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Hyper-polyploid embryos survive after implantation in mice

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Short Communication

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Summary

Polyploids generated by natural whole genome duplication have served as a dynamic force in vertebrate evolution. As evidence for evolution, polyploid organisms exist generally, however there have been no reports of polyploid organisms in mammals. In mice, polyploid embryos under normal culture conditions normally develop to the blastocyst stage. Nevertheless, most tetraploid embryos degenerate after implantation, indicating that whole genome duplication produces harmful effects on normal development in mice. Most previous research on polyploidy has mainly focused on tetraploid embryos. Analysis of various ploidy outcomes is important to comprehend the effects of polyploidization on embryo development. The purpose of this present study was to discover the extent of the polyploidization effect on implantation and development in post-implantation embryos. This paper describes for the first time an octaploid embryo implanted in mice despite hyper-polyploidization, and indicates that these mammalian embryos have the ability to implant, and even develop, despite the harmfulness of extreme whole genome duplication.

Introduction

Polyploidization occurs ubiquitously in nature and is known as an important factor in evolution that brings about various biological advantages. In yeast, the ability to adapt to environmental changes is improved by polyploidization (Selmecki *et al.*, 2015). In plants, ancestral whole genome duplications have resulted in seed and flower improvements, which are driven by gene diversification (Jiao *et al.*, 2011). In vertebrates, multiple polyploidization, acquired by whole genome duplication, has promoted species evolution (Ohno, 1970; Holland *et al.*, 1994), and is supported by recent genomic sequence analysis, propounded as the 2R hypothesis (Panopoulou and Poustka, 2005). Polyploidy exists in fish or amphibians, however polyploid mammals have exhibited various abnormalities during embryogenesis (Eakin *et al.*, 2005; Olzer *et al.*, 2015). Polyploidization triggers failures of cell division or defects in spindle assembly checkpoints (Eakin and Behringer, 2003; Schmid *et al.*, 2014). Abnormal expression of cell division or cell cycle-related genes are also speculated as candidates for the lethality or abnormal development in mammalian tetraploid embryos (Kawaguchi *et al.*, 2009; Park *et al.*, 2012). Other reports have shown that the more polyploidization, the less the number of the inner cell mass in blastocysts (Liu *et al.*, 2004; Lei *et al.*, 2009). Tetraploid embryonic stem cells established from mouse tetraploid blastocysts have shown increment in apoptosis, and decrement of proliferation rate and meiosis-related gene expression (Horii *et al.*, 2015; Imai *et al.*, 2015; Wen *et al.*, 2017).

Most previous studies, focusing on tetraploid embryos or embryonic stem cells, have implied that various factors might be involved in abnormal development of mammalian polyploid embryos. However, how the extent to which polyploidization affects cell function has not yet been elucidated.

In our previous study, we demonstrate that tetraploid, octaploid, and even hexadecaploid, embryos had full capability to develop to the blastocyst stage with a full inner cell mass (Imai *et al.*, 2019). Here we demonstrate for the first time that octaploid embryos might have the ability to implant and even develop in the uterus in mice.

Materials and methods

Recovery of mouse embryos and polyploidization

BDF1 female mice were ovulated by intraperitoneal (i.p.) injection of pregnant mare's serum gonadotropin (Aska Pharmaceutical Co., Ltd) and human chorionic gonadotropin

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Table 1. Implantation rate

	Recipient mice	Transferred embryos	Decidua at 7.5 d.p.c.	Implantation rate (%)
Diploid	9	82	44	53.7^{a}
Tetraploid	12	81	37	45.7$^{a'}$
Octaploid	13	98	36	36.7^{b}

Different letters *a* and *b* indicate significant differences at $P < 0.05$; *a* and *a′* indicate approached significance at $P < 0.1$.

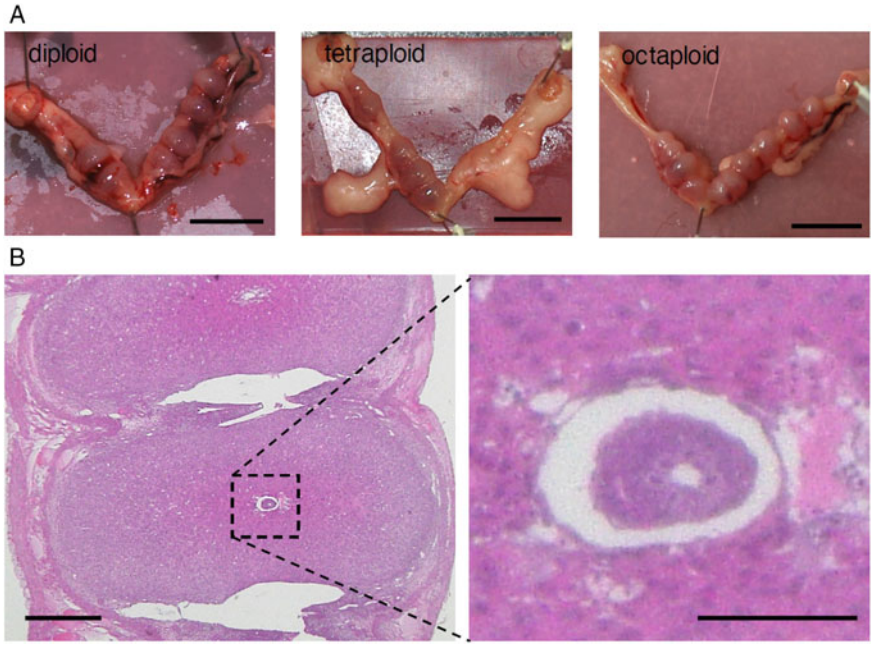


Figure 1. Implantation and decidual formation of polyploid embryos. (*A*) Decidua-formed uteri after polyploid embryo transfer are shown. Uteri transferred with diploid, tetraploid and octaploid embryos are shown left to right. Bars, 1.0 cm. (*B*) Haematoxylin and eosin (H&E) staining of the implantation embryo after octaploid embryo transfer. A higher magnification image of the octaploid implantation embryo is shown on the right. Bar, 500 μm (left); 100 μm (right).

(Aska Pharmaceutical Co., Ltd), and then mated with BDF1 males. Two-cell-stage embryos were collected at 1.5 days post coitum (d.p.c.) and polyploidized using the electrofusion method. Embryos were cultured in M16 medium (Sigma).

Transfer of blastocysts

Blastocyst stage embryos were transferred into the uterine horn of pseudopregnant ICR female mice (at 2.5 d.p.c.; 5–8 embryos for each uterine horn) that had been anaesthetised with a combination of medetomidine, midazoram and butorphanol (Kawai *et al.*, 2011). Following closure of the surgical incision, the mice were roused by i.p. injection of atipamezole. Carprofen was used for pain management. Mice were subjected to euthanasia by cervical dislocation at 7.5 d.p.c. The uterus was fixed with Bouin's solution, embedded in paraffin, and serial sections were prepared and stained with haematoxylin and eosin.

Results and discussion

Decidua were formed at 7.5 d.p.c.in all uteri in which octaploid embryos were transferred, as for diploid and tetraploid embryos (Fig. 1*A*). However, the rate of implantation in octaploid embryos was significantly lower than that in diploid or tetraploid embryos (Table 1). As the total number of cells in polyploid embryos was lower than that of diploid embryos (Imai *et al.*, 2019), we prepared serial sections that allowed comprehensive observation of whole uterine tissues, including in this study the whole concepti, rather than recovering the implanted embryos from decidua. Some octaploid embryos transferred into the uteri were observed in the decidua at the egg cylinder stage (Fig. 1*B*). Previous reports have indicated that octaploid embryos were absorbed and disappeared after implantation, leaving only empty decidua in mice (Wu *et al.*, 2017). Wu and colleagues might have to failed to recover implanted octaploid embryos from the decidua by harvesting, because the polyploidized embryos were smaller than normal implanted embryos. Therefore, we adapted another approach to detect implanted octaploid embryos using whole uterine tissue analysis by serial sectioning. Our present study is the first to report that early octaploid embryos have the ability to implant and develop in the uterus after implantation, despite hyper-polyploidization in mammals.

In haploid embryos, primitive streak formation is absent (Hu *et al.*, 2015) while they develop to the egg cylinder stage (Graham, 1970). In tetraploid embryos, lengthening of cell cycles and growth retardation have been reported in mice (Eakin *et al.*, 2005). In human embryos, chromosomal abnormality causes developmental defects through dysfunction of epithelial–mesenchymal

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transition (Kukharenko, 2018). The lower rate of implantation in polyploid embryos might reflect these abnormalities, in which the maternal immune and endocrine system is related to pregnancy maintenance and post-implantation development in the uterus (Robertson *et al.*, 2018). Although embryo morphology is correlated with chromosomal abnormalities and implantation rates (Gazzo *et al.*, 2019), no report has shown a direct relationship between embryo chromosome number and implantation rates. Detailed mechanisms as to why whole genome duplication occurs and how the extent of polyploidization is involved in aberrational cell function in the early development of implanting embryo are still unknown. There is a need to study hyper-polyploid embryos, produced by repeated whole genome duplication, because most previous studies have only analyzed tetraploid embryos.

Here we describe for the first time that octaploid embryos have the potential to survive after implantation, and indicate that hyper-polyploid embryos have the ability to implant and even develop despite the harmful effect in mammals of larger genome content.

Our results could suggest a new model for analyzing the development of polyploid embryos that could unveil polyploidy development characteristics in mammals.

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Conflicts of interest. All authors declare no conflicts of interest associated with this manuscript.

Ethical standards. All animal experiments were approved by the Experimental Animal Care and Use Committee of Yamaguchi University (protocol number: 267). Mice were housed in groups with *ad libitum* access to water and food.

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