

Gender spotting and chimeric mice

To the editor:

I would like to comment on a recently published article by Wolterink-Donselaar *et al.* on gender determination in newborn dark pigmented mice¹ and to add a point regarding chimeric mice.

Chimeric mice are used to achieve germline transmission of genetic mutations. Typically, chimeric mice are derived from two components: a mouse embryonic stem (ES) cell clone (that carries a desired mutation) and a blastocyst. At least one of the two components carries a dark pigmentation gene that enables investigators to evaluate the degree of chimerism in the mouse. Injection of male ES cell lines into mouse blastocysts generally distorts gender distribution in the progeny, producing a high proportion of males to females instead of an equal ratio². This distortion results from ‘sex conversion’: male ES cells that are injected into a female blastocyst may drive the embryo to develop into a male. Male chimeric mice are subsequently bred with wild-type females to establish a permanent mutant mouse line carrying the introduced mutation.

It is believed that artificial cell culturing conditions can occasionally cause genetically manipulated ES cells to lose their Y chromosomes. Injection of such an XO clone into a blastocyst does not produce male sex conversion in the progeny, resulting

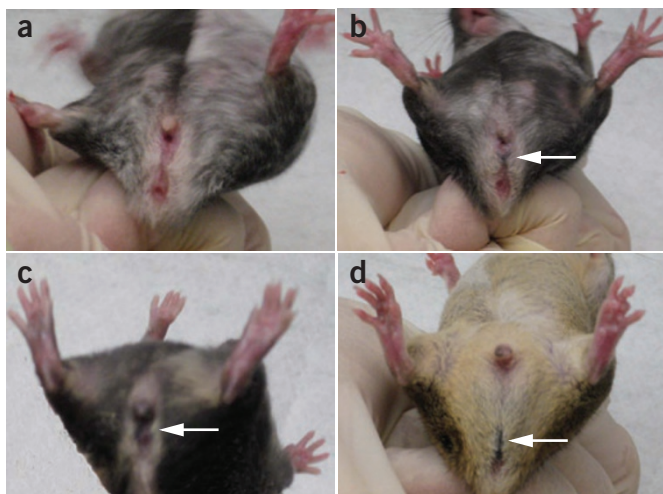


FIGURE 1 | Anogenital pigmentation of chimeric mice that were produced by injecting B6 albino ES cells (**a,b**) or 129 ES cells (**c,d**) into C57BL/6J blastocysts. (**a**) Female chimera with no anogenital spot. The contribution of B6 albino ES cells to coat color is approximately 60% (represented by hair with no pigmentation). (**b**) Female chimera with an anogenital spot (arrow) and approximately 60% coat color contribution of B6 albino cells. (**c**) Female chimera with an anogenital spot (arrow) and low coat color contribution of 129 ES cells (<10%; represented by agouti hair). (**d**) Male chimera with an anogenital spot (arrow), as expected, and 60% coat color contribution of 129 ES cells.

in female mice with a strong ES-cell contribution. Production of female mice is undesirable, as their breeding process takes more time and effort than breeding of male chimeras, and germline transmission might not succeed at all. By identifying the genders of chimeric pups at an early stage, researchers can determine whether it is necessary to produce additional chimeras using alternative ES cell lines and can plan subsequent procedures without delay.

I checked eight litters of chimeric mice that were obtained from ES cell–blastocyst injections. All blastocyst donor females belonged to the C57BL/6J strain. To produce chimeric mice, blastocysts were injected with one of eight genetically altered independent clones, which were obtained from parental ES cell lines from one of two genetic backgrounds: B6 albino (C57BL/6J–Tyrc-2J) or 129 (129S1/SvImJ and 129P2/OlaHsd). Injection of B6 albino ES cells produced chimeras whose coats consisted of white patches on a black background, whereas injection of 129 ES cells produced chimeras with agouti patches on a black background. I noted an anogenital spot in 8 of 19 inspected female chimeras that were produced from ES cells from either genetic background (**Fig. 1**). Presence of the anogenital spot did not necessarily correlate with the percentage of coat color contribution of the strain from which the ES cell lines were derived. This contribution ranged from 10% to 90% in the eight females in which an anogenital spot was observed. Another chimeric female, whose coat color suggested an ES cell contribution of about 60%, did not have an anogenital spot (**Fig. 1a**).

Wolterink-Donselaar and colleagues state that a pigmentation spot consistently appears on the day of birth in male pigmented mice but not in females. Thus, the technique the authors describe for “spotting” the gender of pigmented neonatal mice, though valid for inbred strains, cannot, unfortunately, be extended to chimeric mice. External and internal reproductive organs do not necessarily correspond in chimeric mice; hermaphroditism occurs more frequently in chimeric animals than in non-chimeric animals. **Figure 1c** shows a female chimeric mouse in which male ES cells contributed little to the coat color (<10%; represented by agouti hair). Despite this low contribution, the mouse has a strong anogenital pigment spot, the occurrence of which is controlled by testosterone in several rodent species³.

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1. Wolterink-Donselaar, I.G., Meerding, J.M. & Fernandes, C. A method for gender determination in newborn dark pigmented mice. *Lab Anim. (NY)* **38**, 35–38 (2009).
2. Nagy, A., Gertsenstein, M., Vintersten, K. & Behringer, R. *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 2003).
3. Hamilton, J.B. & Humbert, G. Photographic nature of tanning of the human skin as shown by studies of male hormone therapy. *Science* **88**, 481 (1938).