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ENDOMETRIUM CELL SURFACE ABNORMALITIES IN THE SYRIAN HAMSTER AS A RESULT OF *IN UTERO* EXPOSURE TO DIETHYLSTILBESTROL

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Abstract

Scanning electron microscopy (SEM) was used to observe changes in the hamster endometrium cell surface following in utero pre- and/or postnatal exposure to diethylstilbestrol (DES). Some of the changes in cell surfaces are associated with alterations in cell sizes and shapes (from columnar to cuboidal and/or squamous) and in microvilli and mucous secretion. In all cases, DES treated uteri show mucosal cell surface pleomorphism, apocrine secretion and cystic accumulation of secretory material. Microvillous pleomorphism and peculiar linkages attaching one microvillus to others were investigated. Although the function and nature of such linkages is unclear, their presence seems to be more prominent in the in utero DES treated hamster endometrium. These infrastructures may provide a support for the microvilli distributed on the mucosal cell surfaces, i.e., a morphological compromise between the single microvillous surface and the microridged structures. These interconnections may represent glycocalyx material or remodeling of cell surfaces toward squamous epithelium.

KEY WORDS: Syrian hamster, cell surface, microvilli, interconnections, endometrium, in utero, diethylstilbestrol, scanning electron microscopy.

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Introduction

Scanning electron microscopy (SEM) has been particularly successful in demonstrating endometrial surface changes in the female reproductive tract in response to either normal rhythmical variations in the secretion of ovarian hormones or during implantation in animals (Anderson et al. 1975; Ennis and Davies, 1982; Kühnel and Busch, 1981; Lamb et al., 1981; McLachlan, 1979; McLachlan et al., 1980; Parkening, 1975, 1976, 1979; Plapinger and Bern, 1979; Segalen et al., 1982) and humans (Ferenczy and Richart, 1973, 1974; Schueller, 1973) or to a variety of pathological influences resulting in benign and malignant morphologies (Aycock et al, 1979; Fenoglio et al, 1982; Herbst and Multier-Lajous, 1980; Kenemans et al, 1981; McLennan, 1974; Motta and Andrews, 1976; Nathan et al, 1978; Nilsson et al, 1980; Thom et al, 1981).

Most of the earlier studies have focused on the lower portions of the reproductive tract in both animals and humans, consequently little is known regarding the morphological, histochemical and biochemical changes occurring in the upper genital tract.

This study reports differential morphological changes occurring at the surface of endometrial cells resulting from prenatal and/or postnatal exposure to DES and confirms that some of these changes correspond to those observed in human endometrium (Richart and Ferenczy, 1974). In addition, the uterine changes appear to be more extensive than those previously reported for mice and rats (Lamb et al., 1981; McLachlan et al., 1980; Plapinger, 1981; Plapinger and Bern, 1979).

This study reports peculiar morphological aspects related to the microvillous covering of endometrial cells as a part of more extensive reports describing endometrial cell surface changes resulting from prenatal and/or postnatal exposure to DES (Gilloteaux and Steggles, 1981, also in preparation).

Materials and Methods

Thirty (30) timed pregnant Syrian hamsters (Mesocricetus auratus Waterh, strain lak LVG. SYR) were purchased from Charles River Co.

Twenty (20) were injected subcutaneously with DES in corn oil (100 µg DES/kg bw/day) on days 8-11 (inclusive) of pregnancy. Ten (10) control animals received oil only. After weaning, the female pups were housed three to a cage. The daughters (at 50 days of age) were divided into four groups designated as follows: (i) ♀ from untreated mothers (C.C), (ii) ♀ from DES treated mothers (D.C), (iii) ♀ from untreated mothers then postnatally DES treated (C.D), (iv) ♀ from DES treated mothers then postnatally DES-treated (D.D).

The postnatal DES treatment consisted of the subcutaneous implantation of a DES pellet (15 mg) into the interscapular dorsal region. New pellets were implanted every three months. The effectiveness of this treatment has been discussed previously (Gilloteaux et al., 1982). After 100 days of treatment, 3-5 animals from each group were killed by cervical dislocation, and the uteri removed and divided in two equal parts. One part was fixed in 4% buffered paraformaldehyde (pH 7.3, 0.1 M phosphate) and examined after routine paraffin embedding, followed by topographical hematoxylin eosin and silver reticulin stainings. They were studied using the light microscope and these findings were reported elsewhere. The other part was prepared for either (1) SEM or (2) transmission electron microscopy studies (in preparation). SEM specimens, less than 1 mm in thickness, were soaked 30-45 sec in 0.2% protease (type VI, Sigma) before they were fixed at room temperature in a 3% glutaraldehyde-formaldehyde, 0.1 M cacodylate solution (pH 7.35), washed in 0.2 M Na cacodylate (pH 7.35), then fixed in 1% OsO_4 0.2 M sodium cacodylate solution (pH 7.35). After washing, the SEM fixed specimens were dehydrated using graded ethanol solutions (30% to 100%) and dried in a Polaron E 3000 critical point drying apparatus (Polaron Ltd., England).

As a result of cutting cross-sections through the walls of the uterine horn, the fixation and freeze-drying preparative steps induced shrinkage. This occurred more extensively in the muscular wall than at the level of the endometrium layers. As a consequence, the outer more resilient uterine layers (myometrium and serosa) provoked a reflection of the luminal surfaces toward the outside of the uterine samples. These everted luminal surfaces facilitated the SEM observations. All samples were coated with carbon and gold before examination at 25 kV in a JEOL JSM-35C scanning electron microscope.

Results

Macroscopic changes

When cross-sectional slices from treated uterine horns are compared, we note the following sequence regarding the mean cross-sectional diameter dimensions: D.D (9.80 ± 1.24 mm^2 ± SD) > C.D (8.87 ±. 1.36) > C.C (4.84 ± 1.68) > D.C (2.03 ± 0.26) which correspond to values reported previously (Gilloteaux and Steggles, 1981; Gilloteaux et al., 1982). The untreated C.C (Fig. 1a) and prenatally treated (D.C) uteri (Fig. 2a) are always smaller in size than the postnatally DES treated uteri (C.D and D.D, Fig. 3a and 4a); both treatments show a characteristic combined hyperplastic and hypertrophic response due to the DES exposure. In addition, referring to a previous publication (Gilloteaux, et al., 1982), we know that the D.C uteri are smaller than uteri from any other treatment group of less than 250 days of age. The oldest D.C uteri (350 days old) morphologically resemble 150 day C.D uteri, since cystic structures were observed within the endometrial layers (Gilloteaux et al., 1982). Since the C.D and D.D uteri are hypertrophic and too large to be shown in one illustration, only portions of the cross-section of the uterine horn are shown. In addition both C.D and D.D uteri contain many intraluminal ridges, folds and large polyps. These luminal surfaces, which include ridges, polyps and papillae, are more clearly seen when the uterine horn cross-sections are inverted. These polyps are extensively developed and extend into different size papillae and because they are twisted in the lumen, construct plug-like structures (Fig. 3a and 4a).

Microscopical changes

The changes detected by SEM at the level of endometrium have been histologically described in previous reports (Gilloteaux and Steggles, 1981, 1982, 1983, and Gilloteaux, et al., 1982). Following SEM analysis, general morphological changes can be summarized by the following characteristics:

Control-untreated (C.C) uteri present convex apices and their surface is abundantly covered by microvilli (0.5 µm in height and 0.15 to 0.20 µm in width) while others have only sparse microvilli as secretory cells of the apocrine type (Fig. 1b). In utero DES-treated uteri (D.C) present a hypoplasia and are covered by single cuboidal cylindrical epithelial cells with both types of C.C cells but the D.C uteri show a mucosal membrane apparently unable to produce apocrine or merocrine secretory activity (Fig. 2b). Only later in life, uterine hyperplasia occurs and secretory products are observed in crypts and/or cystic structures (Gilloteaux et al., 1982). Postnatal DES-treated uteri (C.D) present enlargement of uterine horn size compared to C.C. uteri. The endometrial proliferation induces hyperplastic growth with formation of ridges and finger-like polyps which project in the uterine cavity. They may reach 1 mm in height and measure 0.5 x 0.2 - 0.3 mm in cross section and generally contain cystic glands filled with secretory products. The endometrium shows a complex cell surface and microvillous pleomorphisms by presenting squamous smooth to cylindrical, microvillar and merocrine secretory cell mucosa. Microvilli are usually short (0.10 to 0.15 µm in height) except at the cell borders where long microvilli are present (0.25 to 0.30 µm) which appear as ridges enhancing the cell outlines. In many places, an extended single microvillous protrudes from the central apices (Fig. 3b).

Pre- and postnatally DES-treated uteri (D.D) present extensive abnormalities including minute to large papillae (Gilloteaux et al., 1982) which bulge out the uterine ridges, and can reach 2 mm in height. These papillae often appear as finger-like or flat-sheeted shaped expansions

Fig. 1: C.C uteri

1a. SEM aspect of cross-sectioned horn. Bar = 1000 μm.
1b. Depicts a typical epithelial covering with long microvilli, The dense packing of microvilli does not allow a clear view on bridges present at the basal portion of each microvillus. Bar = 1 μm.

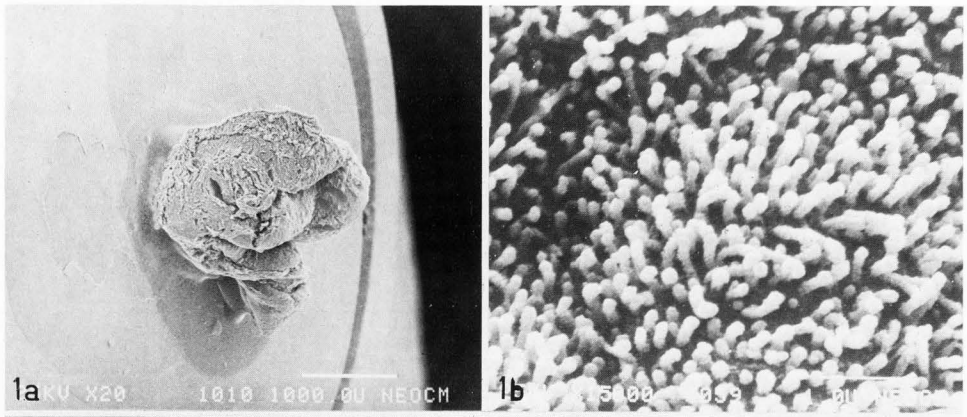


Fig. 2: D.C uteri

2a. Hypoplastic cross-sectioned horn, Bar = 1000 μm.
2b. Note the non-secretory aspect of flat cap-shaped apices. Few intermicrovillar fibrils appear en face or in oblique views Bar = 1 μm.

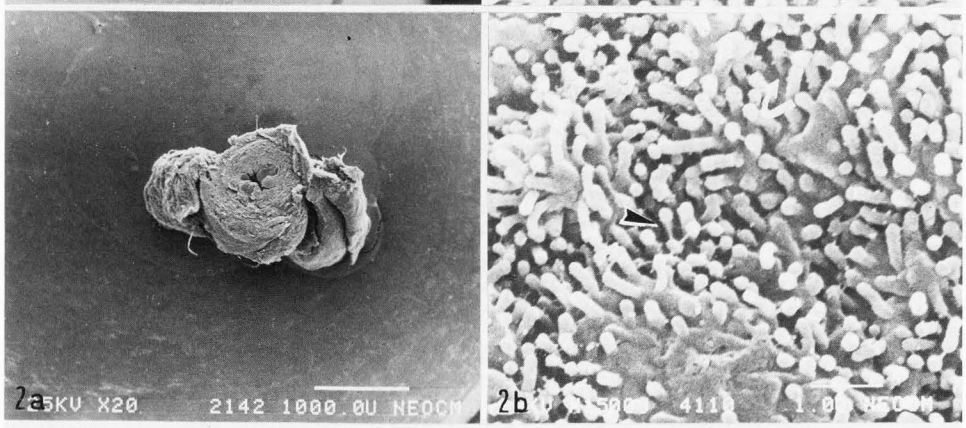


Fig 3: C.D uteri

3a. After dissection of a part of one horn in the longitudinal direction, the luminal surface is everted and the endometrium shows bulging polyps. Bar = 1000 μm.
3b. Endomertial muscosa showing merocrine belbs. Note the short microvilli and their interconnected fibrils. In addition, each cell apex presents one single elongated microvillus (arrow). Bar = 1 μm.

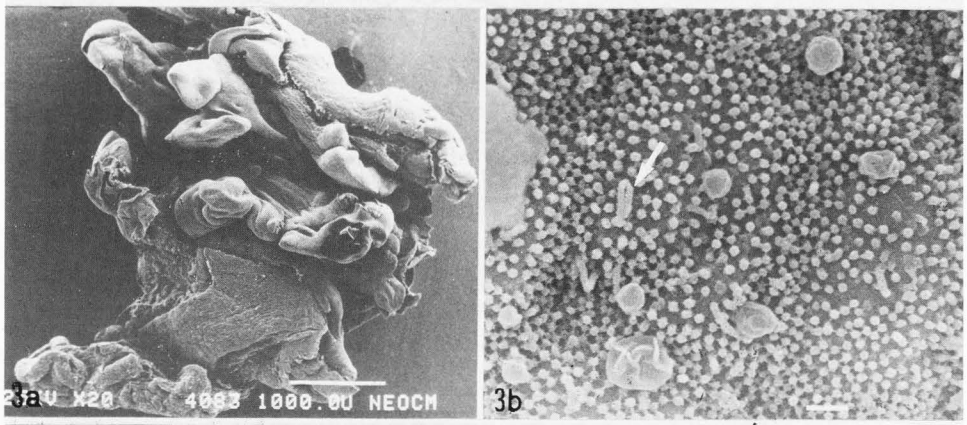
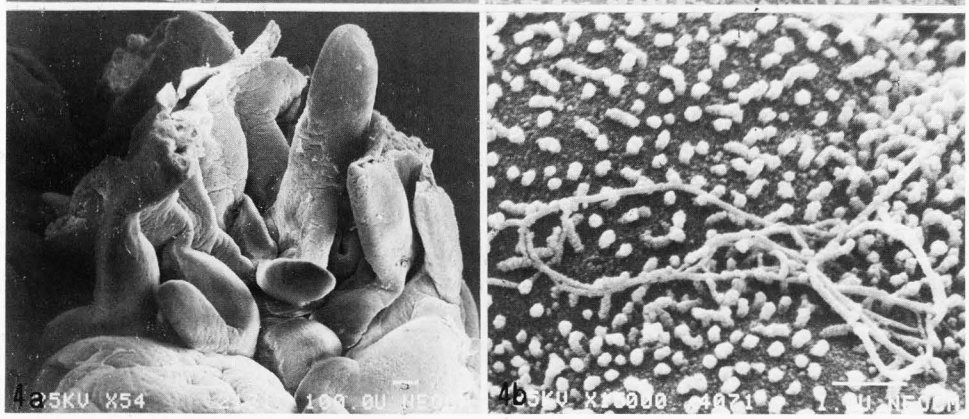


Fig. 4: D.D uteri

4a. Endometrial polyps and finger-like papillae after 300 days postnatal DES treatment. Bar = 100 μm.
4b. Tuft of elongated microvilli localized at the junction of several cell apices covered by short microvilli. Bar = 1 μm.



(0.3 x 0.1 mm in diameter) (Fig. 4a). The surfaces examined by SEM show pleomorphism like in the C.D uteri and often tufts of atypically slender microvilli that could be found at the junction of several cells (Fig. 4b). In addition, many cystic glandular openings are perforated through destruction now protruding squamous mucosa cells. Epithelial cells have swollen bulging surfaces covered by cells with irregular boundaries similar to those noted by Richart and Ferenczy (1974), Stenbäck et al. (1980), Halter et al. (1981) and Gilloteaux and Steggles (1981, also in preparation).

Microvilli peculiarities

Throughout this study we found that C.D and D.D uteri depict microvilli pleomorphism. The cell apices can have either smooth or irregular surfaces due to the presence of short microvilli and sometimes are accompanied by one or more peculiar long slender microvillus (Fig. 3b). Tufts of elongated microvilli are also discovered (Fig. 4b). In addition, we notice that for all the microvillous structures observed, peculiar linkages appear to attach one microvillus to others, either by single or multiple linkages. The treatment by protease to remove mucoproteinaceous secretion and cell debris of the endometrium cell surface helped to reveal these infracellular structures.

Following careful examination of the endometrium cell surfaces, it is remarkable to notice that the covering microvilli are interconnected by anastomoses of less than 0.50 µm in length and 20 to 45 nm in diameter which are freely extended parallel to the outer cell surfaces. Several of these fibers can be observed attaching microvilli to each other. They are localized near the basal portion of each short, long or distorted microvilli (Fig. 5-7).

These microvilli often appear to be equidistantly distributed on the cell apices (about 0.35 to 0.40 µm from center to center). Interestingly enough, these are apices which are devoid of clear microvillous coverage and/or associated with some apical secretory processes. On the secretory epithelial cells, the few microvilli at the edges of the bulging apices are clearly linked by these bridges (Fig. 5). In the cystic glands, where the microvilli appear bent, these linkages are clearly depicted (Fig. 6). At higher magnifications, these bridging structures appear more clearly (Fig. 7a,b).

Discussion

The discovery of rare reproductive tract abnormalities in young women resulting from a prenatal DES-treatment (Haney et al., 1979; Herbst et al, 1979; Robboy et al, 1979; Scully et al., 1978; Stenbäck et al, 1980; Tavassoli and Kraus, 1978) has led to several systematic case reviews and a few animal studies. Some human-like pathologies have been induced in mice (Lamb et al, 1981; Plapinger, 1981; Plapinger and Bern, 1979), rats (Ennis and Davies, 1982; Takewaki, 1964) and hamsters (Leavitt et al, 1980; Rustia and Shubik, 1970); however, few reports have dealt with the upper reproductive tract, or have used SEM to study endometrial abnormalities. This present work complements previous reports (Gilloteaux and Steggles, 1981,1983; Gilloteaux et al., 1982; Steggles and Gilloteaux, 1980) demonstrating the usefulness of the Syrian hamster to study transplacental and postnatal DES carcinogenetic effects on both male and female reproductive tracts, and that the defects resembled some of the described human abnormalities, including hypoplasia (in D.C women) and the lack of uterine secretory activity, which were proposed to contribute to the possible subfertility in women who had been prenatally exposed to DES. Only future SEM studies on uterine tissue biopsies from "D.C" human females could provide an informative comparison.

In this study, we also found that C.D and D.D uterine hypertrophy is associated with polymorphic mucosae, which display hyperplasia and/or abnormalities resembling the carcinoma *in situ* and adenocarcinomatous lesions (Gilloteaux et al., 1982). Among other morphological abnormalities, cell surface pleomorphism accompanied by microvilli alterations has to be mentioned (Ferenczy and Richart, 1974; Halter et al., 1981; Kenemans et al., 1981; Rambo and Szego, 1983). In addition, bridges between microvilli have been noticed throughout this study. Indeed, at this point, it is only possible to speculate about the nature of these filamentous edifices interacting with microvillous surfaces. They can be stress fibers which are parts of the cell subsurface or glycoproteinic bridges produced by the cell surfaces and linking microvilli with each other similar to those presented in illustrations of the epidermal cell surface of the lamprey larva (Fahrenbach, 1975) or the "junctional ridges" found in the lumenal surfaces lining the toad bladder (Davis et al., 1974). These interesting structures have not been observed to be associated with any of the DES treatments (D.C, C.D, and D.D). However, these bridging connections appear to be the most prominent in the D.C uteri. Although the nature and function of these microvillous anastomoses is unclear, we could speculate that these linkages may bring "mechanical" support for these microvilli, i.e., a morphological compromise between the simple microvillous surface and the microridged structures. Finally, the interconnections may represent glycocalyx material or, perhaps, remodeling of cell surfaces toward squamous epithelium. In addition, some of these changes may represent morphological signs for the identification of other pleomorphic changes associated with epithelial metaplasia and other abnormal cell differentiations resulting from DES treatment.

Acknowledgement

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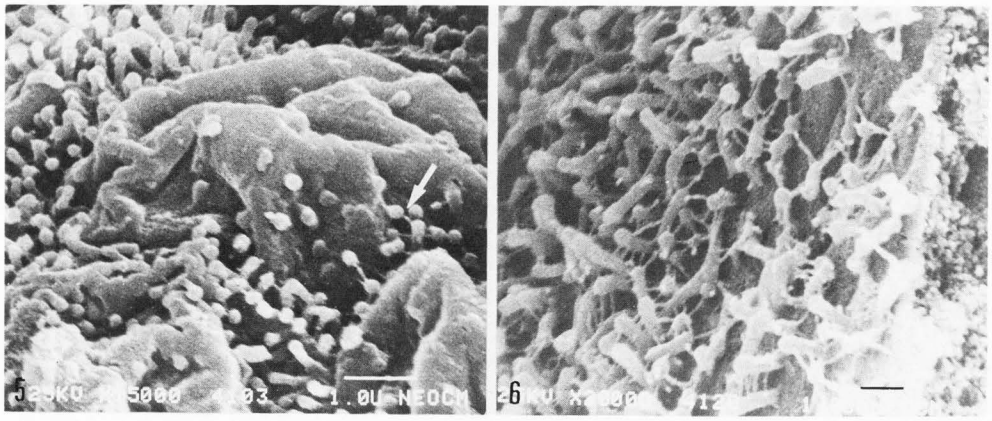
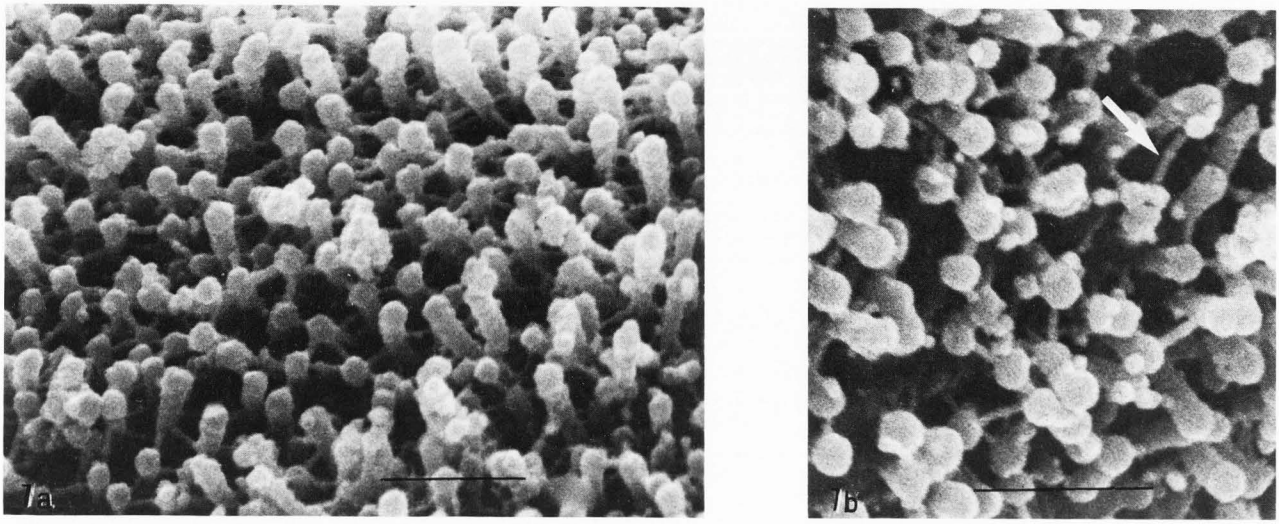


Fig. 5:
Collapsed apices in D.C endometrium. Few short microvilli at the cell edges are observed with their bridging structures (arrow). Bar = 1 μm.

Fig. 6:
Cystic gland surface covered by bent microvilli. Note the obvious bridging structures interconnecting these microvilli. Bar = 1 μm.



Details of microvillous covering in D.C uteri.
7a. Field where the bridging processes are seen obliquely and en face. Bar = 1 μm.
7b. Higher microphotographic enlargement demonstrating intermicrovillar linkages (arrow). Bar = 1 μm.

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Discussion with Reviewers

Reviewer I: Why does DES have a hypoplastic effect prenatally and a hypertrophic or hyperplastic influence when given postnatally? Which uterine compartment (i.e., endometrium or myometrium) is more responsive to DES?
Authors: It is not possible to answer this question since we can only speculate about the differential *in utero* vs. postnatal DES effect. There is, at this time, no explanation available through the literature.
The endometrium is more responsive than the myometrium to DES. The myometrium does not appear to alter unless the treatment is prolonged until 350 days of age or the double prenatal and postnatal DES treatment is applied for at least 200 days. In both cases, inflammatory infiltration and endometriosis are observed within the myometrium.

Reviewer I: Are microridges of DES-treated uterine cells an indication of modulation toward a squamous cell type? Have you observed these cells by transmission electron microscopy and, if so, found conspicuous tonofilaments?
Authors: Yes, the existence of microridges is related to the DES treatment and could be an indication of endometrium modulation toward a squamous cell type. From a preliminary (unpublished) study by transmission electron microscopy, we found intramicrovillar ridges, numerous desmosomes and their accompanying tonofilaments oriented parallel to on in the vicinity of the endometrial cell apices.
These findings, added to our previous observations and those presented in this communication, confirm the transplacental DES effect on the Syrian hamster uterus.

Reviewer I: In this interesting work, you have focused your attention on DES-related uterine changes. Using the same hamster model, have you observed similar or different abnormalities in higher reproductive tract tissues (i.e., oviduct)?
Authors: Yes, by using the same hamster model we have observed similar abnormalities in the oviduct. Occasionally we have found ovarian tumors in the D.D-treated group. These tumors were of the granulosa theca cell type identical to those described by Rustia and Shubik (1976).
Reference: Rustia, M. and Shubik, P. (1976). Transplacental effects of diethylstilbestrol on the genital tract of hamster offspring. Cancer Lett. 1, 139-146.