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Expression of Fatty Acid Synthase Is Closely Linked to Proliferation and Stromal Decidualization in Cycling Endometrium

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Summary: Estrogen-driven proliferative phase growth is the most rapid physiological proliferative process that occurs in the adult. The tissue growth that occurs during this phase of the menstrual cycle requires incorporation of a substantial quantity of fatty acid into the structural lipids of cell membranes. Fatty acid synthase (FAS) is the major biosynthetic enzyme required for de novo synthesis of fatty acids. In this immunohistochemical study, we have observed that human endometrium displays distinct patterns of FAS expression in the proliferative and secretory phases of the normal menstrual cycle. Proliferative endometrial glands and stroma show high FAS expression that closely correlates with expression of Ki-67, estrogen and progesterone receptors, supporting the view that FAS expression plays a role in cellular proliferation in response to estrogen. FAS expression declines during early to midsecretory phase, then reappears in decidualized stromal cells in late secretory phase as well as in the decidua of pregnancy. The second wave of FAS expression correlates with progesterone-receptor localization in the decidual cells, a finding suggesting a second induction of FAS expression in the endometrium, associated with differentiation, that may be regulated by progesterone. **Key Words:** Fatty acid synthase—Ki-67—Proliferation—Endometrium—Menstrual cycle.

The human endometrium undergoes a cyclic process of proliferation, secretory differentiation, and menstrual sloughing under the regulation of estrogen and progesterone that is mediated through binding to their specific receptors. Estrogen-driven proliferative phase endometrial growth is the most rapid physiological tissue expansion that occurs in the adult, with an ~10-fold increase in endometrial tissue mass during a 2-week interval (1,2). In response to progesterone, endometrial proliferation is followed by glandular and stromal differentiation in the secretory phase to provide an appropriate environment for the implanted blastocyst.

Fatty acid synthesis has not been systematically examined in cycling endometrium. The tissue expansion that occurs during the proliferative phase requires incorporation of a substantial quantity of fatty acid into structural lipids; most tissues with high cellular turnover appear to use circulating lipids preferentially. The biosynthetic enzyme, fatty acid synthase (FAS), is one of four enzymes required for the synthesis of fatty acids from acetyl-CoA. It functions normally in the liver in the anabolic conversion of dietary carbohydrate to fat, which is predominantly incorporated into triglycerides for storage in adipose tissue (3). FAS has a specialized physiological function in lactating breast tissue, where it acts to produce milk lipids, but is minimally expressed in most normal adult tissues (4,5).

The cyclic changes in expression of hormone receptors and proliferation antigens that occur in normal endometrium have been described (6–11). In this study, we characterized the expression of FAS in human endometrium during the menstrual cycle in relation to the proliferation antigen, Ki-67, estrogen receptor (ER), and progesterone receptor (PR) expression. In contrast to the

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TABLE 1. *Fatty acid synthase (FAS) expression in endometrial glands throughout the menstrual cycle and in pregnancy*

	Proliferative		Secretory			
	Early	Late	Early	Mid	Late	
FAS score	No. of cases (% of total)	No. of cases (% of total)	No. of cases (% of total)	No. of cases (% of total)	No. of cases (% of total)	Gestational: no. of cases (% of total)
3	6 (100%)	7 (100%)	5 (100%)	0	0	0
2	0	0	0	4 (66%)	0	0
1	0	0	0	2 (33%)	7 (78%)	1 (8%)
0	0	0	0	0	2 (22%)	11 (92%)
Total	6	7	5	6	9	12

Staining for FAS in each case was scored as follows: 0, no staining; 1, low-intensity positive staining; 2, moderate-intensity positive staining; 3, high-intensity positive staining.

liver and lactating breast, where FAS functions in the production of fat for energy storage, in the endometrium FAS expression is associated with cell proliferation and differentiation.

MATERIALS AND METHODS

Tissue Selection

Formalin-fixed, paraffin-embedded nonneoplastic human endometrial tissue obtained for diagnostic biopsy, during evacuation of intrauterine pregnancy, or after hysterectomy for benign disease at the Johns Hopkins Hospital, was used in accordance with institutional guidelines for the use of discarded human tissue. Endometrial dating was performed using standard histological criteria on hematoxylin and eosin–stained sections. The data were correlated with menstrual history when available (12).

Immunohistochemistry

Immunohistochemical localization of target antigens in deparaffinized 8-μm sections was performed on the BioTek 1000 automated immunostainer using the BioTek 1000 kit. The primary antibodies (described in the next section) were followed by biotinylated goat anti-mouse or goat anti-rabbit antibodies, avidin-horseradish peroxidase, and 3,3′-diaminobenzidine as the chromagen with hematoxylin counterstain.

Primary Antibodies

Affinity-purified rabbit polyclonal anti-human fatty acid synthase antibody was used at 50 ng/ml (ChekTec Corporation). Antibodies against Ki-67 (Immunotech), common leukocyte antigen (CD45), human ER (Immunotech), and PR (Novacastra) were used according to the recommendations of the supplier.

Evaluation of Immunoperoxidase Staining

Each stain was scored separately for endometrial glands and stroma. Scoring was of upper functionalis, because zonal variation in immunoreactivity was observed, and upper functionalis was the zone with maximal immunoreactivity. Staining for FAS in each case was scored as follows: 0, no staining; 1, low-intensity positive staining; 2, moderate-intensity positive staining; and 3, high-intensity positive staining. Each case was scored twice independently, with subsequent reconciliation of scored values. Quantitation of positive staining for Ki-67 was obtained using a CAS computer-assisted image analyzer with determinations of percent positive cells based on at least 300 cells per measurement. Staining for estrogen and progesterone receptors was scored as either positive or negative.

TABLE 2. *Fatty acid synthase (FAS) expression in endometrial stroma throughout the menstrual cycle and in pregnancy*

	Proliferative		Secretory			
	Early	Late	Early	Mid	Late	
FAS score	No. of cases (% of total)	No. of cases (% of total)	No. of cases (% of total)	No. of cases (% of total)	No. of cases (% of total)	Gestational: no. of cases (% of total)
3	0	0	0	0	3 (33%)	4 (33%)
2	0	7 (100%)	0	6 (100%)	6 (66%)	8 (66%)
1	6 (100%)	0	5 (100%)	0	0	0
0	0	0	0	0	0	0
Total	6	7	5	6	9	12

Staining for FAS in each case was scored as follows: 0, no staining; 1, low-intensity positive staining; 2, moderate-intensity positive staining; and 3, high-intensity positive staining.

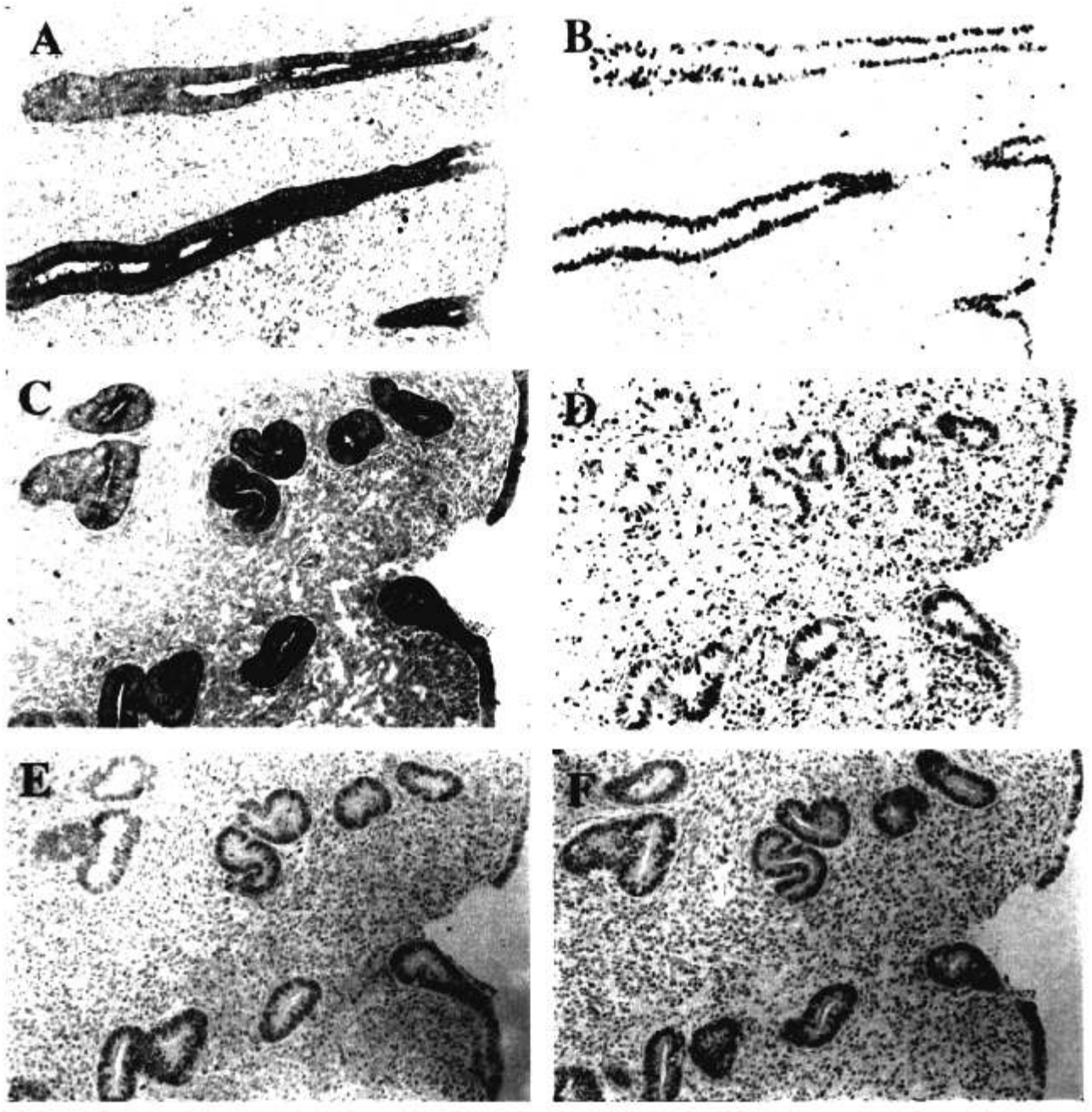

FIG. 1. Proliferative endometrial immunohistochemical stains. A and C: Fatty acid synthase (FAS). B and D: Ki-67. E: Estrogen receptor (ER). F: Progesterone receptor (PR). **A and B:** Early proliferative glands have 3+ immunoreactivity for FAS and high Ki-67 index, whereas expression in stroma is relatively low. Early proliferative glands and stroma are positive for ER and PR as is shown for late proliferative endometrium in E and F. **C–F:** Late proliferative glands and stroma have high FAS expression, high Ki-67 index, and are uniformly positive for ER and PR.

RESULTS

Proliferative Phase

Thirteen proliferative phase samples were analyzed, and in all cases endometrial glands in the functionalis demonstrated strong immunoreactivity (3+) for FAS, with a correspondingly high fraction of Ki-67-positive cells (mean 46%, SD 6%, Table 1). The basalis showed little or no expression of either antigen. Six cases of early proliferative endometrium demonstrated weakly positive (1+) staining for FAS in the endometrial stroma of the functionalis, with a mean Ki-67 index of 17 ± 6% (SD).

Seven cases of late proliferative endometrium demonstrated moderately positive (2+) staining for FAS in endometrial stroma of functionalis, with a mean Ki-67 index of 34 ± 11% (Table 2). Both glandular and stromal cells were positive for ER and PR throughout the proliferative phase (Fig. 1). Four cases with subnuclear vacuoles in <50% of the glands, classified as interval or periovulatory endometrium, had a profile of immunoreactivity similar to that of the proliferative cases, but with scattered glands showing reduced numbers of Ki-67-positive cells such that the mean glandular Ki-67 index in these cases was only 29% (not shown).

Secretory Phase and Gestation

Among secretory phase endometria, five cases were early (days 17–20), six were midphase (days 21–24), and nine were late (days 25–27). As secretory activity appeared in early secretory phase endometrium, pseudostratification, mitotic figures, and Ki-67 reactivity in endometrial glands disappeared in a patchy distribution (Fig. 2). ER and PR expression dropped below detectable levels in parallel. FAS expression in glands remained high through peak secretory activity, then decreased to low or undetectable levels in mid to late secretory phase. Endometrial stromal expression of FAS was low in early secretory phase, but increased to moderate or high levels in the superficial functionalis in cells that became decidualized during the mid and late secretory phases. Stromal Ki-67 expression occurred predominantly in resident lymphoid cells (common leukocyte antigen–positive cells) during secretory phase and in decidua, as has been described (13). Stromal expression of ER was below detectable levels throughout the secretory phase; however, stromal PR expression remained positive (Fig. 3). Twelve cases of decidua from first-trimester gestations had moderate to strongly positive FAS expression and positive PR expression in decidual cells, whereas glan-

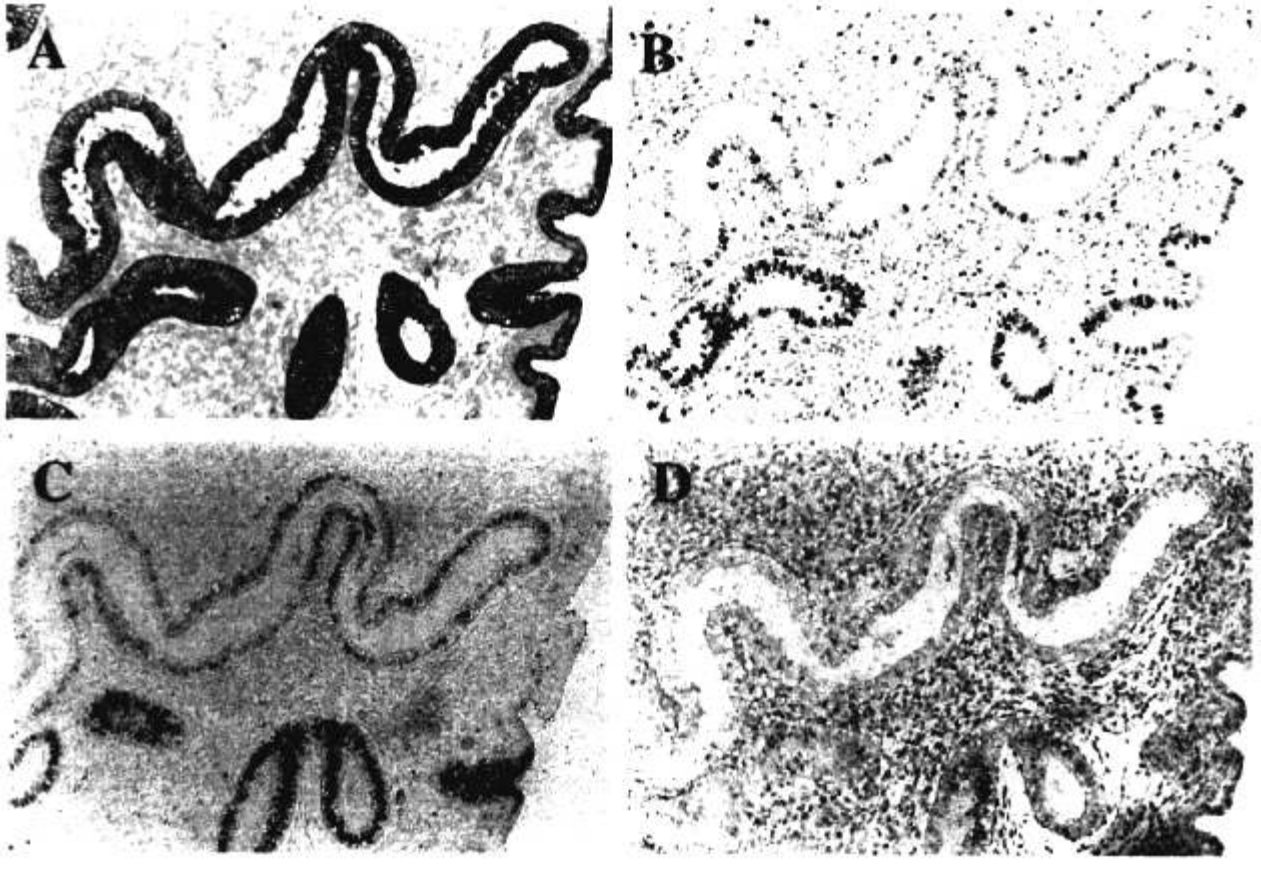

FIG. 2. Early secretory endometrial immunohistochemical stains. **A:** Fatty acid synthase (FAS). **B:** Ki-67. **C:** Estrogen receptor (ER). **D:** Progesterone receptor (PR). Early secretory glands have 3+ immunoreactivity for FAS while stromal expression is low. Ki-67 index and hormone receptor expression in glands decrease in parallel as secretory activity develops with a patchy distribution. Stroma is ER negative, PR positive, with scattered Ki-67-positive lymphoid cells.

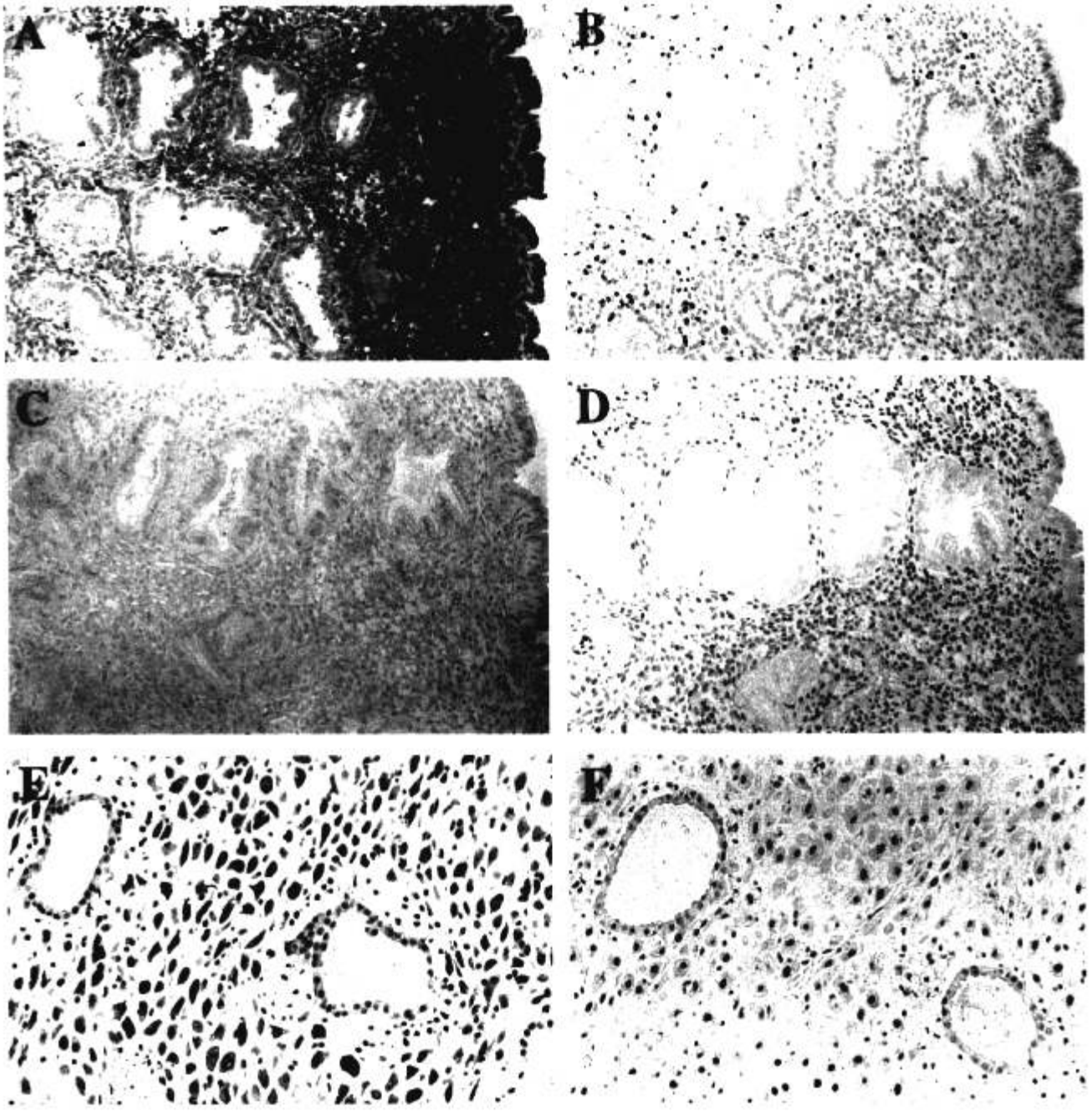

FIG. 3. Late secretory endometrial and decidual immunohistochemical stains. A and E: Fatty acid synthase (FAS). B: Ki-67. C: Estrogen receptor (ER). D and F: Progesterone receptor (PR). Similar staining is seen in late secretory endometrium and in the decidua of pregnancy. Glands of late secretory endometrium **(A–D)** and decidua **(E and F)** show little or no immunoreactivity for FAS, and are negative for Ki-67, ER, and PR. Decidualized stromal cells in both late secretory endometrium and decidua are ER negative, FAS- and PR-positive, with scattered Ki-67-positive lymphoid cells.

dular epithelium was negative for both FAS and PR. Neither ER nor Ki-67 expression was detected in decidua. The cyclic variation of these antigens is summarized in Fig. 4.

DISCUSSION

The expression of FAS immunoreactivity in endometrial glands and stroma during the menstrual cycle oc-

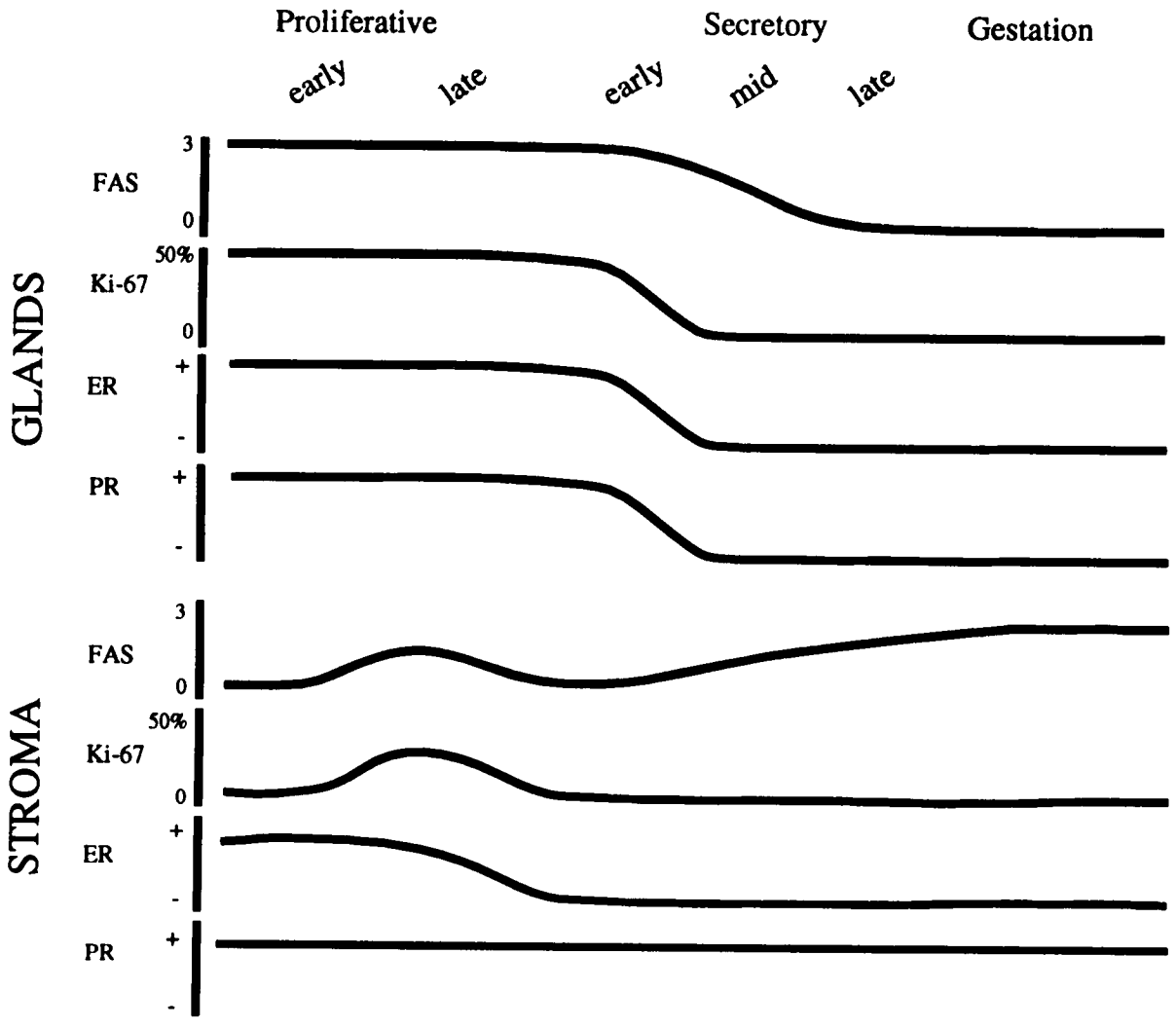

FIG. 4. Profile of variation in antigen expression throughout the menstrual cycle and in pregnancy. During the proliferative phase, glands and stroma express fatty acid synthase (FAS) linked to estrogen receptor (ER), progesterone receptor (PR), and Ki-67, suggesting a relationship to proliferation. In the late secretory phase and pregnancy, FAS is coexpressed with PR, but not ER or Ki-67, in decidualized stromal cells. Staining for FAS was scored as: 0, no staining; 1, low-intensity positive staining; 2, moderate-intensity positive staining; and 3, high-intensity positive staining. Ki-67 was scored as percent of cells stained positively. Staining for ERs and PRs was scored as either positive or negative.

curred in two distinct patterns paralleling endometrial proliferation and secretory differentiation. During the proliferative phase, endometrial glands and stroma of the functionalis were uniformly ER, PR, and FAS positive. This in turn was closely linked to Ki-67 expression, suggesting that FAS expression was estrogen-driven and related to proliferation. Because the process of tissue regeneration and expansion in proliferative-phase endometrium involves higher proliferation rates than any other adult organ or tissue, metabolic demands in endometrium may exceed those of other tissues. In this setting of rapid growth, fatty acid synthesis within endometrial glandular and stromal cells may provide a more abundant and reliable source of fatty acid for structural lipids (phospholipids) than the circulation.

As secretory differentiation proceeded, ER expression dropped below detectable levels in both glands and stroma. Glandular FAS expression disappeared, but stromal expression of FAS increased coincident with decidualization. This was maintained in the decidua of pregnancy. The parallel loss of PR and FAS expression in glandular cells, while both were maintained in the stroma, argues for coordinate regulation of FAS and PR as part of progesterone-stimulated differentiation, as has been shown in breast epithelium during induction of lactational changes (14). It is possible that lipid products of

the fatty acid synthetic pathway in decidua play a role in the implantation and maintenance of pregnancy. In further support of this relationship is the observation of FAS immunoreactivity in decidua induced by exogenous progestational therapy, and in extrauterine decidual reactions (data not shown). The dissociation of hormone receptor expression in glands and stroma may result from epithelial expression of 17β-hydroxysteroid dehydrogenase in the secretory phase, which converts estradiol to the less active estrone, thereby depleting estrogen levels within epithelial cells below circulating levels (15).

Although the link between progesterone-induced differentiation and FAS expression has been previously recognized in breast tissue and suspected in endometrium (16), the proliferation-associated expression described here in the endometrium is novel. This is of particular interest because we have observed a similar linkage of FAS, Ki-67, ER, and PR expression in endometrial hyperplasia and carcinoma (data not shown). Furthermore, elevated FAS expression has recently been described in subsets of human breast, ovarian, colon, and prostate carcinomas with aggressive biological behavior (17–21). FAS expression may therefore play an intrinsic role in proliferation in both normal and neoplastic endometrium.

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