

Editorial

*Corresponding author

Rachel H. Kennedy, PhD

Assistant Professor
Department of Science
Bard Early College-Manhattan;
Department of Psychology
Columbia University
New York, NY 10002, USA
E-mail: rkennedy@bhsec.bard.edu

Volume 1 : Issue 1

Article Ref. #: 1000TFMOJ1e003

Article History

Received: January 25th, 2016

Accepted: January 27th, 2016

Published: January 28th, 2016

Citation

Kennedy RH. *In vitro* toxicology testing: it's time to report the sex of cells. *Toxicol Forensic Med Open J*. 2016; 1(1): e5-e8. doi: [10.17140/TFMOJ-1-e003](https://doi.org/10.17140/TFMOJ-1-e003)

Copyright

©2016 Kennedy RH. This is an open access article distributed under the Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In Vitro Toxicology Testing: It's Time to Report the Sex of Cells

Rachel H. Kennedy, PhD^{1,2*}

¹Department of Science, Bard Early College-Manhattan, New York, NY, USA

²Department of Psychology, Columbia University, New York, NY, USA

In light of evidence taken from numerous fields indicating that males are routinely used more than females as test subjects,¹⁻³ and the resulting poor outcomes of such practice,^{4,5} a conversation on balancing the sex of sample sets is not a new one. In a 2014 meta-analysis of nearly 300 studies, Prendergast and colleagues discovered that females have erroneously been considered more variable owing to their estrous cycle; instead, males were found to be more inconsistent under some experimental conditions.⁶ These results were subsequently confirmed by meta-analysis of microarray datasets.⁷ If we are to agree that sex of the subject (be it animal or human) is important to disclose, then the lack of representation to this day is troubling – and it is furthermore troubling that many studies fail even to report the sex of tissues or cells, meaning we have no idea as to the relevance of sex to measured outcomes. There have been several calls to action on this point in the biomedical field,³ yet we as a toxicology community have not yet been fully persuaded of our failure.

In toxicological studies, cultured cells are routinely used to identify molecular mechanisms driving chemical actions in our environment, the results of which are foundational to designing whole-animal experiments (using so-called “tiered testing”) and developing toxicological profiles that provide guidance by predicting harm.⁸ As part of sound experimental design, researchers often report several specifics of cultured cells, such as species origin and media conditions, but regularly do not report the sex of their cells,⁹ and are seemingly not challenged on this point by peer reviewers and publishing friends.

This invisibility cloak on sex reporting exists and extends beyond any semblance of welcome, despite the fact that every somatic cell indeed has a sex, and sex-specific cell behavior can occur without considering gonadal hormone exposure history. As such, sex chromosome complement, especially in cells studied in culture, has tremendous potential to impact gene expression and resulting mechanistic signaling pathways,¹⁰ and therefore stands to have significant implications for risk assessment, disease susceptibility, and harm prevention.

The sex of cells can clearly matter, and matter in a big way, and the brain has been particularly well studied for its sexual dimorphism at this level. For example, isolated dopaminergic neurons display different morphologies, independent of hormonal status, depending on whether they come from males or females,¹¹ and male hippocampal neurons are more likely than female ones to be killed by the oxidizer peroxynitrite.¹² Still other studies using embryonic neurons from male and female rats have found males to be more sensitive to ischemic environments, while females have been found more sensitive to apoptotic agents.¹³ Regarding exposure to potential toxicants, female murine cells have demonstrated greater sensitivity than their male counterparts to ethanol.¹⁴ This partial list will no doubt continue to grow as more researchers seek to validate or eliminate sex as an experimental factor. Indeed, as others have suggested,¹⁵ the identification of cell sex may explain why observed effects for “identical conditions” may result in dissimilar findings. Should we really expect a cell derived from a female to behave exactly the same as one derived from a male?

Sexual dimorphisms of course exist for cells beyond the brain, and dimorphic cell responses can occur through specific metabolic pathways. For example, researchers have found

evidence that human cells display a wide variety of different metabolites across the sexes, such as the Carbamoyl-phosphate synthase 1 (CPS1) gene, involved in protein and nitrogen metabolism.¹⁶ This is in keeping with other basic research that has repeatedly shown that males and females metabolize drugs differently.^{17,18}

Moving forward, we should hold ourselves to a higher standard of awareness. In a 2015 report by Wan and colleagues,¹⁹ researchers used quantitative Polymerase Chain Reaction (qPCR) – a very common molecular method – to newly identify NIH/3T3 (ATCC No.: CRL-1658) murine embryonic fibroblast cells as female; 3T3 cells have long been used in toxicological experiments, most recently to assess developmental toxicity to therapeutic drugs²⁰ and phototoxic potential,²¹ but also to document cell behavior on nanotubes,²² response to nanoparticles,²³ and antioxidant response.²⁴

Importantly, sex determination using qPCR is now added to the list of several other available tools that can be used to determine the sex type of cultured cells, including immunodetection of H-Y antigen,²⁵ nested PCR,²⁶ Southern blot,²⁷ and enzymatic assays.²⁸ It should also be noted that it is likely that sex of a cell must be determined more than once over prolonged passage in culture – e.g., many “male” cells, such as the T-84 colonic carcinoma epithelial cell line, have been found to have lost the Y chromosome over time.¹⁵

Especially in this era of genomics, understanding sex and gender in science seems fundamental and relevant to basic and applied sciences. Enhanced reporting efforts that clearly identify the cell source as female or male would not only enrich our understanding of risk assessment and mechanisms of action for chemical toxicity, but might also contribute to building a new framework with which to understand complex chemical interactions. In the end, sex is a biological variable that could affect measured endpoints – and is just as relevant as other experimental features such as culture conditions.

In summary, there seems to exist a pervasive assumption that the sex of cells in culture is not important,²⁹ even in the face of mounting evidence that sex influences gene expression in cultured cells, tissues, and beyond.³⁰⁻³² Potential benefits of identifying cell sex have been raised for biomedical science writ large,^{8,33} and here, now, for toxicology more pointedly. Yet, unless it is the focus of their research, most investigators rarely consider whether cells bearing an XY genotype will behave the same as an XX genotype. It is not expected that sex will play a role in every experimental outcome, but at the very least it should be documented as a biological factor and eliminated as having a significant influence on experimental data.

REFERENCES

1. Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev.* 2011; 35: 565-572. doi: [10.1016/j.neubiorev.2010.07.002](https://doi.org/10.1016/j.neubiorev.2010.07.002)
2. Klein SL, Schiebinger L, Stefanick ML, et al. Opinion: sex inclusion in basic research drives discovery. *PNAS.* 2015; 112: 5257-5258. doi: [10.1073/pnas.1502843112](https://doi.org/10.1073/pnas.1502843112)
3. Zucker I, Beery AK. Males still dominate animal studies. *Nature.* 2010; 465: 690. doi: [10.1038/465690a](https://doi.org/10.1038/465690a)
4. Clayrton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. *Nature.* 2014; 509: 282-283.
5. Dhruva SS, Redberg RF. Evaluating sex differences in medical device clinical trials: time for action. *JAMA.* 2012; 307: 1145-1146. doi: [10.1001/jama.2012.254](https://doi.org/10.1001/jama.2012.254)
6. Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci Biobeh Rev.* 2014; 40: 1-5. doi: [10.1016/j.neubiorev.2014.01.001](https://doi.org/10.1016/j.neubiorev.2014.01.001)
7. Itoh Y, Arnold A. Are females more variable than males in gene expression? meta-analysis of microarray datasets. *Biology of Sex Differences.* 2015; 6: 18.
8. National Research Council. Committee on toxicity testing, assessment of environmental agents, board on environmental studies, toxicology, institute for laboratory animal research. Toxicity testing in the 21st Century. A vision and a strategy. National Academies Press, Washington, DC, 2007.
9. Taylor KE, Vallejo-Giraldo C, Schaible NS, Zakeri R, Miller VM. Reporting of sex as a variable in cardiovascular studies using cultured cells. *Biology of Sex Differences.* 2011. 2: 11. doi: [10.1186/2042-6410-2-11](https://doi.org/10.1186/2042-6410-2-11)

10. Wizeman TM, Pardue M-L. Exploring the biological contributions to human health: does sex matter? National Academy Press, 2001.
11. Carruth LL, Reisert I, Arnold AP. Sex chromosome genes directly affect brain sexual differentiation. *Nat Neurosci.* 2002; 5: 933-934. doi: [10.1038/nm922](https://doi.org/10.1038/nm922)
12. Heyer A, Hasselblatt M, von Ahsen N, Hafner H, Siren A-L, Ehrenreich H. In vitro gender differences in neuronal survival on hypoxia and in 17-beta estradiol-mediated neuroprotection. *J Cereb Blood Flow Metab.* 2005; 25: 427-430. doi: [10.1038/sj.jcbfm.9600056](https://doi.org/10.1038/sj.jcbfm.9600056)
13. Du L, Bayir H, Lai Y, et al. Innate gender-based proclivity in response to cytotoxicity and programmed cell death pathways. *J Biol Chem.* 2004; 279(37): 38563-38570. doi: [10.1074/jbc.M405461200](https://doi.org/10.1074/jbc.M405461200)
14. Penaloza C, Estevez B, Orlanski Sm Sikorska M, et al. Sex of the cell dictates its response: differential gene expression and sensitivity to cell death inducing stress in male and female cells. *FASEBJ.* 2009; 23: 1869-1879. doi: [10.1096/fj.08-119388](https://doi.org/10.1096/fj.08-119388)
15. Fuller CM, Insel PA. I don't know the question, but sex is definitely the answer! focus on "in pursuit of scientific excellence: sex matters" and "do you know the sex of your cells?" *Am J Physiol Cell Physiol.* 2014; 306: C1-C2. doi: [10.1152/ajpcell.00342.2013](https://doi.org/10.1152/ajpcell.00342.2013)
16. Mittelstrass K, Ried JS, Yu Z, et al. Discovery of sexual dimorphisms in metabolic and genetic biomarkers. *PLoS Genet.* 2011; 7(8): e1002215. doi: [10.1371/journal.pgen.1002215](https://doi.org/10.1371/journal.pgen.1002215)
17. Anderson GD. Sex and racial differences in pharmacological response: where is the evidence? pharmacogenetic, pharmacokinetics, and pharmacodynamics. *J Womens Health.* 2005; 14: 19-29. doi: [10.1089/jwh.2005.14.19](https://doi.org/10.1089/jwh.2005.14.19)
18. Hughes RN. Sex does matter: comments on the prevalence of male-only investigations of drug effects on rodent behavior. *Behav Pharmacol.* 2007; 18: 583-589.
19. Wan Q, Ni L, Wu L, Zhang L, Liu M, Jiand X. The determination of sex type of the cultured murine cell with quantitative PCR technique. *Human Cell.* 2015; 28: 154-157. doi: [10.1007/s13577-015-0109-3](https://doi.org/10.1007/s13577-015-0109-3)
20. Warkus EL, Yuen AA, Lau CG, Marikawa Y. Use of in vitro morphogenesis of mouse embryoid bodies to assess developmental toxicity of therapeutic drugs contraindicated in pregnancy. *Toxicol Sci.* 2015. doi: [10.1093/toxsci/kfv209](https://doi.org/10.1093/toxsci/kfv209)
21. Heo Y, Pyo MJ, Bae SK, et al. Evaluation of phototoxic and skin sensitization potentials of PLA 2-Free bee venom. *Evid Based Complement Alternat Med.* 2015. doi: [10.1155/2015/157367](https://doi.org/10.1155/2015/157367)
22. Ryoo SR, Kim YK, Kim MH, Min DH. Behaviors of NIH-3T3 fibroblasts on graphene/carbon nanotubes: proliferation, focal adhesion, and gene transfection. *ACS Nano.* 2010; 4(11): 6587-6598. doi: [10.1021/nn1018279](https://doi.org/10.1021/nn1018279)
23. Kafshgari MH, Cavallaro A, Delalat B, et al. Nitric oxide-releasing porous silicon nanoparticles. *Nanoscale Res Lett.* 2004. doi: [10.1186/1556-276X-9-333](https://doi.org/10.1186/1556-276X-9-333)
24. Danihelova M, Veverka M, Sturdik E, Jantova S. Antioxidant action and cytotoxicity on HeLa and NIH-3T3 cells of new quercetin derivatives. *Interdisp Toxicol.* 2013, 6(4): 209-216. doi: [10.2478/intox-2013-0031](https://doi.org/10.2478/intox-2013-0031)
25. White KL. Immunofluorescent detection of a male-specific factor on preimplantation bovine embryos. *Theriogenology.* 1984; 21: 275-284. doi: [10.1016/0093-691X\(84\)90375-3](https://doi.org/10.1016/0093-691X(84)90375-3)
26. Utsumi K, Iritani A. Embryo sexing by male specific antibody and by PCR using male specific (SRY) primer. *Mol Reprod Dev.* 1993; 36: 238-241.
27. Reed KC, Matthews MA, Jones MA. Sex determination in ruminants using Y-chromosome specific polynucleotides. International application published under the Patent Cooperative Treaty (PCT) World Intellectual Property Organization. 1998. Available at: <http://www.google.com/patents/CA1296270C?cl=en10>

28. Williams TJ. A technique for sexing mouse embryos by a visual colorimetric assay of X-linked enzyme, glucose 6-phosphate dehydrogenase. *Theriogenology*. 1986; 25: 733-739. doi: [10.1016/0093-691X\(86\)90131-7](https://doi.org/10.1016/0093-691X(86)90131-7)
29. Nieuwenhoven L, Klinge I. Scientific excellence in applying sex- and gender-specific methods in biomedical and health research. *J Womens Health*. 2010; 19: 313-321. doi: [10.1089/jwh.2008.1156](https://doi.org/10.1089/jwh.2008.1156)
30. Pierce JP, Kievits J, Graustein B, Speth RC, Iadecola C, Milner TA. Sex differences in the subcellular distribution of angiotensin type 1 receptors and NADPH oxidase subunits in the dendrites of C1 neurons in the rat rostral ventrolateral medulla. *Neuroscience*. 2009; 163: 329-338. doi: [10.1016/j.neuroscience.2009.06.006](https://doi.org/10.1016/j.neuroscience.2009.06.006)
31. Wang X, Barber DA, Lewis DA, et al. Gender and transcriptional regulation of endothelial nitric oxide synthase and endothelin-1 in porcine aortic endothelial cells. *Am J Physiol*. 1998; 273: H1962-H1967.
32. Yang X, Schadt EE, Wang S, et al. Tissue specific expression and regulation of sexually dimorphic genes in mice. *Genome Research*. 2006; 16: 995-1004. doi: [10.1101/gr.5217506](https://doi.org/10.1101/gr.5217506)
33. Pollitzer E. Cell sex matters. *Nature*. 2013; 500: 23-24. doi: [10.1038/500023a](https://doi.org/10.1038/500023a)