

Effect of Ovariectomy and Chronic Sex Steroid Administration on Rabbit Skin

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Abstract: The aim of this study was to evaluate the effect of ovariectomy and subsequent chronic estradiol dipropionate (EDP) administration on the thickness of skin layers, percentage fraction of dermal collagen and elastic fibers and area of sebaceous glands in female rabbits. Adult female rabbits were divided into ovariectomized (Ovx), sham-operated controls (So) and ovariectomized treated with estradiol dipropionate (Ovx-EDP). After ovariectomy, the epidermal thickness decreased significantly in all body regions ($P < 0.05$), which was apparently greater in some regions than the others. EDP treatment of Ovx rabbits reversed these changes to the pre-ovariectomy state. The effects of Ovx on dermal thickness at different body sites is markedly more site-dependent than those on epidermal thickness, so that the reduction seen in dermal thickness after Ovx was statistically significant in some regions ($P < 0.05$) and non-significant in the others ($P > 0.05$). Estrogen has a stimulatory effect on the collagen synthesis and dermal collagen content returns completely to normal, after estrogen replacement therapy in all body regions. There is also a direct relationship between skin collagen and dermal thickness. In contrast to the collagen fibers, Ovx and subsequent hormone therapy, have very little if not effects on the percentage fraction of the dermal elastic fibers. Estrogen has a site dependent inhibitory effect on sebaceous glands which varies greatly at different skin regions. It was concluded that gonadectomy exerts reversible body-site dependent effects on the epidermal and dermal thicknesses, dermal collagen content and area of sebaceous glands in female rabbits. Dermal elastic content remains unaffected by gonadectomy.

Key words: Gonadectomy • Estradiol • Skin • Rodents • Epidermis • Dermis

INTRODUCTION

Skin and its appendages being a steroidogenic tissue itself can be affected to a large extent by sex steroids [1]. Estrogen receptor (ER) has been identified in the skin and the concentration of these receptors varies in the different parts of the body. ER α was poorly expressing, being restricted to sebocytes. In contrast, ER β was found to be highly expressed in the epidermis, sebaceous glands (basal cells and sebocytes) and eccrine sweat glands [2]. Estrogen improves skin in more than one way, the collagen content and quality is improved, skin thickness is increased, while vascularisation is enhanced. The extracellular matrix responsible for the tone and appearance of the skin is also improved. It is not just the skin that shows an improvement with estrogen therapy but also skin appendages, such as hair. Estrogens have been shown to increase the hair follicle life cycle [3]. Decreased estrogen slows down mitotic activity in the

epidermal basal layer, reduces the synthesis of collagen and contributes to thickening of the dermo-epidermal junction. Estrogen treatment in post menopausal women has been shown to increase collagen content, dermal thickness and elasticity [4].

Sex steroid hormones have been also implicated in regulating collagen synthesis. Estrogens may not be directly involved in the regulation of collagen synthesis; however, they may play a critical role in regulating organization and stability of collagen fibrils [1].

Although it is well known that sex steroid hormones have very important functions in regulation of skin development and functions, the role of these hormones on skin at different body sites is not well understood. The aims of present study were to evaluate the effects of ovariectomy and subsequent estrogen administration on morphometric properties of the different skin layers and their appendages at different body sites in rabbit skin.

MATERIALS AND METHODS

Fifteen adult female New Zealand white rabbits (3.1-3.5 kg body weight) bred in our colony were used in the experiment. Animals were maintained on normal rabbit pellets and water *ad libitum*. They were randomly distributed into three groups (n=5). Two groups, were bilaterally ovariectomized (Ovx) under ketamin anesthesia (80 mg/kg). Animals of the control group underwent sham operations (SO). One month after ovariectomy, one Oxv group was treated with intramuscular injection of 5mg estradiol valerate. The estradiol valerate was administrated for eight weeks, at five day intervals. Two months later, all the animals were deeply anesthetized with ketamin and fixed by intravascular perfusion via the left ventricle with buffered formalin. After shaving the long hair, the skin from the following defined body regions were excised, flattened and immediately immersed in 10% buffered formalin: pinna of ear, thoracolumbar region, umbilical region and lateral and medial parts of the right forelimb at humeral region. Paraffin-embedded sections were cut at 5 μ m and stained with a sequence of haematoxylin-eosin and phloxine and Verhoeff-van Gieson staining technique was used to distinguish collagen and elastic fibers. Thickness of the epidermis and dermis and area fraction of collagen bundles and elastic fibers were measured using method described by Tapan *et al.* [5]. The surface area of the sebaceous glands was measured according to the method described by Sourla *et al.* [6]. All stereological results were statistically evaluated by One- way ANOVA. Results are reported as mean \pm SEM with a significance level of 0.05.

RESULTS

Body-site-specific morphometric measurements are given in Table 1.

Epidermis: As indicated in Table 1, the thickness of the epidermis varies considerably in the all body regions. Mean epidermal thickness ranged from 10 μ m (umbilical region) to 29 μ m (thoracolumbar region). After Oxv, the epidermal thickness decreased significantly by 18.52, 37.93, 20.0, 16.66 and 31.25% in the pinna of ear, thoracolumbar region, umbilical region, lateral part of the humeral region and medial part of humeral region, respectively ($P < 0.05$). EDP treatment of Oxv rabbits reversed these changes to the pre-

ovariectomy state so that none of the examined morphometric parameters in the Oxv-EDP rabbits was significantly changed in comparison with the SO group ($P < 0.05$).

Dermis: The dermis also varies in thickness depending on the location of the skin. Mean dermal thickness ranged from 180 μ m (lateral part of the humeral region) to 800 μ m (thoracolumbar region).

After Oxv, the dermal thickness decreased significantly by 45.0 and 31.25% in the thoracolumbar region and umbilical region respectively ($p < 0.05$). In contrast, a non significant decrease in dermal thickness by 2.74, 4.25 and 3.58% was observed in pinna of ear, lateral part of of the humeral region and medial part of humeral region respectively. EDP treatment of Oxv rabbits reversed these changes, so that, none of the examined morphometric parameters in the Oxv-EDP rabbits were significantly different from those in the SO controls.

Collagen Bundles and Elastic Fibers: The percentage fraction of dermal collagen and elastic fibers varies considerably at different body sites. Mean percentage fraction of dermal collagen ranged from 6.68% (lateral part of the humeral region) to 77.12% (thoracolumbar region). The greater the dermal thickness, the higher the percentage fraction of dermal collagen at all body sites.

After Oxv, the percentage fraction of dermal collagen decreased significantly by 28.62, 16.83, 28.24, 25.56 and 16.27% in the pinna of ear, thoracolumbar region, umbilical region, lateral part of the humeral region and medial part of the humeral region respectively ($P < 0.05$). EDP treatment of Oxv rabbits reversed these changes to the pre-ovariectomy state.

Mean percentage fraction of dermal elastic fibers ranged from 2.66% (lateral part of the humeral region) to 3.87% (pinna of ear). There was no significant relationship between dermal thickness and the skin elastic content at all body regions.

After Oxv the mean percentage fraction of dermal elastic fibers decreased numerically in all skin regions but the changes were not statistically significant. EDP treatment had no statistically significant effects on the percentage fraction of elastic fibers at different skin sites, so that none of the examined morphometric parameters in the Oxv-EDP rabbits were significantly different from those in the Oxv rabbits.

Table 1: Values of various morphometric measurements (Mean±SD)

Regions	Groups	Epidermal thickness (µm)	Dermal thickness (µm)	Collagen bundles (a.f.)	Elastic fibers (a.f.)	Area of sebaceous glands (sq. µ)
Pina of ear	SO	27.0	401.0	46.47	3.87	29800±2280
	Ovx	22.0	390.0	33.17	3.78	38500±3140
	Ovx-EDP	26.5	398.0	44.66	3.84	30040±2960
Thoracolumbar region	SO	29.0	800.0	77.12	3.07	22500±1880
	Ovx	18.0	440.0	64.14	3.02	34250±3670
	Ovx- EDP	27.0	785.0	75.36	3.02	23200±2480
Umbilical region	SO	10.0	320.0	39.33	3.29	38500±4020
	Ovx	8.0	220.0	28.22	3.18	40625±3900
	Ovx-EDP	9.5	314.0	36.34	3.22	39400±3160
Forelimb (lateral)	SO	21.0	188.0	26.68	2.66	10800±940
	Ovx	17.5	180.0	19.86	2.58	14937±1100
	Ovx-EDP	20.0	183.0	23.96	2.61	11100±880
Forelimb (medial)	SO	16.0	363.0	41.18	3.44	17400±1020
	Ovx	11.0	350.0	34.48	3.37	18270±1400
	Ovx-EDP	15.0	359.0	39.12	3.46	17800±800

µm: micrometer; a.f.:area fraction; sq. µ: square micrometer

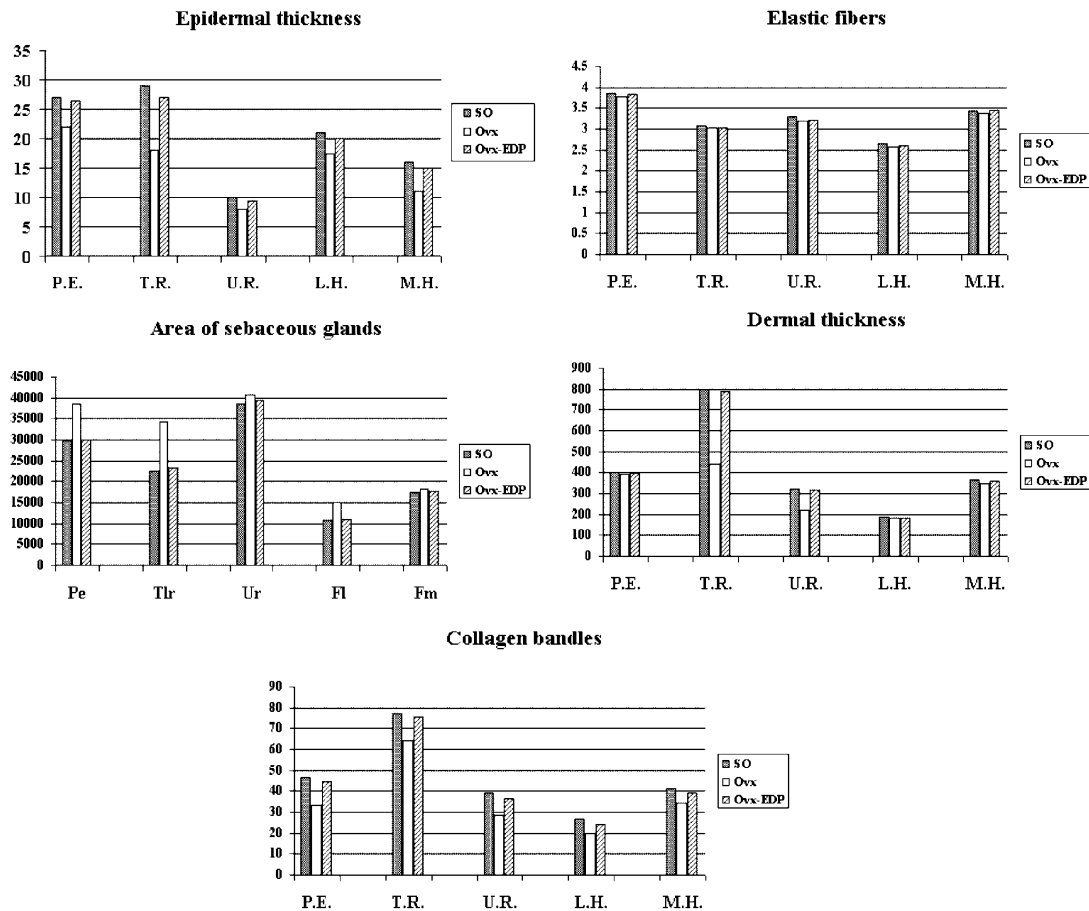


Fig. 1: Effects of gonadectomy and sex steroid administration on the epidermal thickness, dermal thickness, area fraction of collagen bundles, area fraction of elastic fibers and area of sebaceous glands in the skin of pinna of ear (P.E.), thoracolumbar region (T.R.), umbilical region (U.R.), lateral part of humeral region (L.H.) and medial part of humeral region (M.H.). The results are expressed as mean ±SEM, P<0.05.

Sebaceous Glands: The mean total surface area of the sebaceous glands was greatly different at different skin regions, ranged from 10800 sq. μ (lateral part of the humeral region) to 38500 sq. μ (umbilical region).

After Ovx, area of sebaceous glands increased significantly by 29.19, 34.30 and 27.69% in the pinna of ear, thoracolumbar region and lateral part of the humeral region respectively ($P < 0.05$) and non-significantly by 5.23 and 4.76% in the umbilical region and medial part of the humeral region respectively. EDP treatment of Ovx rabbits reversed these changes to the pre-ovariectomy state so that none of the examined morphometric parameters in the Ovx EDP rabbits was significantly changed in comparison with the SO group ($P > 0.05$).

DISCUSSION

Results obtained from the present study showed that the thickness of the epidermis varies considerably in the all body regions. This result is in agreement with previous studies [7-10]. Sex steroid hormones are involved in regulation of skin development and functions. The absence of estradiol slows the mitotic activity of the basal layer of the epidermis [11]. Estrogens have also been shown to increase mitotic activity in the epidermis of mice and women [12, 13], which correlates with epidermal thickening. Keratinocyte growth factor and epidermal growth factor are involved in the regulation of proliferation and differentiation of keratinocytes and it is previously demonstrated that these two factors are up-regulated by estrogen [14, 15]. The significant reduction in epidermal thickness seen in the present study after Ovx, may be due to the decreased mitotic activity of the basal layer of the epidermis resulted from the estrogen deprivation. Results obtained from the present study also showed that Ovx causes a greater reduction in epidermal thickness in some body regions compared to the other ones which may be due to the fact that, the number of estrogen receptors has been reported to vary in different parts of the body [16].

Positive effects of estrogens on the dermal connective tissue are known both for the basic substance, with an increase in acid mucopolysaccharides and hyaluronic acid and the consecutive increase in dermal water storage [17]. The normal water content in the dermis is bound to the hydrophilic glycosaminoglycans. This characteristic may protect the skin against excessive tissue compression while maintaining its suppleness. Estrogens increase dermal hygroscopic properties, probably through enhanced synthesis of dermal hyaluronic acid [18]. Skin dermal thickness appears to

increase in postmenopausal women receiving hormone therapy compared with aged-matched untreated women [19]. The significant reduction in dermal thickness seen in the present study after Ovx, may be due to the negative effects resulted from estrogen deprivation on the dermal connective tissue components and decrease in dermal hygroscopic properties. Results obtained from the present study also revealed that the effects of Ovx on dermal thickness at different body sites are markedly more site-dependent than those on epidermal thickness. This may be due to the fact that the epidermal histological structure is more uniform and similar at the different body sites than that of the dermis which consists of various connective tissue elements with obviously different proportion at different body sites.

Collagen biosynthesis and deposition is a complex, multistep process, which is tightly regulated to maintain proper tissue hemostasis. Sex steroid hormones have been implicated in regulating collagen synthesis. Ovariectomy mainly affects collagen solubility. The absence of estrogens results in decreased expression levels of several of the small leucine-rich repeat proteins and proteoglycans including decorin, fibromodulin and lumican. Estrogens may not be directly involved in the regulation of collagen synthesis; however, they may play a critical role in regulating organization and stability of collagen fibrils in mouse skin [1]. Estrogens stimulate the synthesis, maturation and turnover of collagen in rats [20] and guinea pigs [21] and significantly increase the synthesis of hyaluronic acid in mouse skin [22].

The absence of estradiol reduces the synthesis of collagen and probably that of elastic fibers [11]. In postmenopausal women a decrease in the collagen content of thigh skin at rate of 2% per postmenopausal year has been observed for up to 15 years in women not on hormone replacement therapy [3]. Estrogen treatment in postmenopausal women has been shown to increase collagen content, dermal thickness and elasticity [4]. In agreement with the other studies, this study also revealed that estrogen has an stimulatory effect on the collagen synthesis. Results obtained from the present study also showed that the dermal collagen content returns completely to normal, after estrogen replacement therapy.

We found also a direct relationship between skin collagen and dermal thickness in accordance with Shuster *et al.* [23].

In rats, decreased estrogen levels due to ovariectomy enhance the UVB sensitivity of the skin, resulting in an acceleration of photoaging in terms of an increased formation of significantly deep wrinkles, decreased skin

elasticity and marked damage and advanced curling of the dermal elastic fibers at an early stage [24]. Results obtained from this study showed that, in contrast to the collagen fibers, Ovx and subsequent hormone therapy, have very little if not effects on the percentage fraction of the dermal elastic fibers. This may be due to the fact that the elastic fibers have a very low turnover rate and more time may be required for the fibers to adjust to the new hormonal conditions.

The results of the present investigation demonstrate that estrogen has a site dependent inhibitory effect on sebaceous glands which varies greatly at different skin regions. Estrogen receptor (ER) alpha was poorly expressing, being restricted to sebocytes. In contrast, ERbeta was found to be highly expressed in the sebaceous glands (basal cells and sebocytes) and eccrine sweat glands [2]. It has been known that sebaceous gland activity is stimulated by androgens, but inhibited by estrogens [25-27]. In humans the sebaceous glands enlarge at puberty, with increased production of sebum in both sexes, although sebum production is generally lower in females than males [27]. Furthermore, the subcutaneous administration of estrogen causes a reduction in both size and number of sebaceous glands in the rat [8]. After the menopause, sebaceous activity gradually decreases in the female, while it remains unaltered in men until the 7th or 8th decade before a decrease is seen [27]. Immunostaining for ERb has shown that it is expressed in the human sebaceous gland [28], while a recent comparison of the expression of ERa and ERb in human skin has shown that both receptors are expressed in basal and differentiating sebocytes [29].

In conclusion gonadectomy has reversible body-site dependent effects on the epidermal and dermal thicknesses, dermal collagen content and area of sebaceous glands in female rabbits. Dermal elastic content remains unaffected by gonadectomy.

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