

Adult mice cloned from migrating primordial germ cells

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Primordial germ cells (PGCs) undergo epigenetic modifications as they develop. It is known that pronounced epigenetic modifications commence with the entry of PGCs into the genital ridges [1]. Rapid and possibly active genome-wide demethylation in both male and female PGCs results in the erasure of imprints [2]. To examine the developmental potential of fetal germ cells, we used the nuclear transfer technique. When nuclei of germ cells at 10.5- to 19.5 -days-postcoitum (dpc) were used for cloning, 35-90% of reconstructed oocytes developed to the 2-cell stage. After embryo transfer to surrogate mothers, we examined cloned fetuses in the mid-gestation stage. Fetuses cloned from 10.5 dpc PGCs were morphologically normal (Figs.1 and 2). In contrast, fetuses cloned from the germ cells at later stages (11.5-19.5 dpc) were developmentally delayed in the mid-gestation stage (Figs.1 and 2). The methylation status of the *H19*- and *Snrpn*- imprinting control regions (ICRs) in germ cell clones was similar to that of donor cells. We found that erasure of allele-specific methylation differences was already initiated in PGCs at 10.5 dpc and was complete by 13.5 dpc. None of the embryos cloned from PGCs at 11.5 dpc or later survived to birth [3].

When nuclei of PGCs at 8.5, 9.5 and 10.5 dpc were injected into enucleated oocytes, 35-40% of reconstructed oocytes developed to the 2-cell stage (Table 1). Seven cloned 2-cell embryos developed into full-term offspring (Table 1). Of these, five, all derived from either 8.5 or 9.5 dpc PGCs, developed into healthy adults with normal fertility. Two cloned offspring were also obtained from 10.5 dpc PGCs. One died shortly after birth and the other showed slight growth retardation at weaning although it subsequently developed into a fertile adult. To determine whether migrating PGCs possess normal genomic imprinting, we examined the allele-specific methylation pattern of the *H19*- and *Snrpn*- ICRs in PGCs at 9.5, 10.5 and 11.5 dpc. While the initiation of demethylation was evident at 9.5 dpc, most PGCs at this stage still maintained intact differential methylation pattern. Although erasure of the allele-specific methylation was more evident in PGCs at 10.5 dpc, more than half of the imprinted loci retained allele-specific methylation patterns. By 11.5 dpc, however, the majority of PGCs have completed the erasure of the allele-specific methylation in both the *H19*- and *Snrpn*- ICRs. Somatic cells of mice cloned from PGCs had normal methylated and unmethylated alleles at imprinted loci (Fig. 3). We conclude that PGCs at the early stages of development (until 10.5 dpc) retain intact genomic imprinting, and their nuclei are developmentally totipotent. Those at more advanced stages undergo progressive erasure of allele-specific differences in methylation at imprinted loci and therefore lose developmental totipotency.

[1] J.V. Schmidt et al., *Genes Dev.* 15(2000) 1997.

[2] M.A. Surani, *Nature* 414 (2001) 122.

[3] Y. Yamazaki et al., *Proc. Natl. Acad. Sci. USA* 100 (2003) 12207.

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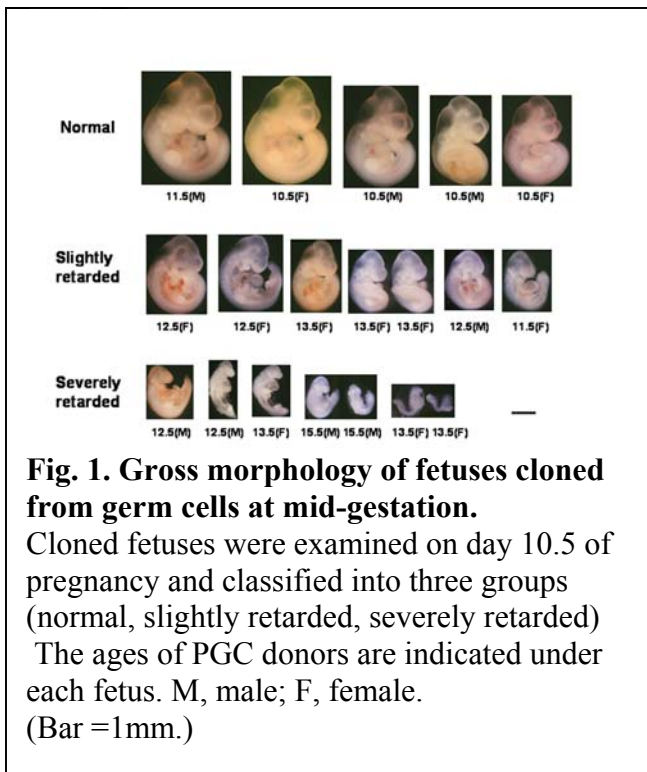


Fig. 1. Gross morphology of fetuses cloned from germ cells at mid-gestation. Cloned fetuses were examined on day 10.5 of pregnancy and classified into three groups (normal, slightly retarded, severely retarded) The ages of PGC donors are indicated under each fetus. M, male; F, female. (Bar =1mm.)

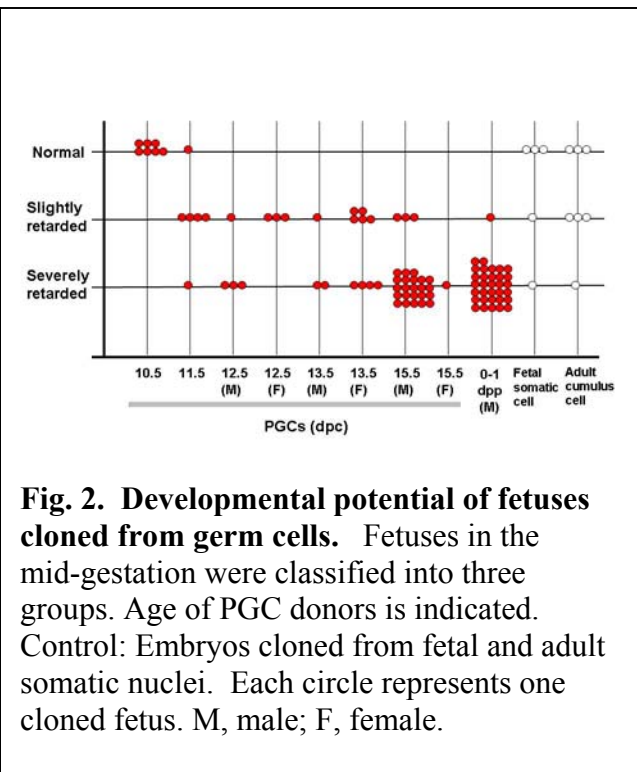


Fig. 2. Developmental potential of fetuses cloned from germ cells. Fetuses in the mid-gestation were classified into three groups. Age of PGC donors is indicated. Control: Embryos cloned from fetal and adult somatic nuclei. Each circle represents one cloned fetus. M, male; F, female.

Table 1. Generation of pups cloned from PGCs at 8.5 -10.5 dpc

PGC donors	Age of PGCs (dpc)	No. of reconstructed oocytes	No. of activated oocytes	No. of 2-cell embryos (%)	Full-term pups (%)	Pups survived
OG2 x DBA	8.5	144	137	50 (36.5)	1 (2.0)	1
CD-1 x OG2	8.5	162	153	67 (43.8)	1 (1.5)	1
OG2x DBA	9.5	191	178	66 (37.1)	3 (4.5)	3
OG2 x DBA	10.5	351	338	149 (44.1)	2 (1.3)	1

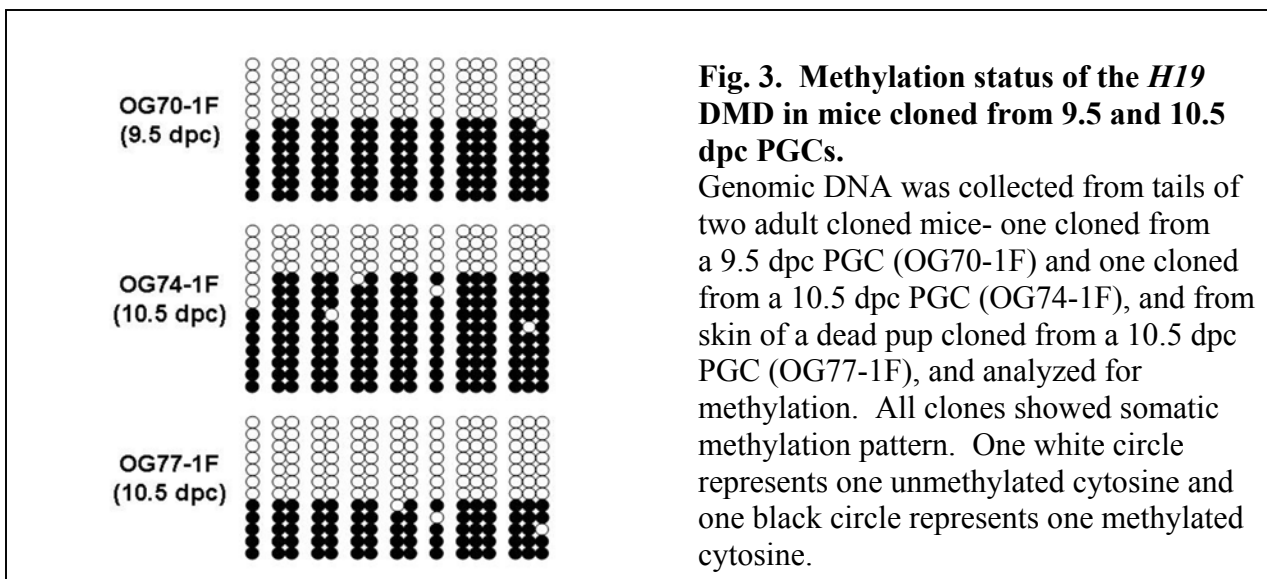


Fig. 3. Methylation status of the H19 DMD in mice cloned from 9.5 and 10.5 dpc PGCs. Genomic DNA was collected from tails of two adult cloned mice- one cloned from a 9.5 dpc PGC (OG70-1F) and one cloned from a 10.5 dpc PGC (OG74-1F), and from skin of a dead pup cloned from a 10.5 dpc PGC (OG77-1F), and analyzed for methylation. All clones showed somatic methylation pattern. One white circle represents one unmethylated cytosine and one black circle represents one methylated cytosine.