Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/7/18/eabe8873/DC1

Supplementary Materials for

Hormone autocrination by vascularized hydrogel delivery of ovary spheroids to rescue ovarian dysfunctions

Hyo-Jin Yoon, Yong Jae Lee, Sewoom Baek, Young Shin Chung, Dae-Hyun Kim, Jae Hoon Lee, Yong Cheol Shin, Young Min Shin, Chungsoon Ryu, Hye-Seon Kim, So Hyun Ahn, Heeyon Kim, Young Bin Won, Inha Lee, Myung Jae Jeon, Si Hyun Cho, Byung Seok Lee, Hak-Joon Sung*, Young Sik Choi*

*Corresponding author. Email: hj72sung@yuhs.ac (H.-J.S.); yschoi08@yuhs.ac (Y. S. Choi)

Published 28 April 2021, *Sci. Adv.* **7**, eabe8873 (2021)
DOI: 10.1126/sciadv.abe8873

This PDF file includes:

Supplementary Figures

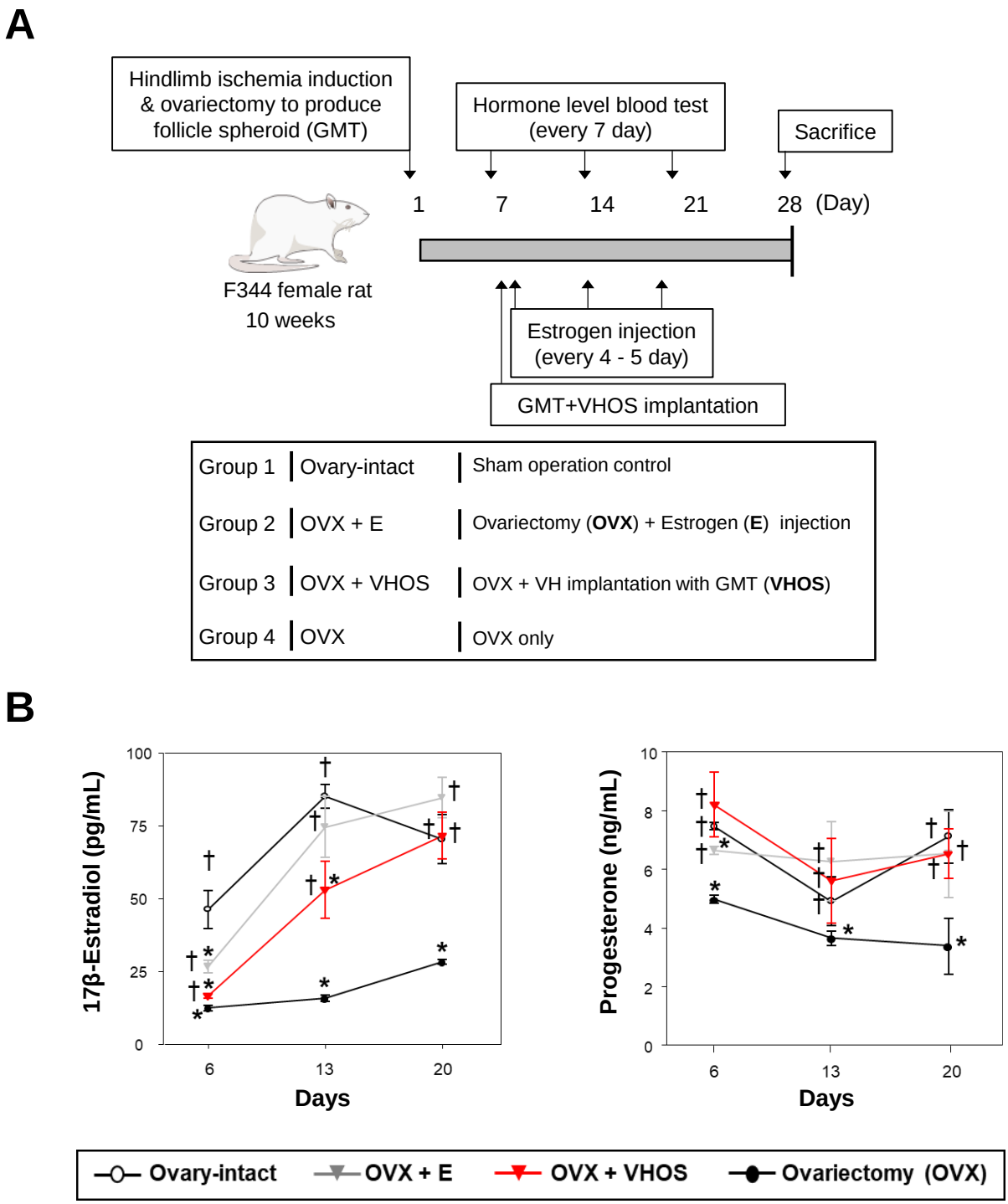

Fig. S1. Low endocrine levels due to an insufficient perfusion connection with host vessels. (Top) Female rats underwent both ovariectomy and hindlimb ischemia at day 7. After 7 more days (day 14), the microchannel hydrogel with GMT spheroids was implanted into the hindlimb ischemia site or estrogen was injected, with repetition every 4-5 days. After 7 more days (day 21), the resultant hormone levels were tested by drawing blood every 7th day, followed by euthanization of rats at day 63. (Bottom) The four test groups are shown in the box (Group 1-sham operation; Group2-OVX + E; Group 3-OVX+VHOS; and Group 4-OVX). The OVX+VHOS group was not able to sufficiently restore the endocrine function to the levels of the Ovary-intact and OVX + E

groups as determined by (B) the circulating plasma levels of 17β-estradiol and progesterone using ELISA analysis of serum. Data=mean ± SEM (Ovary-intact n=3, OVX + E n=5, OVX+VHOS n=6, OVX n=4**)**. * $p<0.05$ versus Ovary-intact; † $p<0.05$ versus OVX.

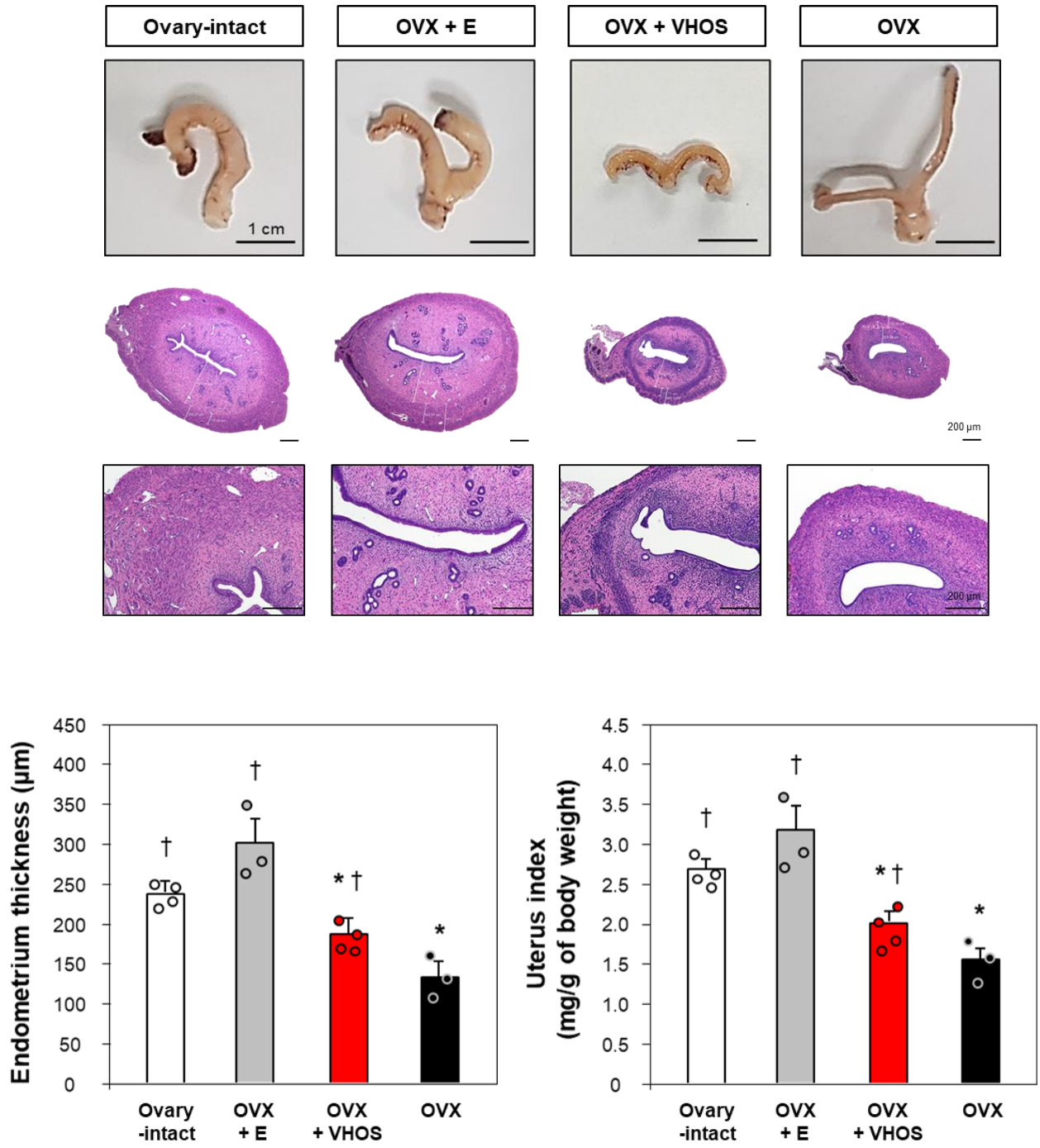

Fig. S2. Poor tissue regeneration due to an insufficient perfusion connection. Comparison of the Ovary-intact with immediate implantation of VHOS indicates the critical need of a 2-week pre-implantation period with the VHOS because of the significantly thinner endometrium and uterus in the OVX+VHOS group, compared to the Ovary-intact group. The thicknesses of the endometrium in the OVX group were significantly thinner than those of the other three test groups. The results with (top) the morphological images (scale bar= cm) were evidenced by (middle) hematoxylin-eosin (H&E) staining (scale bar=200 μm) with (bottom) quantitative analysis (Ovary-intact n=4, OVX + E n=3, OVX+VHOS n=4, OVX n=3**)**. Data=mean ± SEM. * $p<0.05$ versus Ovary-intact; † $p<0.05$ versus OVX. (Photo credit: Hyo-Jin Yoon, Yonsei University College of Medicine.)

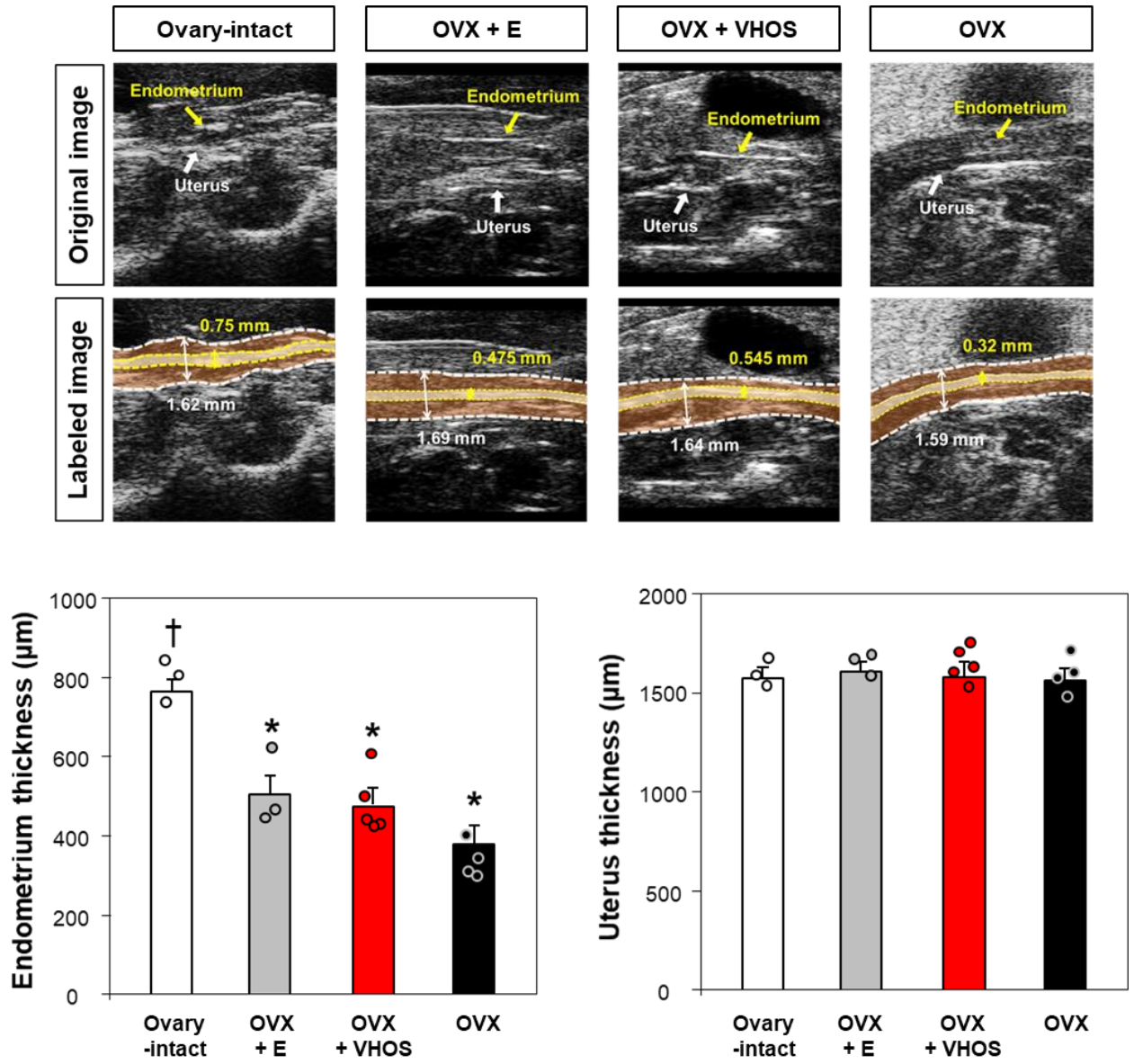

Fig. S3. Slim endometrium by insufficient perfusion connection. When compared with the Ovary-intact group, the OVX+VHOS without a 2-week pre-incubation period, resulted in a significantly thinner endometrium, although the uterus thickness was not significantly different between the two groups. The endometrium thickness of the Ovary-intact group was significantly thicker than those of the other three test groups. The uterus thickness of the OVX group was significantly thinner than those of the other three test groups. The results were evidenced by (top) ultrasonography images of uterus (original, 100×) with magnification and labeling (labeled, 200×), followed by (bottom) quantitative analysis of thicknesses (Ovary-intact n=3, OVX + E n=3, OVX +VHOS n=5, OVX n=4**)**. Data = mean ± SEM. * $p<0.05$ versus Ovary-intact; † $p<0.05$ versus OVX.

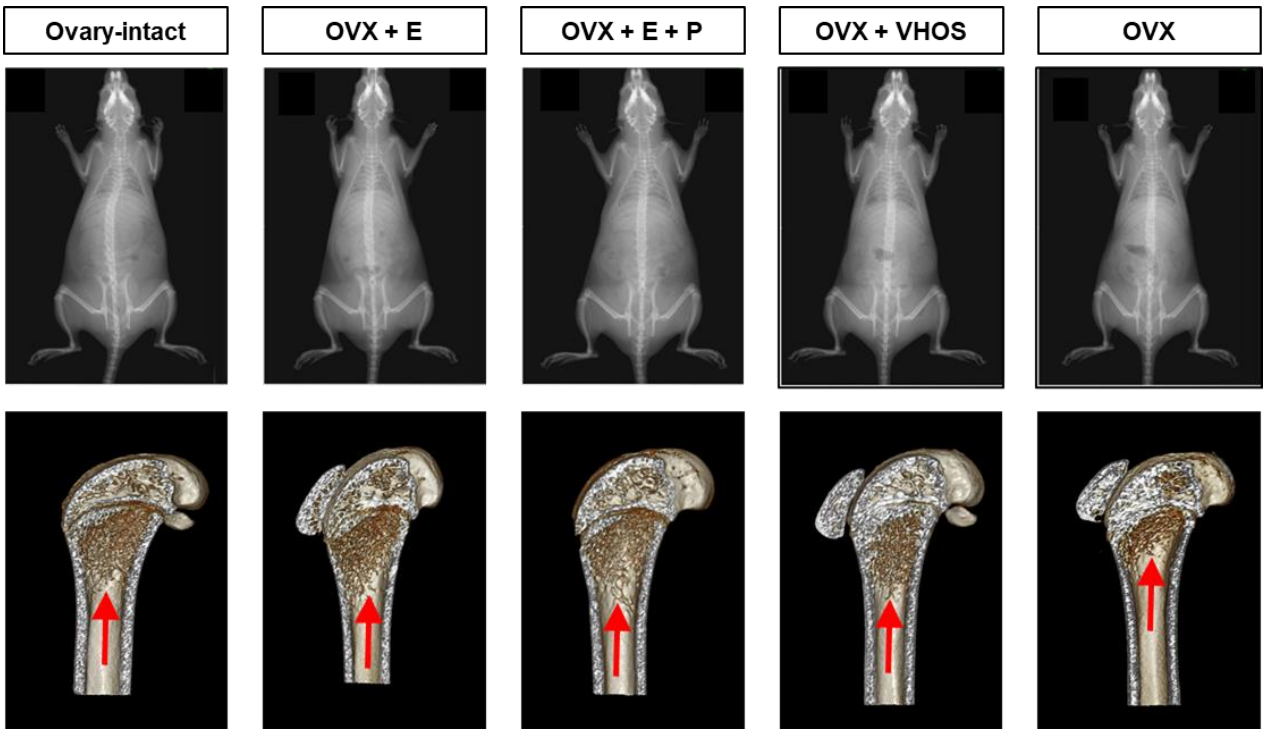

Fig. S4. Dual-energy x-ray absorptiometry (DEXA) and micro-computed tomography (micro-CT) to scan (top) the whole body and (bottom) femoral bone micro-architecture, respectively post positioning in ventral recumbency. Red arrows indicate groove trabecular structures (Ovary-intact n=4, OVX + E n=5, OVX + E + P n=6, OVX+VHO n=5, OVX n=3**)**.