Reproductive semi-cloning respecting biparental origin

A biologically unsound principle

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The original debate article proposed the use of ‘semi-cloning’ as a viable method for assisted reproduction. This debate counters the proposal as being biologically unsound. Given the fundamental limitations of chromosomal segregation and genomic imprinting, the notion of using the MII oocyte to drive haploidization of a somatic cell genome and thereby obtain a substitute for authentic gametes is ill-conceived and untenable.

Key words: assisted reproduction/ cloning/gamete reconstruction/haploidization

Introduction

The recent debate article written by Jan Tesarik (2002) proposes advancing the notion of using what he now calls ‘semi-cloning’ as a viable method for assisted reproduction. He and others have proposed generating substitute gametes with this method, by introducing female somatic cell nuclei into enucleated metaphase II (MII) oocytes, and allowing the polar body to be released, thereby leaving a haploid complement of chromosomes in the oocyte (Kaneko et al., 2001; Lacham-Kaplan et al., 2001; Takeuchi et al., 2001; Tesarik et al., 2001; Palermo et al., 2002a). This oocyte would then be fertilized by a spermatozoon. In a more extreme proposal, a haploidized male somatic cell-derived genome would supposedly substitute for the sperm, and the oocyte would be ‘fertilized’ by introduction of an authentic oocyte-derived haploid genome.

This proposed scheme is biologically unsound. A rudimentary knowledge of the mechanics of meiotic chromosome segregation and the biology of genomic imprinting should suffice to make this apparent.

The odds of a normal haploid complement of chromosomes being retained in an oocyte undergoing its second meiotic division after somatic cell nuclear transplantation are extremely poor (Tateno et al., 2003). We have shown that in mouse MII oocytes extrusion of the polar body fails to leave a haploid number of chromosomes in the oocyte in >90% of the cases, using either murine or Chinese hamster somatic cell donor nuclei (Tateno et al., 2003). In none of the cases where a haploid number of chromosomes remained were these chromosomes comprised of a normal array of chromosomes. Thus, chromosomal segregation in this situation appears to be an entirely random process.

Because of the fundamental differences between mitosis and meiosis, there is no reason to expect normal meiotic segregation of chromosomes from a somatic donor cell in a MII oocyte to yield a normal haploid complement of chromosomes. No opportunity for pairing of homologues exists, and so there is no mechanism by which such segregation can be achieved. Earlier studies suggesting that such segregation occurred presented only limited analysis of a small number of specimens using a method that is of limited resolution. Our data demonstrate clearly that chromosomal segregation of the required nature does not occur.

The subject of imprinting is inadequately and incorrectly addressed in the article by Tesarik. He makes the astounding prediction that the risk of genomic imprinting abnormalities may be less with semi-cloning than with normal somatic cell cloning methods “…because at least one allele of each gene originated from a gamete. …” Although this is true, it can also be predicted that the odds of retaining a given parental homologue in the oocyte is only 50%, making the odds of retaining chromosomes of just one parental origin very remote (for 20 chromosome pairs in mice, the chance is <1 × 10⁻⁶; for the 23 chromosome pairs in the human, the chance is <2 × 10⁻⁷). Thus, the author’s equally astounding prediction that the chromosomal contribution of one of the two parents will be eliminated from the somatic cell genome during the process is clearly in error. It is incorrect, therefore, to suppose that ‘semi-cloning’ offers any advantage over somatic cell cloning approaches; at least in the latter case the embryo begins life with a fully biparental chromosomal constitution.

One could suppose that the oocyte might be able to erase and re-establish imprinting information. It is unlikely that the MII
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Oocyte possesses such ability. It remains to be determined whether germinal vesicle (GV) stage oocytes can fully erase and restore genomic imprinting information. A recent report suggested that normal chromosome segregation may occur in GV stage oocytes (Palermo et al., 2002b). Another study reported that haploidization does not occur with GV stage oocytes (Fulka et al., 2002). Because genomic imprints appear to be established very early during oogenesis (Obata and Kono, 2002), it is unlikely that fully grown GV stage oocytes would retain the capacity to erase and restore genomic imprints. Even if this were to occur, it is unreasonable to propose that a substitute paternal gamete genome could be obtained from an oocyte, as has been suggested (Lacham-Kaplan et al., 2001).

Given the fundamental limitations of chromosomal segregation and genomic imprinting, the notion of using the MII oocyte to drive haploidization of a somatic cell genome and thereby obtain a substitute for authentic gametes is ill-conceived and untenable (<7 × 10^{-13} overall). These two limitations together relegate this proposed methodology to the realm of the fantastic. This is not a matter of controversy, but merely a basic aspect of mammalian reproduction.

Acknowledgements

Research in the authors’ laboratories was supported in part by grants from The Ministry of Education, Culture, Sports, Science, and Technology of Japan (H.T.), the Harold Castle and the Victoria Geist Foundation (R.Y.), and the National Institutes of Health, NIH/NICHD HD38381.

References


