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Immunohistochemical localization of oestrogen receptors and progesterone receptors in the human ovary throughout the menstrual cycle

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Summary. Immunohistochemistry was used to determine the distribution of oestrogen receptors (ER) and progesterone receptors (PR) in the human ovary during folliculogenesis. Primordial and preantral follicles did not contain ER or PR. The granulosa cells of antral follicles had ER, but negligible PR, before the LH surge. In contrast, at the time of LH surge, these cells of the dominant follicle contained PR, but not ER. On the other hand, granulosa cells of the non-dominant follicles had ER, but not PR. After ovulation, the PR persisted in the luteinized granulosa cells and in the corpus luteum during early pregnancy. The theca interna and surrounding stromal cells were ER-negative and PR-positive throughout the menstrual cycle. Thus, the results show that ER and PR are not expressed simultaneously in the granulosa cells, the thecal cells, or the stromal cells during folliculogenesis. Mechanisms controlling the expression of steroid receptors during the normal menstrual cycle and in early pregnancy are discussed.

Key words: Oestrogen receptor – Progesterone receptor – Human ovary – Immunohistochemistry

Introduction

Ovarian functions in various species are regulated by ovarian steroids. Oestrogen and progesterone both may be important local regulators of follicle development (Richards 1980). Oestradiol treatment increases the binding of follicle-stimulating hormone (FSH) to rat granulosa cells (Richards et al. 1979), augments FSH-induced aromatase activity, and induces the formation of luteinizing hormone (LH) receptors on rat granulosa cells (Adashi and Hsueh 1982). This steroid reportedly has a luteotropic effect on the rat and rabbit corpus luteum (Keyes et al. 1983), but a luteolytic effect on the human (William et al. 1979), ewe (Cook et al. 1974) and monkey (Stouffer et al. 1977) corpus luteum. In contrast, progesterone inhibits FSH-stimulated oestrogen production and LH receptor formation on rat granulosa cells (Schreiber et al. 1982). It also suppresses follicular maturation in the hamster (Kim and Greenwald 1987). Progesterone may also have an autocrine luteotropic effect on the human corpus luteum (Rothchild 1981). However, it is not clear whether ovarian steroids act directly on ovarian tissues through their steroid receptors, or indirectly via the hypothalamus and pituitary gland.

Classical radioligand binding techniques have demonstrated oestrogen receptors (ER) and progesterone receptors (PR) in the ovary, suggesting that oestrogen and progesterone may have various autocrine and/or paracrine functions through these specific receptors (Jacobs et al. 1980; Kudolo et al. 1984). However, the precise localization of the ER and PR in the ovary have not been determined.

The relatively recent production of monoclonal antibodies against a specific ER protein (Greene and Jensen 1982) and PR protein (Logeat et al. 1983; Greene et al. 1988) has provided a new immunohistochemical approach for localization of ER and PR in normal and neoplastic tissues of the breast, endometrium and ovary. This method has been used recently to characterize the distribution of ER and PR in the monkey ovary (Hild-Petito et al. 1988). The present study extends this methodology to the human ovary at different stages of the menstrual cycle.

Materials and methods

Either total or biopsy specimens of the human ovary were obtained from 42 women with informed consent at laparotomy for medical indications. The donors (age 32–49 years) had regular menstrual cycles (28–30 days) at surgery, except for four pregnant women. None of them had received exogenous hormones for at least two cycles prior to surgery. The stage of their menstrual cycle at laparotomy was determined by the combination of the following criteria: (1) the days since the onset of the last menstrual period, (2) plasma levels of LH, oestradiol, and progesterone, and (3) histolog-

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Table 1. Oestrogen receptor (ER) and progesterone receptor (PR) expression in follicular cells

Follicular state	Granulosa cells		Theca interna cells		Surrounding stromal cells	
	ER	PR	ER	PR	ER	PR
Primordial ($n=9$)	−	−	−	−	−	−~+
Preantral ($n=6$)	−	−	−	−	−	−~+
Antral ($n=3$)	+~++	−	−	+	−	+~++
Preovulatory (before LH surge) ($n=5$)	++++	−~+	−	+	−	+~++
Preovulatory (during LH surge) ($n=2$)	−~+	+++	−	+~++	−	+~++

Grading by intensity; −, not detectable; +, weak but definitely detectable; ++, strong staining; +++, very intense staining; ++++, most intense staining

ical examination of the endometrium. Endometrial tissues were obtained either from the extirpated uteri or from endometrial biopsies, and the day of the menstrual cycle was estimated histologically according to the method of Noyes et al. (1950). Ovaries were grouped into six categories: early follicular, midfollicular, late follicular, early luteal, midluteal and late luteal phases of the menstrual cycle. On histological examination, individual follices were categorized as primordial, preantral, antral or preovulatory but before the LH surge, preovulatory at the time of the LH surge, and atretic follicles. Corpora lutea were grouped into four categories: early luteal (14–18 days into the menstrual cycle), midluteal (19–24 days into the menstrual cycle), late luteal (25–28 days into the menstrual cycle) and corpus albicans. In addition, several corpora lutea were obtained from patients at 9 weeks of missed abortion, at 7 and 8 weeks into gestation with live fetuses, and with hydatidiform mole. Peripheral LH, oestradiol, and progesterone were measured by specific radioimmunoassays using commercial kits from Green Cross Corporation (Osaka, Japan).

Tissues were cut immediately into small pieces, and three to four were quickly frozen in OCT compound (Ames Co., Elkhart, Ind., USA) and stored at −70° for 1–7 days until use. The remaining tissues were routinely processed for light microscopy.

The immunostaining procedure was performed on cryostat sections by the peroxidase-antiperoxidase method using the ER-ICA and PR-ICA monoclonal kits (Abbott Labs, Chicago, Ill., USA) according to the manufacturer's instructions. Briefly, 4-μm cryostat sections were mounted on glass slides (coated with the tissue adhesive provided in the kits), and placed in 3.7% formaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature and rinsed in PBS for 5 min. Next, they were immersed in 100% methanol (4° C) for 5 min, and then in acetone (4° C) for 3 min. The slides were incubated with normal goat serum to reduce the non-specific binding of primary antibody. Then the slides were incubated with either anti-ER monoclonal antibody or anti-PR monoclonal antibody or control rat IgG (at concentration was 0.1 μg/ml each) for 30 min at room temperature, followed by treatment with goat anti-rat IgG antiserum (bridging antibody) and with the peroxidase-antiperoxidase complex (PAP). Finally, the slides were treated with diaminobenzidine (1.2 mg/ml) in TRIS buffer solution containing 0.02% hydrogen peroxide for 6 min. Counterstaining was carried out with 3% methyl green. The receptor-specific staining appeared as brown-coloured grains in the cells. The control slides treated with control antibodies yielded negative results. For positive controls, cryostat sections of endometrial tissues in the proliferative phase of the cycle (Press et al. 1984; Bergeron et al. 1988) and commercially supplied slides with ER and/or PR-positive cells were used. The intensity of staining was evaluated by repeated staining of the same specimens and by more than two observers. It was graded as (−) for no immunostaining, (+) for weak but definitely detectable, (++) for strong immunostaining, (+++) for very intense immunostaining and (++++) for most intense immunostaining. Histological analyses of the specimens were conducted on both the cryostat sections and the sections that were routinely processed with haematoxylin and eosin stain.

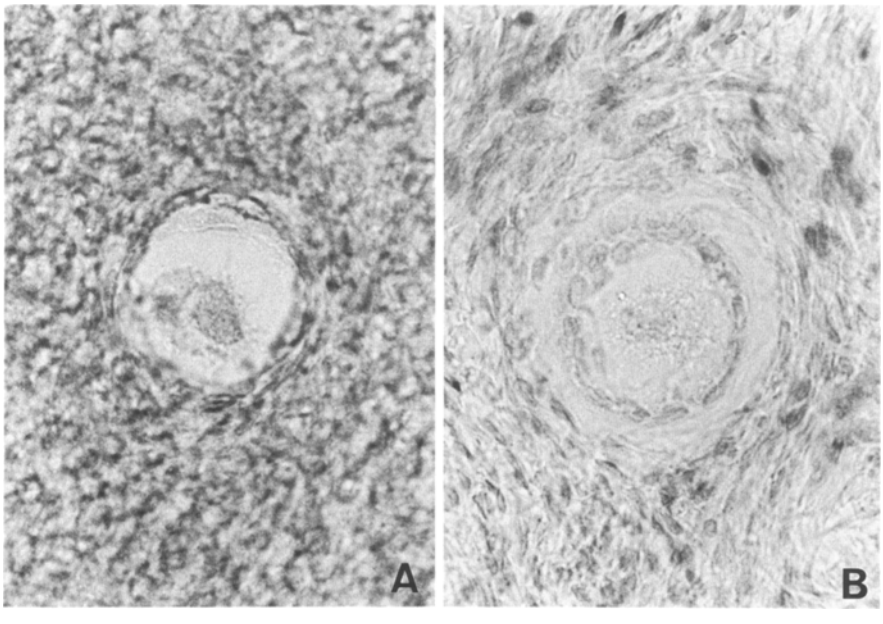

Fig. 1 A, B. Sections of primordial follicles. Neither oestrogen receptor (ER; **A**) nor progesterone receptor (PR; **B**) staining was detected in the granulosa cells. ×600

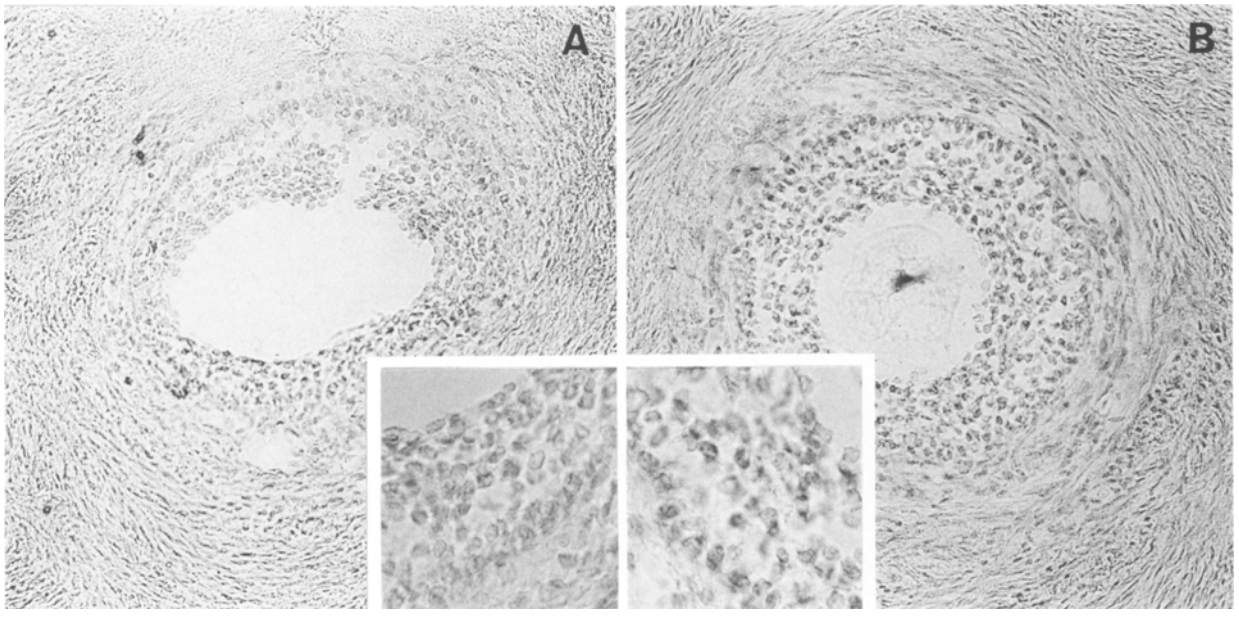

Fig. 2A, B. Sections of preantral follicles. Both ER (**A**) and PR (**B**) staining were negative in the granulosa cells. ×200. *Insets:* Higher magnification of granulosa cells. ×400

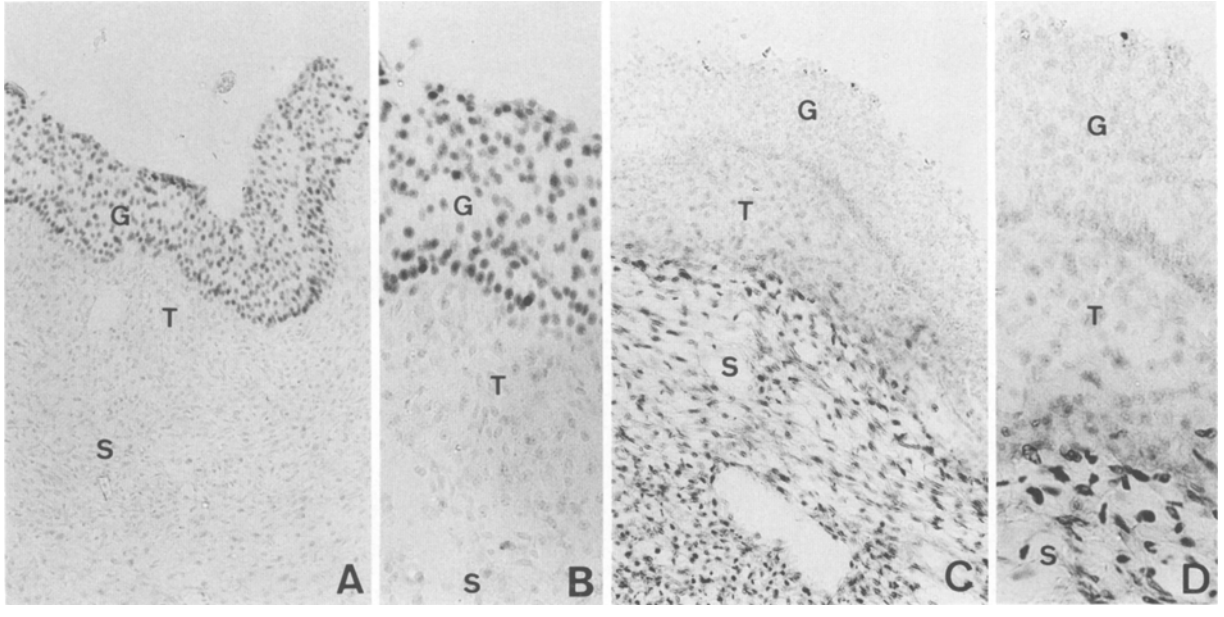

Fig. 3A–D. Sections of antral follicles of midfollicular phase of the menstrual cycle. ER staining (**A, B**) was detected in the granulosa cells (*G*), but not in the other structures of the ovary. PR staining (**C, D**) was not detected in the granulosa cells, but the theca interna cells (*T*) showed weak staining for PR and the surrounding stromal cells (*S*) exhibited comparatively intense staining for PR. **A, C** ×100; **B, D** ×200

Results

For the follicular phase of the menstrual cycle, immunohistochemical localization of ER and PR in the different stages of follicles is given in Table 1. Specific staining with the anti-ER antibody and anti-PR antibody was confined exclusively to the nuclei. The endometrial tissues and commercially supplied ER-positive and/or PR-positive cells for positive controls were also characterized by positive nuclear staining for both ER and PR.

There was no nuclear staining for either type of steroid receptor in the granulosa cells of primordial (Fig. 1A, B) and preantral follicles (Fig. 2A, B) but very weak PR-positive staining was sometimes observed in the ovarian stromal cells surrounding these follicles.

In the midfollicular phase, many of the granulosa

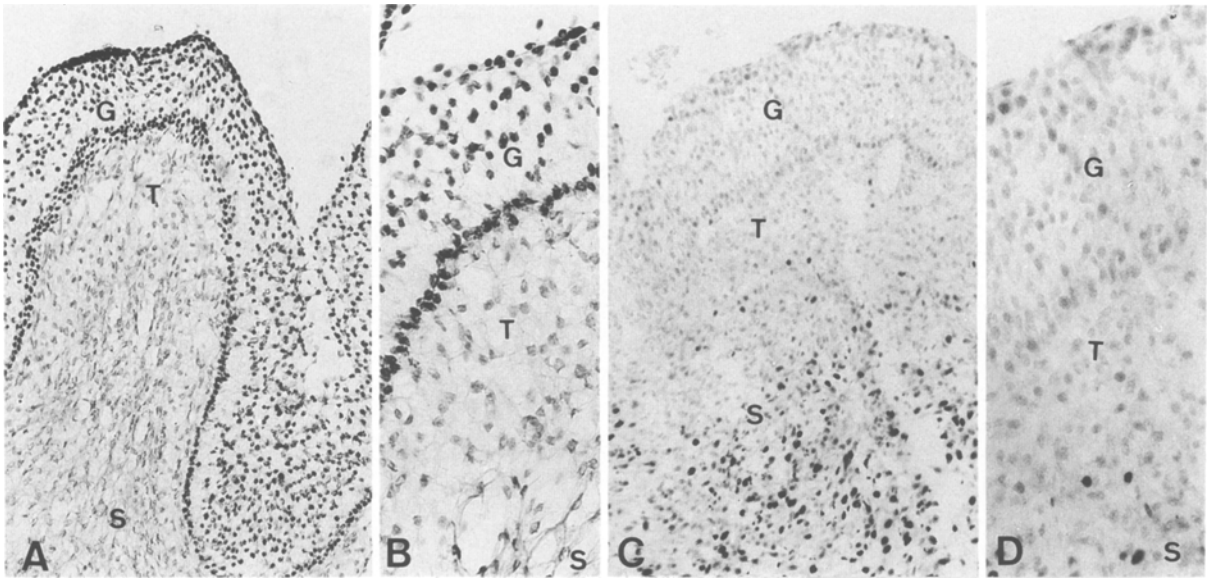

Fig. 4A–D. Sections of the wall of the preovulatory follicles in the late follicular phase and before luteinizing hormone (LH) surge. Intense staining for ER (**A**, **B**) was observed in the granulosa cells (*G*). PR (**C**, **D**) was detected in the theca interna cells (*T*) and the surrounding stromal cells (*S*). **A**, **C** ×100; **B**, **D** ×200

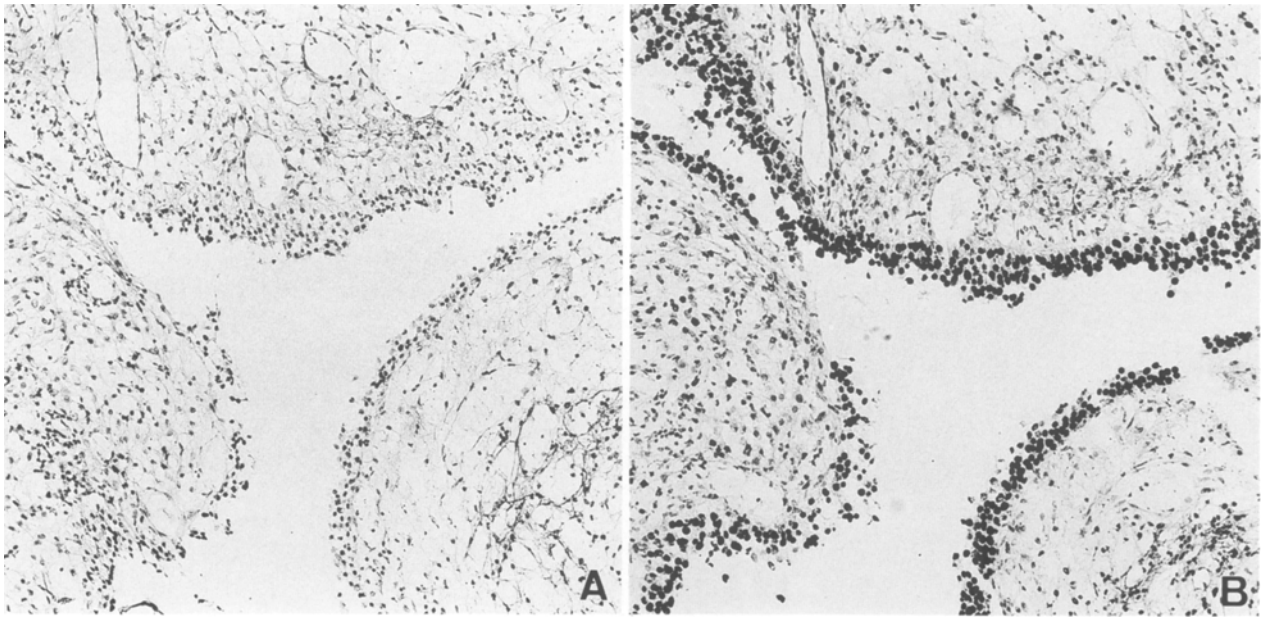

Fig. 5A, B. The wall of the dominant follicles at the LH surge. The ER staining (**A**) of the granulosa cells was weak. In contrast, the granulosa cells displayed intense staining for PR (**B**). ×100

cells of antral follicles showed weak to moderate staining for nuclear ER but not for PR (Fig. 3A–D). In contrast, cells in the thecal layers and the surrounding stromal cells contained PR, but not ER. The staining intensity for PR was more prominent in the stromal cells compared to thecal cells.

In the late follicular phase (before the LH surge), the dominant preovulatory follicle contained granulosa cells that stained intensely positive for ER and faintly positive for PR. In contrast, thecal and stromal cells were more positive for PR, but not for ER (Fig. 4A–D).

Two dominant follicles taken from the women at the time of the LH surge (LH levels: 73.8 IU/ml and 63.4 IU/ml) were the follicles closest to ovulation. The granulosa cells in these follicles were faintly positive for ER (Fig. 5A) and markedly positive for PR (Fig. 5B). However, the staining pattern for ER and PR in the thecal and stromal cells was the same as in preovulatory follicles before the LH surge.

For the luteal phase of the menstrual cycle, Table 2 shows the localization of ER and PR in corpora lutea during the luteal phase of the menstrual cycle. At this

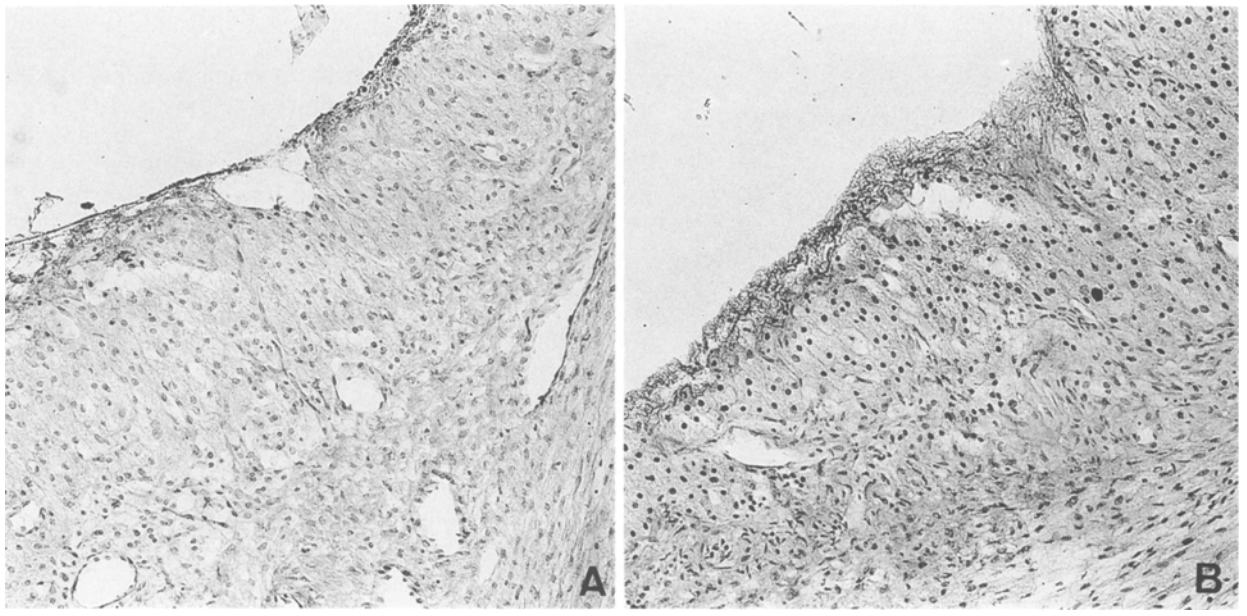

Fig. 6A, B. Sections of the corpus luteum just after ovulation (*cycle date* = 15 day). No nuclear staining for ER (**A**) was observed in the luteal cells; however, PR (**B**) was observed in almost all luteal cells. × 200

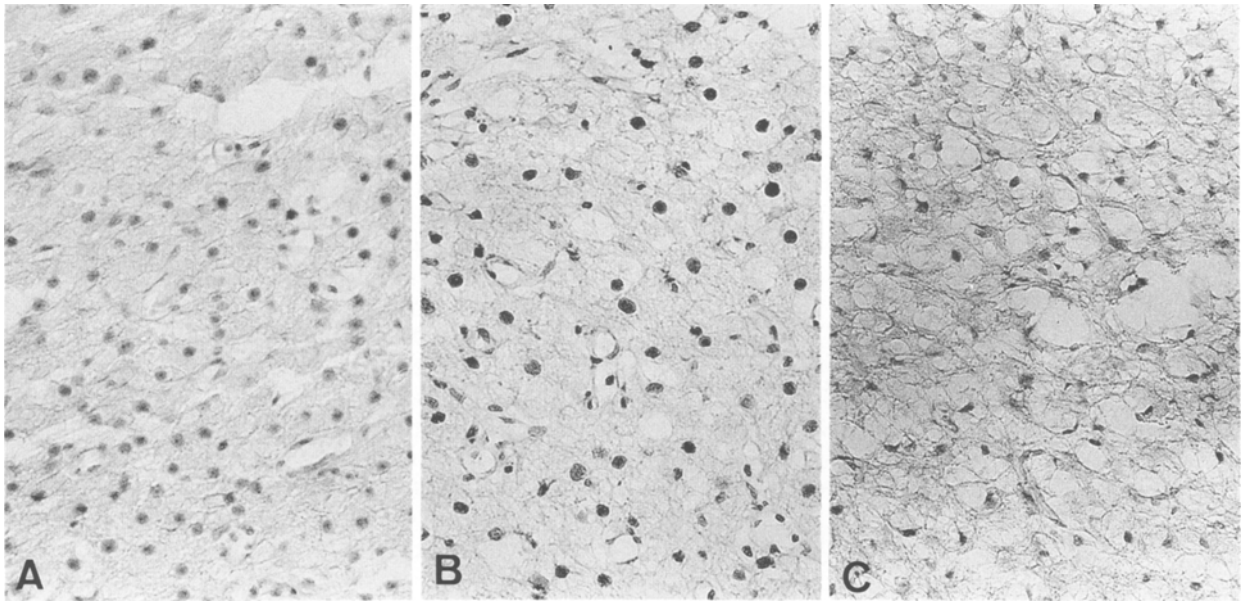

Fig. 7A–C. The localization of PR in the corpora lutea of the early (**A**), mid (**B**) and late (**C**) luteal phases. Almost all luteal cells showed PR-positive staining, but the staining intensity was strongest in the mid luteal phase. × 200

stage, histochemical staining for ER was not observed either in the luteal cells of corpora lutea or in the stromal cells surrounding the corpora lutea. Even in newly formed corpora lutea (taken on days 15 and 16), staining for ER was negative (Fig. 6A). However, almost all luteal cells contained PR (Fig. 6B), with the greatest number of receptors appearing during the midluteal phase and the fewest in the late luteal phase (Fig. 7A–C). The stromal cells around the corpora lutea were also positive for PR staining. Corpora albicanes showed no specific staining for either ER or PR, but only weak staining for PR was observed in the surrounding stromal cells.

For the corpus luteum during pregnancy or associated with hydatidiform mole, the results from immunohistochemical localization of ER and PR in corpora lutea from pregnant women ($n = 3$) and from one associated with a hydatidiform mole are shown in Table 3. At 7 and 8 weeks' gestation, the luteal and stromal cells stained negatively for ER, and less than 25% of these cells stained weakly for PR. However, the ovary from a missed abortion at 9 weeks of gestation showed no

Table 2. ER and PR expression in luteal cells

Luteal phase	Luteal cells		Surrounding stromal cells	
	ER	PR	ER	PR
Just after ovulation ($n=2$)	−	++	−	+~++
Early phase ($n=3$)	−	+++	−	+~++
Mid phase ($n=6$)	−	++++	−	+~++
Late phase ($n=3$)	−	−~+	−	+
Corpus albicans ($n=5$)	−	−	−	−~+

Footnotes as in Table 1

Table 3. ER and PR expression in the corpora lutea of pregnancy

Case	Luteal cells		Surrounding stromal cells		Serum progesterone level (ng/ml)
	ER	PR	ER	PR	
7 weeks' gestation with a live fetus	−	+	−	+	10.1
8 weeks' gestation with a live fetus	−	−~+	−	+	7.7
9 weeks' gestation missed abortion	−	−	−	−	7.3
Hydratidiform mole	−	+	−	+	12.4

Footnotes as in Table 1

Table 4. ER and PR expression in follicles other than the dominant follicle during the menstrual cycle

Non-ovulatory follicle	Granulosa cells		Theca interna cells		Surrounding stromal cells	
	ER	PR	ER	PR	ER	PR
Pre-ovulatory ($n=5$)	−~+	−	−	+	−	+~++
LH surge ($n=2$)	+~++	−~+	−	+	−	+~++
Luteal phase ($n=8$)	−~+	−	−	+~++	−	+~++
Atretic ($n=4$)	−	−	−	−~+	−	−~+

Footnotes as in Table 1

staining for either receptor. However, a corpus luteum from an ovary with a hydatidiform mole showed weak PR staining in most of the luteal cells, but no staining for ER. In each of these cases, the staining intensity for PR was weaker than that in luteal cells during the menstrual cycle.

In follicles other than the dominant follicle during the menstrual cycle taken shortly before the LH surge, there was faint staining for ER, and no staining for PR. During the LH surge, the granulosa cells in the non-dominant follicles showed intense staining for ER, but faint staining for PR. The non-dominant follicles observed in the ovaries with corpora lutea during the luteal phase also showed faint staining for ER, but not for PR. However, in these follicles, the thecal cells and surrounding stroma contained PR, but not ER (Table 4).

The atretic follicles (selected by histological criteria for atresia) showed no specific staining for ER. Several cells in the thecal layers and a few stroma cells surrounding follicles were positive for PR (Table 4).

Discussion

Our current immunohistochemical study demonstrates that ovarian follicular and stromal cells undergo changes in their nuclear ER and PR during the menstrual cycle.

There are reports that oestrogen stimulates both ER and PR synthesis, and progesterone suppresses both ER and PR synthesis in the endometrium, oviduct, vagina, anterior pituitary and hypothalamus, and other target organs (Katzenellenbogen 1980; Leavitt et al. 1983). However, it is unclear why granulosa cells of the antral stage expressed ER, but not PR, staining at a time when ovarian oestrogen levels were rising. One possible explanation is that the two different steroid receptors require different oestrogen levels for their generation, and during the early antral stages of follicular development there may not be sufficient oestrogen to induce the synthesis of PR. Eventually, PR became apparent in the follicles during the preovulatory stage, at a time when the granulosa cells were exposed to the highest levels of oestrogen.

Similarly, the suppression of ER, but not PR, at the time of ovulation and during the luteal phase may be a consequence of differential action by progesterone on receptor expression. Our studies show that PR dominate both the granulosa and the luteal cells when these tissues are producing high levels of progesterone during and after ovulation, respectively. Then towards the end of the luteal phase, the expression of PR decreases as the level of progesterone diminishes. Thus, it appears that the expression of PR correlates with the levels of progesterone. This pattern of PR induction by progesterone is comparable to the results of an immunohistochemical study of endometrial stromal cells which were positive for PR during the late secretory phase (Bergeron et al. 1988). Moreover, during pregnancy, decidua cells are positive for PR (unpublished observations).

In contrast, neither theca interna nor ovarian stromal cells contained ER at any stage of the menstrual cycle. Therefore, it appears that the local oestrogen levels do not induce ER formation in these cells. However, PR were consistently present from the beginning of folliculogenesis through the luteal phase of the cycle. Thus, ER

and PR are expressed differently in granulosa cells when compared with thecal and stromal cells.

It has been suggested that the regulation of a particular steroid receptor is usually under multihormonal control (including such hormones as prolactin and thyroid hormones; Cidlowski and Muldoon 1975; Shafie and Brooks 1977; Katzenellenbogen 1980), and the amount of steroid receptor in different tissues may depend on the action of different hormones. Therefore, the expression of ER and/or PR in the follicular cells of the ovary may be regulated by some unknown factors other than oestrogen and/or progesterone.

LH binding sites exist in the thecal cells throughout the menstrual cycle, but, in granulosa cells, they are present only in the dominant follicles (Shima et al. 1987). Furthermore, LH binding sites have not been observed in the granulosa cells of non-ovulatory follicles. Therefore, the current observations of PR in the granulosa cells of dominant follicles, of PR negativity in the granulosa cells of the non-ovulatory follicles, and of PR expression in thecal cells closely correlate with the distribution of LH binding sites in the ovary. Also, there is evidence that high levels of LH cause a decrease in the ER content of granulosa cells (Richards 1975). Therefore, LH may suppress ER expression in the granulosa cells of the dominant follicles and the thecal cells via LH binding sites.

In the corpora lutea of pregnancy or associated with hydatidiform mole, the staining intensity of PR-positive nuclei appeared to decrease, and some of the luteal cells even had negative staining in spite of their comparatively high state of progesterone synthesis. This absence of receptors may be associated with the degeneration of luteal cells, and/or with the down-regulation of PR by the endogenous progesterone (Isomaa et al. 1979).

In conclusion, ER and/or PR are localized in the follicular cells of the ovary, and through these receptors ovarian steroids may act as local regulators of ovarian function. However, the regulation of ER and/or PR expression in the follicular cells seems to be different from that of other steroid target tissues. Further study is necessary to elucidate the mechanism of the regulation of ER and/or PR expression in the ovary.

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