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Uterine Estradiol and Progesterone Receptor Concentration in Relation to Circulating Hormone Levels and Histoarchitecture During High Endometrial Sensitivity and Induced Decidualization in Guinea Pigs

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Alterations in nuclear and cytosolic estradiol (ER) and progesterone (PR) receptor concentration in the antimesometrial (AM) and mesometrial (M) segments of the uterus in relation to circulating hormone levels, histology and surface topography during the period of high endometrial sensitivity and development of trauma-induced decidualization in cyclic guinea pigs were investigated. The period of high endometrial sensitivity (i.e. day 5 of the estrous cycle) was characterized by elevated plasma estradiol and progesterone and their receptors in the nuclear and cytosolic fractions of the uterus. There was, however, no difference in the concentration of these receptors or the surface ultrastructure in the AM and M segments. Unilateral traumatization by scissor cut along the AM length of the uterus on day 5 of the estrous cycle induced decidual cell reaction resulting in a marked increase in weight of the decidualized (traumatized) uterine horn with advancing decidualization to reach maximum levels (926% of the contralateral nontraumatized uterine horn) 7 days after traumatization. This was associated with decidual transformation and a marked increase in nuclear and cytosolic ER and PR concentration in the AM segment of the traumatized uterine horn. An increase in receptor concentration in the M segment of the traumatized uterine horn or the AM segment of the nontraumatized uterine horn was transitory and of a low order. Receptor concentration in the M segment of the nontraumatized uterine horn remained low throughout days 8–12 of the cycle. Findings indicate a possible role of both estradiol and progesterone in induction of endometrial sensitivity and development and maintenance of decidua in the guinea pig.

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INTRODUCTION

In most mammals, uterus under the influence of a sequential action of ovarian estrogen at estrous and post-ovulatory progesterone enters into a pre-sensitized phase. In species like rat, mouse and gerbil, that exhibit facultative delay of implantation, endometrial sensitivity to blastocyst stimulus for decidual transformation is brought about by the action of the luteal phase nidatory estrogen [1]. In such species, inhibition of the synthesis [2] or action [3] of this estrogen leads to inhibition of decidualization and implantation.

The guinea pig is a unique rodent species since, like the human, it has a long functional luteal phase during each cycle and a completely interstitial type of implantation [4]. Endometrium in this species is maximally sensitive to decidua induction on days 5 and 6 of the estrous cycle [5] (day 0: first day of vaginal opening). Maximum cell proliferation in endometrial stroma in cyclic as well as mated female guinea pigs has also been reported to occur on these days, and is dependent on estrogen action, since a similar response is induced only after estrogen administration in ovariectomized females maintained on daily progesterone therapy [6]. An increase in plasma estradiol (E_2) levels coinciding with high endometrial sensitivity on days 5 and 6 *post-coitum* has also been demonstrated [7]. This is believed to enhance uterine blood flow resulting in increased oxygen supply for the blastocyst and induce regional

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hyperaemia and development of certain preferred sites for implantation in the uterus. While this evidence might be considered as suggestive of the requirement of both luteal phase estrogen and progesterone (P) in implantation in guinea pigs, pioneer investigations [8] reveal that in this species hormones of ovarian origin secreted after day 3 *post-coitum* are not required for implantation, since normal implantation ensues even in animals ovariectomized on or after day 3 without any exogenous hormone therapy.

Thus the precise nature of hormone interplay for induction of endometrial sensitivity, decidualization and implantation in this species remains by and large enigmatic and merits detailed evaluation. This has been attempted here by determining alterations in estradiol (ER) and progesterone (PR) receptor concentration in the antimesometrial and mesometrial segments of the uterus in relation to circulating hormone levels and uterine histoarchitecture during the period of high endometrial sensitivity and development of trauma induced decidua in cyclic female guinea pigs.

EXPERIMENTAL

Animals

Colony bred adult albino virgin female guinea pigs (350–500 g body wt), maintained under standard husbandry conditions with alternate 12 h light and dark periods and a free access to pellet diet (Lipton India Ltd, Bangalore) and tap water, supplemented with leafy vegetables, soaked grams, vitamin C and mineral water were used. Animals were checked every morning for vaginal opening and the first day of its occurrence was taken as day 0 of estrous cycle [6]. Animals exhibiting at least two consecutive regular cycles just before use were included in this study.

Decidua induction and collection of tissues

Decidua was induced in any one of the uterine horns by giving a scissor cut [9] along the entire antimesometrial length of the uterus between 0900–1100 h on day 5 of the estrous cycle [5] under light ether anesthesia. Care was taken not to disturb the contralateral control horn. Animals were autopsied on days 5 (i.e. the day of high endometrial sensitivity) [5], 8, 10 and 12 of the estrous cycle (i.e. 3, 5 and 7 days post-traumatization). Traumatized and nontraumatized (control) uterine horns of each animal were quickly dissected out, stripped free of fat and mesenteries, washed in ice-cold physiological saline, blotted and weighed to the nearest 2 mg on a torsion balance (August Sauter, Germany) (Table 1). Antimesometrial (AM) and mesometrial (M) segments were then separated by cutting along the lateral sides [10] of each uterine horn and stored at −70°C until assayed for receptors and DNA.

Preparation of subcellular fractions

All subsequent steps were carried out in a cold room (0–4°C) unless otherwise specified. Each tissue was homogenized on a polytron (Kinematica, PT 10-35) homogenizer in TMMG buffer (10 mM Tris–HCl, 3 mM $MgCl_2$, 12 mM monothioglycerol, 1 μM cortisol and 10% glycerol; pH 7.4) to achieve a final concentration of 300 mg tissue/ml. Homogenates were filtered through a 100 mesh nylon filter. 0.5–1 ml of each homogenate was saved for DNA [11] determination using calf thymus DNA as the standard. Remaining portions of the homogenates were centrifuged at 800 *g* for 10 min at 0–4°C in an International portable PR-2 centrifuge. Nuclear pellets were washed three times by suspending in 2 ml TMMG buffer and centrifugation before using for binding assay. Supernatants obtained after centrifugation of the whole homogenates at 800 *g* were subjected to ultracentrifugation (IEC, U.S.A., model M-60) at 105,000 *g* for 60 min at 0–4°C to obtain the cytosolic fraction.

Receptor measurement

Total nuclear and cytosolic ER and PR were estimated by the method of Martel *et al.* [12] with slight modifications.

Nuclear receptors

Nuclear pellet was suspended in 5 ml TMMG buffer and 0.3 ml of this nuclear suspension was incubated (v/v) with either (a) 96 nM [^{3}H]E_2 and (b) 96 nM [^{3}H]E_2 + 9.6 μM E_2 or (c) 96 nM [^{3}H]P and (d) 96 nM [^{3}H]P + 9.6 μM P. Samples were incubated in triplicate for 30 min at 37°C for the measurement of ER and 16–18 h at 0–4°C for PR. At the end of the incubation period, nuclear pellet was washed three times with 2 ml cold TMMG buffer to remove unbound [^{3}H]E_2

Table 1. Uterine decidual response in unilaterally traumatized cyclic guinea pigs

Autopsy day		Uterine weight[a]		Uterine weight gain (% of non-traumatized horn)
Day of estrous cycle	Day post-traumatization	Non-traumatized horn	Traumatized horn	
5	0	319 ± 10		
8	3	319 ± 22	660 ± 60[b,d]	107
10	5	242 ± 11[c,f]	1472 ± 97[c,e,f]	508
12	7	242 ± 7[c]	2241 ± 121[c,e,f]	826

[a]Values are ng, mean ± SEM of uteri from 18–21 animals on each day.
[b]$P < 0.05$, [c]$P < 0.01$; vs day 5 of the estrous cycle; [d]$P < 0.05$, [e]$P < 0.01$; vs corresponding non-traumatized uterine horn; [f]$P < 0.01$; vs preceding value. All other relevant comparisons were statistically insignificant.

or [^{3}H]P. Bound [^{3}H]E_2 and [^{3}H]P were extracted (1 ml × 2) with redistilled ethanol [13] and counted for radioactivity in 10 ml xylene-based scintillation fluid in an LKB 1217 RACK BETA Liquid Scintillation Spectrometer at a counting efficiency of 25%. Specific binding was calculated by subtracting the nonspecific binding measured in the presence of excess nonradioactive hormone from the total binding measured in the absence of nonradioactive hormone.

Cytosolic receptors

Prior to binding assay, cytosol was incubated with an equal volume of dextran coated charcoal (DCC, 0.05% dextran and 0.5% charcoal in TMMG buffer) for 60 min at 4°C to remove endogenous steroids. Charcoal was removed by centrifugation at 800 *g* for 10 min and the supernatant was used for binding assay for ER and PR. Parallel incubations were carried out in triplicate. Portions (0.1 ml) of cytosol were incubated (v/v) with either (a) 96 nM [^{3}H]E_2 and (b) 96 nM [^{3}H]E_2 + 9.6 μM E_2 or (c) 96 nM [^{3}H]P and (d) 96 nM [^{3}H]P + 9.6 μM P for 16–18 h at 0–4°C [14]. At the end of the incubation period, unbound steroids were removed by addition of 0.5 ml DCC and incubation at 0–4°C for 15 min followed by centrifugation at 800 *g* for 10 min. Radioactivity was assessed in the supernatants as detailed above.

Plasma E_2 and P concentration

About 2 ml blood samples collected in heparinized tubes from each animal between 0900–1100 h on days 1, 3, 5, 8, 10 and 12 of the estrous cycle were centrifuged and the plasma was stored at −20°C prior to being analyzed for E_2 and P by radioimmunoassay [15] using methods and kits supplied by the W.H.O., Geneva under their Matched Reagent Programme. The intra- and inter-assay variations were within normal limits (Table 2).

Histology

Representative tissue samples from traumatized and nontraumatized uterine horns on each day were fixed in freshly prepared Bouin's fixative for histological (5 μ) sectioning. Sections were stained with haematoxylin and eosin.

Surface ultrastructure

For scanning electron microscopy, the uterus was immediately fixed at 0–4°C in a freshly prepared 0.1 M sodium cacodylate buffer (pH 7.3 ± 0.1) containing 3% glutaraldehyde and 20% paraformaldehyde [16]. After brief fixation, each uterine horn was cut close to the M side under a stereomicroscope (American Optics, model 561 C-HI) while still immersed in the fixative, and then left in the fixative for a further 4 h at 4°C. The samples were then washed in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetraoxide in 0.1 M cacodylate buffer for 2–3 h and dehydrated in alcohol and isoamylacetate. Finally the tissues were dried in a Balzer critical point drier using CO_2 as the critical point drying medium. The specimens were fixed on aluminium stubs, coated with gold palladium alloy in a sputter coater (Polaron E 5000) and photographed on Philips 515 Scanning Electron Microscope.

Chemicals and reagents

[2,4,6,7-^{3}H]E_2 (104 Ci/mmol) and [1,2,6,7-^{3}H]P (101.7 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, England). All other chemicals were purchased from Ernest F. Fullam Inc., Sigma Chemical Co. and Laad Research Industries Inc. (U.S.A.).

Statistical analysis

The data were analyzed by the Duncan's Multiple Range Test [17].

Table 2. Alterations in peripheral plasma E_2 and P concentration in guinea pigs subjected unilaterally to artificially induced uterine decidualization on day 5 of the estrous cycle

Day of estrous cycle	Day post-traumatization	Peripheral plasma concentration	
		E_2 (pmol/l)	P (nmol/l)
1		168.2 ± 8.2	16.1 ± 1.2
3		151.6 ± 10.0	11.1 ± 0.9
5	0	247.3 ± 14.6[b,e]	24.9 ± 1.0[a,e]
8	3	194.5 ± 6.0[c,d]	21.5 ± 1.1
10	5	200.4 ± 12.4	21.6 ± 1.6
12	7	245.9 ± 10.1[b,d]	31.6 ± 1.8[b,c,e]

Values are mean ± SEM of 6 determinations.
[a]$P < 0.05$, [b]$P < 0.01$; vs corresponding day 1 concentration; [c]$P < 0.05$; vs corresponding day 5 concentration; [d]$P < 0.05$, [e]$P < 0.01$; vs corresponding preceding value.

RESULTS

Decidual response

The extent of decidual response was assessed on the basis of uterine weight gain and histology.

Uterine weight

Unilateral traumatization on day 5 of the estrous cycle induced uterine decidualization as evidenced by a marked ($P < 0.01$, Table 1) increase in weight of the traumatized uterine horn 3, 5 and 7 days post-traumatization and the weight gain (over corresponding nontraumatized uterine horn) was 107, 508 and 826%, respectively. The nontraumatized uterine horn, in comparison, exhibited a significant decrease in weight on days 10 and 12 of the cycle ($P < 0.01$, vs days 5 and 8 of the cycle).

Histology

On day 5 of the estrous cycle, i.e. the day of high endometrial sensitivity, the lumen was lined with cuboidal epithelium and the stroma consisted primarily of fibroblast cells. Some edema was apparent on the AM side. Glands were prominent along the M and

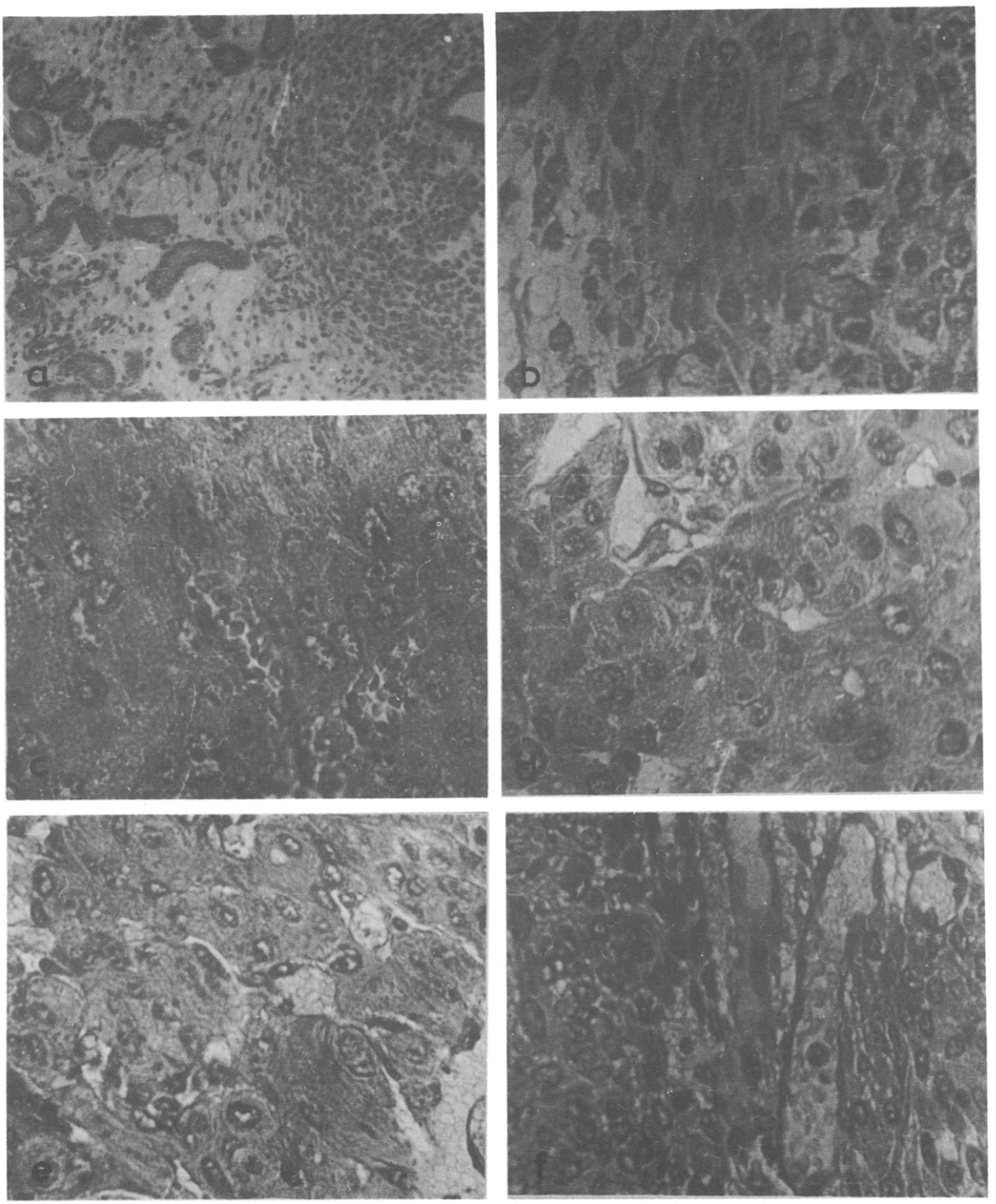

Fig. 1. (a and b) Transverse section of the guinea pig uterus on day 5 of the estrous cycle. (a) M side × 310 and (b) AM stroma × 1290. (c–e) Uterine peripheral endometrial stroma with advancing induced decidualization at 3(c), 5(d) and 7(e) days post-traumatization. Note increasing vascularization with advancing decidualization × 1290. (f) Some differentiating endometrial stroma near the central region at 7 days post-traumatization × 1290.

lateral sides. The histological picture 3 days after trauma (i.e. day 8 of the estrous cycle) showed initiation of decidual reaction along the periphery on the AM side. On day 5 post-trauma, an increase in number of differentiated decidual cells was observed. On day 12 of the cycle (i.e. 7 days post-trauma), a large number of decidual cells and mitotic figures were present on both the AM and M sides and demarcation between these segments was less distinct, except for a small zone of endometrial glands along the M side (Fig. 1).

Fig. 2. (Opposite)

Fig. 2. (a) Scanning electron micrograph of guinea pig uterus on day 5 of the estrous cycle. Note demarcation in the AM and M areas × 1000. Antimesometrial (b) and mesometrial (c) sides covered with a thick carpet of microvilli. Note almost similar surface ultrastructure and absence of pinopods and cilia. Glandular openings are visible × 3000.

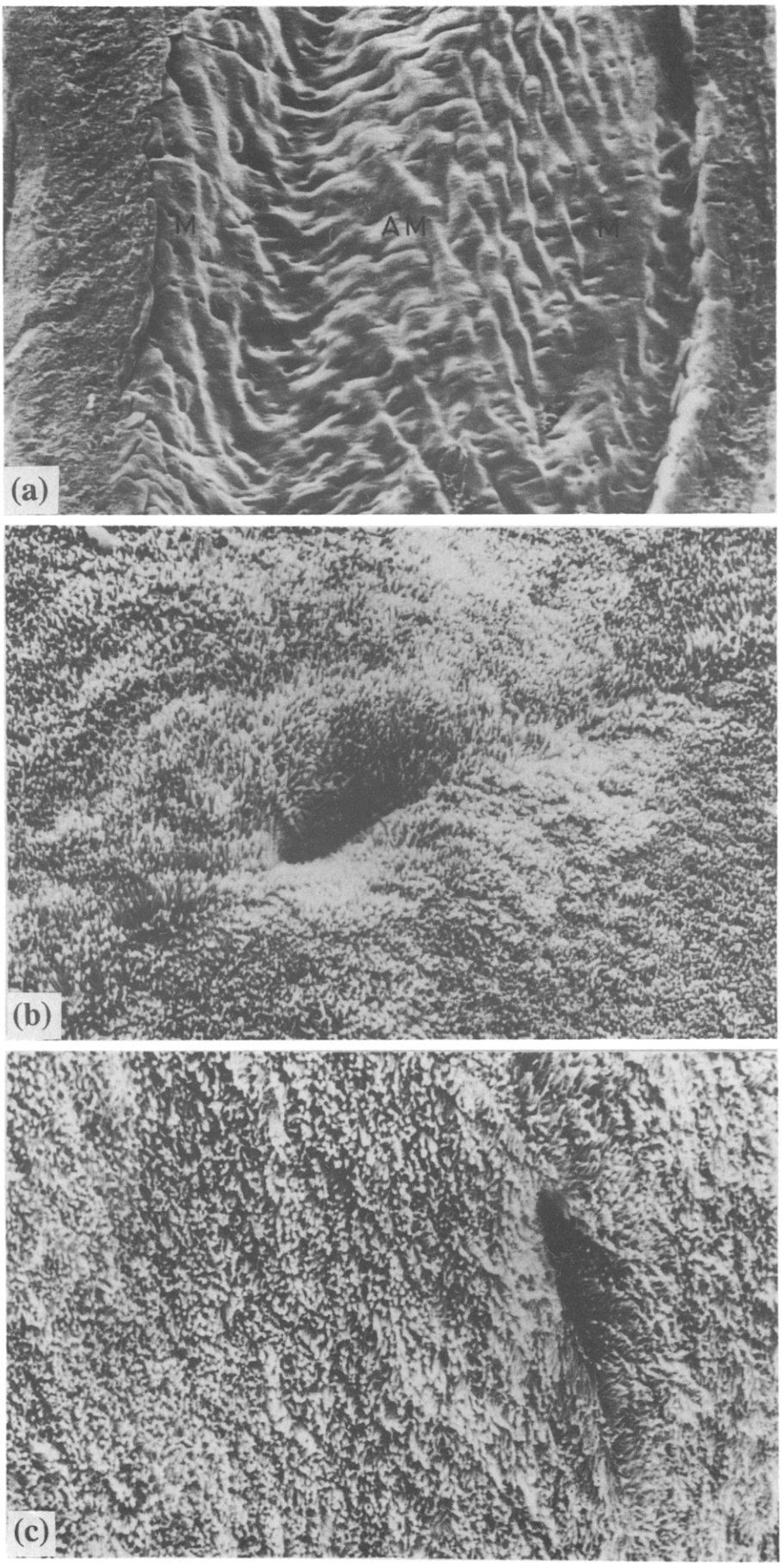

Fig. 2—*legend opposite.*

Surface ultrastructure

Ultrastructurally, the entire endometrial surface on day 5 of the estrous cycle was uniformly undulated with numerous openings of endometrial glands [Fig. 2(a)]. At higher magnification, both the AM and M surface were covered with a thick carpet of short microvilli and there was no apparent difference in their surface ultrastructure. Cell boundaries were not visible. Pinopods and cilia were totally absent [Fig. 2(b and c)].

Peripheral plasma E_2 and P concentration

Maximum plasma E_2 concentration was observed on day 5 of the estrous cycle ($P < 0.01$, vs days 1 or 3, Table 2). This corresponds to the period of high endometrial sensitivity to decidualizing stimulus of the blastocyst. A transient but statistically significant ($P < 0.05$, vs day 5) decrease was observed on day 8, followed by an increase to reach almost the same levels on day 12 ($P < 0.05$, vs days 8 or 10) as observed on day 5 of the cycle.

In comparison, high levels of plasma P observed on day 5 of the estrous cycle ($P < 0.05$, vs day 1; $P < 0.01$, vs day 3, Table 2) were maintained until day 10. This was followed by a further increase to reach peak levels on day 12 of the cycle ($P < 0.05$, vs day 5; $P < 0.01$, vs days 8 and 10).

ER concentration

The concentration of nuclear and cytosolic ER and PR on day 5 of the estrous cycle, i.e. the day of high endometrial sensitivity to a decidualizing stimulus was almost similar in the AM and M segments of the uterus. With advancing decidualization, however, significant differences in the concentration of these receptors in the two segments of the uterus were observed (Figs 3 and 4).

The ER concentration remained almost unchanged until day 3 post-traumatization (i.e. day 8 of the estrous cycle). This was followed by a marked increase in the nuclear as well as cytosolic ER concentration in the AM segment of the decidualized (traumatized) uterine horn to reach peak levels on day 12 of the cycle. The increase on days 10 and 12 was significantly more ($P < 0.01$) when compared to the concentration on days 5 and 8 or to the corresponding M segment or to the AM segment of the nontraumatized uterine horn (Figs 3 and 4). Some increase was also observed in the M segments of the traumatized uterine horn on days 10 and 12, which though markedly lower ($P < 0.01$) than the corresponding AM segment, was significantly more ($P < 0.05$, nuclear ER on day 10 and $P < 0.01$, cytosolic ER on days 10 and 12) when compared to the M segment of the nontraumatized uterus. The non-decidualized (nontraumatized) uterine horn generally presented a low concentration of ER in both the AM and the M segments. A transient increase ($P < 0.01$, vs day 8) observed in only the cytosolic ER concentration in the AM and M segments on day 10 of the estrous cycle was not maintained (days 10 vs 12, $P < 0.01$, Fig. 4).

PR concentration

The concentration of nuclear as well as cytosolic PR registered a significant decrease ($P < 0.01$) in both the AM and M segments 3 days after traumatization (Figs 5 and 6). Except for the nuclear PR concentration, which was significantly more in the AM segment of the traumatized uterine horn than the corresponding M segment ($P < 0.05$) or the AM segment of the nontraumatized uterine horn ($P < 0.01$), the concentration of PR was almost similar in all the other segments. This was followed by a marked increase ($P < 0.01$, vs days 8 or 10) in the cytosolic as well as nuclear PR in the AM segment of the decidualized (traumatized) uterus on day 12 to reach levels even higher ($P < 0.05$) than that observed on the day of traumatization (i.e. day 5 of the cycle). Some increase in cytosolic as well as nuclear PR was also observed on day 12 in the M segment of the traumatized uterine horn ($P < 0.05$, vs day 10; $P < 0.01$, vs corresponding segment of the nontraumatized uterine horn) and the AM segment of the nontraumatized uterine horn ($P < 0.01$, vs corresponding M segment), but the levels were markedly lower ($P < 0.01$) than that in the AM segment of the traumatized uterus. The nuclear and cytosolic PR concentration in the M segment of the nontraumatized uterine horn remained low throughout days 8–12 ($P < 0.01$, vs corresponding segment on day 5) of the estrous cycle.

DISCUSSION

Results of this study provide evidence of high peripheral plasma E_2 and P concentration coinciding with the period of maximal endometrial sensitivity of the uterine endometrium to decidualizing stimulus, as well as during trauma induced decidualization. Findings also reveal an almost similar concentration of nuclear and cytoplasmic ER and PR and surface ultrastructure in the AM and M segments of the uterus on the day of endometrial sensitivity. The receptor concentration altered with advancing decidualization, being highest in the AM segment of the decidualized (traumatized) uterine horn and lowest and almost unaltered in the M segment of the nontraumatized uterine horn.

In species like the rat, mouse and gerbil that exhibit facultative delay of implantation, endometrial sensitivity for decidualization is brought about by the luteal phase nidatory estrogen secreted shortly before implantation, which induces proliferation of the endometrial stroma conditioned by the luteal progesterone [cf. 3, 21]. This has been confirmed in ovariectomized animals, where estrogen given after several days of P treatment induces mitotic activity in the stroma, and priming with estrogen reduces the time necessary for the effect of P to manifest [18]. In guinea pigs, too, significant stromal mitoses, similar to that occurring normally on days 5 and 6 of the estrous cycle,

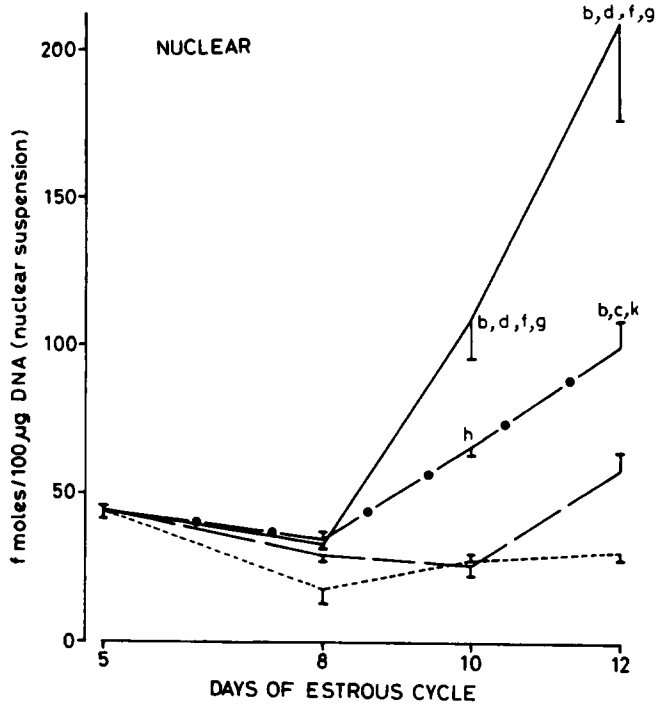

Fig. 3

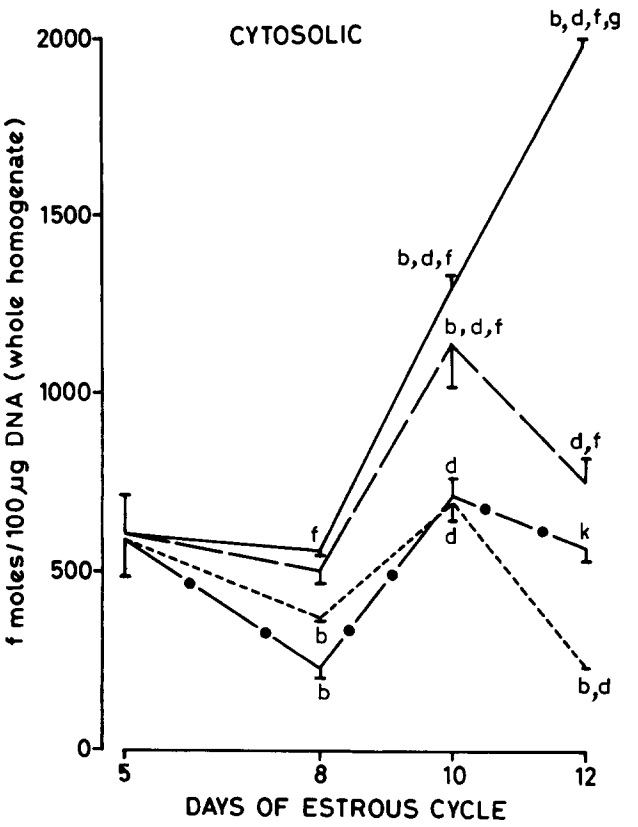

Fig. 4

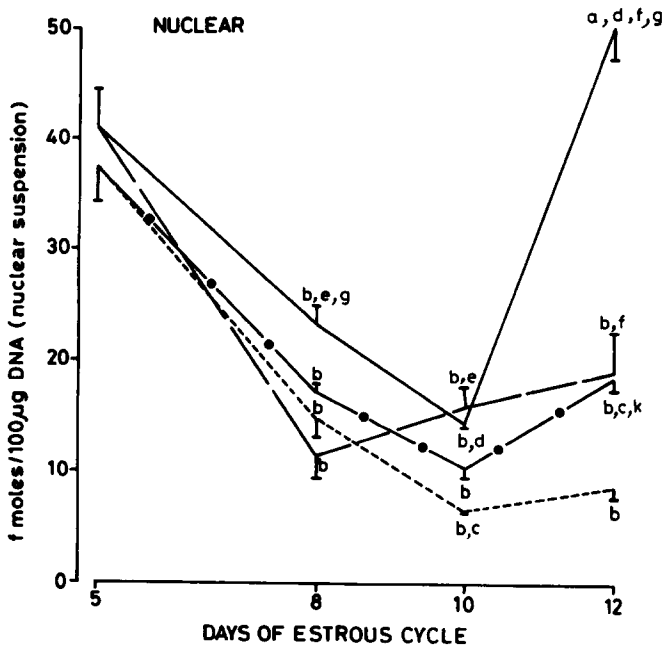

Fig. 5

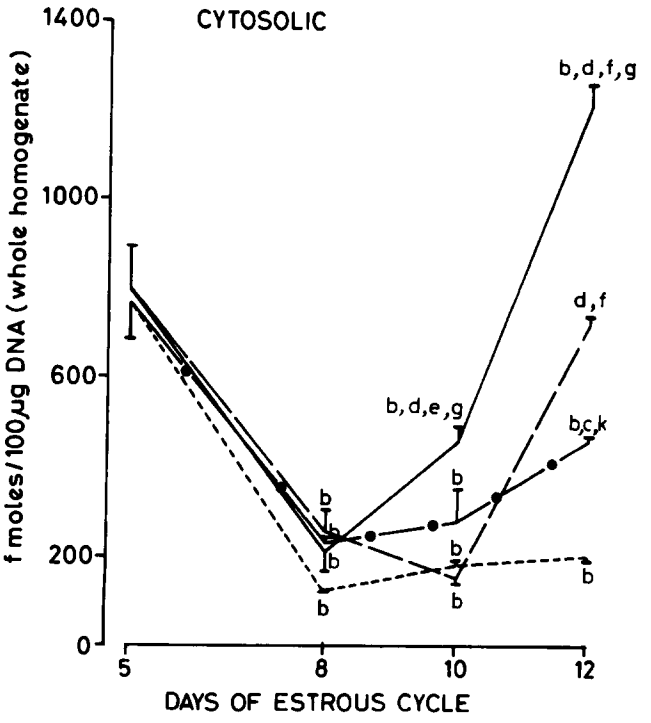

Fig. 6

Fig. 3–6. Uterine nuclear and cytosolic ER (Figs 3 and 4) and PR (Figs 5 and 6) concentration during high endometrial sensitivity and development of induced decidualization following unilateral scissor-cut traumatization. Trauma was given on day 5 of the estrous cycle and animals autopsied 3, 5 and 7 days post-traumatization (i.e. on days 8, 10 and 12 of the estrous cycle). Each point represents mean of three determinations. Cross bars indicate standard error of means. $^{a}P < 0.05$, $^{b}P < 0.01$; vs corresponding value on day of high endometrial sensitivity, i.e. day 5 of the estrous cycle; $^{c}P < 0.05$, $^{d}P < 0.01$; vs corresponding preceding day concentration; $^{e}P < 0.05$, $^{f}P < 0.01$; vs corresponding M segment; $^{g}P < 0.01$; vs corresponding AM segment of nontraumatized uterine horn; $^{h}P < 0.05$, $^{k}P < 0.01$; vs corresponding M segment of nontraumatized uterine horn. (——), AM segment of traumatized uterine horn; (—●—), M segment of traumatized uterine horn; (– – –), AM segment of nontraumatized uterine horn; (- - -), M segment of nontraumatized uterine horn.

could be induced in ovariectomized animals only if estrogen was administered at the end of P treatment, suggesting secretion and a role of estrogen in stromal proliferation during the luteal phase of the cycle [6]. While this differential response has been related to lack of proper estrogen priming [19], peak mitotic activity in the stroma and glandular epithelium coinciding with increased estrogen secretion have been observed during high endometrial sensitivity in intact animals [20]. It is also postulated that luteal estrogen though not obligatory for implantation in this species, probably synergizes with P in the normal preparation of endometrium. However, under the experimental conditions [8], limited preparation obtained with P alone appears sufficient to allow implantation [2, 6]. Evidence of a similar facilitatory role of estrogen in implantation and pregnancy maintenance is available in species like the rabbit [21] and the ewe [22], where, too, implantation does not appear to depend upon the presence of prenidatory estrogen. It is also reported that in some species like the hamster [23] and the guinea pig ([5, 24] and this study), high levels of circulating estrogen, sometimes even higher than in species like the rat which requires luteal estrogen for implantation [3] have been observed at the expected time of implantation. Others like the rhesus monkey, exhibit a shift in the ratio of P to E_2 levels in the endometrium at the time of implantation and an increased occupancy of nuclear ER and PR during the fertile cycles, suggestive of subtle estrogenism in the process of endometrial preparation for ovo-implantation [25].

Like most laboratory rodents, implantation in the guinea pig initiates on the AM side [26]. It is also only along the AM side that insertion of glass or paraffin beads into the subepithelial stroma of the endometrium [27] or a complete scissor cut along the entire length of the uterus [9] induced optimal decidual response in this species. In the present study, however, we did not observe any significant difference in the concentration of E_2 or P receptors (although their concentration was high) or the surface ultrastructure between the AM and the M segments of the uterus on day 5, i.e. during the period of high endometrial sensitivity in this species. Interestingly, in most species including the guinea pig [9] and the human [28], the period of maximal endometrial sensitivity to the blastocyst stimulus for decidualization is well defined. In the mouse, it has also been demonstrated that when oil is injected intra-luminally, the decidual reaction is always initiated on the AM side and the pieces of tumor or muscle placed in the uterine lumen always attach on the AM surface [1]. It is, however, not clear whether the luminal surface on the AM side of the endometrium is in any way preferentially attractive to the blastocyst. The available information on the morphological and biochemical alterations associated with endometrial sensitivity is limited and includes better vascularization of the AM than the M side in rats [1], appearance of abundant large ectoplasmic projections or pinopods on the luminal surface in rats [29] and humans [30] and appearance of specific proteins in the uterine fluid in species like rabbit [31], ferret and mink [1].

It is also reported that in the cyclic guinea pigs, peripheral plasma P concentration starts decreasing after day 9 [32, 33]. In the present study, however, high peripheral plasma P concentration attained on day 5 was not only maintained but showed a further increase on day 12 of the cycle, i.e. 7 days after unilateral uterine decidualization in cyclic animals. These findings confirm the reported [9] luteotrophic action of the decidual tissue in the guinea pig like that in the rat [34].

We have, however, observed a marked increase in the concentration of both ER and PR in the AM segment of the traumatized (decidualized) uterus with advancing decidualization. Comparatively low receptor concentration in the M segment of the traumatized uterus and AM segment of the nontraumatized uterus and almost basal levels in the M segment of the nontraumatized uterus also correlate well with the lack of decidual response in these segments.

The findings of this study, together with the reported increase in certain estrogen responsive parameters such as uterine blood flow and intra-uterine oxygen tension [7] and high circulating estrogen and P concentration during the maximal endometrial sensitivity and induced decidualization ([7, 20] and this study), suggest a possible role of both estrogen and P in induction of endometrial sensitivity and decidual growth in this species.

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REFERENCES

1. Finn C. A. and Porter D. G.: Implantation of ova (Chapter 6) and The control of implantation and the decidual cell reaction (Chapter 8). In *The Uterus* (Edited by C. A. Finn and D. G. Porter). Elek Science, London (1975) pp. 57–73 and 86–95.
2. Aitken R. J.: The hormonal control of implantation. *Ciba Foundation Symposium 64* (new series) (1978) 53–83.
3. Singh M. M. and Kamboj V. P.: Fetal resorption in rats treated with an antiestrogen in relation to luteal phase nidatory estrogen secretion. *Acta Endocr. (Kbh)* **126** (1992) 444–450.
4. Motta C. M. and Hutchinson J. S. M.: Effects of clomiphene citrate on early pregnancy in guinea pigs. *J. Reprod. Fert.* **92** (1991) 65–73.
5. Mitchell J. A. and Garris D. R.: Deciduoma formation in response to uterine trauma in the guinea pig. *Biol. Reprod.* **19** (1978) 1135–1141.
6. Mehrotra S. N. and Finn C. A.: Cell proliferation in the uterus of the guinea pig. *J. Reprod. Fert.* **37** (1974) 405–409.
7. Garris D. R. and Whitehead D. S.: Uterine blood flow and timing of blastocyst implantation in the guinea pig. *Am. J. Physiol.* **241** (1981) E142–E145.
8. Deanesly R.: Retarded embryonic development and pregnancy termination in ovariectomized guinea pigs: progesterone deficiency and decidual collapse. *J. Reprod. Fert.* **28** (1992) 241–247.
9. Garris D. R.: Decidual tissue growth and regression in the guinea pig: regulation by uterine blood flow and relation

to circulating progesterone concentrations. *Endocrinology* **114** (1984) 1599–1604.
10. Chen T. J., Hwang T. C., Huang C. C., Jow G. M. and Yu J. Y. L.: Deciduomal estrogen and progesterone receptors in unilateral pregnant hamsters. *Endocrinology* **125** (1989) 302–307.
11. Burton K.: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric examination of deoxyribonucleic acid. *Biochem. J.* **62** (1956) 315–323.
12. Martel M., Moniev M. N., Psychoyos A. and De Feo V. J.: Estrogen and progesterone receptors in the endometrium, myometrium and metrial gland of the rat during the decidualization process. *Endocrinology* **114** (1984) 1627–1634.
13. Anderson J., Clark J. H. and Peck E. J. Jr: Oestrogen and nuclear binding sites: Determination of specific sites by ^{3}H-estradiol exchange. *Biochem. J.* **126** (1972) 561–567.
14. Castellano-Diaz E., Gonzalez-Quijano M. I., Liminane J. M. and Diaz-Chico B. N.: Tamoxifen decreases the estradiol induced progesterone receptors by interfering with nuclear estrogen receptor accumulation. *J. Steroid Biochem.* **33** (1989) 133–139.
15. Singh G., Singh M. M., Maitra S. C., Elger W., Kalra V., Upadhyay S. N., Chowdhury S. R. and Kamboj V. P.: Luteolytic action of two antiprogestational agents (RU-38486 and ZK-98734) in the rat. *J. Reprod. Fert.* **83** (1988) 73–83.
16. Singh G., Singh M. M., Maitra S. C., Elger W. and Kamboj V. P.: Effect of antiprogestins RU 486 and ZK 98734 on uterine decidualization: Histological and surface topographic studies. In *Hormone Antagonists for Fertility Regulation* (Edited by C. P. Puri and P. F. A. Van Look). Indian Society for the Study of Reproduction and Fertility, Bombay, India (1988) pp. 99–104.
17. Duncan D. B.: Multiple range and multiple F tests. *Biometrics* **11** (1955) 1–42.
18. Weitlauf H. M.: Biology of implantation. In *The Physiology of Reproduction* (Edited by E. Knobil, J. Neill *et al.*). Raven Press, New York (1988) pp. 231–263.
19. Marcus G. J.: Hormonal control of proliferation in the guinea pig uterus. *J. Endocr.* **63** (1974) 89–97.
20. Garris D. R.: Postpartum regeneration of the guinea pig endometrium: relationship to serum estradiol and progesterone concentrations. *Anat. Rec.* **210** (1984) 41–44.
21. Singh M. M. and Booth W. D.: Origin of oestrogen in preimplantation rabbit blastocysts. *J. Steroid Biochem.* **11** (1979) 723–728.
22. Miller B. G. and Moore N. W.: Effect of progesterone and estradiol on RNA and protein metabolism in the genital tract and survival of embryos in the ovariectomized ewe. *Aust. J. Biol. Sci.* **29** (1976) 565–571.
23. Joshi H. S. and Labhsetwar A. P.: The pattern of ovarian secretion of oestradiol and oestrone during pregnancy and the post-partum period in the hamster. *J. Reprod. Fert.* **31** (1972) 299–302.
24. O'Grady J. E. and Bell S. C.: The role of endometrium in blastocyst implantation. In *Development in Mammals, Vol. 1* (Edited by M. H. Johnson). Elsevier, Amsterdam (1977) pp. 165–244.
25. Sengupta J. and Ghosh D.: Estradiol and progesterone levels in the endometrium and the reproductive tract luminal washing during the pre-implantation stages of gestation in the rhesus monkey. *Acta Endocr. (Copenh.)* **120** (1989) 649–654.
26. Blandau R. J.: Implantation. In *Proceedings of the Symposium on the Use of Non-Human Primates for Research Problems in Human Reproduction*. Sukhumi, USSR, 13–17 Dec. (1971) pp. 69–92.
27. Blandau R. J.: Experimental production of endometrial polyps in the guinea pig. *Cancer Res.* **9** (1949) 526–531.
28. Shettles L. B. and Schmidt-Mathiesen H.: Endometrium and nidation. In *Des Normale Menschliche Endometrium* (Edited by H. Schmidt-Mathiesen). G. Thieme Verlag, Stuttgart (1963) pp. 294–315.
29. Sarantis L., Roche D. and Psychoyos A.: Displacement of receptivity for nidation in the rat by the progesterone antagonist RU 486: a scanning electron microscopy study. *Human Reprod.* **3** (1988) 251–255.
30. Martel D., Frydman R., Glissant M., Maggioni C., Roche D. and Psychoyos A.: Scanning electron microscopy of postovulatory human endometrium in spontaneous cycles and in cycles stimulated by hormone treatment. *J. Endocr.* **114** (1987) 319–324.
31. Hegele-Hartung C. and Beier H. M.: Distribution of uteroglobin in the rabbit endometrium after treatment with an antiprogesterone (ZK 98.734): an immunocytochemical study. *Human Reprod.* **1** (1986) 497–505.
32. Challis J. R. G., Heap R. B. and Illingworth D. V.: Concentrations of oestrogen and progesterone in the plasma of nonpregnant, pregnant and lactating guinea pigs. *J. Endocr.* **51** (1971) 333–345.
33. Singh M. M., Fahnrich M., Hasan S. H. and Elger W.: Reversibility of antigestagenic action of antiprogestin ZK 98299 during early pregnancy in guinea pigs by exogenous gestagens. *Proc. 2nd A. Meet. Indian Soc. for Study of Reprod. Fert.*, 3–4 Feb. (1990) p. 48.
34. Rothschild I. and Gibori G.: The luteotrophic action of decidual tissue: the stimulating effect of pseudopregnant rats. *Endocrinology* **97** (1975) 838–842.