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ORIGINAL PAPER

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The effect of estrogen-replacement therapy on clitoral-cavernosal tissue in oophorectomized rats: a histo-quantitative study by image analyzer

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Abstract Sexual dysfunction is an important problem for aging females. However, little attention has been paid to female sexual dysfunction. The clitoris is an important organ for physiological sexual function in females. There is a close relationship between the presence of sexual complaints and levels of estrogen. Using the rat as an experimental model, we evaluated the effect of estrogen-replacement therapy and its timing on clitoral-cavernosal collogen fiber content after oophorectomy. Four-month-old female Wistar rats ($n=36$) weighing 230–250 g were used. They were categorized into four groups: oophorectomized (Group 1: $n=10$); oophorectomized + delayed estrogen replacement (group 2: $n=10$); oophorectomized + immediate estrogen replacement (group 3: $n=10$); and sham operated (group 4: $n=6$). The estrogen replacement used was 17-β-estradiol. All rats were euthanized at the same age. The specimens were stained with Masson's trichome technique, and computerized image analysis was used to quantify the collagen-fiber content of clitoral-cavernous tissue. The clitoral collagen-fiber percentages in the different groups were as follows: group 1: $64.17 \pm 5.01\%$; group 2: $62.57 \pm 5.37\%$; group 3: $56.33 \pm 3.85\%$; group 4: $51.48 \pm 6.37\%$, respectively. Although there was a tendency in the untreated group for a higher collagen-fiber content, no statistically significant difference was found among groups ($P > 0.05$). Although the results of this study were not statistically significant, estrogen did appear to decrease clitoral-cavernosal collagen-fiber content. These findings may be important in the pathophysiology of postmenopausal female sexual dysfunction.

Keywords Female sexual dysfunction · Estrogen · Oophorectomy · Clitoral-cavernosal tissue

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Introduction

Sexual dysfunction is a common problem in females and males [3]. Female sexual dysfunction is a multicausal and multidimensional problem, involving various anatomical, physiological and psychological factors. It is a significant age-related, progressive and highly prevalent problem that affects 20–50% of women [16].

The clitoris and vagina are important organs for physiological sexual function in females [15]. The clitoris, an embryologic homologue of the penis, is a sensory organ arising from the embryological genital tubercle [30]. Tarcan et al. showed that clitoral-cavernosal erectile tissue undergoes fibrosis and loss of smooth muscle with increasing age. They suggested that chronic ischemia due to atherosclerosis-induced clitoral-cavernosal arterial insufficiency may play a role [29].

In females, sexual arousal and frequency of coitus decreases with of age. This condition may be due to endocrinological or psychological disorders [2, 4]. Aging, menopause and a decline in circulating estrogen levels cause an increase in sexual complaints [24]. There is a direct correlation between the presence of sexual complaints and levels of estriol < 50 pg/cm^3 [7].

The aim of this study was to evaluate the hormone-related sexual disorders and the effect of early and late estrogen-replacement therapy on clitoral-cavernosal tissue after oophorectomy using the rat as an experimental model.

Materials and methods

Four-month-old female Wistar rats ($n=36$) weighing 230–250 g were used. Anesthesia was induced with an intraperitoneal injection of xylazine 5 mg/kg plus ketamine 25 mg/kg at room temperature. The rats were fed a standard pellet diet and water ad libitum and were housed in cages holding three animals. Bilateral oophorectomy was performed via dorsal flank incision, as described previously [31]. They were categorized into four groups: oophorectomized (group 1: $n=10$); oophorectomized + delayed estrogen replacement (group 2: $n=10$); oophorectomized + immediate estrogen replacement (group 3: $n=10$); and sham operated (group 4: $n=6$). For treatment, 17-β-estradiol (Premarine intramuscular, 1 mg/kg/day, Wyeth Comp, USA) was used. Oophorectomy (groups 1 and 3) and sham operation were performed on the same day. Group 2 was oophorectomized 6 weeks before that day. Estrogen replacement started on the same day for groups 2 and 3 and lasted 6 weeks. All rats were killed at the same time. The study design is demonstrated in Table 1.

Tissue procedure

Tissue samples were fixed in 10% neutral buffered formalin overnight. The fixed tissues were processed, embedded in paraffin, and sectioned at 5 μm. Five sections for each of the paraffin-embedded tissues were obtained. Tissue sections were stained with hematoxylin-eosin and Masson's trichrome technique. Slides were examined by the same pathologist (ME) under the light microscope, in this blind study (Olympus B×50).

Computerized image analysis

Quantitative analysis of clitoral-cavernosal collagen-fiber contents was done with a Zeiss Vision KS400 (version 3.0, Germany) image-analyzer system and a Zeiss Axioscope light microscope equipped with a Sony (3-chip) color video camera on Masson's trichrome stained slides. On the image analyzer, five sections for each animal were examined. At least 25 fields were randomly selected and examined from each tissue section. The percentage of collagen-fiber content was calculated for every field.

Statistical analysis

The percentage of collagen-fiber content was expressed as mean ± SEM (Standard error of mean). The percentages of clitoral-collagen-fiber content in the four groups were compared by Kruskal-Wallis one-way analysis of variance.

Results

The mean percentage ± SEM of the clitoral collagen-fiber contents by computerized image analysis in the different groups is shown in Table 2. Although there was a tendency for the untreated group to have a higher collagen-fiber content, no statistically significant difference was found among groups ($P>0.05$), (Fig. 1, Fig. 2 and Fig. 3).

Table 1 Study design, *Ox* oophorectomy, *ERT* estrogen replacement therapy, *K* killing time

Weeks	0	6	12
Group 1	Ox	Ox	K
Group 2		(ERT)	K
Group 3		Ox (ERT)	K
Group 4		Sham	K

Table 2 The mean collagen-fiber percentages of clitoral-cavernosal tissue in groups (*SEM* standard error of mean)

Groups	Collagen-fiber content (% ± SEM)
1 ($n=10$)	64.17 ± 5.01
2 ($n=10$)	62.57 ± 5.37
3 ($n=10$)	56.33 ± 3.85
4 ($n=6$)	51.48 ± 6.37

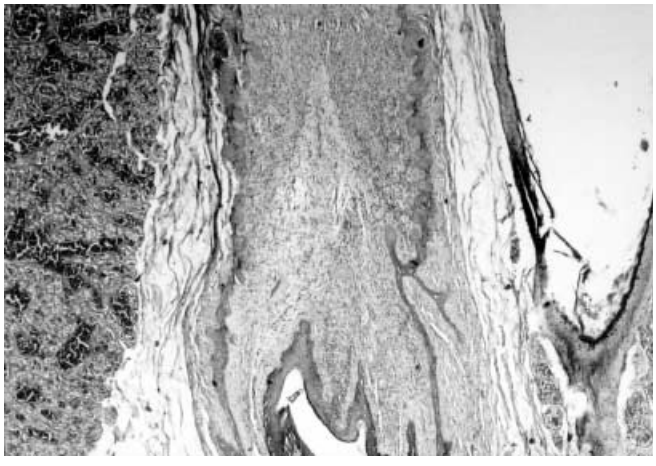

Fig. 1 Normal clitoral-cavernosal tissue. Thinly keratinized stratified squamous epithelium, loose connective tissue and one of pair corpora cavernosa was seen. Masson's trichrome ×100

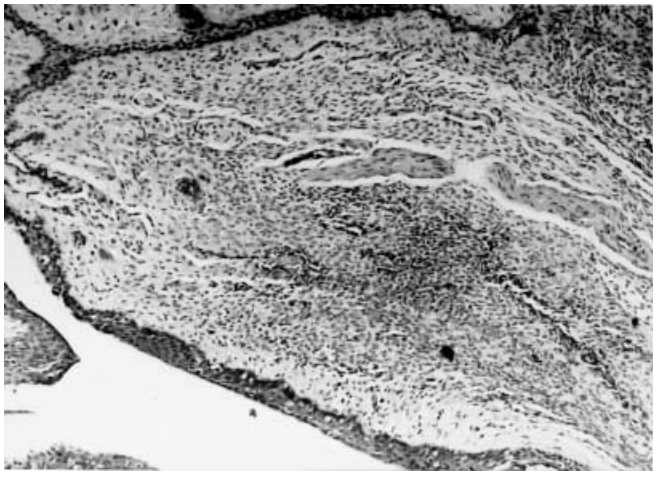

Fig. 2 Early estrogen-replacement therapy, minimal increase in connective tissue as compared with Fig. 3 (green stain). Surface is covered by stratified squamous epithelium and beneath the epithelium, nerve corpuscles among the slightly increased connective tissue, Masson's trichrome ×100

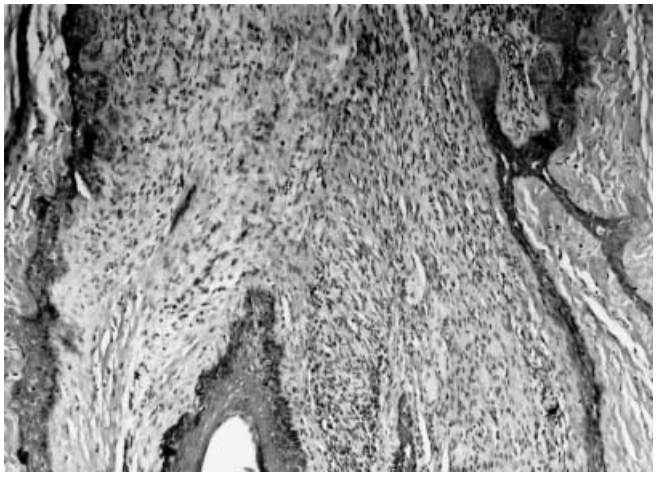

Fig. 3 Late estrogen-replacement therapy, severe increase in connective tissue (green stain), Stratified squamous epithelium and heavily increased collagen fibers were seen, Masson's trichrome ×100

Discussion

Female sexual dysfunction is a significant problem affecting the quality of life of 20–50% of women [16, 28, 23, 22]. Until recently, little attention has been paid to female sexual dysfunction. The study of female dysfunction is gradually increasing.

The prevalence of female sexual-arousal disorders increases following menopause. As sexual problems observed during aging and menopause are related to the functional and anatomical changes of the vaginal mucosa and submucosa [27, 8], little research has focused on clitoral-cavernosal tissue. The clitoris is composed of the glans, right and left cavernosal bodies and tunica albuginea [32]. Clitoral erection develops as a result of hemodynamic changes within the clitoral-cavernosal erectile tissue [17]. During female sexual arousal, clitoral length and diameter, vaginal lubrication and vaginal-wall engorgement increase, as a result of neurotransmitter-mediated vascular and non-vascular smooth-muscle relaxation [5, 15].

Female sexual function is assumed to be closely related to the hormonal milieu of the body. Estrogen is given first consideration in treating complaints of sexual dysfunction. Many studies have been done to evaluate the availability of different preparations that could replace estrogen and androgen for sexual dysfunction. Estrogen-replacement therapy has been shown to enhance sexual desire in a significant portion of women [26]. This therapy also results in improved clitoral sensitivity, increased libido and decreased pain during intercourse [11, 1]. However, the effects of estrogen on female sexual dysfunction are still controversial. Omu et al. suggested that although estrogen-replacement therapy gives significant relief to symptoms of sexual dysfunction, other contributory factors should always be evaluated [20]. In a longitudinal study, Dennerstein found that most aspects of female sexual functioning were not affected by age, menopausal functioning or hormone levels [13]. Coope also showed that libido is not increased by estrogen therapy [12].

In vitro studies have shown that proliferation of vascular smooth muscles and depositions of extracellular matrix proteins are inhibited by estrogen [6,9]. Berman et al. suggested that estrogen appears to play a significant role in maintaining vaginal mucosal integrity, vaginal blood flow and vascular lumen patency [7]. Dubey et al. also found that 17-β-estradiol and progesterone inhibit cardiac fibroblast growth and collagen synthesis [14].

Tarcan et al. reported that aging women undergo histological changes in clitoral-cavernosal erectile tissue. Using computer-assisted histomorphometric analysis, they found an age-related increase in clitoral-cavernosal connective tissue and loss of clitoral-cavernosal smooth muscle. They proposed that these changes might be the result of alterations in the circulating levels of estrogen or other sex hormones [29]. It has been reported that estrogen has vasoprotective and vasodilator effects that increase vaginal, clitoral and urethral arterial flow [18]. Nitric oxide synthase expression, the enzyme responsible for the production of nitric oxide (NO), is regulated by estrogen. Sarrel et al. showed that estrogen-replacement therapy improves vaginal mucosa, increases vaginal NO levels and decreases vaginal apoptosis after aging and/or surgical castration [25]. Recently, NO and phosphodiesterase type V have been identified in clitoral smooth muscle [10].

Estrogen might play a critical role in preventing clitoral-cavernosal fibrosis during aging and menopause. In this study, we aimed to evaluate the effectiveness of estrogen-replacement therapy on clitoral-cavernosal fibrosis secondary to oophorectomy. To our knowledge, this is the first study on this subject. Although there was a steady tendency among groups in terms of decreasing collagen-fiber content in response to estrogen replacement, these differences were not statistically significant. It also appeared that immediate estrogen replacement was more effective in preventing the process of fibrosis. These results could be more significant if the duration of estrogen ablation were longer.

As oophorectomy is a surgical castration, other age-related physiological factors affecting female sexual function during menopause may not have been represented. High incidence of vascular risk factors in the elderly population may also play a role. Previous studies also showed that chronic cavernosal insufficiency causes fibrosis in the clitoris and penis [21, 19]. This study evaluated only the hormonal effect on the clitoral-cavernosal tissue.

Clitoral-cavernosal fibrosis may be important in the pathophysiology of postmenopausal sexual dysfunction. The effect of treatment options of this condition may be increased if this fibrosis can be prevented. Our preliminary study indicates that estrogen appears to decrease clitoral-cavernosal collagen-fiber content. Further

studies may be helpful in demonstrating this effect of estrogen more definitively.

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