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Hypoxanthine phosphoribosyltransferase (HPRT)-deficiency is associated with impaired fertility in the female rat

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Purine metabolites play critical roles in regulating early embryonic development in mammals. The levels of cyclic AMP, cyclic GMP and hypoxanthine regulate meiotic arrest of mouse oocytes in vivo, whilst elevated levels of hypoxanthine, adenine, or inosine can disrupt the first cleavage stages during embryonic development in vitro (Dienhart & Downs, 1996; Wigglesworth et al., 2013). The enzyme hypoxanthine phosphoribosyltransferase (HPRT) is an essential component of the purine salvage pathway, involved in recycling hypoxanthine and guanine to provide substrates for the synthesis of nucleic acids and key metabolites including second messengers. The HPRT gene is located on the X chromosome and when mutated in humans causes the debilitating neurological disorder Lesch–Nyhan disease in males (Lesch & Nyhan, 1964). Here, we report that absence of HPRT disrupts early embryonic development leading to impaired fertility in female Hprt knockout (KO) rats.

We previously described the generation of Hprt KO rats using targeted rat DA embryonic stem cells (Meek et al., 2016). The Hprt mutant rats lack exons 7 and 8 of the Hprt gene and do not express HPRT protein. Although the Hprt KO rats appeared generally healthy, they exhibited reduced levels of dopamine in the midbrain, in line with previous observations made in Hprt KO mice and in human Lesch–Nyhan patients (Meek et al., 2016). To examine the requirement for HPRT function during rat embryonic development we crossed Hprt KO rats, but repeated matings failed to produce any offspring (Figure 1a). Although fertilized 1-cell embryos were recovered from KO × KO matings, only fragmented embryos were recovered at day E4.5, when wild-type embryos would normally reach the blastocyst stage (Figure 1b,c). Hprt KO males were fertile and could produce normal sized litters typical of rats with a similar DA/Sprague Dawley mixed genetic background (Figure 1a; Meek et al., 2020). In contrast, Hprt KO female rats mated with wild-type males produced many fragmented embryos and <50% expanded blastocysts at day E4.5, which corresponded with reduced litter sizes at term (Figures 1b and 1e). Interestingly, male pups were represented in these litters, albeit at slightly reduced numbers, indicating that “rescue” did not rely on the contribution of an intact Hprt allele from X-chromosome-bearing sperm (Figure 1a). In matings with wild-type males from a transgenic line carrying a Rex1-EGFP knock-in reporter gene that is first expressed at the 4–8-cell stage (Meek et al., 2020), Rex1-EGFP fluorescence was evident in almost all fragmented embryos (Figure 1d,c). This confirmed that fertilization of most HPRT-deficient oocytes had taken place, and zygotic gene activation had begun in the majority of the degenerating embryos.

The failure to recover intact blastocysts from crosses between Hprt KO rats demonstrated that HPRT activity is essential for proper progression through the initial cleavages of early embryonic...
FIGURE 1 Disruption of early embryonic development in hypoxanthine phosphoribosyltransferase (HPRT)-deficient rat embryos. (a) Numbers of genotypes and sex of offspring in litters generated by matings between Hprt heterozygous (HET) females and wild type (WT) or Hprt knock-out (KO) males. There was no statistically significant reduction in the number of KO animals or males in any of the crosses (t test), although it was noticeable that fewer males were generally present in KO(f) × WT(m) litters. Reduced numbers of offspring were obtained, however, in litters produced by matings between KO females and WT males, compared with either HET females × WT males (p < 0.05) or HET females × KO males (p < 0.05). Repeated attempts to interbreed KO animals by co-housing animals for up to 4 months produced no offspring (*). Males designated (m), females (f) and instances where genotypes could not be determined (n.d.). (b) The mean numbers of total embryos (Et: solid bars) and the sub-set that had developed into blastocysts (BL: hatched bars) were counted in litters recovered at day E4.5 from timed matings between different WT and Hprt mutant rats (means ± SD). The numbers in brackets refer to numbers of litters analyzed. Reduced numbers of identifiable blastocysts were obtained in matings between KO females and WT males, and KO females and KO males (p < 0.05). The dotted line shows the percentage of timed matings that generated no embryos. (c) Bright-field images of typical embryos recovered at E4.5 from matings between KO rats. Note all embryos were either fragmented or arrested during early cleavage, and none had developed into blastocysts. (d) UV /bright-field composite of E4.5 blastocyst embryos generated by mating a HET female with a WT male carrying the Rex1-egfp knock-in reporter gene. The Rex1-egfp transgene is expressed in all cells of the blastocysts. (e) UV /bright-field composite images of E4.5 embryos generated by mating a KO female with a WT male carrying the Rex1-egfp knock-in reporter. Only 3 of the embryos have developed into blastocysts, whilst the remainder are fragmented. Scale bars represent 50 μM.

development in the rat. This requirement for HPRT activity did not exclusively depend upon expression of the Hprt gene in the early embryo as development was rescued by fertilization with Y chromosome-bearing sperm lacking the intact Hprt gene. Indeed, Hprt contribution from the paternal X chromosome within the female embryo at the 2-4 cell stage might be limited in any case if, as in the mouse, this chromosome is partially inactivated before fertilization and only becomes fully active in the early epiblast of the blastocyst. Moreover, zygotic transcription in rats initiates around the 4-cell stage (Zernicka-Goetz, 1994), possibly too late to alleviate disruption of development observed at 2–4 cell stages observed in HPRT-deficient oocytes.

Normal embryo development of some KO oocytes after fertilization with wild-type sperm suggests that Hprt-derived products carried by either sperm or the seminal fluid can rescue the HPRT-deficient embryos. The retention of cytoplasmic bridges between developing spermatids allows equilibration of mRNA, protein, and metabolites between maturing sperm, making post-meiotic spermatids phenotypically equivalent (Braun, Behringer, Peschon, Brinster, & Palmiter, 1989). In this way, Hprt-derived RNA, protein, or HPRT-dependent purine metabolites provided by any sperm could rescue HPRT-deficient embryos. Alternatively, metabolites or factors in the seminal fluid or in sperm-associated extracellular microsomes could be provided in trans by Hprt-expressing somatic support cells of wild-type males.

Rescue by wild-type sperm was incomplete pointing to a sensitized state in the HPRT-deficient embryos, either due to variation in the sensitivity of HPRT-deficient embryos or the penetrance of rescue factors. The nature of the early HPRT-deficient sensitized state and the identity of the rescue factor(s) requires further investigation. However, based on previous experiments in mouse embryos (Dienhart & Downs, 1996; Wigglesworth et al., 2013), it is tempting to speculate that imbalances in the levels of purines or derivative metabolites contribute to a sensitized state that compromises early embryonic development in the rat. HPRT-deficient rat embryos may, therefore, provide a new in vivo experimental system in which to investigate how purine metabolism and the uterine environment influence early embryo development in mammals.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

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REFERENCES
Braun, R. E., Behringer, R. R., Peschon, J. J., Brinster, R. L., & Palmiter, R. D. (1989). Genetically haploid spermatids are phenotypically diploid. Nature, 337, 373–376.
Dienhart, M. K., & Downs, S. M. (1996). Cyclic AMP reversal of hypoxanthine-arrested preimplantation mouse embryos is EDTA-dependent. Zygote, 4, 129–137.
Lesch, M., & Nyhan, W. L. (1964). A familial disorder of uric acid metabolism and central nervous system function. American Journal of Medicine, 36, 561–570.
Meek, S., Thomson, A. J., Sutherland, L., Sharp, M. G. F., Thomson, J., Bishop, V., … Singh-Dolt, K. (2016). Reduced levels of dopamine and altered metabolism in brains of HPRT knock-out rats: A new rodent model of Lesch-Nyhan Disease. Scientific Reports, 6, 25592.
Meek, S., Wei, J., Oh, T., Watson, T., Olavarrieta, J., Sutherland, L., … Joshi, A. (2020). A stem cell reporter for investigating pluripotency and self-renewal in the rat. Stem Cell Reports, 14, 154–166.
Wigglesworth, K., Lee, K.-B., O’Brien, M. J., Peng, J., Matzuk, M. M., & Eppig, J. J. (2013). Bidirectional communication between oocytes and ovarian follicular somatic cells is required for meiotic arrest of mammalian oocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 110, E3723–E3729.

Zernicka-Goetz, M. (1994). Activation of embryonic genes during preimplantation rat development. *Molecular Reproduction and Development*, 38, 30–35.

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