



Libraries and Learning Services

University of Auckland Research Repository, ResearchSpace

Version

This is the Accepted Manuscript version of the following article. This version is defined in the NISO recommended practice RP-8-2008

<http://www.niso.org/publications/rp/>

Suggested Reference

Camlin, N. J., McLaughlin, E. A., & Holt, J. E. (2017). The use of C57Bl/6 × CBA F1 hybrid cross as a model for human age-related oocyte aneuploidy.. *Molecular reproduction and development*, 84(1), 6-7. doi: [10.1002/mrd.22766](https://doi.org/10.1002/mrd.22766)

Copyright

Items in ResearchSpace are protected by copyright, with all rights reserved, unless otherwise indicated. Previously published items are made available in accordance with the copyright policy of the publisher.

For more information, see [General copyright](#), [Publisher copyright](#), [SHERPA/RoMEO](#).

Correspondence

The use of C57Bl/6xCBA F1 hybrid cross as a model for human age-related oocyte aneuploidy[†]

Running Title: C57Bl/6xCBA F1 hybrid and age related aneuploidy

Nicole J. Camlin^{1,2*}, Eileen A. McLaughlin^{1,2,3} and Janet E. Holt^{2,4}

1. School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW 2308, Australia
2. Priority Research Centre for Reproductive Science, University of Newcastle, Callaghan, NSW 2308, Australia
3. School of Biological Sciences, University of Auckland, Auckland 1010, New Zealand
4. School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was supported by Australian Research Council (ARC) grants to E A McLaughlin (DP110100418) and J E Holt (DP120100946/DE120101242) and an Australian Postgraduate award to N J Camlin.

*Corresponding Author(s):

Ms. Nicole J. Camlin

Professor Eileen A. McLaughlin

PRC for Reproductive Science

School of Environmental and Life Sciences

University of Newcastle, CALLAGHAN, NSW, 2308

Ph: +64 9 923 2769

Fax: +61 249216308

Email: Nicole.Camlin@uon.edu.au; eileen.mclaughlin@auckland.ac.nz

[†]This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/mrd.22766]

Received 2 November 2016; Revised 29 November 2016; Accepted 5 December 2016

Molecular Reproduction & Development

This article is protected by copyright. All rights reserved

DOI 10.1002/mrd.22766

Oocyte numbers decrease whereas the incidence of aneuploidy increases as women age. The molecular mechanisms underpinning this age-related decline in oocyte quality are not completely understood. Human oocytes are particularly error prone, with reports of aneuploidy rates as high as 50-60% (Fragouli et al. 2011; Kuliev et al. 2011). Mouse oocytes, in contrast, are generally more resilient to age-related aneuploidy, with different strains harboring disparate susceptibilities to chromosome segregation errors. This is clearly observed with aneuploidy rates as low as 9% (C57Bl/6 mice, 17-19 months) to 25% (B6D2F1/J mice, 16-19 months), but may be as high as 33% (MF1 mice, 15-17 months) to 43% (CD1 mice, 19-25 months) (Chiang et al. 2010; Sebestova et al. 2012; Shomper et al. 2014; Yun et al. 2014). Such variability in murine aneuploidy rates has hampered investigations into the causes of aged-related aneuploidy, with even the highest aneuploidy rates failing to reach those presented in older women.

The aim of this study was to determine if the C57Bl/6(female) x CBA(male)F1 hybrid mouse is an appropriate animal model for investigating human age-related oocyte aneuploidy. This strain of mouse was chosen due to its prevalent use for investigating oocyte meiosis – a result of the robust nature of their oocytes, which exhibit high rates of survival and maturation following microinjection and/or in vitro culture.

Assuming that mice and humans age in a linear relationship, extrapolation estimates suggest that 12 and 17 months correspond to 34 and 48 years in humans, respectively. Therefore, prophase I-arrested, germinal vesicle (GV) oocytes were collected from antral follicles of euthanized mice at 1, 12, or 17 months of age. As observed in humans, oocyte numbers decreased with increasing age (Table 1). A significant reduction in the number of retrievable good-quality GV oocytes was noted at 12 months ($P<0.0001$), and this further decreased at 17 months ($P<0.0001$).

Collected GV oocytes were allowed to mature in vitro at 37°C and 5% CO₂ for 16 hours until metaphase II (MII), in Minimum Essential Medium supplemented with fetal calf serum. The ploidy

status of MII eggs was investigated using immunofluorescence confocal microscopy, as previously described by Camlin et al. (2016). The spindles of MII eggs were collapsed with monastrol to spread chromosomes prior to fixation. Immunocytochemistry was then used to label DNA and kinetochores, followed by confocal imaging to create a collation of z-stack images. Kinetochores pairs were then counted to determine chromosome content, with 20 kinetochore pairs considered euploid. As expected, aneuploidy rates were low in oocytes from 1-month-old animals (5.1% oocytes aneuploid). By 12 months and beyond, however, chromosome segregation errors increased to approximately half of all oocytes examined (Table 1).

Oocyte aneuploidy can result from either the premature individualization of sister chromatid pairs (individual chromosomes are gained or lost) or bivalent nondisjunction (whole chromosome pairs are gained or lost), so we next determined the type of chromosome segregation errors in oocytes from aged mice. Analysis of confocal images revealed that mis-segregation errors were evenly divided between bivalent nondisjunction (NDJ) and premature sister separation chromatid (PSSC) (Table 1).

Chromosome separation is extremely error prone in human oocytes; indeed, aneuploidy is a leading cause of miscarriage and birth defects, particularly in women over the age of 35 years. Our results highlight, once again, the variability observed between mouse strains in relation to their vulnerability to age-related oocyte aneuploidy. Specifically, we established that the C57Bl/6 x CBA F1 hybrid mouse is a highly suitable strain to investigate the causes of NDJ and PSSC that associate with age-related oocyte aneuploidy. We hope to use this model to improve our understanding of the molecular mechanisms underpinning this phenomenon in humans.

References

- Camlin NJ, Sobinoff AP, Sutherland JM, Beckett EL, Jarnicki AG, Vanders RL, Hansbro PM, McLaughlin EA and Holt JE. 2016. Maternal Smoke Exposure Impairs the Long-Term Fertility of Female Offspring in a Murine Model. *Biology of Reproduction* 94(2):39, 31-12.
- Chiang T, Duncan FE, Schindler K, Schultz RM and Lampson MA. 2010. Evidence that Weakened Centromere Cohesion Is a Leading Cause of Age-Related Aneuploidy in Oocytes. *Current Biology* 20(17):1522-1528.
- Fragouli E, Alfarawati S, Goodall N-n, Sánchez-García JF, Colls P and Wells D. 2011. The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. *Molecular Human Reproduction* 17(5):286-295.
- Kuliev A, Zlatopolsky Z, Kirillova I, Spivakova J and Cieslak Janzen J. 2011. Meiosis errors in over 20,000 oocytes studied in the practice of preimplantation aneuploidy testing. *Reproductive BioMedicine Online* 22(1):2-8.
- Sebestova J, Danylevska A, Dobrucka L, Kubelka M and Anger M. 2012. Lack of response to unaligned chromosomes in mammalian female gametes. *Cell Cycle* 11(16):0--1.
- Shomper M, Lappa C and FitzHarris G. 2014. Kinetochore microtubule establishment is defective in oocytes from aged mice. *Cell Cycle* 13(7):1171-1179.
- Yun Y, Holt JE, Lane SIR, McLaughlin EA, Merriman JA and Jones KT. 2014. Reduced ability to recover from spindle disruption and loss of kinetochore spindle assembly checkpoint proteins in oocytes from aged mice. *Cell Cycle* 13(12):1938-1947.

Table 1. The impact of maternal aging on oocyte numbers and aneuploidy rates in a C57Bl/6 x CBA F1 hybrid

Endpoint	1 month	12 month	17 month
Average number of oocytes [†] (number of animals)	27.4±9.3 (n=35)	17.5±2.7* (n=6)	6.8±3.2* [#] (n=36)
% aneuploidy rate (%) (n=aneuploidy oocytes/ total)	5.1% (n=4/78)	53.2%* (n=25/47)	47.5%* ^{##} (n=29/61)
oocytes with bivalent nondisjunction (%) (n= NDJ oocytes/ total)	5.1% (n=4/78)	21.3%** (n=10/47)	24.6%** ^{##} (n=15/61)
oocytes with premature sister chromatid separation (%) (n= PSSC oocytes/total)	0% (n=0/78)	31.9%* (n=15/47)	22.9%* ^{##} (n=14/61)

ANOVA with Tukey post-hoc testing used for oocyte number. Fisher Exact test used for aneuploidy rates.

[†], mean ± standard deviation reported.

*, $P < 0.0001$ compared to 1-month-old oocytes.

**, $P < 0.05$ compared to 1-month-old oocytes.

[#], $P < 0.0001$ compared to 12-month-old oocytes.

^{##}, $P > 0.01$ compared to 12-month-old oocytes.