

1 **Vitrified canine testicular cells allow the formation of**
2 **spermatogonial stem cells and seminiferous tubules following**
3 **their xenotransplantation into nude mice**

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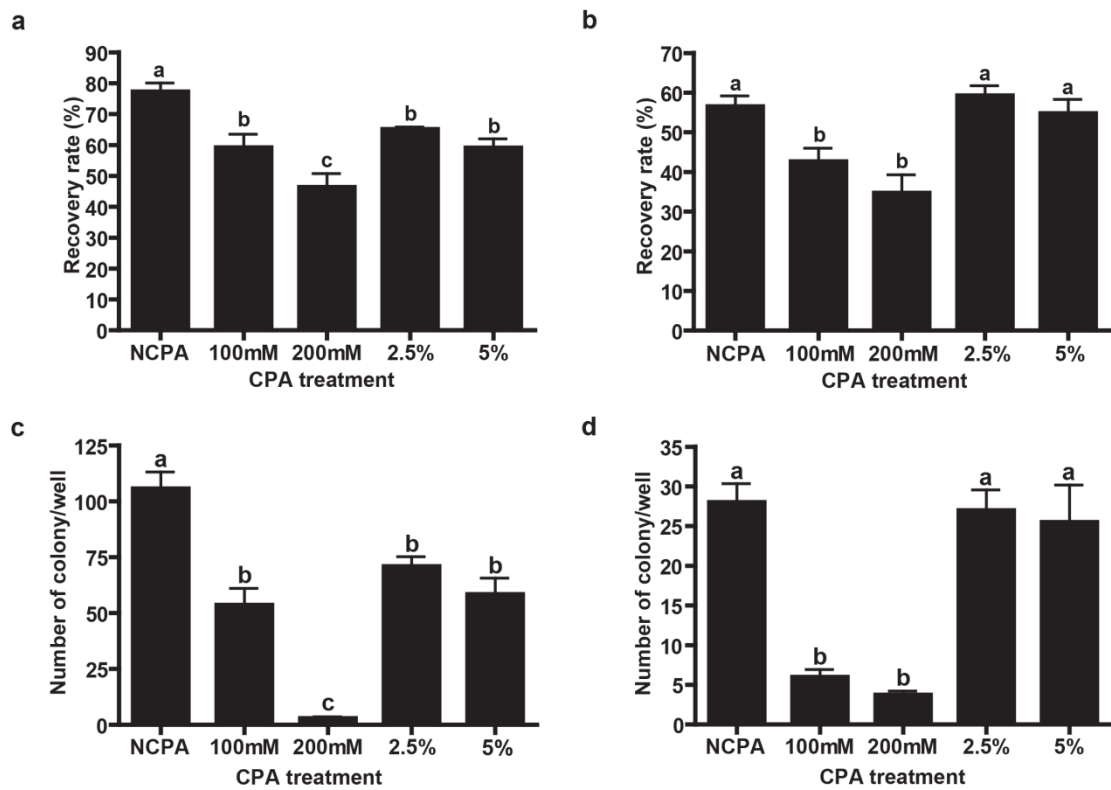
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19 Short title: Germ cell culture and xenotransplantation in BM

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21 **Supplementary figure legend**



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23 **Supplementary Figure 1.** Effect of cryoprotective agents on the recovery rate of

24 cryopreserved testicular cells and the colony number in two-month-old beagle testis.

25 Total testicular cells were thawed after cryopreservation for three months. Recovery rate

26 of testicular cells cryopreserved in StemPro-34 (a) and DMEM-FBS (b) medium with

27 DMSO (final concentration=10%) and different cryoprotective agents. The number of

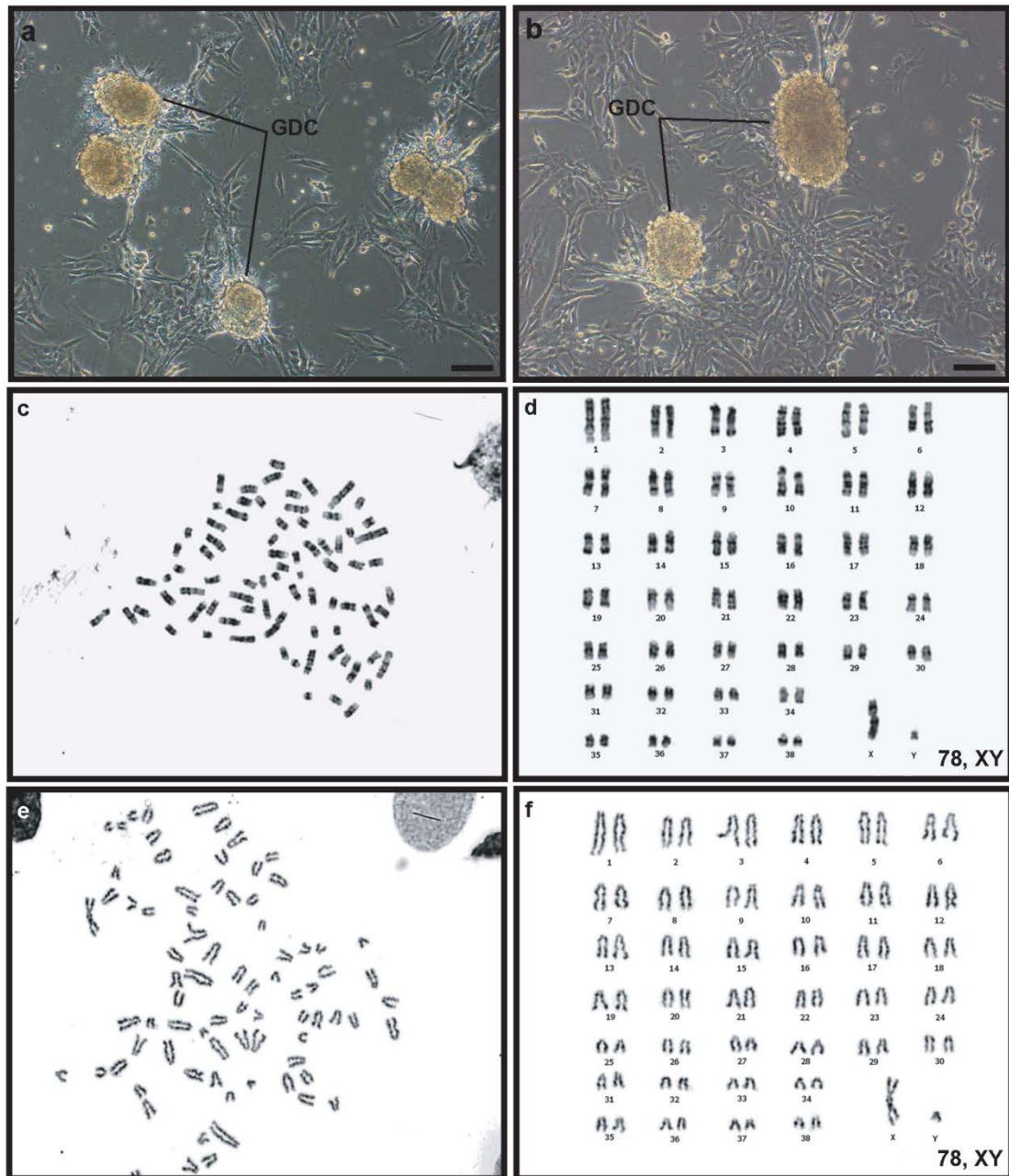
28 colonies in StemPro-34 medium after thawing from StemPro-34 (c) and DMEM-FBS (d)

29 medium with DMSO and different cryoprotective agents. NCPA, non-cryoprotective

30 agent group; 100mM, 100mM trehalose-added group; 200mM, 200mM trehalose-added

31 group; 2.5%, 2.5% PEG-added group; 5%, 5% PEG-added group. $P < 0.05$.

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34 **Supplementary Figure 2.** Colony culturing using primary and cryopreserved testicular
 35 cells and karyotyping of cryopreserved two-month-old beagle testis testicular cells
 36 colony after thawing.

37 Morphology of primary cells GDCs in culture (a) and testicular cells previously
 38 cryopreserved in StemPro-34 and DMSO (b). Karyotypes of colonies from primary
 39 cultures (c and d) and cryopreserved (e and f) testicular cell cultures after thawing.
 40 Panel c and e indicate G-banded metaphase spread of the primary and cryopreserved

41 testicular cells culture, respectively. Scale bars are 100 μm and magnification is $\times 100$ in
42 panel A and B. GDC, germ cell-derived colony.