On the Effect of Estrogen Receptor Agonists and Antagonists on the Mouse Hair Follicle Cycle

To the Editor:

We were quite surprised to read the Letter to the Editor by Stenn *et al* in the *Journal of Investigative Dermatology* (110:95, 1998), stating that the estrogen receptor agonist, 17-β-estradiol, and the estrogen receptor antagonist, ICI 182,780, failed to alter mouse hair cycling. This result is surprising as we have reported that: (i) the topical application of 17-β-estradiol arrests hair follicles in telogen, (ii) the topical application of ICI 182,780 causes telogen follicles to enter anagen, and (iii) the estrogen receptor is expressed in the nuclei of the dermal papilla cells of the telogen follicle in CD-1 mice (Oh and Smart, 1996). In their letter, Stenn *et al* stated that they "made a concentrated attempt in two independent and widely separated laboratories to reproduce the experiments as described." They further indicated that they "treated animals following the Oh/Smart protocol precisely" and both of their studies failed to demonstrate any effect of the agonist or antagonist. It was most unfortunate that we were not afforded an opportunity to read and respond to this letter before its publication because it is obvious that these investigators did not conduct their experiments as we described. The most profound difference we noted was that they used doses of 17-β-estradiol and ICI 182,780 that were 5000-fold lower than the doses we used. Dr. Stenn has confirmed that both his laboratory and Dr. Paus's laboratory used a 10 nM dose, which is 5000-fold lower than the correct dose of 10 nmol per 200 μl of acetone vehicle. This dose is clearly stated in our paper.

In addition, Stenn *et al* did not use the CD-1 strain of mice that was used in our studies, but instead used C3H and C57BL/6 mice because they have "found that careful hair cycle studies using a nonpigmented animal such as the CD-1 mouse, are difficult to interpret." Many high quality, seminal hair follicle cycle studies have been conducted in nonpigmented mice. While it is of interest to determine the efficacy of 17-β-estradiol and ICI 182,780 in other strains of mice, it is regrettable that CD-1 mice were omitted from their experimental design. The inclusion of CD-1 mice as a positive control would have allowed these investigators the chance to detect their dose error, as a 5000-fold lower dose would not be effective in altering the hair follicle cycle in CD-1 mice, thus indicating that something was wrong. The use of the correct dose of 17-β-estradiol and ICI 182,780 would alter the hair follicle cycle in CD-1 mice just as we reported in our studies. Although we do not know if 17-β-estradiol and ICI 182,780 will function in C3H and C57BL/6 mice as they do in CD-1 mice, we do know that other strains of mice such as SENCAR and TG.AC mice respond to 17-β-estradiol in a manner similar to CD-1 mice (unpublished results). Experiments to examine the efficacy of 17-β-estradiol and ICI 182,780 in C57BL/6 and C3H mice are currently underway in our laboratory.

Addendum We have completed the experiments on the effect of 17-β-estradiol and ICI 182,780 on the hair follicle cycle in C3H and C57BL/6 mice. Using the experimental conditions described in our previous publication (Oh and Smart, 1996) we found that in both C3H and C57BL/6 mice, ICI 182,780 caused telogen follicles to enter anagen and 17-β-estradiol arrested the hair follicles in telogen. Thus, if the correct dose (10 nmol per 200 μl acetone vehicle) of estrogen agonist and antagonist are employed, these agents alter the hair follicle cycle in C3H and C57BL/6 mice in a manner similar to that previously described in CD-1 mice.

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REFERENCE

Oh HS, Smart RC: An estrogen receptor pathway regulates the telogen-anagen hair follicle transition and influences epidermal cell proliferation. *Proc Natl Acad Sci USA* 93:12525–125230, 1996

Reply:

The experiments we reported in our letter were described as executed, and the mouse strains investigated were chosen for the reasons stated. The experiments were planned and conducted completely independent of one another but, unfortunately, the same mistake was made in both cases – the concentrations used were not as reported in the original report. Although we take responsibility for this oversight, we also recognize that the dosage listing is not entirely conventional to the field. Before starting the repeat experiments we consulted several independent researchers outside our respective laboratories; in all cases the understood dosage was interpreted exactly as we had.

When we repeated the work using the twice weekly protocol and the concentrations used originally by Oh and Smart (*Proc Natl Acad Sci* 93:12525), β-estradiol did indeed inhibit the normal progression of spontaneous anagen in pigmented mice (C57B16). From additional and subsequent studies that we have since executed, we have learned several important features about the role of estrogen receptor-mediated signaling in murine hair growth control that we did not formerly appreciate. We would hope to share these data in a future report.

We are indebted to Drs. Oh and Smart for calling our attention to this interesting phenomenon and regret the confusion our mistake might have caused.

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Note from the Editor: Due to an editorial office error, Drs. Smart and Oh were not given a chance to reply to the original letter about their paper by Drs. Stenn, Paus, and Filippi (*J Invest Dermatol*, 110:95, 1998). We apologize to all the authors for this mistake.