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ORIGINAL PAPER

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Estradiol receptor binding to the epithelium of uterine lumen and glands: region- and time-related changes during preimplantation and periimplantation periods studied by autoradiography

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Abstract The presence and changes of estradiol nuclear binding and related functions in uterine luminal and glandular epithelium were studied before and after blastocyst implantation using receptor autoradiography with ^{3}H-estradiol-17β in association with ^{3}H-thymidine incorporation and immunocytochemical binding of antibody to estrogen receptor ER-α. ^{3}H-estradiol nuclear binding is present but variable during days 1.5–7.5 of pregnancy. Sites of strong nuclear binding of ^{3}H-estradiol exhibit strong immunocytochemical staining with ER-α antibody. Qualitative and quantitative evaluation of autoradiograms reveal that there is a general increase of nuclear ^{3}H-estradiol binding during the first 3 days after fertilization in both luminal and glandular epithelium. The binding of estradiol is stronger in glandular epithelium from day 2.5 to day 7.5, paralleled by a rise in ^{3}H-thymidine incorporation on day 2.5. By comparison, in the epithelium of the uterine lumen ^{3}H-estradiol nuclear binding is low, but relatively high in epithelial cells at lateral branching of the lumen where the increase in ^{3}H-estradiol binding corresponds to an increased labeling index with ^{3}H-thymidine. A highly differentiated binding of ^{3}H-estradiol to luminal and glandular epithelium was demonstrated with region- and time-specific changes of related effects on cell proliferation, differentiation, and secretion, probably involving involution and remodeling. The strong ^{3}H-estradiol binding to glandular epithelium suggests that estradiol exerts pronounced effects on glandular activities in the periimplantation period.

Keywords ^{3}H-estradiol · Autoradiography · Receptor binding · Uterus · Pregnancy

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Introduction

After conception, extensive morphological and functional changes take place in the uterus, which can be followed through the preimplantation and periimplantation stages (review in Abrahamshon and Zorn 1993). Estradiol is one of the ovarian hormones that play an important role in the proliferation, differentiation, and function of uterine cells during early pregnancy (for review see Weitlauf 1994).

Estradiol acts on the uterine luminal and glandular epithelia by promoting changes in cell shape and cell surface components, on the number and shape of glands, and the glandular–stromal ratio. Moreover, estradiol induces synthesis of many secretory molecules and may facilitate their secretion (Martin et al. 1973; Weitlauf 1994; Chen et al. 1999).

The action of estradiol is mediated through intracellular estrogen receptors (ERs), which regulate gene transcription via the estrogen-responsive element (Mangelsdorf et al. 1995). Estrogen receptors have been localized by "in situ" radiolabeled hormone binding and by immunohistochemistry in the epithelial, stromal, and myometrial compartments of the uterus in different species (Stumpf 1968; Prasad et al. 1974, 1976; Sartor 1977; Ward et al. 1978; Ennis and Stumpf 1988; Yamashita et al. 1989; Brenner et al. 1991; Li et al. 1992; Tibbets et al. 1998; Tan et al. 1999; Tessier et al. 2000). Each one of these studies was focused on a particular stage of pregnancy so that the picture of the period of pregnancy remained fragmented.

Changes in ER levels in the endometrium during early pregnancy and pseudopregnancy have been quantified by biochemical methods (Talley et al. 1977; Ward et al.

1978; Logeat et al. 1980; Martel and Psychoyos 1981; Moulton and Koenig 1981). In most cases, however, the ER quantitative evaluations have been made with the whole uterus and neither the dynamic morphological adaptation of the pregnant uterus nor the ER distribution in different compartments or individual cellular populations of the endometrium were considered (Martel and Psychoyos 1981; Weitlauf 1994). This may explain the difficulties in establishing a comprehensive relationship between the ER concentration and modifications observed in the different compartments of the uterus during the postfertilization and blastocyst implantation periods.

In this study, we followed the distribution and changes in ER levels in the mouse uterine luminal and glandular epithelium during the preimplantation and periimplantation periods. The binding of ^{3}H-estradiol to nuclear receptor was demonstrated by receptor microautoradiography. ^{3}H-estradiol nuclear binding was characterized by time-related topographic and quantitative analysis of autoradiograms, by comparisons with patterns of ^{3}H-thymidine incorporation, as well as the localization of antibody to ER-α. Receptor microautoradiography has been applied because of its high sensitivity and resolution, its quantitative nature, and its informative value demonstrated in previous studies with radiolabeled estradiol and other steroid hormones (Stumpf et al. 1981; Shughrue et al. 1992).

Materials and methods

Animals and tissue preparation

Forty-nine adult virgin female Swiss albino mice, aged 3–4 months, ranging in weight from 30 to 37 g, were used. The animals were housed in a 12 h/12 h light/dark schedule at 22°C, with food and water available at all times. To know the precise time of pregnancy, females were mated during a 2-h period and then examined for copulation plugs. When plugs were found, this was considered zero hour postcoitum and the beginning of pregnancy. Animals at 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 days of pregnancy were used, with at least three each for receptor microautoradiographic studies and two each for immunocytochemical studies. The chemical identity of nuclear radioactivity as being ^{3}H-estradiol has been established in previous experiments (Stumpf 1971; Martel and Psychoyos 1981; Moulton and Koenig 1981). National and international principles of laboratory animal care were followed, and experiments were approved by the Institute of Biomedical Sciences' Animal Ethics Committee.

Autoradiography

3H-estradiol localization

[2,4,6,7,16,17-^{3}H]–estradiol-17β (E2–3H), specific activity 140 Ci/mMol (New England Nuclear), was dissolved in ethanol–water 1:10 and, under ether anesthesia, injected into the tail vein, 0.2 μg/100 g body weight. Mice were killed 1 h afterwards. Uterine horns were excised and transversal and longitudinal fragments of 1–2 mm length were placed on tissue holders and freeze-mounted by immersion into isopentane cooled by liquid nitrogen. Four-micron frozen sections were cut in a cryostat (Microm 500) and thaw-mounted on emulsion-coated slides (Stumpf 1971). The mounted slides were stored in a desiccator box at −15°C for 35, 70, or 100 days. Short-exposure (35-day) autoradiograms were used for silver grain counting, and long-exposure autoradiograms for qualitative and low-magnification surveys. At the end of exposure, slides were fixed in buffered 2% paraformaldehyde for 30 s, then photographically processed, and stained with methyl green–pyronin, specific for RNA (red) and DNA (blue-green).

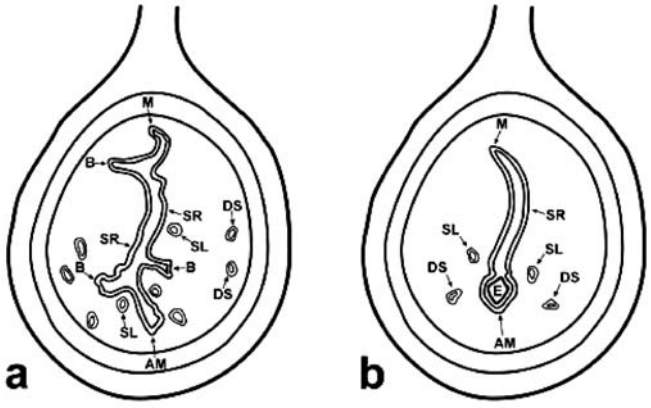


Fig. 1a, b Schematic representation of a transverse section of uterine horns. Days 1–3 (**a**) and day 4 of pregnancy (**b**). *M* Mesometrial extensions of the lumen, *AM* antimesometrial extension of the lumen, *SR* straight region of the central part of the lumen, *B* branched extensions, *SL* glands in subluminal location, *DS* glands in a deep stromal location, *E* embryo

Quantitative evaluation of autoradiograms with 3H-estradiol

Silver grains over nuclei were counted interactively with a Computer System Pro Plus Scan connected to a Nikon photomicroscope fitted with a digital camera at 100× magnification.

During the process of embryo implantation, the uterine lumen changes markedly. Consequently, the epithelial cells that line the uterine crypt usually die by apoptosis. Because of this fact, quantitative evaluation was done only in the period in which the luminal epithelium was still present, from day 1.5 to day 4.5 of pregnancy.

For quantitation, regions of the luminal and glandular epithelium were selected from transverse sections according to their position in the uterus (see schematic representation of the selected areas in Fig. 1). Before counting, in the selected regions nuclear areas of individual cells were determined and the mean nuclear area was assessed. For silver grain counting, only nuclei with an area above the mean were selected. For each epithelial region, at least ten nuclei were evaluated per section in three slides each per animal in three animals. Silver grains over nuclei were counted, entered, and semiautomatically evaluated with a computer program.

3H-thymidine incorporation

^{3}H-thymidine (New England Nuclear), specific activity 60 Ci/mM, dissolved in distilled water, was injected i.p. 1 μCi/g body weight into two mice at the same periods of pregnancy as described above. One hour after the injection, the animals were killed and uterine tissues dissected and immersion-fixed in Methacarn (methanol, chloroform, glacial acetic acid; 6:3:1) for 3 h, washed, dehydrated, and embedded in Paraplast. Five micron sections were placed on glass slides, deparaffinized, hydrated, and then dipped in liquefied nuclear emulsion, air-dried and placed in desiccator boxes for exposure in a refrigerator. After exposure for 60 days, slides were photographically processed, stained with methyl green–pyronin and coverslipped.

The regional distribution of ^{3}H-thymidine-labeled cells was compared to that of ^{3}H-estradiol-labeled cells. The comparison was based on a qualitative analysis of the patterns of regional labeling without counting individual cells.

Immunohistochemistry

Affinity-purified rabbit polyclonal antibody for mouse ER-α isoform (MC-20; Santa Cruz Biotechnology, USA) was raised against a peptide mapping the C-terminus of the ER-α of mouse origin.

Small pieces of uterus were take from pregnant animals at the same stages mentioned for ^{3}H-estradiol localization. Samples were fixed with Methacarn fixative, rinsed with absolute ethanol, and embedded in Paraplast. Five-micrometer sections were cut (Microm HM-200), adhered to glass slides with 0.1% poly-L-lysine (Sigma, USA), and then air-dried at room temperature. Each of the succeeding steps was followed by a thorough rinse with PBS.

All steps were performed in a humid chamber at room temperature and care was taken to avoid drying. Sections were treated with 3% H_2O_2 (Merck) in PBS for 30 min to block endogenous peroxidase activity. Non-specific reaction was blocked by incubating the sections for 30 min with normal goat serum diluted 1:1 in PBS-10% bovine serum albumin followed by incubating overnight with undiluted Super Block (Pierce) blocking buffer. After this, sections were incubated overnight at 4°C in primary antibody ER-α diluted 1:100 in PBS–0.3% Tween 20. Sections were then washed thoroughly with PBS followed by incubation with biotinylated goat anti-rabbit IgG (Vector) diluted 1:100 in PBS for 1 h at room temperature. After rinsing in PBS, sections were treated with Vectastain ABC kit (Vector) for 1 h at room temperature. The reaction was visualized using 0.03% 3,3′-diaminobenzidine (Sigma) plus 0.03% H_2O_2 in PBS. Control sections were prepared omitting the primary antibody. After immunostaining, sections were lightly stained with Mayer's hematoxylin and examined with a Nikon Eclipse E800, and images were captured with Image Pro Plus software (Media Cybernetics, L.P. Maryland, USA) and subsequently printed with an Epson Stylus 777.

Results

Morphology and ^{3}H-estradiol nuclear distribution

Preimplantation period

In this period, a single layer of tall columnar epithelium lined the uterine lumen. On day 1.5 of pregnancy, the uterine lumen was very branched and distended and contained cell debris and mucus, and the cells displayed many luminal protrusions of apical cytoplasm (Fig. 2a). On this day and on day 2.5, the apical region of the epithelium was intensely pyronin (RNA)-positively stained (Fig. 2a, b). Qualitative and quantitative evaluations showed that the nuclear concentration of ^{3}H-estradiol was weak on day 1.5 and increased from day 2.5 to day 3.5 of pregnancy (Figs. 2a–c, 4a). The binding of ^{3}H-estradiol was always conspicuous in certain areas of the epithelial layer, particularly in the extremities of luminal branches (Figs. 2a, 4a). On day 3.5 the increases in the ^{3}H-estradiol binding by luminal epithelial cells situated in the mesometrial pole of the uterus were notable (Figs. 2c, 4a).

Glands were numerous and well developed particularly on day 1.5 of pregnancy. On this day and in the following days, the majority of the glands were present in the middle and antimesometrial stroma. Qualitative and quantitative evaluation showed that nuclear concentration of radioactivity was clearly stronger in the glands than in the luminal epithelium throughout that period (Figs. 2a–f, 4a, b). Moreover, ^{3}H-estradiol binding increased from day 1.5 to day 2.5 (compare Fig. 2d and e, and see Fig. 4b). In serial sections, the heterogeneity of uterine glands was apparent. Some of these profiles seemed to indicate strong secretory activity. Some of the glands were strongly distended with low cuboidal epithelium, while some of the larger glands even had squamous epithelium. The retention of ^{3}H-estradiol was likewise heterogeneous among the various glandular profiles particularly on day 3.5 (Fig. 2f). Squamous epithelial cells observed mainly on day 1.5, sometimes were weakly labeled or unlabeled. On the contrary, on day 2.5 many intensely labeled glandular segments were observed near or in contact with the uterine lumen (Fig. 2b). Qualitative and quantitative evaluation showed that on days 1.5 and 2.5 nuclear labeling was stronger in the glands situated in the deep stroma than in the glands situated in the superficial stroma (Fig. 4b)

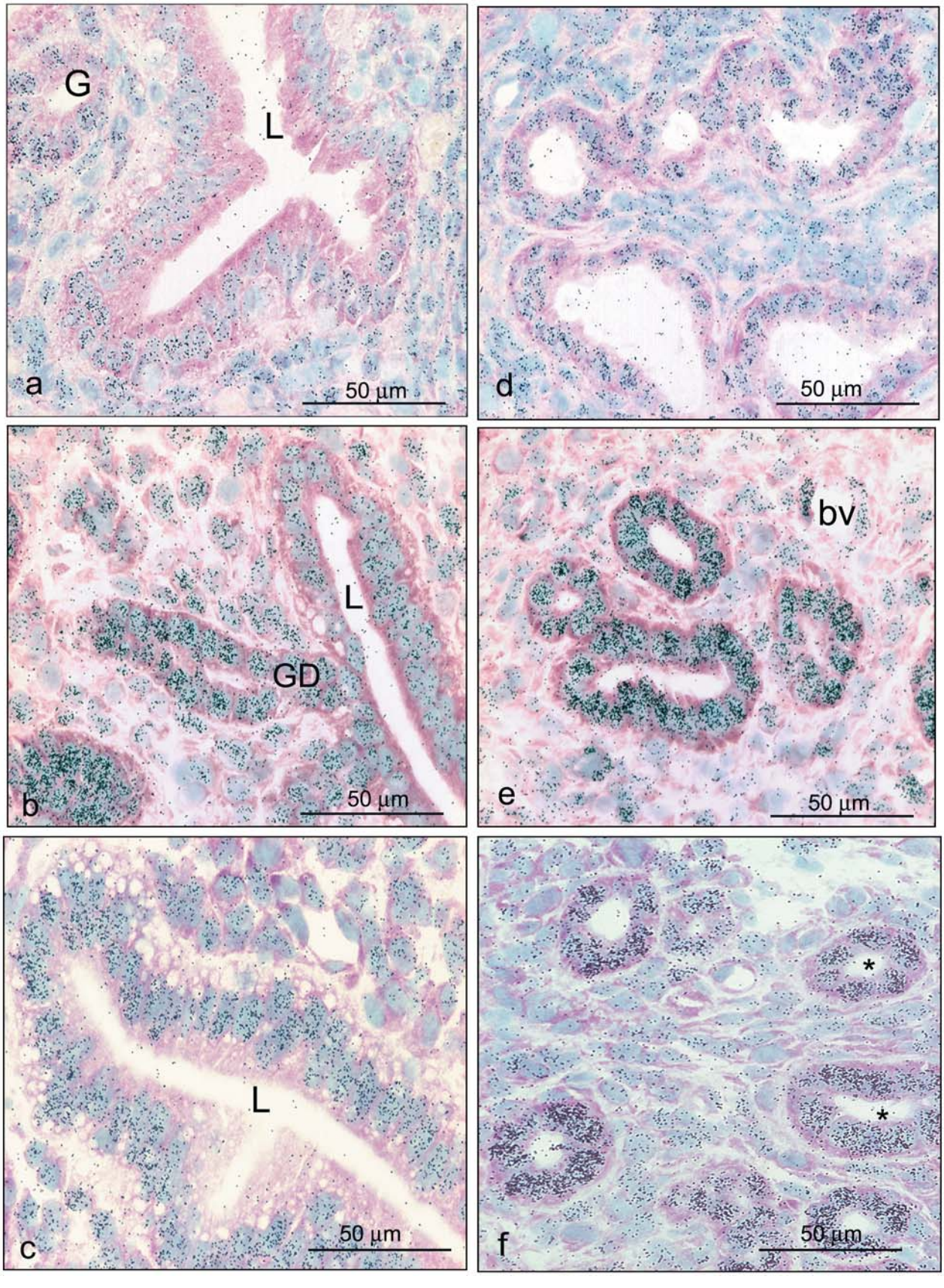
Periimplantation period

On day 4.5 of pregnancy, a few hours before the embryo implantation starts, the architecture of the uterine lumen changed markedly. On this day, no branched extensions were observed in the uterine lumen and blastocysts were lodged in deep crypts formed into the antimesometrial stroma (Fig. 3). Qualitative and quantitative evaluation showed that the ^{3}H-estradiol binding in the epithelium decreased compared to that on day 3.5. The binding was very low, particularly in epithelial cells contacting trophoblast cells and increased toward the mesometrial region of the lumen (Figs. 3, 4a).

Glands were in general smaller compared to the previous day and formed by cuboidal epithelium. Glands were very few in the implantation sites (IS; Fig. 3) and abundant in the interimplantation sites (IIS). Although smaller, the glandular cells showed generally strong pyronin-positive staining. The binding of ^{3}H-estradiol decreased compared to that on days 2.5 and 3.5 (Fig. 4b), however, strongly labeled glandular segments crossed the decidualized area of the stroma and established contact with a very weakly labeled luminal epithelium (Fig. 3). No significant difference was observed in the ^{3}H-estradiol binding when glands situated in the superficial stroma were compared with those in the deep stroma (Fig. 4b)

On day 5.5 the blastocysts were already implanted into uterine crypts (Fig. 5a). In serial longitudinal sections of the uterine horns, the uterine lumen displayed multiple folds at the mesometrial pole, from which a main crypt lodging the implanted embryo was formed toward the antimesometrial region (Fig. 5a, b). After the embryo

Preimplantation Period



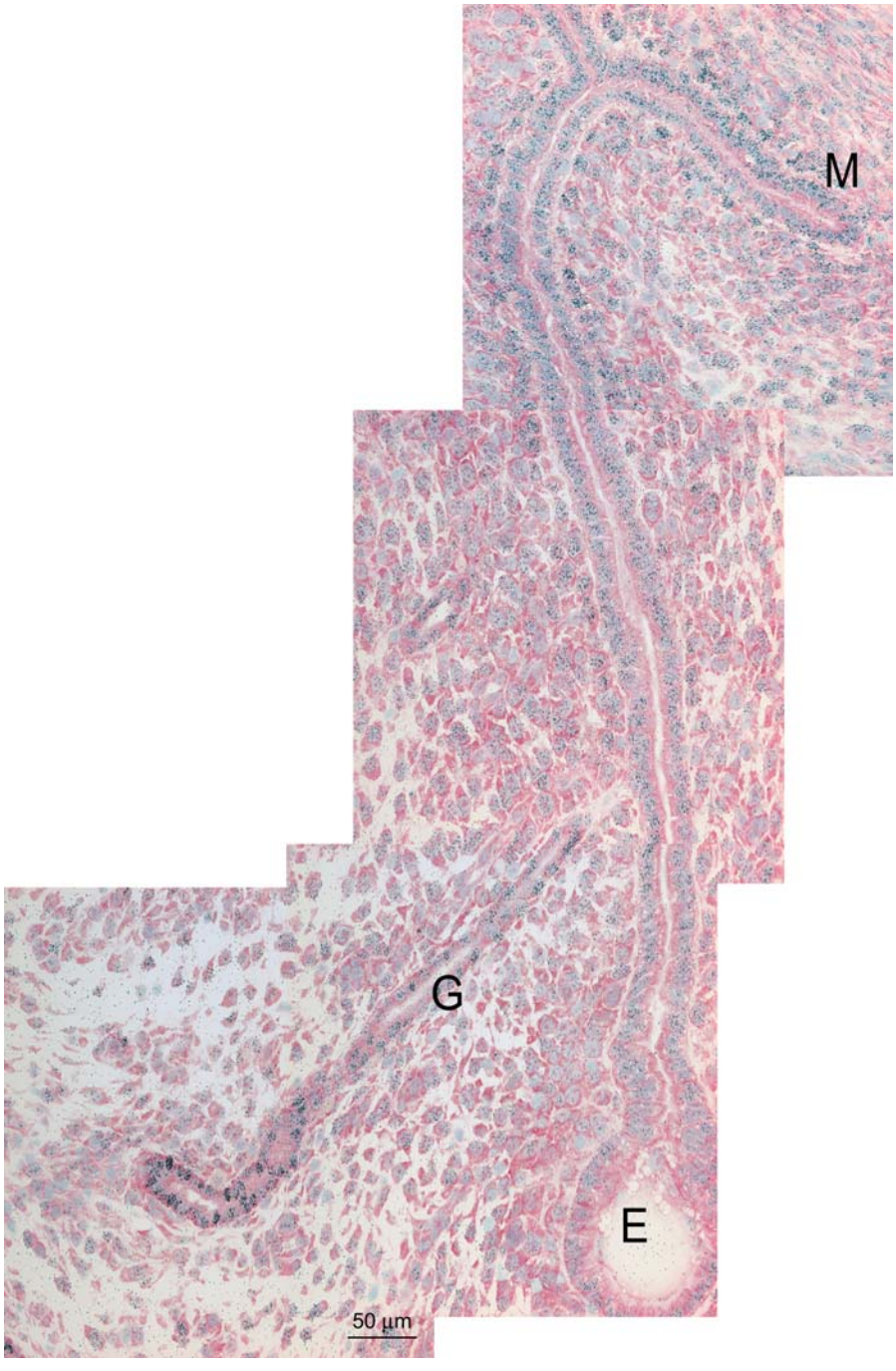


Fig. 3 ^{3}H-estradiol concentration on day 4.5 of pregnancy is very low in the epithelial cells lining the implantation crypt and progressively increases toward the mesometrial extension of the lumen. The concentration of ^{3}H-estradiol is higher in glands than in luminal epithelium. Note variations among individual cells and changes along the course of the gland. *G* Gland, *E* embryo, *M* mesometrial region. Exposure time: 70 days

Fig. 2a–f ^{3}H-estradiol concentration on days 1.5 (**a**, **b**), 2.5 (**c**, **d**), and 3.5 (**e**, **f**) after fertilization. In the luminal epithelium, nuclear labeling is higher at luminal branches and at mesometrial and antimesometrial extensions (**a**, **c**, **e**). The concentration of ^{3}H-estradiol is higher in glands than in luminal epithelium (**a–f**). Among glands, epithelial height, diameter of lumen, and nuclear silver grain density vary (**c**, **d**, **f**). *Asterisks* indicate low-labeled glandular profiles. *G* Gland, *GD* glandular duct, *L* uterine lumen, *bv* blood vessel. Exposure times: 70 days for **c–f**; 100 days for **a**, **b**

implantation, the surface of the implantation crypt was devoid of epithelial layer, whereas the epithelium was maintained at the mesometrial pole of the IS and in the lumen of the IIS. In these regions, the luminal epithelial cells were low-cubical. ^{3}H-estradiol binding was strong in the luminal epithelium of IIS, moderate in the mesometrial region of IS, and decreasing markedly toward the implantation crypt (Fig. 5a, b). No binding of 3H-estradiol was observed in the embryo.

In the IS, glands were sparse or mostly absent in decidualized areas of the endometrial stroma. Examination of serial sections, however, showed long glandular

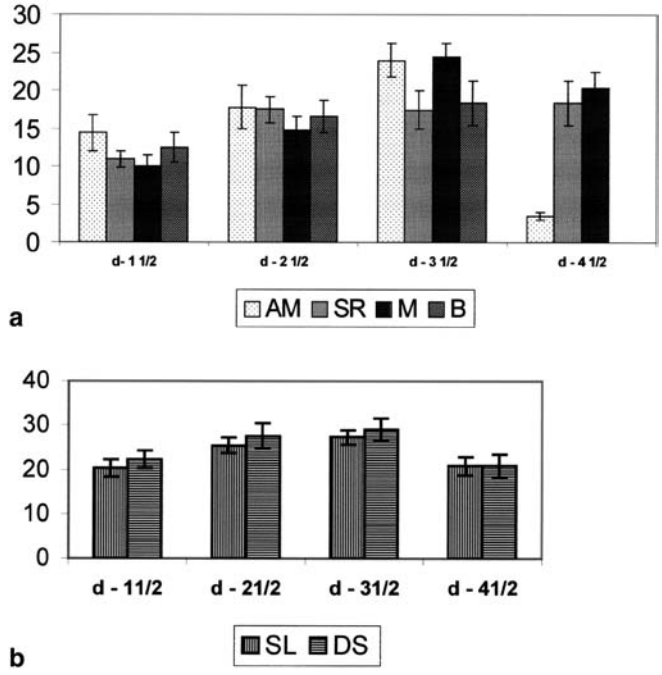


Fig. 4a, b Quantitative evaluation of autoradiograms. Silver grain counts over luminal (**a**) and glandular (**b**) epithelial cell nuclei at days 1.5–4.5 of pregnancy from three animals per time period and the mean (±SD) of over 30 cells per epithelial region per animal. *M* Mesometrial extensions, *AM* antimesometrial extensions, *SR* straight region of the central part of the uterine lumen, *B* branched extensions, *SL* glands in the subluminal stroma, *DS* glands in the deep stroma, *d* day

tubes that cross the entire extension of the stroma and terminate very close to the implantation chamber (Fig. 5a, c). Glands situated in the mature decidua and predecidua were always strongly labeled (Fig. 5c, d). A small number of glands was present in the non-decidualized stroma situated near the myometrium. These glands were tortuous and morphologically heterogeneous showing a low binding of ^{3}H-estradiol.

On days 6.5 and 7.5, in general, both the morphology of the uterus as well as the ^{3}H-estradiol binding and distribution were similar. The majority of epithelial cells continued to retain ^{3}H-estradiol. However, there were notable differences regarding the ^{3}H-estradiol binding between epithelial sheets situated at different places of the uterus. In both IS and IIS the labeling by ^{3}H-estradiol was higher at the mesometrial end of the uterine lumen (Fig. 5e). On day 7.5, serial longitudinal sections of the uterus clearly showed the formation of the new lumen, crossing the uterine wall from the mesometrial to antimesometrial direction and running parallel to the interface between IIS and IS (Fig. 5f). In the new lumen, the epithelial layer that covers the IIS side was strongly labeled, while the epithelial layer of the opposite side in contact with decidua was only weakly labeled (Fig. 5f). The number of glands decreased progressively from day 5.5 to day 7.5 in both IIS and IS. In the regions of the decidua and predecidua, glands were sparse and inconspicuous and, because of the alterations of the glandular epithelium, may not be recognizable, except in serial sections that permitted to follow them and recognize their connections. The nuclear labeling in glands, however, continued to be higher than in luminal epithelium.

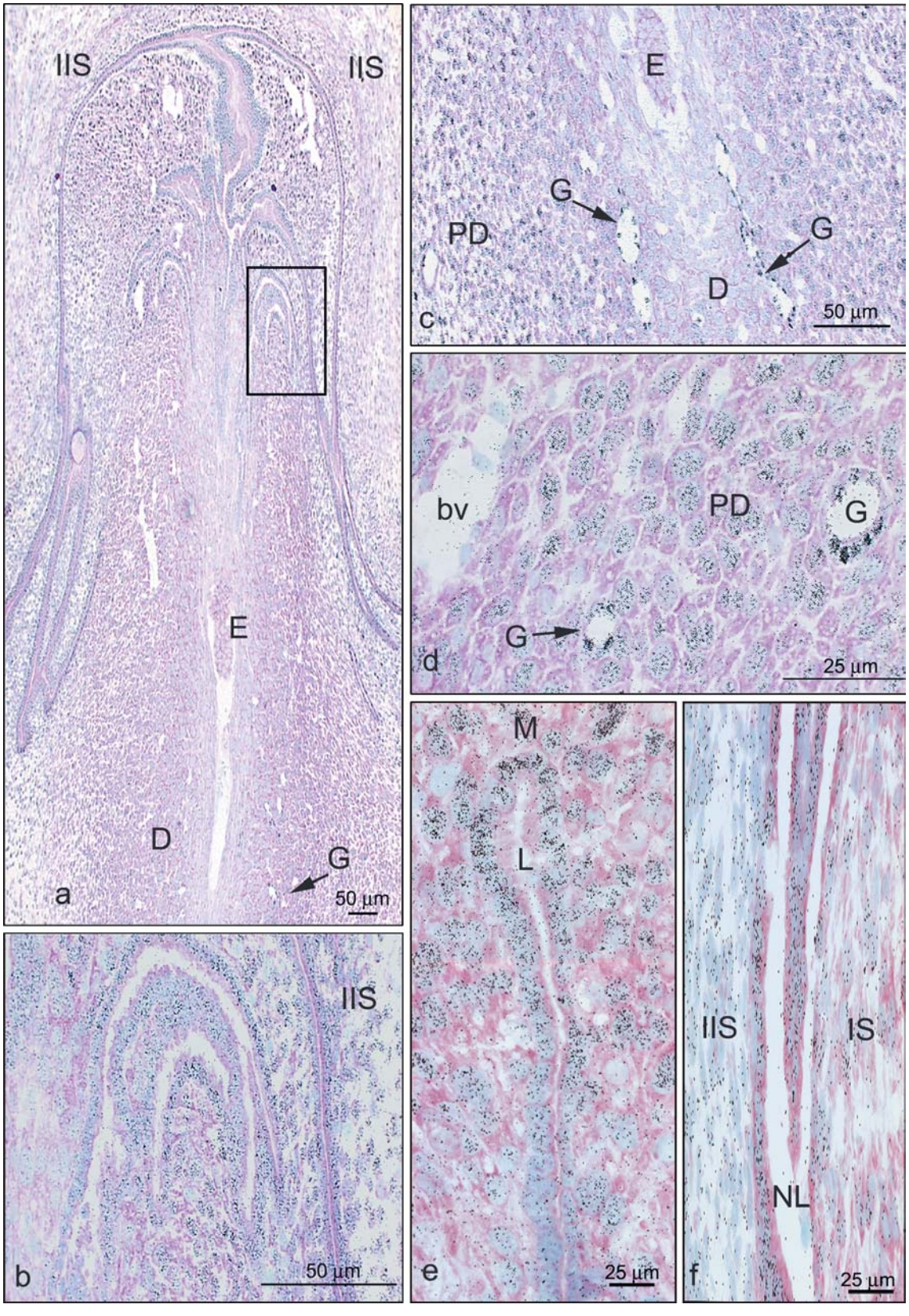
Comparison between ^{3}H-estradiol nuclear binding and ^{3}H-thymidine incorporation

On day 1.5, exclusively epithelial cells of the luminal epithelium incorporated ^{3}H-thymidine. Similar to ^{3}H-estradiol, tritiated thymidine incorporation was present mostly in epithelial cells situated in the extremities of the branched areas of the uterine lumen (Fig. 6a). On day 2.5, besides the luminal epithelium a few cells of the uterine glands were also labeled (Fig. 6b). On day 3.5, ^{3}H-thymidine incorporation stopped in both luminal and glandular epithelium and started in the stromal compartment (Fig. 6c). This pattern of ^{3}H-thymidine incorporation was maintained during days 4.5, (Fig. 6d), 5.5, and 6.5. On day 7.5 ^{3}H-thymidine incorporation was observed in luminal epithelial cells and corresponded to that of ^{3}H-estradiol binding. In both IS and IIS the number of labeled cells was highest in the mesometrial end of the uterine lumen (Fig. 6e). At the IIS site, similar to ^{3}H-estradiol, the epithelial layer that covers the new lumen was strongly labeled with ^{3}H-thymidine while in the epithelial layer in contact with the decidua, only rare cells were labeled (Fig. 6f). In glands, no incorporation of ^{3}H-thymidine was observed whereas glandular cells maintained a sustained binding of ^{3}H-estradiol during all periods.

Comparison between ^{3}H-estradiol nuclear binding and immunolocalization of ER-α

The immunocytochemical result of ER-α distribution was in general similar to that obtained for ^{3}H-estradiol binding using autoradiography. Positive reaction for ER-α was observed in both luminal and glandular epithelium during all days of pregnancy. Figure 7 shows a comparison

Fig. 5a–f On day 5.5 of pregnancy (**a–d**) ^{3}H-estradiol concentration in the luminal epithelium varies according to the position in the uterine horn. It is diminished toward the implantation chamber, but increased toward the mesometrium and the lateral interimplantation regions as seen in the longitudinal sections (**a**). Enlargement of the region in the *rectangle* (**b**). Strongly labeled ducts of glands cross the decidualized endometrium toward the embryo (**c**). Glands in the predecidua are small and show distinct degree of ^{3}H-estradiol binding (**d**). On day 7.5 of pregnancy (**e**, **f**) ^{3}H-estradiol concentration in the luminal epithelium is strong at the mesometrial pole of the uterus with a gradient of decreased labeling toward the implantation crypt (**e**). The luminal epithelium is strong at the surface of the interimplantation sites but weak or abolished along the adjacent decidua (**f**). *IIS* Interimplantation site, *IS* implantation site, *E* embryo, *G* gland, *D* mature decidua, *PD* predecidua, *bv* blood vessel, *M* mesometrial region, *L* uterine lumen, *NL* new lumen. Exposure time: 100 days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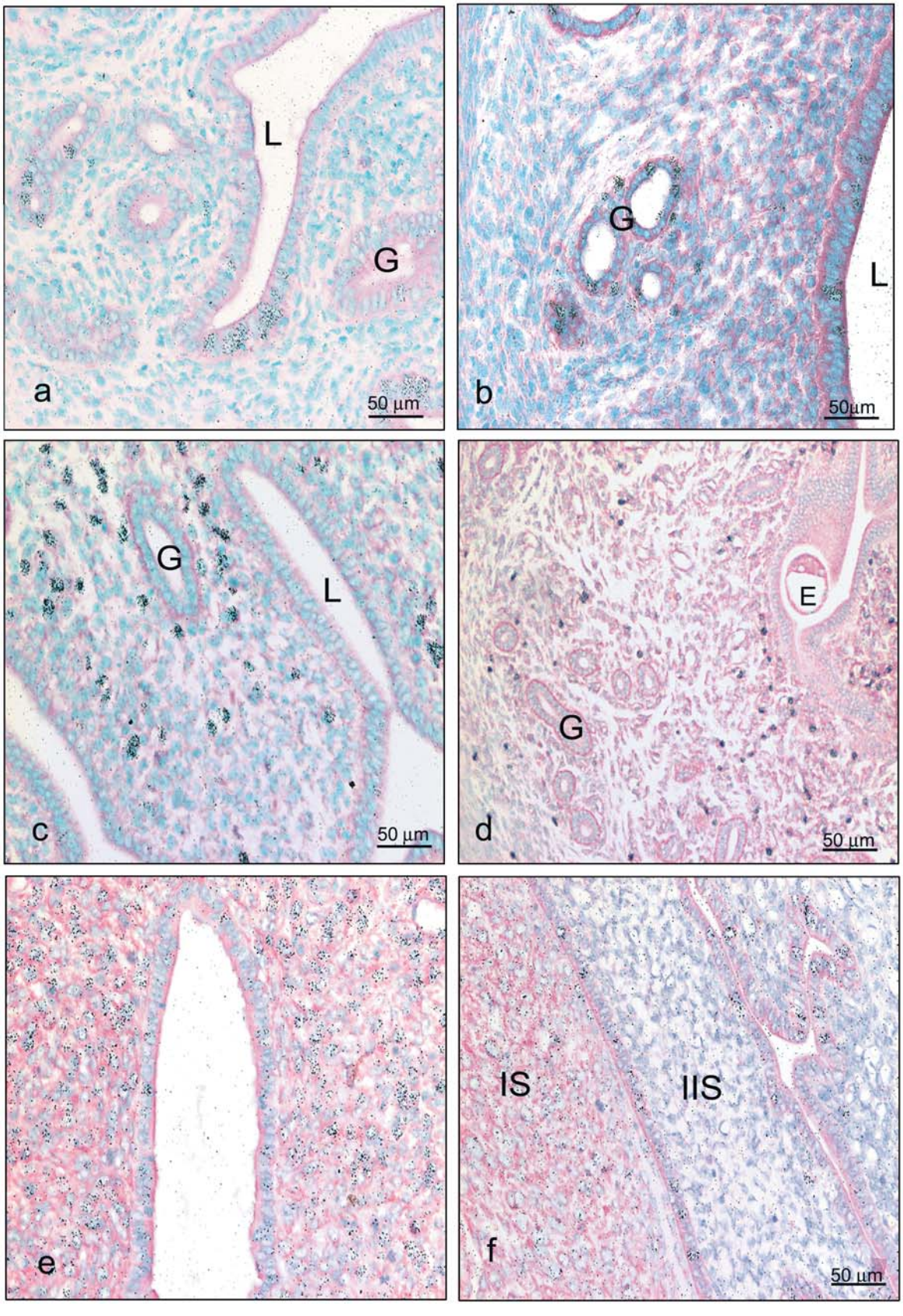
^{3}H-THYMIDINE



between immunocytochemistry and autoradiographs of uteri on day 3.5 of pregnancy.

The strongest radiolabeled glands also were strongly immunopositive, and the luminal epithelium with relatively weak radiolabeling was also weakly immunopositive. When results of immunocytochemistry were compared with those of autoradiography, differences in sensitivity and resolution became apparent. For instance: (a) immunocytochemistry did not reveal subtle but clear differences between superficial and deep glands, (b) immunocytochemistry did not reveal differences among different regions of the luminal epithelium, and (c) immunocytochemistry showed unstained nuclei in the luminal epithelium that appeared labeled with ^{3}H-estradiol.

Discussion

For the identification of in vivo hormone target tissues, receptor microautoradiography provides cellular–subcellular resolution and high sensitivity that is impossible or difficult to achieve by biochemical and immunocytochemical approaches alone. After injection of tritium-labeled estradiol with high specific activity, sites of uptake and binding can be identified, qualitatively and quantitatively, and then further characterized. The localized radioactivity reflects the acting hormone that binds to specific nuclear receptors with different affinities and capacities related to tissue-specific functions (Stumpf 2003).

After injection of radiolabeled estradiol-17β, uptake and retention of the hormone occurs in cell nuclei of specific uterine tissues with varying intensities and changes during the pre- and periimplantation periods of the embryo. These changes are complex and characteristic not only for each tissue, but also within the same tissue according to its location, its association with other tissues, predominantly the blastocyst, and the regions of the mesometrium and antimesometrium. In the autoradiograms, nuclear uptake of ^{3}H-estradiol is demonstrated and substantiated by quantitative evaluation. In all of these target cell populations, ^{3}H-estradiol uptake increases during the first 3 days after fertilization. Throughout the whole period the ^{3}H-estradiol binding is highest in glandular epithelium compared with luminal epithelium.

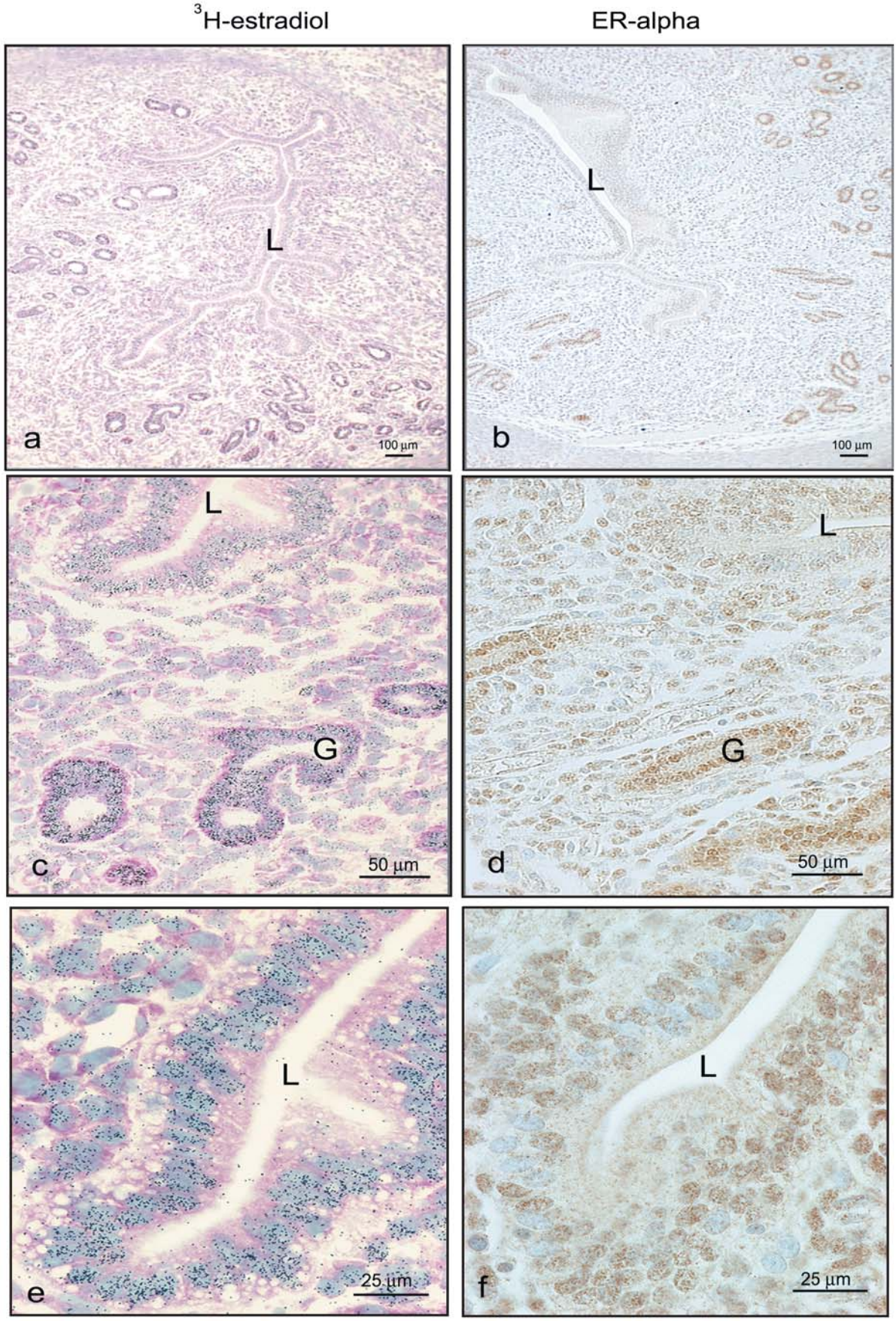
The results of our immunocytochemical studies from day 1.5 to day 7.5 of pregnancy as well as those published in the literature for days 4–7 (Tan et al. 1999) indicate that in the uterine tissues estradiol binds to the subtype ER-α. When the results obtained by autoradiography are compared with those obtained by immunocytochemistry, there appears to be general agreement in low magnification surveys. Careful and detailed examination, however, and especially quantification that is possible only with high-resolution autoradiograms, reveal differences that pertain to different sensitivity and resolution of these two approaches.

After fertilization in the epithelium of the uterine lumen and glands morphological changes occur in rapid succession paralleled by differences in ^{3}H-estradiol nuclear uptake. On days 1.5, 2.5, and 7.5 of pregnancy, in the luminal epithelium nuclear concentration of ^{3}H-estradiol is correlated with ^{3}H-thymidine incorporation. The concentration of proliferative activity at the edges of the uterine lumen may reflect remodeling of the lumen that occurs in particular during the preimplantation period but also subsequently. The gradual increases in the ^{3}H-estradiol binding by luminal epithelial cells observed from day 1.5 to day 3.5 are probably related to successive morphological and functional changes optimizing conditions for reception and nidation of the conceptus (Parr and Parr 1989; Weitlauf 1994). During the early periimplantation period on day 4.5 of pregnancy, the decrease of ^{3}H-estradiol binding is probably influenced by progesterone dominance in this phase (Psychoyos 1973), which has an inhibitory effect on the ER-α expression in the luminal epithelium (Wang et al. 1999). Our results indicate that the inhibitory effect on ER expression is not homogeneous throughout the epithelial cells since the distribution of ^{3}H-estradiol receptor binding is not uniform but varies along the uterine lumen. The binding of ^{3}H-estradiol by epithelial cells lining the uterine crypt was very low, probably because these cells will die by apoptosis several hours after embryo implantation (Parr and Parr 1989).

During the later periimplantation period, the pattern of ^{3}H-thymidine incorporation by luminal epithelial cells is again very similar to that observed for ^{3}H-estradiol. In both IS and IIS, labeled cells are concentrated mainly in the mesometrial end of the uterine lumen. The sustained proliferative activity exhibited by the luminal epithelial cells at the mesometrial pole of the uterus may be related to the formation of the new uterine lumen that starts on day 7 of pregnancy. The proliferative activity expressed by epithelial cells that line the new lumen differs from one side to the other. A similar distribution is observed for ^{3}H-estradiol receptor binding in these regions. These results demonstrate the existence of a close association between cell proliferation and ER expression in the luminal epithelium during this period of pregnancy.

Classic studies by Finn and Martin (1967) showed that mitotic activity of endometrial glands in mice tends to be

Fig. 6a–f Autoradiograms of endometrium of pregnant mice 1 h after injection of ^{3}H-thymidine. Paraffin sections stained with methyl green–pyronin. On day 1.5 of pregnancy, only luminal epithelial cells are labeled. Note a concentration of labeled cells in the branched areas of the lumen (**a**). On day 2.5, both luminal epithelium and glandular epithelium are labeled (**b**). On day 3.5, many stromal cells are labeled while both luminal epithelium and glandular epithelium are unlabeled (**c**). On day 4.5, labeled cells continue to be observed only in the endometrial stroma (**d**). On day 7.5, at the mesometrial pole, many luminal epithelial cells are labeled (**e**). Epithelial cells lining the surface of decidua at the IS are not labeled while most of the epithelial cells lining the IIS surface are labeled (**f**). *L* Uterine lumen, *G* uterine glands, *E* embryo, *IS* implantation site, *IIS* interimplantation site. Exposure time: 60 days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f
25 µm

low before ovulation but increases 3 days later. Our results of ^{3}H-thymidine incorporation largely agree and extend these former data and demonstrate that ^{3}H-thymidine incorporation by glandular cells on day 2.5 of pregnancy parallels a strong ^{3}H-estradiol binding in these cells. Thus, our results corroborate those from Finn and Martin (1973) who suggested that the proliferative activity in glands during that period is hormone dependent. However, from day 3.5 of pregnancy onward, ^{3}H-thymidine incorporation is no longer observed in the glandular epithelium, while a strong ^{3}H-estradiol concentration continues to exist. The results of the present studies provide several indications for strong secretory activity of uterine glands during the preimplantation and periimplantation periods. This is apparent in the intense pyronin (RNA)-positive stain of the cytoplasm of epithelial glandular cells. It is noteworthy that some of the observed occasional drastic changes in glandular morphology resemble those seen in apocrine mammary glands. These changes all are associated with strong nuclear concentration of ^{3}H-estradiol, with the exception of some squamous cells in the interrupted lining of distended portions of, perhaps, exhausted portions of the glands.

Different studies published in the literature provide evidence for glandular secretory capabilities during the periimplantation period (reviewed by Given and Enders 1989) and that this secretory activity is under the control of estradiol (Aitken 1977; Pratt 1977; Surani 1977; Fishel 1979). In vitro studies indicate that the secretory activity of cultured endometrial cells changes if progesterone or estrogen is added to the incubation medium (Bell et al. 1986). Glycoproteins such as uteroglobin (Beir 1968; Atger et al. 1980) and insulin-like growth factor-I (Person et al. 1997) change in the uterine flush according to the hormonal profile of the animal.

Recent studies with adult uterine gland knockout ewes demonstrated that endometrial glands and, by inference, their secretion are required for periimplantation conceptus survival and development (Gray et al. 2001). Moreover, it is known that a cytokine, leukemia inhibitory factor (LIF) is expressed in the uterine endometrial glands of mice, specifically on day 4 of pregnancy. The LIF secretion is under maternal control and its gene expression is estrogen-dependent (Bhatt et al. 1991; Shen and Leder 1992; Stuart et al. 1992). There is direct evidence obtained from mice deficient in a functional LIF gene that this molecule is an absolute requirement for embryo implantation. According to Cullinan et al. (1996) LIF acts through an autocrine/paracrine interaction with its receptor at the luminal epithelium. The presence of highly ^{3}H-estradiol-labeled glandular profiles in the decidua in close proximity to the implantation cavity is shown in the present work, reinforce the concept that the uterine glands may have a role in the regulation of embryo implantation and development.

In summary, the present data indicate strong estrogen action upon glandular epithelium, however, with various changes of emphasis on proliferation and differentiation earlier, then secretion, and involution and remodeling later. Incipient cell involution and death appear to be associated with a reduction or abolition of nuclear estradiol binding. What causes the target tissue-specific changes in estrogen binding, morphology, and function remains to be clarified, involving perhaps progesterone and local tissue factors as well. The present data together point to a hierarchy of estrogen effect on uterine epithelia in which the glandular epithelium appears to play a predominant role during the postfertilization–periimplantation period.

Fig. 7a–f Comparison between autoradiograms of ^{3}H-estradiol injection (**a**, **c**, **e**) and immunocytochemistry for estrogen receptor (ER)-α (**b**, **d**, **f**). At low magnification, no differences are observed between the distribution of ^{3}H-estradiol and ER immunolocalization. In both images, glands appear strongly labeled while the luminal epithelium and stroma appears negative (**a**, **b**). High magnification autoradiograms (**e**), however, clearly show that the ^{3}H-estradiol binding occurs in almost all cells of the luminal epithelium, some of which remain unstained with antibody (**f**). *L* Uterine lumen, *G* uterine glands. Exposure time: 70 days

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