Journal of Dermatological Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Letter to the Editor

The ovariectomized mouse simulates the pathophysiology of postmenopausal female pattern hair loss

To the Editor,

The pathophysiology of female pattern hair loss (FPHL; Fig. 1A) remains elusive, and treatment options supported by solid evidences are extremely limited, making its management challenging [1]. The prevalence of FPHL increases in women aged >50 years [2]. Considering that menopause transition starts at around 47 years of age [3] and that hair diameter or density is distinct to some extent between pre- and postmenopausal women [4], we hypothesized that ovariectomized (OVX) mice recapitulating the menopause may manifest a condition similar to FPHL. This study aimed to evaluate the advantage of OVX mice as a postmenopausal FPHL model.

Three-week-old female C57BL/6 mice underwent either ovariectomy or sham surgery (Fig. 1B). All animal experiments were approved by the Intramural Animal Care and Use Committee of the LION Corporation (Receipt No. 2014-184, 2014-237).

Around the transition from the first to the second hair cycle (12 weeks after the birth) OVX mice started to demonstrate sparse hair areas, whereas sham-operated control mice exhibited no such change (Fig. 1B, C). To precisely assess hair density, dorsal skin samples were collected from 24-week-old sham-operated control and OVX mice (Fig. 1B). Skin color investigation revealed that all hair follicles were in telogen phase (data not shown). A stereomicroscopic investigation revealed that the average number of hair/mm^2 in OVX mice ($n = 8$, 69.9 ± 18.7) was significantly lower than that observed in sham-operated control mice ($n = 8$, 104.6 ± 16.2) ($P < 0.01$; *t*-test) (Fig. 1D, E). The increase in kenogen hair follicles (hair follicles without hair shafts) represents another characteristic FPHL feature [5], which were more frequently observed in OVX mice at the level of isthmus than in sham-operated control mice (Fig. 1F). In additon, a quantitative analysis revealed that a significantly lower number of hair shafts were present in OVX mice ($n = 8$, 1.40 ± 0.32) than in sham-operated control mice ($n = 8$, 1.68 ± 0.12) ($P < 0.05$; *t*-test), accounting for decreased hair density (Fig. 1F, G).

To investigate whether these hair phenotypes in OVX mice was indeed associated with the absence of a female hormone, OVX mice were supplemented with 0.05 or 0.36 mg of estradiol (E2) via sustained-release pellets (Innovation Research of America, Sarasota, FL, US) subcutaneously implanted twice at 4 and 17 –week old to cover 180 days. A microscopic investigation detected sparse hair areas in placebo-treated OVX mice which were hardly observed in sham-operated control mice and E2-supplemented OVX mice (Fig. 2A). A quantitative analysis confirmed that the hair density in placebo-treated OVX mice ($n = 8$) was 53.3 ± 14.4 hairs/mm^2, whereas that in low and high E2-supplemented OVX mice ($n = 8$ and 7) was 91.0 ± 17.9 and 95.0 ± 23.6 hairs/mm^2, respectively (Fig. 2B). Thus, E2 supplementation proportionally inhibited OVX-induced hair loss ($P < 0.01$, Dunnett's test). A high E2 supplementation maintained a hair density, which was comparable with that in sham-operated control mice ($n = 8$, 103.1 ± 25.1 hairs/mm^2, Dunnett's test) (Fig. 2B). In histometric analysis (Fig. 2C), the number of hair shafts per follicular unit in placebo-treated OVX mice ($n = 8$) was 1.24 ± 0.37, whereas that in low and high E2-supplemented OVX mice ($n = 8$ and 7) was 1.69 ± 0.16 and 1.84 ± 0.16, respectively (Fig. 2D). The counts were significantly higher than that in placebo-treated mice and the increase was E2 dose dependent ($P < 0.01$, Dunnett's test; Fig. 2D). Considering that sham-operated control mice ($n = 8$) had 1.57 ± 0.23 hair shafts per follicular unit, a low dose of E2 supplementation was sufficient to cancel out the effect of ovariectomy on hair follicles and maintained hair density in OVX mice.

To further understand the mechanism of hair loss in OVX mice, the hair cycle was evaluated by assessing the color of the dorsal skin [6]. Compared with sham-operated control mice, placebo-treated OVX mice had more accelerated hair cycles, characterized by the shortened first telogen phase and early emergence of the second catagen phase after the operation (Fig. 2E). Hair cycles were more synchronized in E2-supplemented OVX mice than in placebo-treated OVX mice. Interestingly, telogen phase was more prolonged in E2-supplemented OVX mice than in sham-operated control mice. In high E2-supplemented mice, larger number of hair follicles remained in telogen phase compared to other mice groups (Fig. 2E). These findings might suggest an adjustive role of E2 in telogen-anagen transition during hair cycling.

FPHL has been considered to comprise heterogeneous patient groups with a distinct etiopathogenesis. Thus, OVX mice cannot be used as a model for all subtypes of FPHL but only for those with a postmenopausal onset. We are aware that there are some limitations. Most human scalp hair follicles are in anagen, whereas telogen hair follicles predominate in mouse pelage. Intrinsic hair and menstrual cycles are clearly distinct between two species [7,8]. A previous study reported accelerated catagen development in estrogen receptor-beta knockout mice partially supporting our observation [9]. However, phenotypic hair loss was not described and, unlike in OVX mice, a short-term evaluation of hair growth after birth was conducted in these mice. Therefore, they cannot be directly compared with OVX mice.

In practice, ovariectomized patients usually receive hormone replacement therapy and the effect of ovariectomy on human hair follicles can hardly be assessed, however, our findings suppored, if not all, a clinical observation that a decrease in estrogen results in hair loss [1,4]. An increase in kenogen hair follicles has been

http://dx.doi.org/10.1016/j.jdermsci.2017.04.002

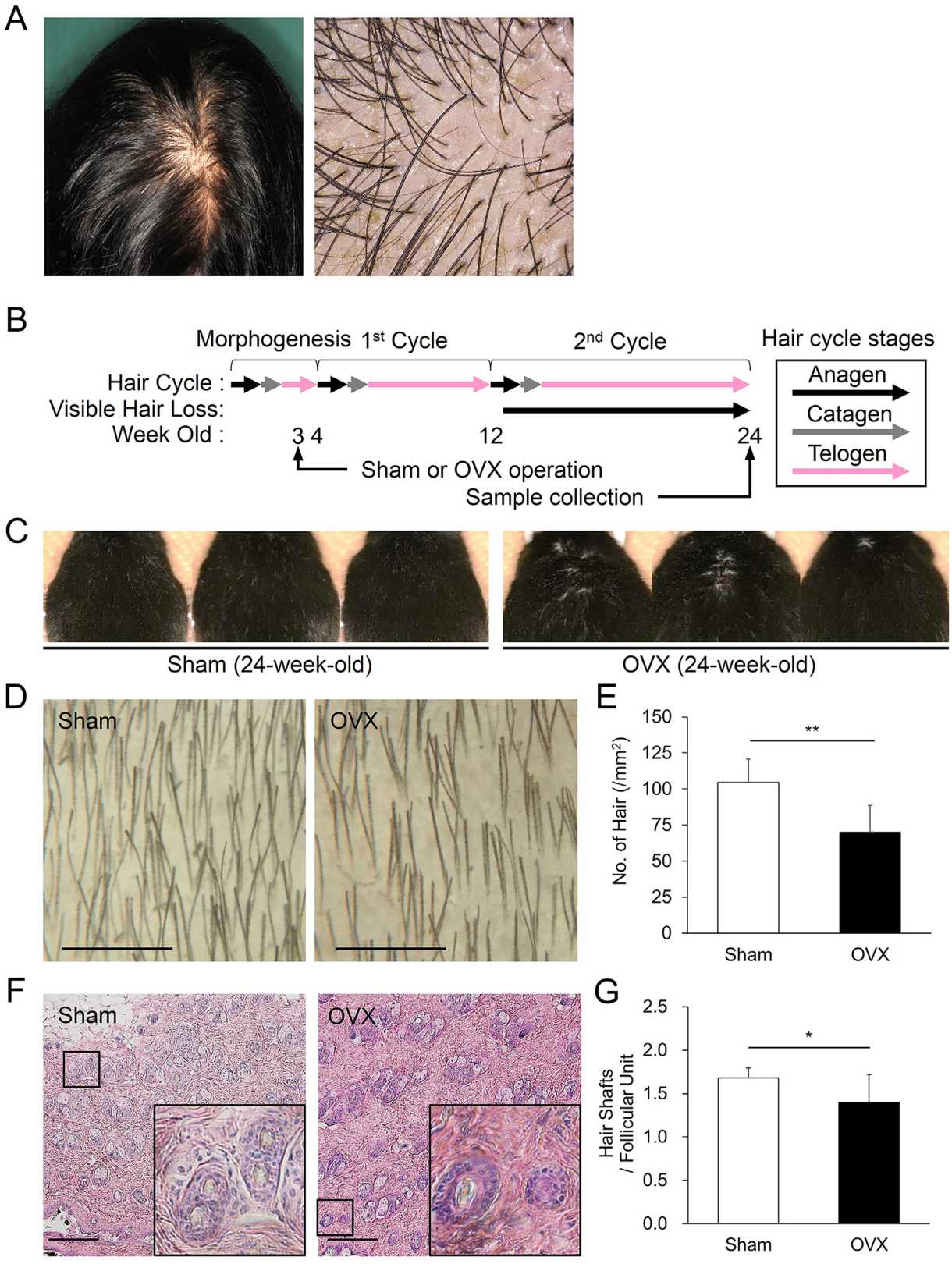

Fig. 1. Ovariectomized (OVX) mice demonstrated hair loss resembling female pattern hair loss (FPHL) (A) Clinical and dermoscopic manifestations of FPHL patient. Pattern hair loss (left), hair diameter diversity and kenogen hair follicles were noted (right). (B) The design of the study. (C) The sparse hair areas observed in OVX mice. (D, E) Decreased hair density in OVX mice compared with sham-operated control mice. Scale bar = 500 μm. (n = 8). (F) Increase in kenogen hair follicles in OVX mice. Hematoxylin and eosin staining. Scale bar = 200 μm. (G) OVX mice had lower number of hair shafts per a follicular unit when compared to sham-operated control mice (n = 8). The values in (E) and (G) are the mean ± SD. *$P < 0.05$, **$P < 0.01$..

Please cite this article in press as: Y. Endo, et al., The ovariectomized mouse simulates the pathophysiology of postmenopausal female pattern hair loss, J Dermatol Sci (2017), http://dx.doi.org/10.1016/j.jdermsci.2017.04.002