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EPIDERMAL GROWTH FACTOR (EGF) IN THE QUAIL OVARY

by

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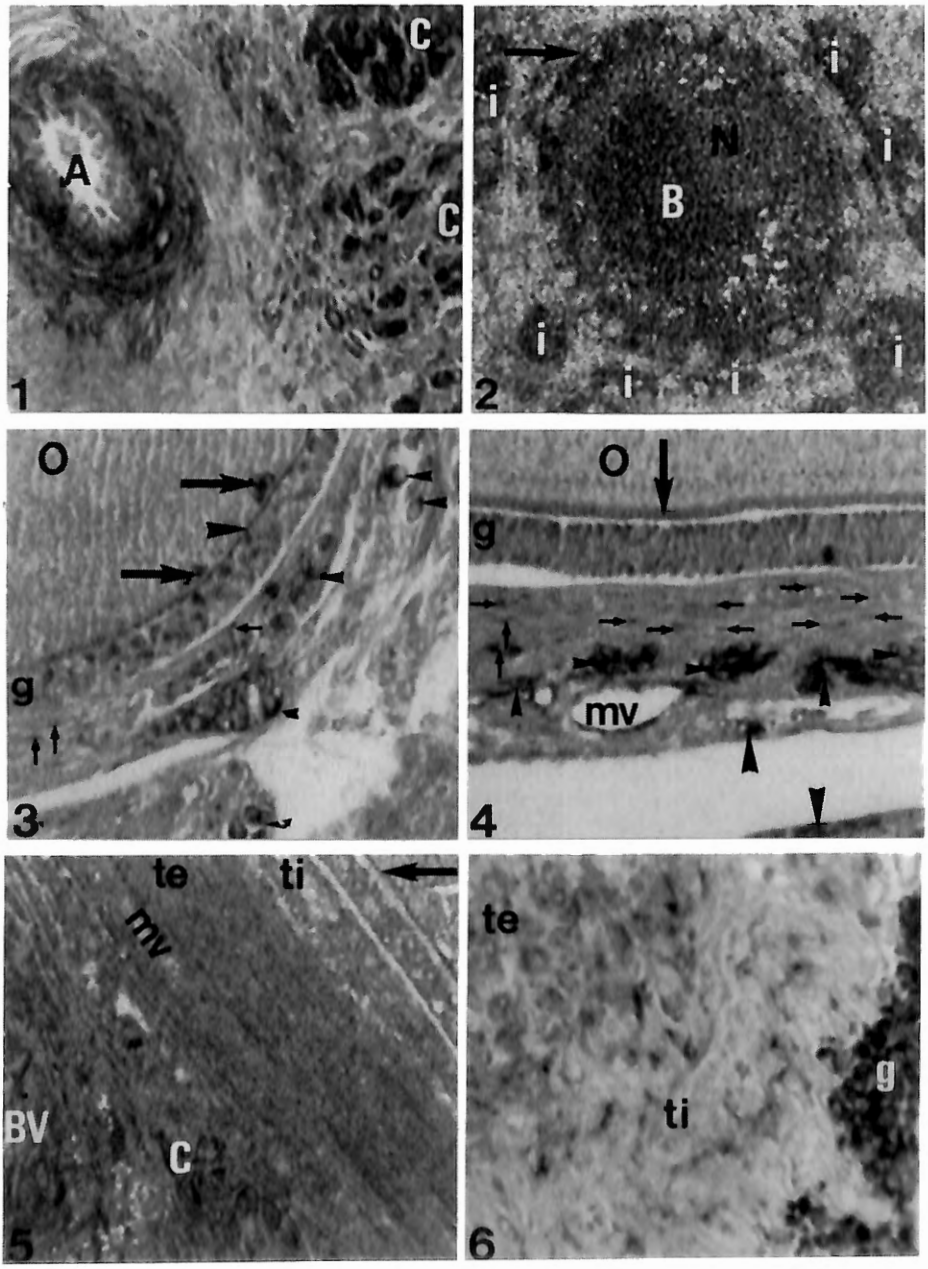
SUMMARY

Using immunocytochemical techniques for the localization of epidermal growth factor (EGF) in the quail ovary, we observed a major amount of EGF in smooth muscle cells of blood vessels and of chordae, in interstitial cells, in granulosa cells of small follicles, in the Balbiani complex of prelampbrush oocytes, in nerve cells, and in the cells of granulosa and theca externa of postovulatory follicles. In general, the staining intensity of granulosa cells decreased during folliculogenesis, and increased after ovulation. In the oocyte, immunoreactivity was shifted from the Balbiani complex to the zona radiata during development. These results support the hypothesis that EGF primarily acts on less differentiated follicles. It is also suggested that EGF can modulate ovarian contractile processes.

Keywords : Epidermal growth factor, quail, ovary.

INTRODUCTION

Ovarian folliculogenesis is a dynamic and complex process, which is regulated by the interplay of several factors, including growth factors. The present study focuses on EGF, a small single-chain polypeptide (MW 6043) originally detected during a search for nerve growth-promoting factors in the mouse (LEVI-MONTALCINI and COHEN, 1960). It is best recognized for its mitogenic activity. Distribution and role of EGF in the ovary have mainly been investigated in mammals (reviewed by MULHERON and SCHOMBERG, 1993). In this study, we have localized EGF in the quail ovary.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MATERIAL AND METHODS

Female adult Japanese quails (*Coturnix coturnix japonica L.*) were killed by decapitation. Their ovaries were fixed in EACH fixative (PERRY-O'KEEFE *et al.*, 1990), Carnoy's fluid, methacarn (PUCHTLER *et al.*, 1970), or Bouin's fixative. After tissue processing, the tissue blocks were embedded in paraffin or in ImmunoBed™ (Polysciences Inc., Warrington, PA), a plastic embedding medium. We used commercially available antibodies : rabbit polyclonal antibodies directed against mouse EGF (SIGMA Chemical Co., St.-Louis, MO) or against human EGF (Santa Cruz Biotechnology Inc., Santa Cruz, PA), and a mouse monoclonal antibody directed against human EGF (Oncogene Science Inc., Uniondale, NY). EGF was localized in paraffin sections using the unlabelled antibody peroxidase-anti-peroxidase technique. Immunoreactivity (IR) was revealed by the method of GRAHAM AND KARNOVSKY (1966) or of SHU *et al.* (1988). In semi-thin plastic sections, EGF was localized using the immunogold-silver staining procedure. The oocytes and follicles were classified in stages according to CALLEBAUT (1973).

Method specificity was controlled by incubation with primary antibody preabsorbed with recombinant human EGF (Santa Cruz Biotechnology Inc.). Antibody specificity was tested using immunoblotting.

RESULTS

The three primary antibodies yielded the same results. IR was predominantly found in smooth muscle cells of blood vessels and of chordae (Fig. 1), in granulosa cells of prelampbrush follicles (Fig. 2), in interstitial cells (Figs 2-4), in nerve cells, in the Balbiani complex of prelampbrush oocytes (Fig. 2), in the zona radiata of developing oocytes (Figs 4, 5), and in granulosa cells and cells of the theca externa of postovulatory follicles (Fig. 6). The staining intensity of granulosa cells decreased during follicular maturation, and increased after ovulation. In the oocyte, IR was shifted from the Balbiani complex to the zona radiata during oocyte development. IR was also detected in some cells of atretic follicles, of the superficial epithelium, of the tunica albuginea, and of the theca of developing follicles (Figs 3-5). In one

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Figs 1-6. — Micrographs of the quail ovary (× 446). — 1. Medulla : IR in smooth muscle cells of blood vessels (A : artery) and of chordae (C). — 2. Cortex : IR in granulosa (arrow) of prelampbrush follicles, in interstitial cells (i), and in the Balbiani complex (B). N : nucleus. — 3. Early lampbrush follicle : IR in interstitial cells (small arrowheads), in thecal cells (small arrows), in granulosa (g), in cortical ooplasm (arrowhead), and in cells (arrows) engulfed by the oocyte (O). — 4. Stalked follicle : IR in zona radiata (arrow), in granulosa, in thecal cells (small arrows), in cell clusters in thecal periphery (small arrowheads), in smooth muscle cells of the middle venous layer (mv), and in branches of chordae (arrowheads). — 5. Mature preovulatory follicle : IR in wall of blood vessels (BV), in chordae, in thecal cells, and in zona radiata (arrow). ti : theca interna ; te : theca externa. — 6. Postovulatory follicle : IR in granulosa cells, in cells of theca interna, and in contracted cells of theca externa.

of the ovaries, positively stained engulfed cells in the ooplasm of developing oocytes, were observed (Fig. 3).

DISCUSSION

In a previous study, ONAGBESAN *et al.* (1993) demonstrated the presence of an EGF-like peptide in the theca of preovulatory follicles of the hen. In the present study, we revealed that EGF can be found in several ovarian cell types. We noticed that in some mammals, EGF is also detected in interstitial cells (GÖRITZ *et al.*, 1994 ; KANNO *et al.*, 1994), in thecal and granulosa cells (ROY and GREENWALD, 1990 ; MARUO *et al.*, 1993 ; GÖRITZ *et al.*, 1994 ; KANNO *et al.*, 1994), and in oocytes (KASSELBERG *et al.*, 1985 ; ROY AND GREENWALD, 1990 ; MARUO *et al.*, 1993). Moreover, ROY and GREENWALD (1990) showed in the hamster that staining intensity in granulosa cells fades during folliculogenesis.

A few *in vitro* experiments, examining the role of EGF in the hen ovary, were performed (reviewed by PEDDIE *et al.*, 1993). It was found that EGF stimulates proliferation of the granulosa and theca. EGF attenuates gonadotropin action and inhibits steroidogenesis. It prevents premature differentiation of granulosa cells. The effects of EGF are decreasing with follicular maturity.

These results and data support the hypotheses that EGF primarily acts on less differentiated follicles (MULHERON and SCHOMBERG, 1993), and that EGF potentially produced by thecal interstitial cells (paracrine) or granulosa cells (autocrine) acts on granulosa cells *in vivo* (JOHNSON, 1994).

Relying on the presence of EGF in ovarian smooth muscle cells, abundantly present in the avian ovary (VAN NASSAUW *et al.*, 1994), the existence of ovarian contractility (VAN NASSAUW *et al.*, 1994), and the data concerning contractile effects of EGF (HOLLENBERG *et al.*, 1989 ; PETITCLERC *et al.*, 1994), it is suggested that EGF may modulate ovarian contractile processes. Finally, the presence of positively stained engulfed cells in the ooplasm of developing oocytes is a rare event, the meaning of which remains unclear.

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