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Dose-dependent effects of exogenous gonadotrophins on the endometrium of the rat

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Abstract **We compared the serum levels of oestrogen and progesterone and the endometrial morphology of normal pregnant rats at 5,5 days' gestation with those of pregnant rats given either low (10 IU) or high (20 IU) doses of two gonadotrophins: follicle-stimulating hormone (FSH) and human chorionic gonadotrophin (HCG). Evidence of ovarian hyperstimulation was observed in the high- but not the low-dose group; both treatment regimens caused significant changes in the endometrial surface, epithelial height, the microvillous border, the glycocalyx, the subepithelial stromal cells and the mitotic activity of the surface epithelial and stromal connective tissue cells. The effects of the high-dose treatment were more severe than those of the low-dose treatment. The serum oestradiol and progesterone levels of the treated groups were not significantly different from those of the control group. The changes in the endometrium after both treatment regimens may interfere with normal trophoblastic-endometrial interactions and could influence the maintenance of pregnancy. This investigation demonstrated that even low doses of gonadotrophins, which do not cause obvious ovarian stimulation, affect uterine morphology. The findings have important implications for *in vitro* fertilisation and embryo transfer programmes.**

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The high failure rate of human *in vitro* fertilisation (IVF) and embryo transfer (ET) programmes has been ascribed to many factors including maternal age, the type of infertility and the pattern of oestradiol rise during the follicular phase.[1-3] Recently, Chen[4] found that the number of embryos transferred in a particular cycle can be positively correlated with a successful outcome in IVF and ET. In order to obtain the required number of oocytes for IVF and ET, exogenous gonadotrophins are routinely administered. However, despite the transfer of numerous embryos, the success rate of IVF and ET remains low. Ovarian hyperstimulation alters the hormonal profile and the influence of oestrogens and progesterones on the uterus modifies the morphology of the endometrium.[5] In rats even low doses of exogenous gonadotrophins (10 IU) when given out of phase with the sexual cycle, have deleterious effects on the morphology of the endometrium.[6] A study simulating human IVF situations[5] has suggested that the sustained high oestradiol levels following hyperstimulation are responsible for the severely disrupted morphology. The effects on the endometrium may well interfere with the normal endometrial-trophoblastic interactions that are a prerequisite for embryo implantation. This investigation compares the effects of low doses of gonadotrophins given in phase with the sexual cycle with those of higher doses and discusses the implications for IVF and ET.

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Materials and methods

Daily vaginal smears were taken from adult female virgin Sprague-Dawley rats (200 - 250 g body weight) to establish the oestrous cycle. The phases of the oestrous cycle are: oestrous, metoestrous, di-oestrous and pro-oestrous. Animals with regular 4-day cycles were randomly divided into two main groups.

Hyperstimulated rats (group 1)

10 IU in phase

Six rats were injected with 10 IU of follicle-stimulating hormone (FSH) (Folligon, Intervet, Johannesburg) at midday of the mid-di-oestrous phase (i.e in phase with the oestrous cycle), followed by 10 IU of human chorionic gonadotrophin (HCG) (Pregnyl; Organon, Holland) 24 hours later, i.e. between the late di-oestrous and pro-oestrous phases.

20 IU in phase

Six rats were injected with 20 IU of FSH at midday of the mid-di-oestrous phase followed by 20 IU of HCG 24 hours later.

All animals were mated on the evening of the second injection with proven fertile males.

Control rats (group 2)

Six rats were mated with proven fertile males on the night of the pro-oestrous phase.

Mating was confirmed by the presence of a mucous plug or by spermatozoa in the vaginal smear. Pregnancy was diagnosed by a pregnancy-type vaginal smear (i.e. predominantly leucocytic and with an abundance of mucus) and failure to return to the oestrous phase within 4 days.

Animals were sacrificed by cervical dislocation after 5,5 days of pregnancy (the time of embryo implantation).[7] Blood was collected from the carotid vessels for radio-immunoassay (RIA) of progesterone and 17 ß-oestradiol.[8] The ratio of progesterone to oestradiol ($P:E_2$) was calculated.

The number of developing follicles and corpora lutea in the ovaries was qualitatively assessed at the time of sacrifice. The uterine horns were rapidly removed and processed for light and electron microscopy. Areas of dilatation (probable implantation sites) were fixed in Bouin's solution for light microscopy, while tissue for electron microscopy was fixed in 2,5% glutaraldehyde in 0,1M sodium cacodylate buffer (pH 7,2 - 7,3) at 4°C for 2 hours and post-fixed in 1% osmium tetroxide for 1 hour. The tissues were dehydrated, embedded and sectioned for viewing.[6]

Morphometric analysis of the endometrium was performed using a semi-automatic measuring device (Kontron Videoplan). The mucosal depth and the heights of the surface and glandular epithelia were measured.[6]

The mitotic index (MI) of the surface and glandular epithelium and of the stromal cells was determined by counting the number of mitotic figures per 1 000 cells. A minimum of 10 non-consecutive sections per animal (taken at 50 μm intervals) were used.

The ultrastructure of the epithelium, including the surface modifications, i.e. the microvilli and the glycocalyx, and the cells and fibres of the stroma were examined.

Statistical analysis of the data was performed using Student's *t*-test as well as non-parametric statistical tests (Wilcoxon signed ranks test, the Mann-Whitney *U*-test and the Kruskal-Wallis test).

Results

At post-mortem the ovaries of the 10 IU group appeared similar to those of the control animals. In contrast, the ovaries of the 20 IU group were enlarged and showed more developing follicles and more numerous corpora lutea than the control ovaries. The uterine horns of the 20 IU rats, but not those in the 10 IU group, were dilated and contained large amounts of fluid.

RIA

The results of the RIA for progesterone and oestradiol are given in Fig. 1. Although there were no statistically significant differences between the oestradiol levels for any of the groups there was an increasing trend with maximum levels reached in the 20 IU group (Fig. 1a). Progesterone levels (Fig. 1b) and the P:E_2 ratio (Fig. 1c) did not differ between groups.

General morphology of the endometrium

Early embryos were seen attached to the surface epithelium of the uterus in the control rats (Fig. 2a). Unattached embryos, often appearing degenerate, were found in the uterine lumina of both groups of hyperstimulated rats (Fig. 2b). In control animals the contour of the surface epithelium was smooth and even (Fig. 3a), while that of both the 10 IU and the 20 IU groups was uneven (Figs 3b and 3c). A low cuboidal epithelium lined the luminal surface of the endometrium in control rats (Fig. 3a). In both the hyperstimulated groups there was a tall columnar surface epithelium (Figs 3b and 3c). Decidualisation of the subepithelial stromal cells was apparent in the control rats (Fig. 3a) but absent after hyperstimulation (Figs 3b and 3c). The stroma of the 10 IU hyperstimulated group appeared to be more oedematous than that of controls or of the group hyperstimulated with 20 IU of exogenous gonadotrophins. The surface epithelial cells of the control rats appeared generally healthy (Fig. 4a), but varying degrees of degeneration in these cells were observed in both the experimental groups (Fig. 4b). Short sparse microvilli with a thick glycocalyx covered the luminal surface of the epithelial cells of the control animals (Fig. 5a). In both the hyperstimulated groups there were large numbers of long and branching microvilli with a deficient or absent glycocalyx (Figs 5b and 5c).

Morphometric analysis

The means and the standard errors of the means (SEM) of the endometrial mucosal depth and the surface and glandular epithelial heights of control and hyperstimulated rats are given in Table I. The mucosal depth of the 10 IU group was significantly higher than that of the control group, but that of the 20 IU group was significantly reduced. After hyperstimulation with both regimens the surface epithelial height was significantly greater than in the controls; the 20 IU animals also had a significantly higher epithelium than the 10 IU rats. There were no differences in the glandular epithelial heights of any of the groups.

TABLE I.
Endometrial mucosal depth, surface epithelial height and glandular epithelial height of control and hyperstimulated rats (mean ± SEM)

	Control	Hyperstimulated 10 IU	Hyperstimulated 20 IU
Mucosal depth (mm)	0,518 ± 0,01	0,569[a] ± 0,01	0,471[a] ± 0,01
Surface epithelial height (μm)	10,1 ± 0,1	17,0[a] ± 0,1	23,0[a,b] ± 0,4
Glandular epithelial height (μm)	12,5 ± 0,1	12,7 ± 0,1	12,6 ± 0,1

a and b: $P < 0,0001$, Student's *t*-test, Mann-Whitney *U*-test and Wilcoxon signed rank test. a = hyperstimulated group v. control, b = 20 IU group v. 10 IU group.

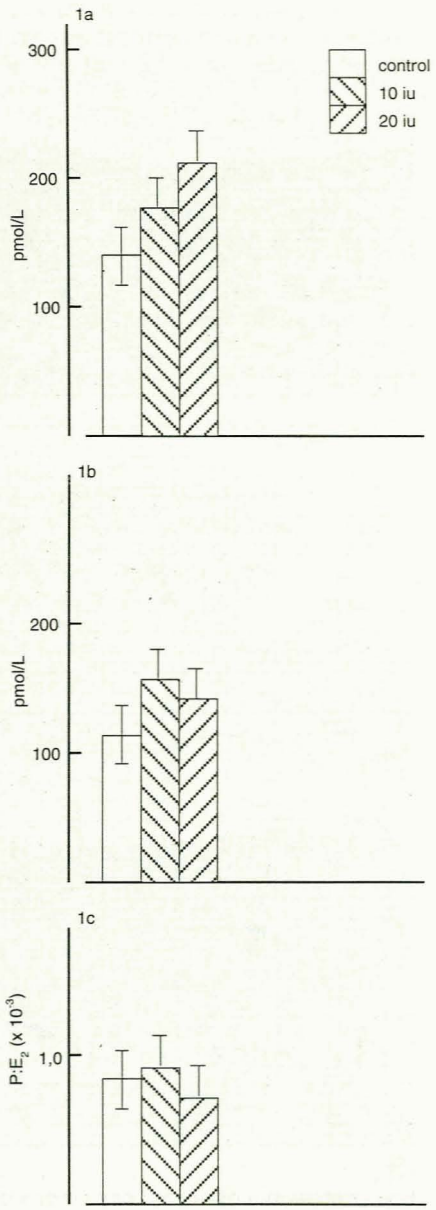

FIG. 1.
Bar graphs showing serum levels of (a) oestradiol, (b) progesterone and (c) the P:E_2 ratio in control and experimental rats at 5,5 days after mating.

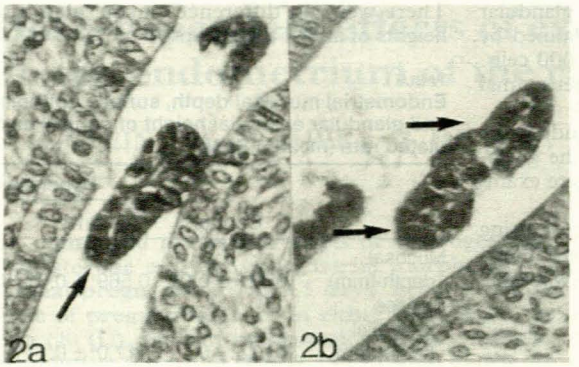

FIG. 2.
Embryos in (a) 5,5-day control and (b) 20 IU hyperstimulated rat (arrows). **The embryo in (a) is attached to the endometrium while the embryos in (b) lie free in the lumen (a × 110; b × 90).**

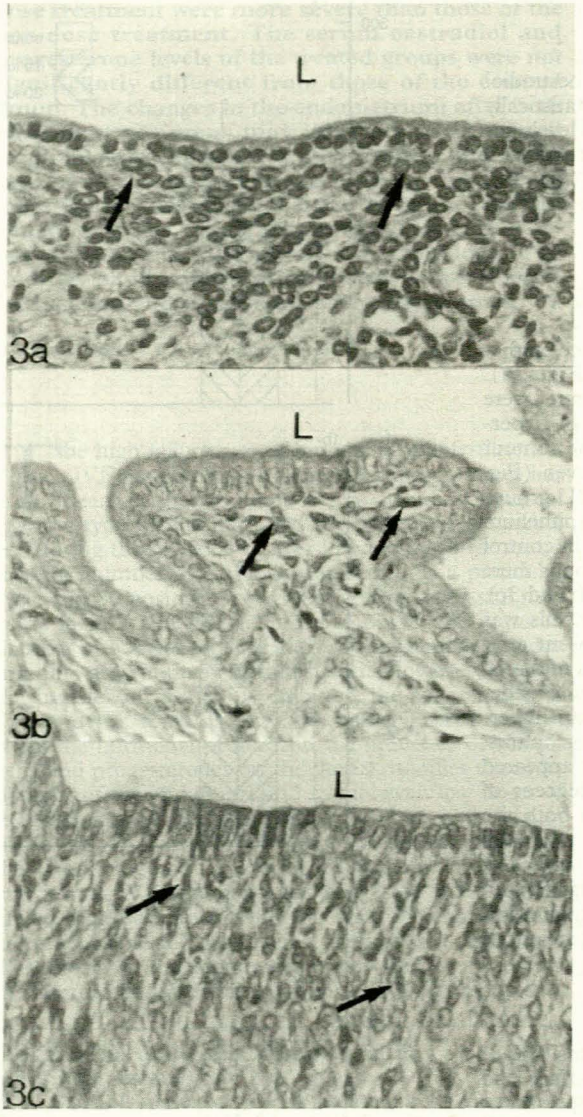

FIG. 3.
Endometrial mucosa of 5,5-day pregnant (a) control, (b) hyperstimulated 10 IU and (c) 20 IU rats. Note the low cuboidal epithelium and decidualised subepithelial stromal cells in (a) and the columnar cells and flattened subepithelial stromal cells in (b) and (c) (arrows) **(L = lumen) (× 110).**

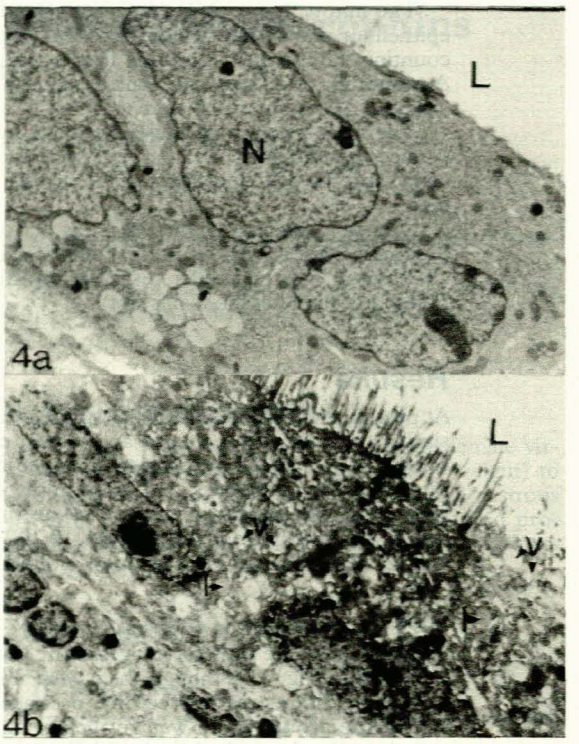

FIG. 4.
Electron micrographs of surface epithelial cells from 5,5-day pregnant (a) control and (b) 20 IU hyperstimulated rats. In (b) the cells appear to be degenerating; the chromatin of the nucleus is denser than in the control cells, dilated vesicles (v) and numerous lipid droplets (l) are present (L = lumen) (× 3 600).

Mitotic activity

The MI of the surface and glandular epithelial cells and the stromal cells of the control and hyperstimulated groups are given in Table II. Both hyperstimulation regimens caused significant reductions in the mitotic activity of the surface epithelial cells compared with the control group. Stromal cell MI showed a tendency to decrease between the control and 10 IU groups. The MI of the 20 IU rats was significantly different from that of the controls. The MI of glandular epithelial cells was similar in all three groups.

TABLE II.
Mitotic indices of the surface and glandular epithelium and the stromal cells of control and hyperstimulated rats at 5,5 days of pregnancy (mean ± SEM)

		Hyperstimulated	
	Control	10 IU	20 IU
Surface epithelium	3,93 ± 0,44	0,6[a] ± 0,36	1,14[a] ± 0,27
Glandular epithelium	1,11 ± 0,66	0,7 ± 0,46	1,48 ± 0,37
Stromal cells	1,98 ± 0,44	1,25 ± 0,30	0,59[a] ± 0,19

a: $P < 0,05$, Student's *t*-test, Mann-Whitney *U*-test and Kruskal-Wallis test, hyperstimulated group v. control.

Discussion

Blastocyst attachment to and invasion of the maternal endometrium is a critical period in early pregnancy. Recent studies have emphasised the importance of the

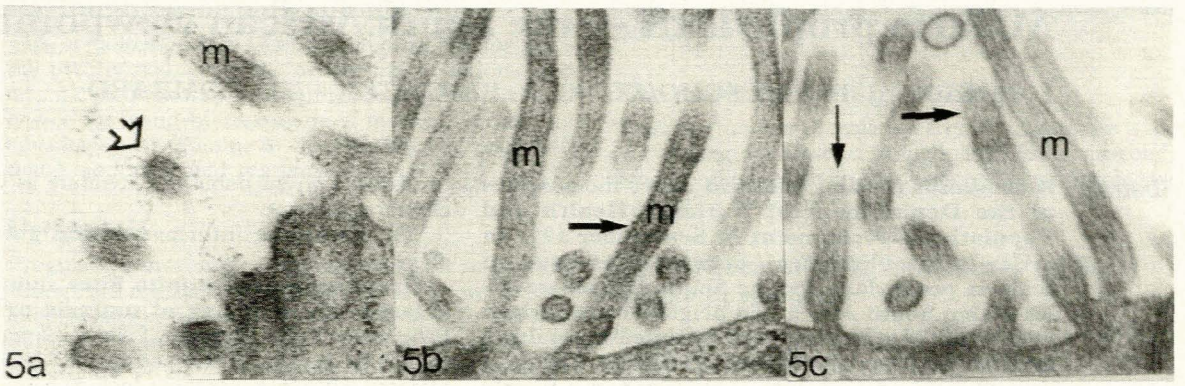

FIG. 5.
Microvillous border of the surface epithelium of (a) control, (b) hyperstimulated 10 IU, and (c) 20 IU rats. Note the *numerous long microvilli (m) and absent to scanty glycocalyx in* (b) *and* (c) *compared with the short microvilli (m)* and prominent glycocalyx in (a) (arrows) (× **50 000**).

intra-uterine environment in implantation,[9] and the relevance of a structurally normal endometrium in this process has been reported.[5] In IVF and ET programmes it is important to establish dosage regimens for gonadotrophins which will produce superovulation while causing the minimum of change in the endometrial morphology; these regimens would presumably use the lowest possible doses of gonadotrophins. We have shown that when low doses of two commonly used gonadotrophins (FSH and HCG) are given to rats out of phase with the oestrous cycle, severe endometrial disruption results.[6] In the present study, the same low doses of gonadotrophins (10 IU) given in phase with the oestrous cycle did not appear to cause superovulation(excessive numbers of corpora lutea were not observed). Nevertheless, there were deleterious effects on the morphology of the endometrium. The surface epithelium, and particularly those regions involved in embryo attachment, the microvilli and the glycocalyx, were severely affected. Furthermore there was no decidualisation of the subepithelial stromal cells as well as reduced mitotic activity in the surface epithelial and stromal cells. All the endometrial changes observed in the low-dosage group were present and exaggerated in the high-dosage group. The effects described here could result in a poor prognosis for both embryo attachment and the maintenance of pregnancy. It should be noted that neither the actual number of embryos produced by these subjects nor the number of corpora lutea present in the ovaries were determined; therefore although the gross anatomical evidence of superovulation was lacking in the 10 IU group, there may well have been some degree of ovarian stimulation in these animals.

Statistically significant differences between the serum oestradiol and progesterone levels of the three groups studied could not be demonstrated. However, the trend appeared to be toward an increase in oestradiol concentration between the controls and the two hyperstimulated groups. The highest levels occurred after administration of 20 IU of the gonadotrophins. In this investigation only the hormone levels at 5,5 days of gestation were measured, and it is possible that the hormonal levels prior to this stage (i.e. at 1,5 - 4,5 days) were raised. Indeed, we have previously shown that after hyperstimulation with 20 IU of gonadotrophins in rats, oestradiol levels are significantly raised during the pre-implantation period but fall to levels similar to those in control animals at 5,5 days.[5] Although data are not available on animals injected with 10 IU of gonadotrophins at 1,5 - 4,5 days of gestation, it is highly likely that the oestradiol level would be raised at least enough to disrupt the $P:E_2$ ratio. Gidley-Baird *et al.*[10] have reported that the $P:E_2$ ratio is a more important predictor of the success of implantation than the absolute levels of either hormone. It is possible that early sustained rises in the oestrogen concentrations following both gonadotrophin treatments may have affected the pre-implantation $P:E_2$ ratio thereby contributing to the abnormalities seen in the endometrium in this study.

We have demonstrated that increasing doses of gonadotrophins in rats have increasingly deleterious effects on the endometrium. Negative effects are apparent even after administration of very low doses. These results are of great relevance to IVF and ET programmes as they indicate that even low doses, which do not cause gross ovarian changes, affect the endometrium in ways which could interfere with the establishment and maintenance of pregnancy.

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