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# Ultrastructure of Human Uterine Epithelium at the Time of Implantation after Postovulatory Administration of Norethindrone

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## ABSTRACT

**The present investigation was performed to elucidate the mechanism of action of the previously demonstrated contraceptive effect in women of postovulatory administration of a synthetic gestagen, norethindrone (NET). Seven women participated during 3 control cycles and during 8 treatment cycles, in which NET was given orally after ovulation. Daily peripheral plasma levels of progesterone, estradiol and NET were assayed. An endometrial biopsy was taken in all cycles at about the expected time of implantation. Light microscopy revealed no consistent differences between non-treatment and treatment cycles but electron microscopy indicated that, after NET treatment, the mitochondria had grown larger and that nucleolar channel system had appeared. These changes suggest an increased progesterone-like influence upon the epithelium, despite the decreased progesterone plasma levels, caused by NET. It is assumed that these structural changes, caused by the postovulatory NET treatment, might change the functional properties of the endometrium and thereby impair the possibilities for normal implantation.**

## INTRODUCTION

Postovulatory administration of synthetic gestagens to women has been shown to markedly reduce peripheral plasma levels of progesterone (14). Estradiol levels are also decreased but this decrease is not so pronounced (23). It was proposed that the "luteolytic" effect of such treatment might be utilized for postovulatory contraception (14). The concept was that the hormonal effects of the treatment would induce changes in the endometrium significant enough to impair the possibility of normal implantation of the blastocyst (23).

When tested in clinical trials, in which a synthetic gestagen, norethindrone (NET), was given in various doses and dose schedules (23, 24) the treatment was found to have a certain contraceptive effect although the efficacy was not sufficiently high to merit its use in clinical practice.

One possible reason for the relatively low contraceptive efficacy of the NET treatment, in spite of the drastic effect upon the progesterone levels, could be that the drug itself substitutes for the decreased progesterone levels (23). From previous investigations (1, 2, 7, 8, 10, 17, 26) it is known that NET influences the endometrium, but in these studies the administration of NET did not follow the present schedule. Therefore, the present investigation was designed to study the impact of postovulatory NET treatment upon the human uterine epithelium of normal women at the time of implantation, as visualized by light- and electron-microscopy, in relation to the peripheral plasma levels of the ovarian steroids and of NET.

## MATERIAL AND METHODS

### *Volunteers*

Seven healthy regularly menstruating women, in the age range 25–30 years, participated in this investigation. Five of the women had previously been pregnant and the remaining 2 women have both become pregnant since the present study. No pathological changes were found upon pelvic examination (including a vaginal smear for cytology) before the start of the investigation.

### *General design of the investigation*

A total of 11 menstrual cycles were investigated. Daily peripheral venous blood samples were collected, commencing before the expected day of ovulation until after the day when implantation is expected to occur in a normal pregnancy. The concentrations of progesterone and estradiol were assayed in each blood sample. From the results of these assays the probable day of ovulation was calculated: After the mid-cyclic peak the first significant decrease in the estradiol level and the second day with a progesterone level above

1 ng/ml plasma was called the day of ovulation (14). Progesterone levels of 5 ng/ml plasma or more were regarded as postovulatory levels, i.e. as an indication of ovulation (15).

Three of the eleven cycles served as controls. In eight of the eleven cycles oral NET treatment was given postovulatory and the NET plasma levels were assayed. The treatment was aimed to start on the third postovulatory day, when the corpus luteum is functioning well and when, therefore, peripheral blood levels of progesterone have become high enough to permit the conclusion that ovulation has taken place (15). However, for practical reasons the results of both the progesterone and the estradiol assays were sometimes not available until a couple of days after the day when the blood samples were taken. Therefore, preliminary calculation of the day of ovulation, which was in some cycles based upon only one or two increased progesterone plasma levels, sometimes required correction when all the results of the hormonal assays were available. In three of the cycles, treatment had actually been started on the fourth and in one cycle on the fifth postovulatory day, as indicated in the Table.

In order to study the influence of varius schedules of treatment the doses of NET ranged in total from 150 to 300 mg and the period of treatment from 2 to 4 days. This is also indicated in the Table.

The women were instructed to take their tablets in the evening and to give their blood sample in the morning. However, one woman (woman D, indicated in the Table) took her NET dose in the morning about one hour before the blood sample was taken.

During each control and treatment cycle an endometrial biopsy was obtained for light- and electron-microscopy. The timing of biopsy removal was aimed to coincide with the probable time when implantation occurs in a normal pregnancy, i.e. about 1 week after the time of ovulation (9). For practical reasons the period of time elapsing from the calculated day of ovulation until the biopsy was taken varied between 6 and 8 days.

All women were instructed to use mechanical and/or chemical contraceptives during the investigation.

### *Hormonal assays*

*Progesterone in plasma* was estimated by a competitive protein binding assay, as described by Johansson (13).

*Estradiol in plasma* was determined by a radioimmunoassay as described by Hotchkiss et al. (11), modified as described by Edqvist & Johansson (4).

*Norethindrone (NET) in plasma* was assayed by a radioimmunoassay as described by Nygren et al. (25).

### *Endometrial morphology*

The endometrial biopsies were obtained with a Genell curette without anesthesia and without dilation of the cervix. The biopsies were taken from the middle part of the anterior wall of the corpus uteri.

The biopsies were immediately fixed by immersion in a 2.5% solution of glutaraldehyde in Soerensen phosphate buffer (pH 7.4) and were kept in the fixative for periods of one day up to several months. At embedding, small specimens were cut from the biopsies, rinsed in the buffer, and post-fixed for 3 hours in a solution of 1% osmium tetroxide in Soerensen phosphate buffer, pH 7.4. Dehydration was performed in ethanol, and the specimens were embedded in Epon 812.

For light-microscopy, the sections were stained in a basic solution of Toluidine Blue. After microscopical examination appropriate areas of glandular or luminal epithelium were selected and trimmed for sectioning for electron-microscopy. For transmission electron-microscopy, the sections were stained with uranyl acetate followed by lead citrate. For scanning electron-microscopy (21), specimens with an endometrial surface area of about 1×1 mm were cut, carefully rinsed in distilled water, and taken to 10% ethanol in water for freeze-drying. The specimens were frozen in isopentane cooled by liquid nitrogen and dried in $10^{-3}$ Torr for 3 days at −79°C (solid carbon dioxide). The dry specimens were mounted for scanning electron-microscopy and coated with carbon followed by gold. A Jeol JSM-U3 scanning microscope was used.

## RESULTS

### *Hormonal assays*

All cycles in this investigation were found to be ovulatory, as judged by the levels of progesterone in plasma. The results of the assays of progesterone, estradiol and NET are shown in the Table, which summarizes the daily plasma levels of these hormones from the day after the calculated day of ovulation until the day when the endometrial biopsy was taken. This gives an estimation of the total amount of the hormones in plasma between the time of ovulation and the date of the biopsy.

Woman A had, initially, high progesterone levels and low estradiol levels compared with those found in most of the other treatment cycles, but responded as expected to the NET treatment. Woman B had comparatively low estradiol levels in both cycles investigated, while in her second treatment cycle progesterone levels were exceptionally low. The remaining 5 women, C through G, showed a more consistent pattern.

Comparing control cycles and treatment cycles from the same women (E, F and G) it was found that the summarized progesterone levels were lower in the treatment cycles. Estradiol levels were not consistently changed but it should be noted that in 2 of the women (F and G) the

Table. *Peripheral plasma concentrations of progesterone, estradiol, and NET, summarized from the day after ovulation until the day when the endometrial biopsy was taken. The day of ovulation is called day 0*

| | Control cycles | | | Treatment cycles | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | NET treatment | | | | | |
| Woman | Biopsy on day | Progesterone (ng/ml plasma) | Estradiol (pg/ml plasma) | mg | on day | Biopsy on day | Progesterone (ng/ml plasma) | Estradiol (pg/ml plasma) | NET (ng/ml plasma) |
| A | | | | 100 | 4–6 | 7 | 138 | 445 | 456 |
| B | | | | 100 | 5–7 | 8 | 52.5 | 310 | 696 |
| | | | | 100 | 3–4 | 6 | 18.8 | 645 | 332 |
| C | | | | 100 | 3–4 | 7 | 49.5 | 1285 | not assayed |
| D | | | | 100 | 4–5 | 7 | 43.8 | 1305 | 1413[a] |
| E | 7 | 87.1 | 1475 | 50 | 3–6 | 7 | 41.7 | 1445 | 349 |
| F | 7 | 66.9 | 1226 | 50 | 4–6 | 8 | 49.2 | 1328 | 219 |
| G | 6 | 73.2 | 1032 | 50 | 3–5 | 7 | 41.4 | 995 | 114 |

[a] Woman D took her NET tablets about one hour before the blood sample was taken.

hormonal levels were summarized from one more day in the treatment cycle as compared with the corresponding control cycle. On the day of the biopsy the progesterone plasma level in woman E decreased from 14.5 ng/ml in the control cycle to 0.6 ng/ml in the treatment cycle, in woman F from 9.1 ng/ml to 0.9 ng/ml and in women G from 15.5 ng/ml to 5.5 ng/ml. Corresponding estradiol plasma levels were decreased from 245 to 110 pg/ml in woman E, from 210 to 60 pg/ml in woman F and from 250 to 135 pg/ml in woman G.

To illustrate the hormonal patterns, the daily plasma levels of progesterone, estradiol and NET in the control cycle and the treatment cycle of woman E are shown in Fig. 1.

*Endometrial morphology*

*Light microscopy*. The biopsies were examined with regard to the amount of glycogen, stromal oedema, and decidual changes (9, 22, 27). Microscopy demonstrated slight differences among the biopsies, but no difference was sufficiently consistent to serve as a criterion of NET-induced change. However, the glycogen content of the uterine epithelium appeared to increase somewhat after the NET treatment.

*Electron microscopy*. The ultrastructure of the luminal and glandular epithelium from biopsies obtained on the 6th–8th postovulatory day of the normal untreated women corresponds rather well to what has been previously reported (3, 5, 6, 12, 18, 20, 21, 28, 29, 31): Apical protrusions were present although they contained only a few glyco-

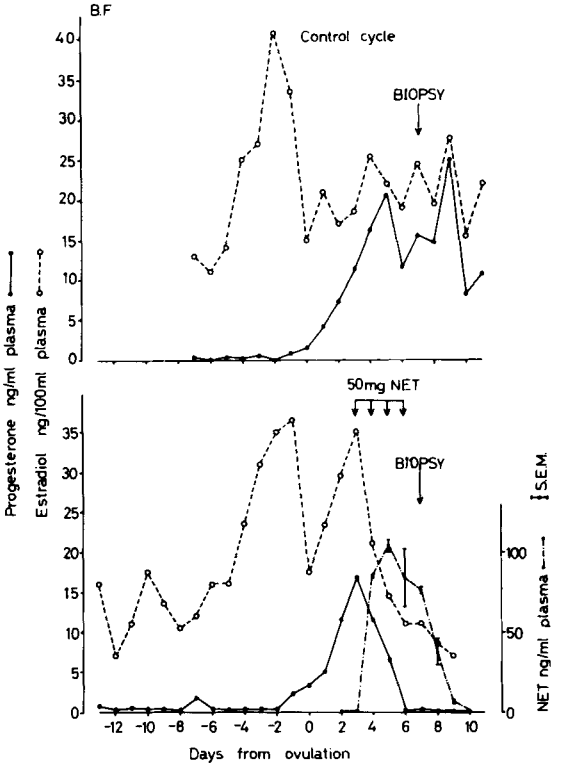


*Fig. 1.* Woman E. Daily peripheral plasma levels of progesterone, estradiol and NET during one control cycle and one NET treatment cycle. In both cycles an endometrial biopsy was taken 7 days after the calculated day of ovulation (=day 0).

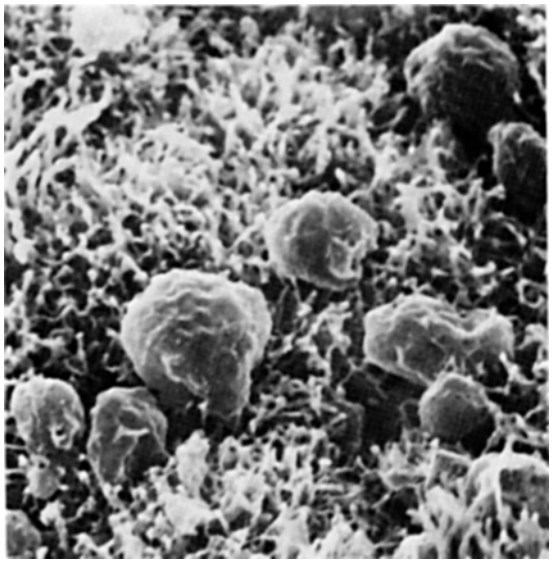

*Fig. 2.* Luminal surface of normal endometrium, day 7 postovulatory. The microvilli of the secretory cells are noticed. Several cells also possess apical protrusions. ×10 000.

gen granules (Fig. 2). The endoplasmic reticulum was well developed and the Golgi apparatus was rather large. Most mitochondria were in the normal size range and only a few giant mitochondria were observed. Lysosome-like bodies occurred sparsely. The amount of glycogen granules varied but never reached the high levels present a few days earlier in the cycle when glycogen content reaches its peak (28).

The ultrastructure of the cells of the luminal and glandular NET-influenced uterine epithelium differed in some aspects from that of the control biopsies. Apical protrusions were present but they generally contained more vesicles and glycogen granules than were observed in biopsies from untreated women (Fig. 3). The mitochondria were generally larger (Fig. 4), and giant mitochondria were more often present basally. The glycogen granules seemed to be more numerous, often occurring in groups containing glycogen vacuoles. Nucleolar channel systems were rather frequently observed (Fig. 5).

No marked differences related to the various dose schedules were found. Obviously, this does not exclude the existence of functional differences although the technique used in the present investigation did not reveal any structural differences.

The biopsies from woman A showed a glandular epithelium containing more dense granules than were usually observed.

## DISCUSSION

The duration between the ingestion of NET tablets and the blood sampling for the determination of plasma NET levels was not entirely consistent, for practical reasons. After oral ingestion, NET has been found to have a plasma half-life, after its initial peak, of about 9 hours only (25). Thus, the plasma levels of NET recorded in the present investigation cannot be used for strictly quantitative comparison but merely as an indicator that the women took their tablets as intended.

A decrease in progesterone and estradiol plasma levels was found during treatment cycles at the day of biopsy, as anticipated from previous experience with postovulatory NET treatment (14, 23). One woman (A) had extremely high progesterone levels as compared with the other women in this study. On examining the electronmicrographs, the uterine epithelium of this woman was found to contain more dense granules than did the epithelium from the other NET-treated women. However, since this is an observation from one single woman, no conclusion can at present be drawn from the finding. Further, it must be remembered that our knowledge of the inter-relationship between a hormonal plasma concentration and its effect upon the target tissues is meager. For instance, we do not yet know the time lag between a change in the plasma level of a hormone and the structural response it may induce in the uterine epithelium. Therefore, in the present communication only general trends in hormonal and structural changes have been considered.

Light microscopical findings indicating that the glycogen content of the gland cells increased after NET treatment are not particularly conclusive, considering the inconsistency of the findings and the relatively few biopsies available. Although electron microscopical observations imply various difficulties (19), changes were found which seem to be sufficiently consistent to warrant the conclusion that NET did affect the uterine epithelium. The changes comprised the appearance of large mitochondria and nucleolar channel systems. Both

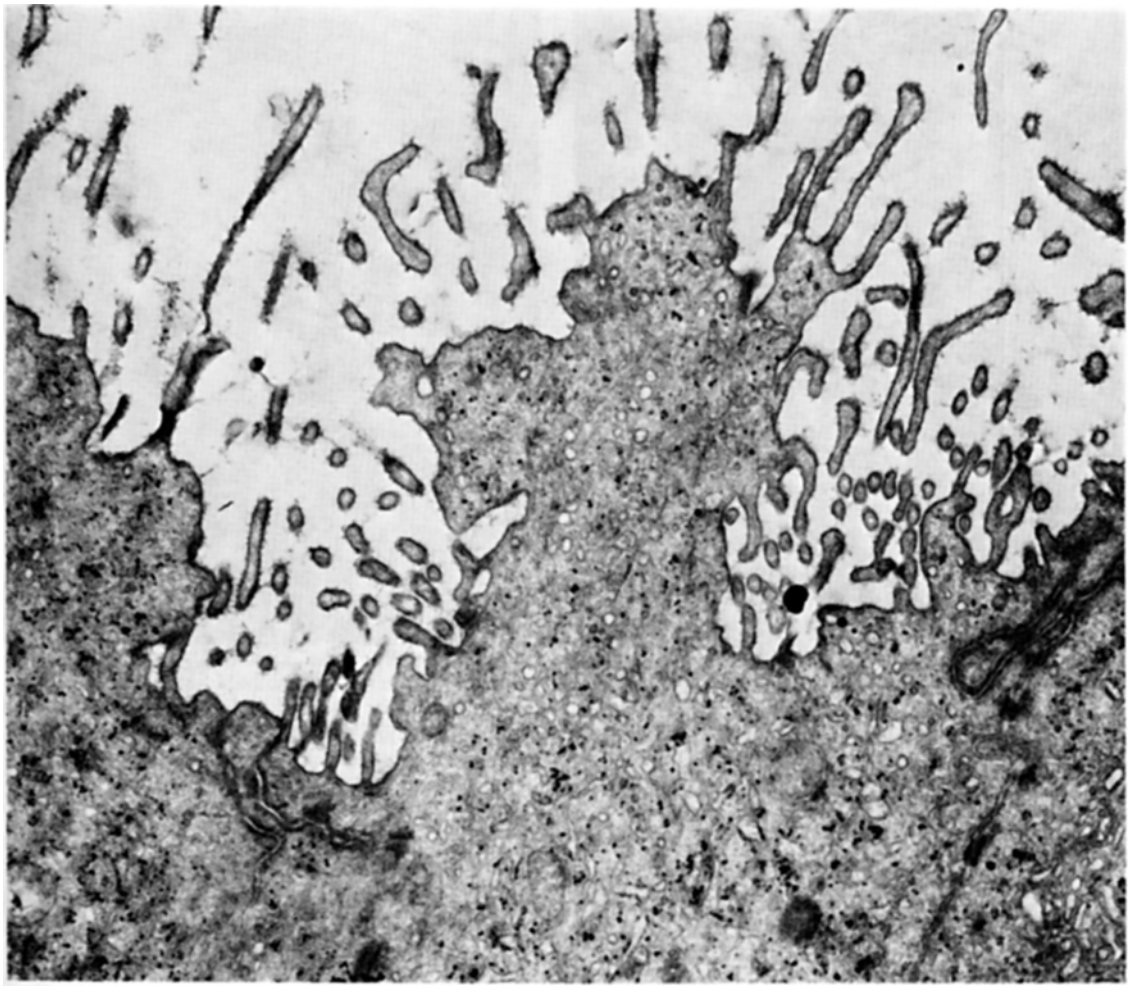

*Fig. 3.* Luminal part of uterine epithelium, day 7 postovulatory after NET treatment. An apical protrusion is observed. It contains vesicles and glycogen granules. ×20000.

these features are regarded, by most authors, as an effect of progesterone (1, 7, 17, 30). Since the serum levels of both progesterone and estradiol were lower in the treatment cycles at the time of biopsy, the progesterone-like action on the epithelium is probably due to the synthetic gestagen. Results from animal studies (16) suggest that the relation between the action of progesterone and NET upon uterine hormone receptors might be of great importance in this respect.

Several explanations for a contraceptive effect of the NET treatment are possible. The tubal function might be affected. The proportion of tubal pregnancies, found in previous clinical trials with postovulatory NET treatment was unexpectedly high (24), even though the actual number was too low to provide conclusive statistical evidence of an increased incidence of tubal pregnancies. Furthermore, the luteotrophic effect of human chorionic gonadotropin (HCG) from the blastocyst upon the corpus luteum after successful implantation, might be affected by the presence of NET. However, both experiments with exogenous HCG administered to women treated with NET after ovulation (14) and the normal hormonal and histological development of pregnancies occurring after such treatment (24) suggest that this is not a probable explanation. The most likely explana-

*Fig. 4.* Basal part of glandular uterine epithelium, day 7 postovulatory after NET treatment. The mitochondria generally are larger than at a similar stage of a control cycle. ×30000.

tion is perhaps that the NET-induced changes observed in the uterine epithelium might result in a secretion which is not optimal for the survival of the blastocyst or in an extra-cellular coat on the luminal surface, counteracting the attachment of the blastocyst.

It is possible that the use of a drug with a more potent progesterone-like action might influence the uterine epithelium more drastically, thereby giving a better contraceptive efficacy than postovulatory NET treatment. On the other hand a drug having the same depressive effect as NET upon the function of the corpus luteum but which does not exhibit progesterone-like actions on the uterine epithelium might also induce a more pronounced disturbance in the development of the endometrium.

*Fig. 5.* Nucleolar channel system of glandular uterine epithelium, day 8 postovulatory after NET treatment. ×25000.

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