ORIGINAL RESEARCH

THE EFFECT OF A LEVONORGESTREL-RELEASING INTRAUTERINE DEVICE ON OVARIECTOMIZED RAT ENDOMETRIUM UNDER ESTROGEN REPLACEMENT THERAPY

Murat Api[1], Feriha Ercan[2], Serap Arbak[2], Olus Api[3]

[1] Department of Obstetrics and Gynecology, Haseki Education and Research Hospital, Istanbul, Turkiye [2] Department of Histology and Embryology, School of Medicine, Marmara University, Istanbul, Turkiye [3] Department of Obstetrics and Gynecology, Kartal Education and Research Hospital, Istanbul, Turkiye

ABSTRACT

Objective: Our aim was to investigate the effects of levonorgestrel-releasing intrauterine system on the endometrium of ovariectomized rats under estrogen replacement therapy.

Methods: Twenty-four Sprague-Dawley rats were divided into four groups and operated for the insertion of levonorgestrel-releasing (3 µg/day) or placebo-bearing intrauterine devices (IUD). Following the operation, the rats were randomly assigned to take estrogen (0.01 mg. per kg. rats) or placebo replacement therapy for 30 days. Endometrial biopsies were collected at the end of the study period. The sampled endometrial tissues have been analyzed for morphologic criteria under the light microscope. Group I was assigned to take levonorgestrel-releasing IUD and systemic estrogen whereas Group II was assigned for levonorgestrel-releasing IUD and systemic placebo and Group III for placebo-bearing IUD and systemic estrogen; finally Group IV was treated with placebo-bearing IUD and systemic placebo.

Results: The endometrial morphology of Group I revealed inactive endometrium in 50%, edema in 33% and atrophy in 16% whereas the endometrial morphology of Group II revealed inactive endometrium in 66%, and atrophy in 33%. The endometrial morphology of Group III revealed epithelial hyperplasia in 66%, edema in 16% and myometrial hyperplasia in 16% while inactive endometrium was identified in 16%, and atrophy in 83% of rats in Group IV.

Conclusion: Our results revealed that progestagen containing intrauterine systems are sufficient enough to protect the endometrium from hyperplasia in rats under systemic estrogen replacement therapy. This is somehow encouraging data to support that the endometrium of postmenopausal women under systemic estrogen can be sufficiently protected by an intrauterine gestagen-containing device. The use of such an IUD will serve to protect them from the adverse effects of systemic gestagens.

Keywords: *Rat endometrium, Levonorgestrel, Intrauterine device, Estrogen replacement therapy*

ÖSTROJEN REPLASMAN TEDAVİSİ ALTINDAKİ OVARİEKTOMİZE SIÇANLARDA LEVONORGESTREL-SALAN RAHİMİÇİ ARACIN ETKİLERİ

ÖZET

Amaç: Levonorgestrel salan rahimiçi sistemin, östrojen replasman tedavisi altındaki ovariektomize sıçanların endometriumu üzerindeki etkilerini araştırmak.

Yöntem: Yirmidört adet Sprague-Dawley sıçanı 4 gruba bölünerek, levonorgestrel salan intrauterin sistemin (3 µg/day) ve plasebo-içeren rahimiçi aracın (RİA) yerleştirilmesi için operasyona alındı. Operasyonu takiben, sıçanlar 30 gün süreyle östrojen (0.01 mg./kg.) ve plasebo replasman tedavisi almak üzere gruplara randomize edildi. Çalışmanın sonunda deneklerden endometrial örnekleme yapıldı. Alınan endometrial dokular morfolojik kriterler açısından incelenmek üzere ışık mikroskopisi ile incelendi. Grup I levonorgestrel salan RİA ve sistemik östrojen alan, Grup II levonorgestrel salan RİA ve plasebo alan, Grup III plasebo içeren RİA ve sistemik östrojen alan, Grup IV ise plasebo içeren RİA ve plasebo alan sıçanlar olarak belirlendi.

Bulgular: Grup I'e ait endometrial morfoloji %50'sinde aktif olmayan endometrium, %33'ünde ödem, %16'sında atrofi olarak görülürken. Grup II'ye ait morfoloji %66'sında aktif olmayan endometrium, %33'ünde atrofi olarak saptandı. Grup III'te ise % 66'sında epitelyal hiperplazi, %16'sında ödem, %16'sında myometrial hiperplazi bulunurken, Grup IV' ün. %83'ünde atrofi, % 16'sında ise aktif olmayan endometrium tespit edildi.

Sonuç: Sistemik östrojen replasman tedavisi altındaki sıçanların endometriumlarını hiperplaziden korumada, progestagen içeren rahimiçi sistemin yeterli olduğu sonucuna ulaşılmıştır. Elde edilen veriler, sistemik östrojen alan postmenopozal kadınların endometriumlarını korumada gestagen içerem rahimiçi aracın yeterli olabileceği yönünde cesaret vericidir. Bu tip RİA kullanımı ile hastalar sistemik gestagenlerin istenmeyen etkilerinden korunmuş olacaklardır.

Anahtar Kelimeler: *Sıçan endometriumu, levonorgestrel, intrauterin sistem, östrojen replasman tedavisi*

Corresponding author: *Murat API Acelya Sok. No: 12 / 2. Postal code: 31865 Dragos/ Kartal/ İstanbul/ Türkiye Tel:(0090) 05424241807 (0090) 02163622280* ***E-mail:*** *muratapi@hotmail.com*

INTRODUCTION

The intrauterine system (IUS) can be used to supplement estrogen replacement therapy (ERT), in which estrogen is used to relieve such menopausal symptoms as hot flashes, sweating, sleep disturbances and vaginal dryness. Estrogen replacement therapy also helps to prevent osteoporosis and cardiovascular disease.

However, ERT also stimulates the endometrium. Adding a progestin at menopause, such as the levonorgestrel in the IUS, counteracts endometrial stimulation and helps protect against the overgrowth of endometrial tissue, precancerous endometrial changes and endometrial cancer [1,2].

The sustained release of low-dose levonorgestrel directly into the uterus via the IUS may result in more endometrial protection, less irregular bleeding, and fewer systemic side effects than the release of progestins via pills or implants [3,4].

Postmenopausal women should use progesteron in order to protect their endometrium while taking ERT. The progesterons in use have variable systemic adverse effects [5,6]. In order to minimize these adverse effects, the local use of gestagens by the help of intrauterine device seems to be a logical option. Nevertheless, the efficacy of this route of administration is still under investigation. For this purpose, the endometrial effects of intrauterine use of levonorgestrel with systemic estrogen therapy have been investigated at the light microscopic levels.

MATERIALS AND METHODS

Animals

Twenty-four Spraque-Dawley rats (mean weight: 240 gr.) were subdivided into four groups containing six rats in each. The University Ethic Commitee approved the trial to be performed according to the rules of Strazburg declarations (Marmara University Faculty of Medicine Ethic Commitee No: 657/ 2). Table I shows the distribution of rats according to their subdivisions.

Table I: Distribution of the rats according to their subdivision

Groups	Intrauterine device	Therapy applied
Group I	Levonorgestrel (IUD)	Estrogen
Group II	Levonorgestrel (IUD)	Placebo (Olive oil)
Group III	Placebo silicon (IUA)	Estrogen
Group IV	Placebo silicon(IUA)	Placebo (Olive oil)

In the first group, an intrauterine device -releasing levonorgestrel-(LNg-IUD) has been applied by hysterotomy and estrogen replacement therapy was given for a period of one month. In the second group, an intrauterine device-releasing levonorgestrel-was applied by hysterotomy and placebo treatment was given for a period of one month. In the third group, an intrauterine device -releasing nothing- was applied by an operation and estrogen replacement therapy was given for one month. In the fourth group, an intrauterine device -releasing nothing- was applied by an operation and placebo treatment was given for one month. At the end of one month, by a second intervention, endometrial tissue samples were collected for light and transmission electron microscopic investigation and bones from the right femurs were extracted for bone mineral densitometric measurements. The revealed data were statistically analysed.

Calculation of rat dosages of estrogen and progesteron-releasing intrauterine devices

Each flacon with a total volume of 1 ml. and containing 0.738 mg estradiol benzoate and olive oil vehicle was supplied by the company. Organon® . Same volume of olive oil flacons were used for placebo injections. As the estimated ethinyl estradiol dosages used for hormone replacement in the ovariectomized rats was reported to be 0.01 mg. per kg. rats per day, the equivalent estradiol benzoate dosage was calculated by the usage of estradiol potency.

From this point, it was estimated that 0.1 ml. of daily estradiol benzoate intramuscular injections was the appropriate dose for ovariectomized rats with a mean weight of 240 grams.

The appropriate LNg-IUDs for rats have been supplied by Lerias Pharmaceuticals, Huhtamaki, Oy Turku, Finland, Norplant®. These IUDs for human hormonal contraception contain 36 mg of

levonorgestrel in each of 6 rods, 2.4 mm in diameter in width and 3.4 cm in length. These rods release 13.33 mcg/24 hour levonorgestrel for the first 6-18 months in humans. It has been calculated that one third of each rod can release a sufficient amount of levonorgestrel for ovariectomized rats (3 mcg/day) according to relative dose equivalency of gestagens.

The Operation procedures

Before surgery, the rats were weighed and anesthesia was achieved by injections of a mixture of chlorpromazine (0.15 mg/ 100gr. per rat) and ketamine (0.1 mg/ 100gr. per rat) into the peritoneal cavity.

The abdominal wall of the rats was depilated, cleaned and a 2 cm. of vertical incision was made for laparotomy. The rats were ovariectomized by ligation and dissection of the utero-ovarian and suspensory ligaments of the ovaries. Fig. 1 depicts the operation applied to the rats internal genitalia (Fig. 1).

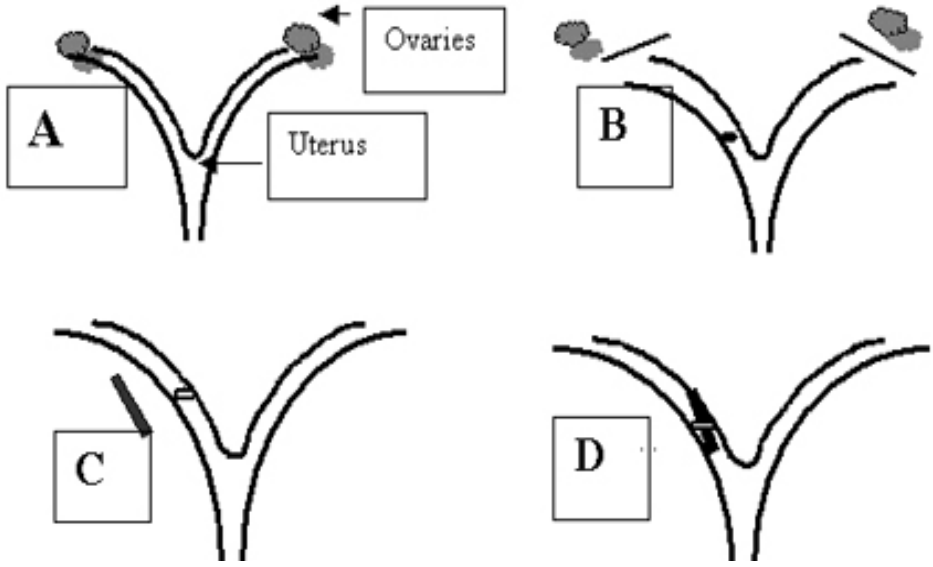

Fig. 1. The operation applied to the rats internal genitalia A: Normal anatomy of the rats. B: Ovariectomy and left uterine horn incision site C: Intrauterine device and uterus ready for application D: The IUD has been applied and operation ended.

After the operation, the rats were injected with placebo or estrogen, according to their groups, for 30 days and reoperated for endometrial sampling from the left uterine horn.

Light microscopy

For light microscopy, the endometrial specimens were fixed in Bouin's solution and embedded in paraffin. The paraffin sections were cut at 5 µm thickness and stained with hematoxylene and eosin (H&E) for routine examination and Masson's Trichrome for fibrous tissue details. Sections were examined with Olympus BH-2 photomicroscope.

Statistical analysis

Statistical evaluation was performed with computer assisted SPSS version 11.5 (Statistical Package for Social sciences) package programme. The difference between the histopathologic findings were computed by Fisher's Exact test. Non-parametric tests were used for statistical evaluation because of the limited numbers of samples. Significance level was accepted at $p<0.05$.

RESULTS

All morphologic findings under the light microscopy in the endometrial samples of the groups were been classified into five different histopathological criteria according to a veterinarian pathology specialist. Table II depicts this distrubution among the groups.

The endometrial morphology of Group I revealed inactive endometrium in 50%, edema in 33% and atrophy in 16% whereas the endometrial morphology of Group II revealed inactive endometrium in 66%, and atrophy in 33%. The endometrial morphology of Group III revealed epithelial hyperplasia in 66%, edema in 16% and myometrial hyperplasia in 16% while inactive endometrium was identified in 16%, and atrophy in 83% of rats in Group IV (Fig. 2).

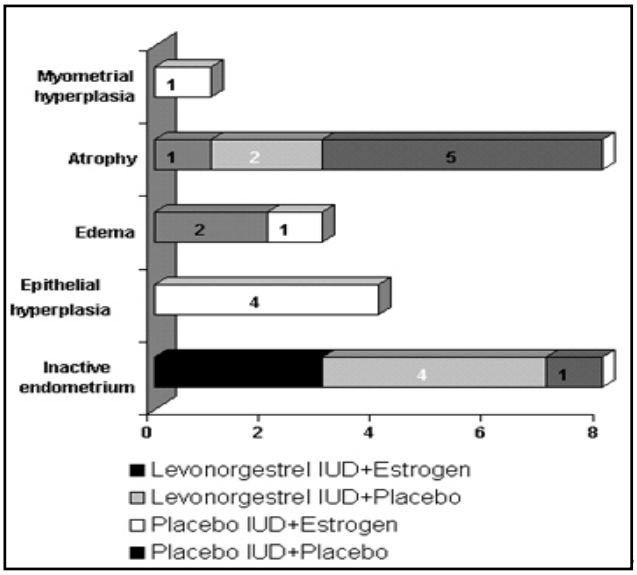

Fig 2: The bar chart of histologic findings of the rats

Table II: The distribution of groups according to histologic findings. Among the histological findings there were statistically significant difference established for epithelial hyperplasia (p=0.02) and atrophy (p=0.015) findings. Other findings did not differ in each group (p>0.05).

	Histology				
Group	**Inactive endometrium**	**Epithelial hyperplasia**	**Edema**	**Atrophy**	**Myometrial hyperplasia**
I	3 (50%)	-	2 (33%)	1 (16%)	-
II	4 (66%)	-	-	2 (33%)	-
III	-	4 (66%)	1 (16%)	-	1
IV	1	-	-	5 (83%)	-

Histologically, the cellular components of the endometrium could be clearly subdivided into glands and surrounding stroma cells comprising the vasculature.

As ovariectomy was performed for all subjects, the hormonal production from the gonads was seized for the study period. It was clearly demonstrated that atrophic changes occurred in 83% of the fourth group (Fig. 6) which was revealed at the end of the study period. This atrophic endometrial change was proportionally more frequent than the other groups and this difference was found to be statistically significant (p<0.05). The first group has been served as a sample group to answer the study question. So the histologic findings of this group was extensively evaluated. The endometrium remained inactive in 50% of the this group and 33% of them featured stromal edema (Fig. 3). This histologic result seems to reflect the relative dominance of local gestagenic effect of intrauterine device on the endometrium. In 16% of this group atrophy was revealed; which represents the local progestojenic effect of intrauterine device was strong enough to supress endometrium against the circulating level of estrogen. There was a substantial reduction (p<0.05) in both the atrophy and the proportion of cells with epithelial hyperplasia in the first group. The latter features are representitive for hormonal abstinance or estrogenic dominance, respectively. In the second group, a form of gestagenic dominance was more prominent as the 66% of

inactive endometrium and 33% of atrophy were demonstrated (Fig. 4). On the other hand in the third group, the unopposed estrogen influenced the endometrium and myometrium while 83% of the subjects revealed some extent of adverse events (66% epithelial hyperplasia and 16% myometrial hyperplasia). Only 16% of this group featured edema which has an unknown significance at the rat endometrium but most probably it should be related to steroidal stimulation. The hyperplasia seen in the third group (Fig. 5) was statistically more frequent than the other groups ($p<0.05$).

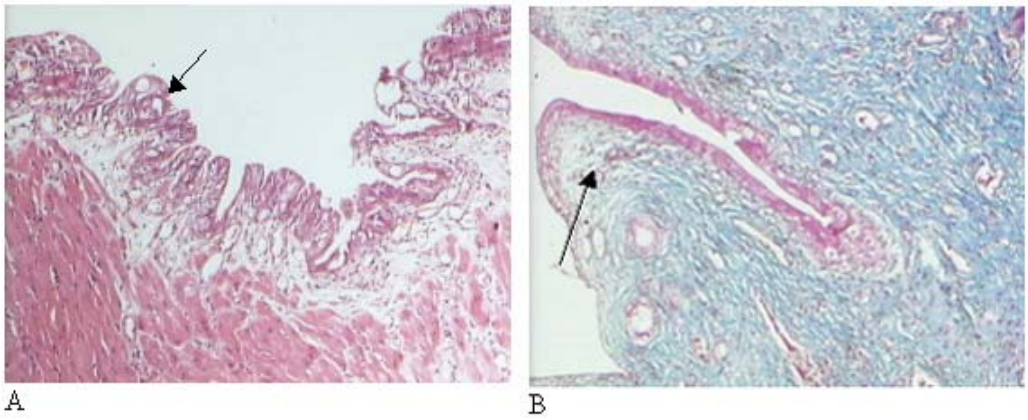

Fig. 3: Group I: Light micrographs indicate A-) inactive endometrium (→) H+EX 40; B) Mild stromal edema (→). Masson's Trichrome X40;

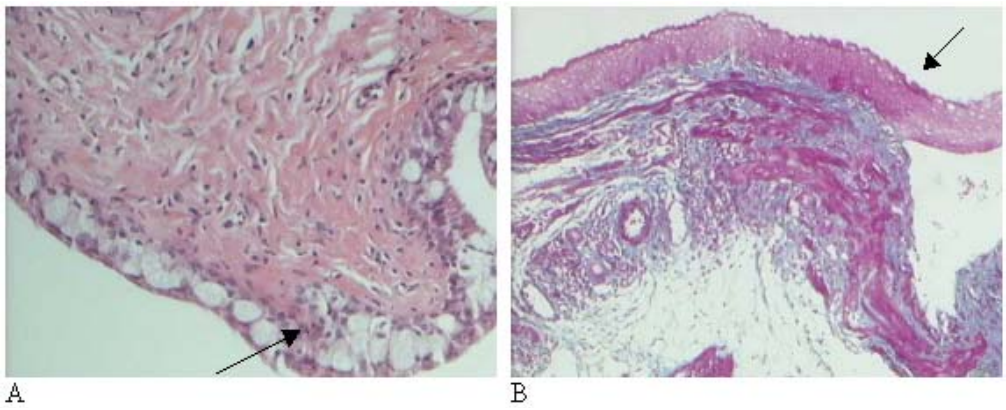

Fig. 4: Light micrographs of Group II: A) Inactive endometrium and thin epithelial cells with stromal atroply (→) H+E X 40 and B) Inactive endometrium with no glandular structure (→) Masson's trichrome X 40;

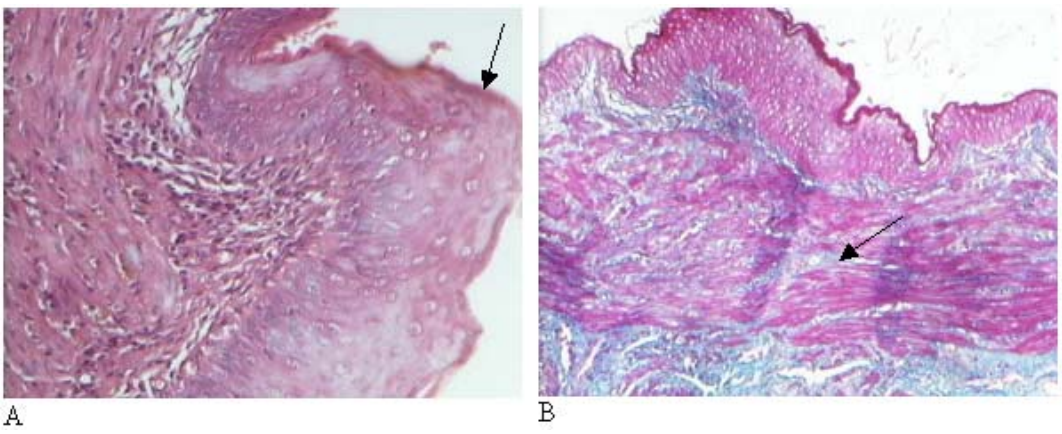

Fig. 5: Light micrographs of Group III indicate A) epithelial hyperplasia (→) H+EX 40; B) myometrial hyperplasia (→) Masson's trichrome X 40

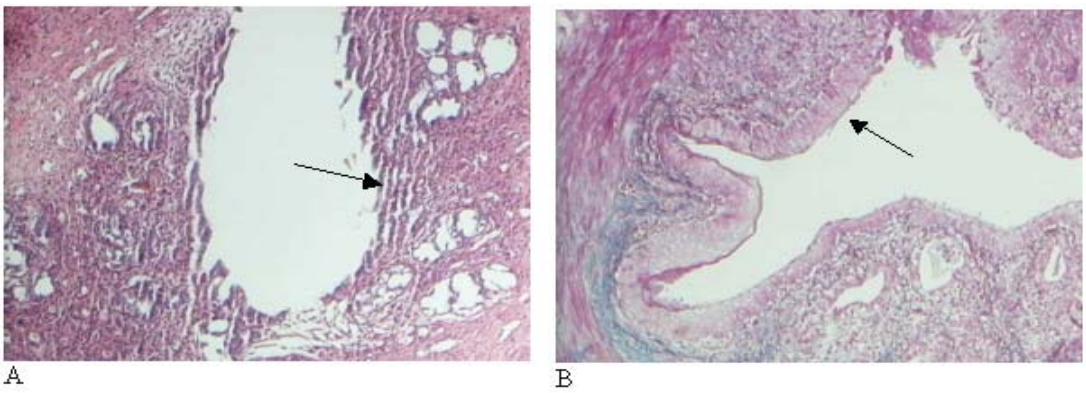

Fig. 6: Light micrographs of Group IV indicate A) atrophic epithelial structure (→) and desquamation H+EX 40; B) inactive endometrium (→) Masson's trichrome X 40;

DISCUSSION

The addition of a gestagen to estrogen replacement therapy is necessary to protect the endometrium against hyperplasia and to minimize the risk of endometrial cancer. In order to minimize the systemic adverse effects of gestagens, they were administered in a sequential fashion. The major disadvantage of such therapy is cyclic menstrual bleeding which further diminutes patients' compliance for the estrogen replacement therapy. This drawback can be avoided by continuous gestagen administration along with estrogen replacement which leads to amenorrhea after initial periods of spotting [7]. On the other hand, the disadvantageous effect of continuous administration of gestagen on lipid metabolism may be even more pronounced than that of progestogen administered cyclically. This concept has gained support from the observations that the continuous administration of 19-nortestosteron derivative has counteracted the beneficial effect of estrogen on serum high density lipoprotein (HDL) and cholesterol level [8,9] whereas the estrogen-induced reduction in total cholesterol and low density lipoprotein (LDL) cholesterol has remained unaffected by continuous administration estrogen and progestin[10,11].

One option for minimizing these possible systemic effects of progestins while preventing endometrial hyperplasia is to administer the progestin locally in the uterine cavity. A progesteron-releasing intrauterine device developed for contraception may thus be a logical alternative for gestagen administration along with postmenopausal hormone replacement therapy [12].

With a daily release rate of 20 µg. of levonorgestrel for 5 years, LNg-IUD effectively prevents the proliferative effect of estrogen on the endometrium in humans. Its indication for use was expanded to the treatment of menorrhagia and it was reported to be a promising method for the administration of gestagen in peri-menopausal patients on oral estrogen replacement therapy [13,14].

Some amount of the levonorgestrel released from the LNg-IUD is absorbed from the uterine cavity into the systemic circulation, however its effect on lipids and lipoproteins has been reported to be insignificant [15].

The use of unopposed estrogens in women with climacteric complaints increases the incidence of endometrial hyperplasia and cancer [16,17]. In our first group of rats, no hyperplasia or cancer was encountered. On the other hand, in the third group treated with unopposed estrogen, keratinized squamous metaplasia and myometrial hyperplasia were seen more commonly. Even it is not statistically significant, in the third group, one case of glandular hyperplasia was detected as the sole abnormal pathological finding. This finding of glandular hyperplasia may reflect the unopposed estrogenic effect on the rat uterus, nevertheless, further studies with larger sample sizes are needed to confirm these findings. In 2003, Philips et al evaluated the endometrial effects of levonorgestrel releasing intrauterine device in women of reproductive age and found out that the effects were characteristic, relatively constant and in keeping with the effects of both a progestogenic compound and a mechanical device. Morphological features found in most of the endometria were decidualisation of stroma (72

of 75 cases), atrophy of endometrial glands (65 of 75 cases), a surface papillary pattern (38 of 75 cases), and a stromal inflammatory cell infiltrate (59 of 75 cases). Additional common histological features were the presence of foci of stromal myxoid change (29 of 75 cases) and stromal haemosiderin pigment (24 of 75 cases). Reactive atypia of surface glands, glandular metaplastic changes, stromal necrosis, and stromal calcifications were found in small numbers of cases [18]. In year 2002, Raudaskoski et al evaluated the clinical and endometrial efficacy and lipid response of two different doses of intrauterine levonorgestrel assessed in comparison with sequential oral medroxyprogesterone acetate in postmenopausal women receiving continuous oral E2-valerate [19]. Endometrial hyperplasia was not observed in any of the treatment groups during the 12-month study and they concluded that both 10 microg and 20 microg levonorgestrel systems provided good endometrial protection in postmenopausal women on estrogen replacement therapy. Furthermore, Wildemeersch evaluated the endometrial safety with a low-dose intrauterine levonorgestrel-releasing system (releasing 14 microg of LNG per day) after 3 years of estrogen substitution therapy [20]. They found out that the endometrial histology specimen showed profound endometrial suppression with glandular atrophy and stroma decidualization in all women.

Our animal data is in keeping with the above mentioned human data and the results from this study in a small group of rats are promising, since it is important to study the rat endometrium at light microscopic levels, providing pioneer data for further investigations. To our knowledge, this is the first such study in the literature.

As a result, light microscopical investigations - both in animal and human models - suggest that it is possible to supply a safe and beneficial hormone replacement therapy to postmenopausal non-hysterectomized women; taking systemic estrogen replacement therapy by the use of locally active levonorgestrel-releasing intrauterine device in order to protect them from the systemic adverse effects of gestagens and to supply the efficient and safe protection in their endometrium.

REFERENCES

1. Wollter-Svensson LO, Stadberg E, Andersson K, et al. Intrauterine administration of levonorgestrel 5 and 10 microg/24 hours in perimenopausal hormone replacement therapy, a randomized clinical study during one year. Acta Obstet Gynecol Scand 1997; 76(5):449-54
2. Taddei GL, Bargelli G, Scarselli B, et al. Precancerous lesions of the endometrium and medical treatment. Eur J Contracept Reprod Health Care 1997 ;2(4):239-41.
3. Suhonen SP, Holmström T, Allonen HO, et al. Intrauterine and subdermal progestin administration in postmenopausal hormone replacement therapy. Fertil Steril 1995; 63(2):336-42
4. Suhonen SP, Allonen HO, Lähteenmäki P. Sustained-release estradiol implants and a levonorgestrel-releasing intrauterine device in hormone replacement therapy. Am J Obstet Gynecol 1995; 172(2 Pt 1):562-67.
5. Kim CJ,Jang HC, Cho DH, Min YK. Effects of hormone replacement therapy on lipoprotein (a) and lipids in postmenopausal women. Arterioscler Thromb 1994; 14(2):275-81.
6. Kim CJ, Min YK, Ryu WS, Kwak JW, Ryoo UH. Effect of hormone replacement therapy on lipoprotein (a) and lipid levels in postmenopausal women. Influence of various progestogens and duration of therapy. Arch Intern Med 1996 12-26; 156(15):1693-700.
7. Staland B. Continious treatment with natural estrogens and progestagens. A method to avoid endometrial stimulation. Maturitas 1981; 3:145-56
8. Mattsson LA, Cullberg G, Samsioe G. A continuous estrogen-progestogen regimen for climacteric complaints effects on lipid and lipoprotein metabolism. Acta Obstet Gynecol Scand 1984; 63:673-7
9. Farish E, Fletcher CD, Dagen MM et al. Lipoprotein and apolipoprotein levels in postmenopausal women on continuous estrogen-progestogen therapy. Br J Obstet Gynecol 1989; 96:358-64.
10. Sporrong T, Hellgren M, Samsioe G, Mattsson LA. Metabolic effects of continuous estradiol-progestin therapy in postmenopausal women. Obstet Gynecol 1989; 73:754
11. Jensen J, Riis BJ, Strom V, Christiansen C. Longterm and withdrawal effects of two different estrogen-progestogens combination on lipid and lipoprotein profiles in postmenopausal women. Maturitas 1989; 11:117-28
12. Rybo G, Anderson K, Odlind V. Hormonal intrauterine devices. Ann Med 1993;25:143-7
13. Andersson K, Rybo G. Levonorgestrel releasing intrauterine device in the treatment of menorrhagia. Br J Obstet Gynecol 1990; 97:690-4

14. Andersson K, Mattsson LA, Rybo G, Stadberg E. Intrauterine release of levonorgestrel- a new way of adding progestogen in hormone replacement therapy Obstet Gynecol 1992; 79:963-7.

15. Raudaskoski T, Tomas EI, Paakkari IA, Kauppila AJ, Laatikainen TJ. Serum lipids and lipoproteins in postmenopausal women receiving transdermal estrogen in combination with levonorgestrel relasing intrauterine device. Maturitas 1995; 22:47-53.

16. Schiff I, Sala HK, Cramer D, Tulchinsky D, Ryan KJ. Endometrial hyperplasia in women on cyclic or continuous estrogen regimens. Fertil Steril 1982; 37:79-82.

17. Ziel HK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. N Engl J Med 1975; 293:1167-70.

18. Phillips V, Graham CT, Manek S, McCluggage WG. The effects of the levonorgestrel intrauterine system (Mirena coil) on endometrial morphology. J Clin Pathol. 2003; 56(4):305-7.

19. Raudaskoski T, Tapanainen J, Tomas E, Luotola H, Pekonen F, Ronni-Sivula H, Timonen H, Riphagen F, Laatikainen T. Intrauterine 10 microg and 20 microg levonorgestrel systems in postmenopausal women receiving oral oestrogen replacement therapy: clinical, endometrial and metabolic response. BJOG. 2002; 109(2):136-44.

20. Wildemeersch D, Schacht E, Wildemeersch P, Calleweart K, Pylyser K, De Wever N. Endometrial safety with a low-dose intrauterine levonorgestrel-releasing system after 3 years of estrogen substitution therapy. Maturitas. 2004; 48(1):65-70.