

# Histopathological changes in the uterus of rats treated neonatally with clomiphene citrate

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## Summary

Clomiphene citrate has estrogenic and teratogenic effects on the developing rat and human fetal genital tract. Clomiphene citrate was given subcutaneously to newly born rats by daily injections of 100 µg/kg and changes caused by clomiphene in female rats were investigated at 28 and 35 days. Depending upon the effect of clomiphene citrate, histopathological changes such as the observation of polygonal shaped nodular cells in the uterus epithelium and presence of erythrocytes in free nature in the stroma were considered as important findings.

**Key words:** Clomiphene citrate; Uterus; Neonatal rat; Histopathological changes.

## Introduction

There has been considerable interest in the mechanism of action of triphenylethylene antiestrogens because of their ability to antagonise many of the actions of estrogen. Clomiphene citrate, a synthetic estrogen agonist-antagonist, has been extensively used in clinical medicine for the induction of ovulation in ovulatory women [1, 2]. Triphenylethylene -derived antiestrogens-tamoxifen, nafoxidine and clomiphene result in a long retention of the estrogen receptor in the nuclei of uterine cells, resulting in a long-lasting increase in uterine weight [3]. Clomiphene citrate has estrogenic and teratogenic effects on the developing rat and human fetal genital tract [4, 5]. Abnormalities have been observed in the genital organs of rats treated neonatally with antiestrogen; adenosis-like lesions in the cervicovaginal epithelium, suppression of uterine glands and uterine hypoplasia and ovarian dysgenesis [5, 6, 7, 8].

Moreover, hypertrophy in the epithelium and stroma of uterine glands was observed in mature and ovariectomized mature rats due to an antiestrogen effect, whereas atrophy was seen in the epithelium and stroma with long-term antiestrogen effects [6, 7, 8]. The present paper deals with the histopathological changes in the uterus of rats treated neonatally with clomiphene citrate.

## Materials and Methods

Rats were kept at room temperature in animal houses (four groups of female litters); 28-day and 35-day-old treated rats and the corresponding controls were subcutaneously given daily injections of 100 µg/kg clomiphene citrate suspended in 0.02 ml

of saline for 5 days starting on the day of birth. The animals were killed by ketamine anesthesia at 28 and 35 days of age. The dissected tissues were fixed in 10% formaldehyde dehydrated and embedded in paraffin and then sectioned transversally at 4-6 µm. The resulting paraffin sections were stained with staining methods such as hematoxylin eosin and Masson tripple. The stained sections were observed under an illuminated microscope, and photographed with a photomicroscope. The changes were then histopathologically evaluated.

## Results

The histopathological changes in rats treated neonatally with clomiphene citrate were examined in comparison to controls. The uterine epithelium cells acquired a cylindrical shape, proliferation in the epithelium and were elongated in height at 28 days in rats given clomiphene citrate (Fig. 1). The accumulation of cells in free nature of the basal membrane and the musculature layer however, appeared to be normal (Fig. 2). The uterine epithelium of the age-matched controls was composed of a single layer of cuboidal cells (Fig. 3). At 35 days there was elongation in the uterine epithelium, papillary development toward the lumen, dilation in blood vessels and an increase in erythrocytes in free nature beneath the epithelium connective tissues in clomiphene injected animals (Fig. 4). The uterine epithelium of 35-day-old controls preserved its cuboidal shape (Fig. 5).

## Discussion

The potential teratogenic and cardiogenic effects of exogen estrogens on the development of various genital canals in human and experimental animals are well known [6, 7, 8]. In animal models, exogenous hormones

Received August 6, 1997

revised manuscript accepted for publication September 15, 1997

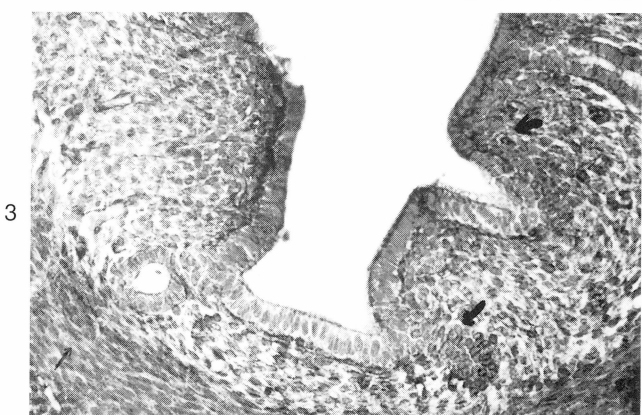
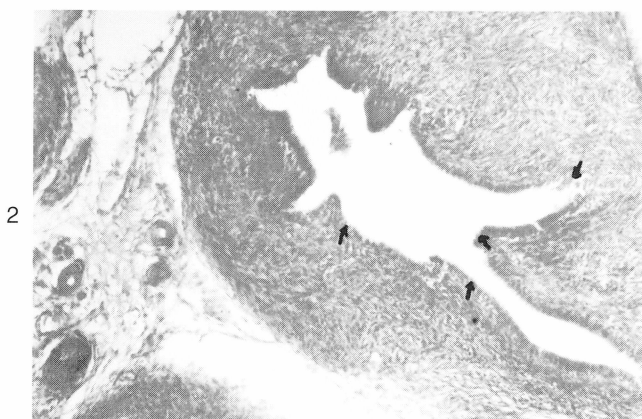


Fig. 1. — The uterus of a 28-day-old rat given clomiphene citrate injections. Proliferation in epithelium and elongation of cellular height (indicated by thick arrow); H.E original magnification  $\times 41$ .

Fig. 2. — The uterus of a 28-day-old rat given clomiphene citrate injections. The accumulation of cells in free nature of basal membrane (indicated by thick arrow) and normal appearance of musculature layer (indicated by thin arrow) Masson 3 original magnification  $\times 41$ .

Fig. 3. — The epithelium and stroma of a 28-day-old control (H.E original magnification  $\times 41$ ).

have been shown to perturb uterine gland formation. Cunha *et al.* [5] observed glandular cystic hyperplasia in uteri. But in our study no glandular cystic hyperplasia in the uterus was observed. Branham *et al.* [9, 10] have

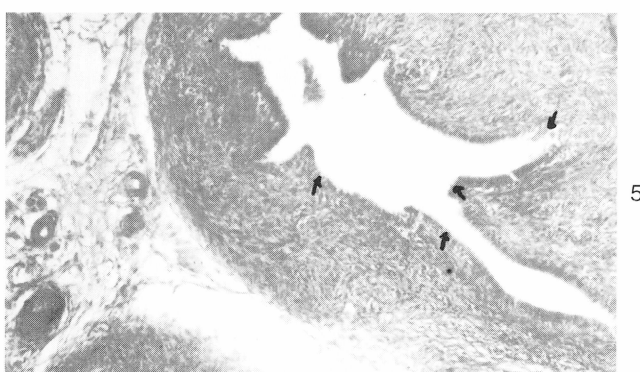
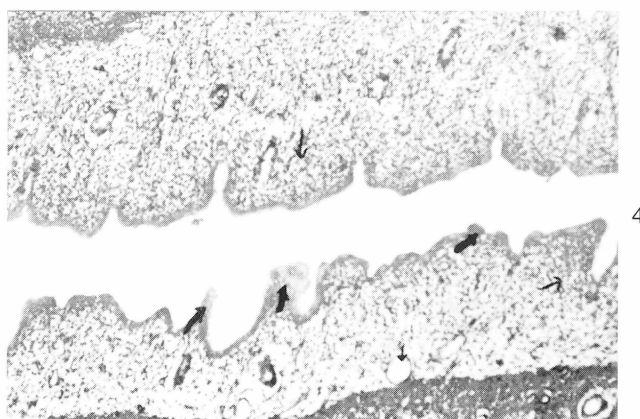


Fig. 4. — The uterus of a 35-day-old rat given clomiphene citrate injections. Proliferation in epithelium and papillary development (indicated by thick arrow). Increased accumulation of cells in free nature of basal membrane and dilation in blood vessels (indicated by thin arrow) Masson 3 original magnification  $\times 41$ .

Fig. 5. — The epithelium and stroma of the uterus in a 35-day-old control rat (H.E original magnification  $\times 41$ ).

recently shown that administration of either estradiol-17 $\beta$  or antiestrogen to neonatal rats inhibits uterine gland formation; an effect reported earlier in neonatal mice treated with diethylstilbestrol (DES) [11]. The inhibitory effects of estradiol and antiestrogen are clearly age-dependent [9, 10]. Iquichi *et al.* [6] observed elongation in the uterine epithelium at 5 and 30 days due to antiestrogen effects, whereas this finding was encountered at 28 and 35 days in the present study. They have also noted the disorganisation of the mesenchymal tissues in the uterine stroma, considered an important finding, and the involution of musculature. However, such a finding was not obtained (Fig. 2) in the present study. The important findings of our study were the observation of epithelium cells of cylindrical shapes and elongated in height at 28 days (Fig. 1) and also an increase in cells in free nature of basal membrane at 28 days and 35 days, depending upon the effects of clomiphene citrate.

We conclude that clomiphene citrate, which is one of the antiestrogens used to induce ovulation, had positive effects on estrogen receptors and caused some histopathological changes in tissues.

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