). Also, the distributional pattern of glycoconjugates in the oviductal epithelium of pseudopregnant rabbits, investigated both by conventional methods of carbohydrate histochemistry (Menghi et al., 1992) and HRP-conjugated lectin labelling (Menghi et al., 1995), proved to depend specifically on the time elapsing from HCG injection. In particular, between 9 and 13h after the experimental treatment, modifications of the estrous histochemical patterns accounted for the progressive decrease up to loss of some secretory glycoconjugates in the ampulla and isthmus epithelium, in agreement with the declining expression of the relative secretory patterns. Following the ovulation time, increase of distinct sialylated glycoconjugates in the isthmus epithelium correlates well with the appearance of specific features in the isthmic secretory granules.

Collectively, the temporal and regional specificity of the structural changes here reported substantiates the concept of differential segmental roles of the oviduct in the reproductive process. It seems, indeed, reasonable, according to Kapur and Johnson (1988), that events hosted in the ampulla, such as egg-sperm interaction, cumulus cell dispersal, and fertilization, may benefit from epithelial secretions different from those important for events occurring in the isthmus, such as sperm migration, embryo transport and cleavage.
Rabbit oviduct after HCG injection

Acknowledgements: We express our gratitude to Miss S. Cammerton and Mr S. Riccioni for technical assistance. This work was supported by the Italian M.U.R.S.T. (40% and 60%) grants.

References


Harper M.J.K. (1965). Transport of eggs in cumulus through the ampulla of the rabbit oviduct in relation to day of pseudopregnancy. Endocrinology 77, 114-123.


Rabbit oviduct after HCG injection


Accepted August 14, 1996