Food for Thought ...

Sex and Media: Considerations for Cell Culture Studies

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Abstract

Cell culture has enhanced our understanding of cellular physiology and constitutes an important tool in advancing mechanistic insight. Researchers should be reminded, however, that there are limitations in extrapolating data derived from cultured cells to questions focusing on the impact of sex. In this *Food for Thought*, we highlight two underappreciated aspects of cell culture systems regarding sex: how cell culture media alter the sex hormone environment, and how the innate sex of the cell is often not factored into the overall analysis. By paying careful attention to these areas, researchers can facilitate reproducibility of their cell culture models, which is consistent with the mandate of the National Institutes of Health to improve scientific rigor and reproducibility in research.

1 Cell sex and rigor in biomedical research

The National Institutes of Health (NIH) has called upon the biomedical research community to enhance scientific rigor in experimental design, analysis, and reporting, and, at the same, has mandated that all research must now include sex as a biological variable[1]. A major goal of enhanced rigor is improving the transparency of experimental approaches and, consequently, the reproducibility of experimental data. However, there are many factors related to the study of sex in cell culture models that can confound reproducibility. In this paper we will highlight two general areas of concern: the effect of culture media components on the hormonal environment of cultured cells, and the contribution of chromosomal sex to cellular phenotypes (Fig. 1). Better integration and reporting of these factors will improve not only the reproducibility of cell culture studies, but also the fidelity of *in vitro* models to *in vivo* systems. Ultimately, this will aid in their useful translation to whole animal biology.

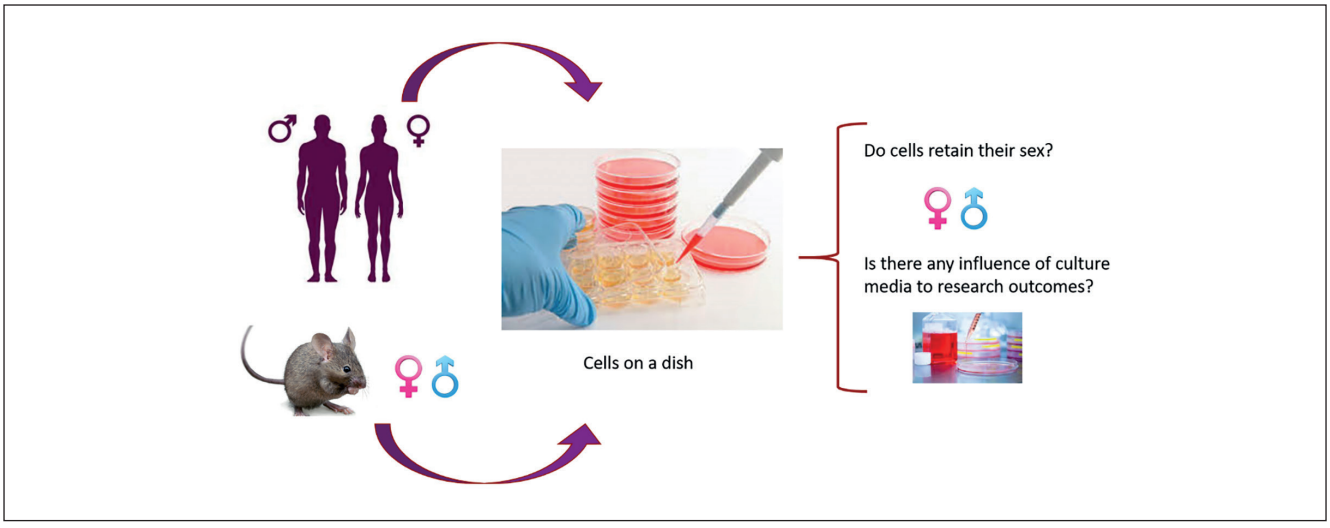

Fig. 1: Factors related to the study of sex in cell culture models that can confound reproducibility

[1] Grants & Funding, https://grants.nih.gov/reproducibility/index.htm (accessed 14.06.2018).

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2 Cell culture media

Like plasma and interstitial fluid *in vivo*, cell cultures employ media to facilitate the exchange of nutrients, gases, and wastes from cells. Many medium formulations exist with varying mixtures of nutritive and non-nutritive components, including glucose, amino acids, vitamins and minerals, as well as inorganic salts, and buffers to maintain the osmolality and pH of the extracellular environment (Freshney et al., 2011). While classical synthetic medium formulations support viability of a wide range of cell types, it is important to realize that medium still provides an artificial environment compared to physiologic conditions. For example, a comparison of the most widely used "classical" synthetic media, Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute 1640 (RPMI1640), to normal human or mouse plasma revealed significant disparities in glucose, micronutrient, and electrolyte concentrations (Arigony et al., 2013; McKee and Komarova, 2017).

3 Estrogenic activity and cell culture media

Similarly, many media contain components that exert estrogen-like actions, and cell culture models that do not account for these effects are susceptible to bias caused by alteration of the hormonal milieu. For example, estrogen receptors (ER) mediate the effects of estrogens and are present in most cell types. A wide variety of compounds can bind ERs and initiate both genomic and non-genomic signaling to influence cell proliferation, differentiation, and metabolism (Farzaneh and Zarghi, 2016). Sex differences with respect to ER sensitivity have also been shown; for example, Hong et al. (2009) demonstrated that male bone marrow derived mesenchymal stem cells (MSC) were sensitive to a wider range of concentrations of estradiol than female MSC's. These studies demonstrate that the *in vitro* hormonal environment influences cells in a sex-specific manner and may confound the reproducibility and, ultimately, translatability of cell culture studies. Indeed, male cells cultured in "estrogenic" media may be exposed to a non-physiologic hormonal milieu that often is not taken into consideration when analyzing and extrapolating the data. Given the widespread use of cell culture, close assessment of how culture media *per se* can change the outcome of experiments by influencing sex hormone-responsive elements *in vitro* is important. Below, we discuss three common constituents of media that may influence the sex hormone milieu of cells in culture.

Serum

Commercially available cell culture media often require supplementation with animal-derived serum in order to support cell viability. Serum contains hormones (including sex steroids), proteins, carbohydrates, lipids, vitamins, and growth factors required for cell growth, metabolism, attachment and proliferation (Brunner et al., 2010; van der Valk et al., 2018). Fetal bovine serum (FBS) and bovine calf serum are among the most commonly used serum supplements. Despite its ubiquity, the use of serum in cell culture media is problematic for several reasons. First, serum composition is variable. Serum is taken from individual fetal cows at the time of their mother's slaughter; as such, the composition of the serum can vary from fetus to fetus, and depends on the age of the mother, feed composition, season of slaughter, and geographic location of the slaughterhouse. Additionally, FBS batches are produced by combining serum from multiple fetuses. In the past, it was assumed that this mixing would "smooth" out variability in serum composition; however, researchers rarely analyze or account for the chemical makeup of their serum lots (due to time and expense). Studies that have done so have demonstrated variability with respect to, specifically, concentrations of estrogens and phytoestrogens (Stubbings et al., 1989).

The use of serum remains popular and widespread. In order to avoid the potential influence of serum hormones on outcome measures, estrogen researchers minimize sex hormone concentrations by charcoal filtering or dialyzing serum (Moreno-Cuevas and Sirbasku, 2000). However, the extent of charcoal stripping is also variable and depends on multiple factors, such as the amount of charcoal used and the stripping time (Sikora et al., 2016). Many commercial suppliers of charcoal-stripped serum have proprietary stripping protocols that are not reported in the literature. Sikora et al. (2016) demonstrated lot to lot variability in charcoal stripped FBS by showing changes in proliferation of MCF-7 cells when exposed to different batches or lots of serum. It is also important to note that stripping the serum not only diminishes the concentration of sex hormones but also alters other components of the serum such as vitamins and growth hormones, which could influence the success of cell culturing and the outcome of the experiments.

Due to the variable nature of both complete and stripped serum, serum-free media systems have been developed. For investigators using animal-derived serum, stringent reporting of the serum brand and lot number will increase transparency and be consistent with the NIH mandate[1] to enhance scientific rigor in experimental design, analysis, and reporting.

Phenol red

Phenol red is a common pH indicator that is present in most commercially available media. Appearing bright red in a cell culture at a pH of 7.4, phenol red yellows in response to acidification of the medium during cell growth. Structurally, it resembles some nonsteroidal estrogens (Berthois et al., 1986) and has been shown to bind and activate ERs in multiple cell types in a dose dependent manner (Berthois et al., 1986; de Faria et al., 2016). Phenol red is considered a weak estrogenic compound (Welshons et al., 1988) by some, while others consider it a potential endocrine disrupting compound that can interfere with the natural production and metabolism of hormones and may be car-

[1] Grants & Funding, https://grants.nih.gov/reproducibility/index.htm (accessed 14.06.2018).

Tab. 1: Concentration of phenol red in different media formulations from different sources (in µM)

Source	DMEM	MEM	DMEM/F-12	RPMI 1640	IMDM
Thermo-Fisher®	39.85	26.57	21.57	13.28	39.85
Lonza®	40.76	26.57		13.55	40.76
ATCC®	42.33		22.85	14.11	42.33
Sigma-Aldrich®	44.87	31.04	24.35	14.96	45.15

DMEM, Dulbecco's Modified Eagle's Medium; MEM, Minimum Essential Medium; DMEM/F-12, DMEM/Ham's F12; RPMI, Roswell Park Memorial Institute; IMDM, Iscove's Modified Dulbecco's Medium.

cinogenic (Wesierska-Gadek et al., 2007). The effect of phenol red on cellular phenotypes has been demonstrated in cancer cells lines. For example, MCF-7 cells are more proliferative in media containing more phenol red compared to media with less phenol red, suggesting an estrogen-like proliferative effect. Additionally, when MCF-7 cells were treated with rascovitine, a pro-apoptotic therapeutic cancer agent in medium lacking phenol red, the growth of the cells was inhibited when compared to cells treated with the same compound in phenol-containing media, indicating that the phenol media may modulate the therapeutic efficacy of anti-cancer drugs (Wesierska-Gadek et al., 2007).

Estrogenic effects of phenol red are not restricted to cancer cells. Markers of proliferation and differentiation in primary cultures of immature pituitary cells and rat uterine cells were increased when cells were cultured in phenol red media compared to culturing cells in non-phenol media (de Faria et al., 2016). In another example of how the culture medium influences the phenotype of the cells, ovarian surface epithelium cells (OSE) obtained from women and cultured in phenol red media developed into large oocytes, while OSE cells cultured in phenol-free media differentiated into multiple stem cell types (Bukovsky et al., 2005). Additionally, Liu et al. (2013) characterized phenol red effects in neurons, demonstrating that phenol red in neurobasal media inhibited neuronal depolarization similar to when the cells were exposed to 17 β-estradiol, again suggesting an estrogenic effect of the phenol red. These examples highlight the importance of factoring the presence of phenol red and its estrogen-like properties into the final analysis and understanding possible confounding effects of phenol red in cell culture media. Additionally, it is important to note that common commercial medium preparations contain different concentrations of phenol red (Tab. 1). For example, DMEM contains approximately 40 µM phenol red, while DMEM/F12 and RPMI 1640 contain approximately 22 µM and 13 µM, respectively[2] (Wesierska-Gadek et al., 2007). Welshons et al. (1988) observed that the estrogenic activity of phenol red with respect to a particular cell type is not always proportional to its concentration in the medium. Serum added to medium binds phenol red, thereby reducing its activity, and different manufacturing processes may introduce impurities that contribute to differences in these effects. While these observations were made decades ago, the ubiquity of phenol red in today's culture media suggests that comparison of cell culture studies using the same cell lines but different commercial media (or even lots of media) with different concentrations and brands of serum should be approached with caution, especially regarding studies of estrogens or other estrogenic compounds. As such, investigators need to be aware of the concentrations of phenol red in their media systems, as well as any possible confounding effects that may occur due to its presence, and report both the brand and lot number of all media used.

Estrogenic contribution of plastics

Plasticware used for most cell cultures contains polystyrene, which releases phenolic compounds into the medium (Soto et al., 1991) and may additionally influence estrogenic pathways. Specifically, it is speculated that when using certain plastics, the weak estrogens released into the medium may attenuate the effects of exogenous estrogens (Moreno-Cuevas and Sirbasku, 2000). Therefore, cell culture plasticware needs to also be regarded as a possible confounding estrogenic element. At this time, it is unreasonable to suggest that modern cell culture studies move away from the use of plastic micro-tubes, petri dishes, and flasks. Rather, investigators are encouraged to test a range of plastics with their cells of interest and note differences in estrogen-linked phenotypes, including proliferation and differentiation ability.

4 Knowing the sex of the cell is important

Despite the NIH mandate[1] to include sex as a biological variable in all studies (Institute of Medicine, 1994, 2012; Morselli et al., 2016), few cell-based assays report the sex of their cells (Shah et al., 2014). Reasons for this are varied: investigators may judge that sex is not relevant to their research or they simply may not be aware of the sex of their cells (Ritz et al., 2014). Furthermore, caution is warranted even when cell sex is acknowledged in the experimental design: over the course of standard cell culture passaging the original sexual identity can

[2] Gibco Media Formulation Tool, https://www.thermofisher.com/us/en/home/life-science/cell-culture/mammalian-cell-culture/classical-media/gibco-media-formulation-tool.html, 2017.

Tab. 2: Sex of common cell lines (compiled from[a,b,c])

Name	Description	Sex	Note
HEK 293	Human embryonic kidney	X	
C2C12	Mouse muscle	X	
CHO	Hamster ovary	X	
COS7	African green monkey kidney, SV40 transformed	XY	
VERO	African green monkey	X	
HeLa	Human cervical carcinoma	X	
Jurkat E6.1	Human leukemia T cell lymphoblast	XY	
NIH 3T3	Mouse embryo	XY	
3T3L1	Mouse embryo	XY	
Panc-1	Human pancreatic ductal adenocarcinoma	X*	Derived from male, Y chromosome lost
1.4E7	Insulin secreting islets	X	Fusion, primary islet with Panc-1
CACO-2	Human Caucasian colon adenocarcinoma	X*	Derived from male, Y chromosome lost
PC-3	Human Caucasian prostate adenocarcinoma	X*	Derived from male, Y chromosome lost
U-87 MG	Human glioblastoma astrocytoma	X*	Derived from male, Y chromosome lost
U937	Human Caucasian histiocytic lymphoma	X*	Derived from male, Y chromosome lost
BHK21 (clone 13)	Syrian hamster kidney		ECACC reports line as unsexed

[a] ATCC. *Cell lines*, https://www.atcc.org/en/Products/Cells_and_Microorganisms/Cell_Lines.aspx (accessed 14.06.2018).
[b] CACC. *Culture collections*, https://www.phe-culturecollections.org.uk/collections/ecacc.aspx (accessed 14.06.2018).
[c] ExPASy. *Cellosaurus – a knowledge resource on cell lines*, https://web.expasy.org/cellosaurus/ (accessed 14.06.2018).

be lost, even in established cell lines. For example, the lung cell line CRL-5873, which was originally procured from a female donor, now harbors Y chromosome fragments (Durkin et al., 2000). Loss of the Y chromosome from cell lines originally procured from males also occurs. In fact, the American Type Culture Collection (ATCC) reports that approximately 100 cell lines in their collection originally derived from male humans or mice have now "lost" the Y chromosome[3] (Tab. 2; reviewed by Shah et al., 2014), and this phenomenon is particularly common in cancer cell lines.

While sex chromosomes have been understood as critical for the development of the gonads, their contribution to autosomal gene regulation has been difficult to characterize and often overlooked. Now, new paradigms have emerged emphasizing that sexual identity begins at the cellular level. Both sex hormones and sex chromosomes regulate the phenotypes of cells and tissues, as well as the expression and regulation of autosomal traits in a sexually specific manner (Institute of Medicine, 2011; Mauvais-Jarvis et al., 2017). Sexually dimorphic gene expression patterns that are independent of sex hormones have been shown in neuronal cell populations, as well as stem cell lines derived from the liver, kidney, and muscle (Shah et al., 2014). Additionally, studies have demonstrated that there is a contribution to the development of the nervous system as well as to cardiac and kidney function that is unique to the Y chromosome (Meyfour et al., 2017). Epigenetic inactivation of one X-chromosome in embryonic female cells functions to equalize the dosage of X-linked genes with that of male cells. As noted by Shah et al. (2014), the X-chromosome expresses a large number of genes associated with metabolic and cell function, as well as immune response. Interestingly, a significant portion of genes residing on the "inactivated" X-chromosome can be actively expressed in the artefactual cell culture environment. It is therefore important that investigators become aware of the sex of their cells and design cell culture studies that account for possible contributions of sex chromosomes by including cells of each sex.

General chromosomal instability is also common in transformed cell lines. Introduction of viral oncogenes is an established method of producing an immortalized line; however, as passages accumulate, fundamental changes to the genotype and phenotype occur and transformed cell lines are susceptible to loss of both autosomal and sex chromosomes. Importantly, these losses do no occur uniformly; cultures of transformed lines tend to be a mosaic of chromosomal patterns. Therefore, transformed cell lines do not allow for determination of sex as a biological variable. The sex of transformed cell lines must not be assumed

[3] Loss of Y-chromosome FAQs, https://www.atcc.org/Global/FAQs/0/C/Loss%20of%20Y-chromosome-1252.aspx (accessed 14.06.2018).

based on reports in the literature, nor should data from one strain of a particular sex be taken as representative of that sex in general. Authentication of both primary and immortalized cell lines and strains should be carried out regularly in order to assess the sex chromosome configuration for a particular line/strain. Cell authentication (for example, by employing short-tandem repeat profiling for human cells and cells lines) not only verifies the sex of the cell line, but also the presence of contaminating cells or other evidence of phenotypic drift. As such, it represents a necessary step to improving the transparency of cell culture experimental design. Another aspect that deserves attention is the fact that cells from different sexes can respond differently to the same treatment, and this is important for the outcome of the research. For example, in the study of Ferrario et al. (2008), human hematopoietic cord blood cells and murine bone marrow progenitors from males and females were exposed *in vitro* to different concentrations of inorganic arsenic. Interestingly, inorganic arsenic was toxic on male and female colony forming units from both species, but it increased the proliferation rate of both human and murine female cells only, while male cells showed no significant modulation of proliferation, indicating a cell sex difference.

5 Concluding remarks

To begin to appreciate the contribution of sex hormones and sex chromosomes, we are reminded that sexual differences exist with respect to health and disease; indeed, women are 50-75% more likely than men to present with an adverse drug reaction, and nearly 80% of the drugs withdrawn by the Food and Drug Administration were removed in part because they posed a greater health risk or caused adverse health effects in women. Yet, much of the data on mechanisms of action of drugs has been generated using cell-based assays that do not account for the sex of the cell. Since sex influences responses to both drugs and nutrients and influences the determination of the safety and efficacy of emerging treatments, initial characterization of the pharmacodynamics and kinetics of drugs should be carried out in both male and female cells in a culture environment that, ideally, recapitulates physiologic conditions *in vivo*. It should also be noted that a lack of an apparent sex difference does not preclude its existence. Indeed, males and females may share similar phenotypes that arise from dissimilar molecular mechanisms; as such, it is important that *in vitro* model systems accurately retain these sex-based mechanisms.

Cells in culture are exposed to conditions that add significant variables and complicate the interpretation of data obtained from cell-based assays. Decades of data have been obtained using classic cell culture media and cell-based systems that do not strictly control the hormonal environment *in vitro* or adequately consider the sex of the cell. With renewed appreciation as to how sex influences biology, it is important to remember that cells in culture also have a sex and that the media and culturing conditions interact with their "cell" sex. Cell culture systems are capable of generating high quality, mechanistic data in a cost-effective manner; however, cell culture systems and medium formulations are artificial and do not recapitulate *in vivo* conditions. These caveats should be factored into the overall analysis and interpretation of the data. Using *in vitro* approaches without accounting for possible effects of cell sex and medium components raises a number of questions regarding the validity and reproducibility of results. Accounting for these factors will help to avoid this and improve the quality and applicability of *in vitro* models to increasingly complex biological questions.

References

Arigony, A. L., de Oliveira, I. M., Machado, M. et al. (2013). The influence of micronutrients in cell culture: A reflection on viability and genomic stability. *Biomed Res Int 2013*, 597282. doi:10.1155/2013/597282

Berthois, Y., Katzenellenbogen, J. A. and Katzenellenbogen, B. S. (1986). Phenol red in tissue culture media is a weak estrogen: Implications concerning the study of estrogen-responsive cells in culture. *Proc Natl Acad Sci U S A 83*, 2496-2500. doi:10.1073/pnas.83.8.2496

Brunner, D., Frank, J., Appl, H. et al. (2010). Serum-free cell culture: The serum-free media interactive online database. *ALTEX 27*, 53-62. doi:10.14573/altex.2010.1.53

Bukovsky, A., Svetlikova, M. and Caudle, M. R. (2005). Oogenesis in cultures derived from adult human ovaries. *Reprod Biol Endocrinol 3*, 17. doi:10.1186/1477-7827-3-17

de Faria, A. N., Zancanela, D. C., Ramos, A. P. et al. (2016). Estrogen and phenol red free medium for osteoblast culture: Study of the mineralization ability. *Cytotechnology 68*, 1623-1632. doi:10.1007/s10616-015-9844-2

Durkin, A. S., Cedrone, E., Sykes, G. et al. (2000). Utility of gender determination in cell line identity. *In Vitro Cell Dev Biol Anim 36*, 344-347. doi:10.1290/1071-2690(2000)036<0344:UOGDIC>2.0.CO;2

Farzaneh, S. and Zarghi, A. (2016). Estrogen receptor ligands: A review (2013-2015). *Sci Pharm 84*, 409-427. doi:10.3390/scipharm84030409

Ferrario, D., Croera, C., Brustio, R. et al. (2008). Toxicity of inorganic arsenic and its metabolites on haematopoietic progenitors "in vitro": Comparison between species and sexes. *Toxicology 249*, 102-108. doi:10.1016/j.tox.2008.04.008

Freshney, R. I. (2011). *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*. New Jersey: John Wiley & Sons, Inc. doi:10.1002/9780470649367

Hong, L., Sultana, H., Paulius, K. and Zhang, G. (2009). Steroid regulation of proliferation and osteogenic differentiation of bone marrow stromal cells: A gender difference. *J Steroid Biochem Mol Biol 114*, 180-185. doi:10.1016/j.jsbmb.2009.02.001

Institute of Medicine (1994). *Women and Health Research: Ethical and Legal Issues of Including Women in Clinical Studies*. Volume I. Washington, D.C.: National Academies Press (US). doi:10.17226/2304

Institute of Medicine (US) Forum on Neuroscience and Nervous System Disorders (2011). *Sex Differences and Implications for Translational Neuroscience Research: Workshop Summary*.

Washington, D.C.: National Academies Press (US). ISBN-13: 978-0-309-16124-4.

Institute of Medicine (US) Board on population health and public health practice (2012). *Sex-Specific Reporting of Scientific Research: A Workshop Summary*. Washington, D.C.: National Academies Press (US). ISBN-13: 978-0-309-22524-3.

Liu, X., Chen, B., Chen, L. et al. (2013). U-shape suppressive effect of phenol red on the epileptiform burst activity via activation of estrogen receptors in primary hippocampal culture. *PLoS One 8*, e60189. doi:10.1371/journal.pone.0060189

Mauvais-Jarvis, F., Arnold, A. P. and Reue, K. A. (2017). A guide for the design of pre-clinical studies on sex differences in metabolism. *Cell Metab 25*, 1216-1230. doi:10.1016/j.cmet.2017.04.033

McKee, T. J. and Komarova, S. V. (2017). Is it time to reinvent basic cell culture medium? *Am J Physiol Cell Physiol 312*, C624-C626. doi:10.1152/ajpcell.00336.2016

Meyfour, A., Pooyan, P., Pahlavan, S. et al. (2017). Chromosome-centric human proteome project allies with development biology: A case study of the role of Y chromosome genes in organ development. *J Proteome Res 16*, 4259-4272. doi:10.1021/acs.jproteome.7b00446

Moreno-Cuevas, J. E. and Sirbasku, D. A. (2000). Estrogen mitogenic action. III. Is phenol red a "red herring"? *In Vitro Cell Dev Biol Anim 36*, 447-464. doi:10.1290/1071-2690(2000)036<0447:EMAIIP>2.0.CO;2

Morselli, E., Frank, A. P., Santos, R. S. et al. (2016). Sex and gender: Critical variables in pre-clinical and clinical medical research. *Cell Metab 24*, 520. doi:10.1016/j.cmet.2016.07.017

Ritz, S. A., Antle, D. M., Côté, J. et al. (2014). First steps for integrating sex and gender considerations into basic experimental biomedical research. *FASEB J 28*, 4-13. doi:10.1096/fj.13-233395

Shah, K., McCormack, C. E. and Bradbury, N. A. (2014). Do you know the sex of your cells? *Am J Physiol Cell Physiol 306*, C3-18. doi:10.1152/ajpcell.00281.2013

Sikora, M. J., Johnson, M. D., Lee, A. V. and Oesterreich, S. (2016). Endocrine response phenotypes are altered by charcoal-stripped serum variability. *Endocrinology 157*, 3760-3766. doi:10.1210/en.2016-1297

Soto, A. M., Justicia, H., Wray, J. W. and Sonnenschein, C. (1991). P-nonyl-phenol: An estrogenic xenobiotic released from "modified" polystyrene. *Environ Health Perspect 92*, 167-173. doi:10.1289/ehp.9192167

Stubbings, R. B., Liptrap, R. M. and Basrur, P. K. (1989). Estradiol and progesterone concentrations in fetal bovine serum and their implication in maturation and fertilization in vitro. *Theriogenology 31*, 260. doi:10.1016/0093-691X(89)90668-7

van der Valk, J., Bieback, K., Buta, C. et al. (2018). Fetal bovine serum (FBS): Past – Present – Future. *ALTEX 35*, 99-118. doi:10.14573/altex.1705101

Welshons, W. V., Wolf, M. F., Murphy, C. S. and Joran, V. C. (1988). Estrogenic activity of phenol red. *Mol Cell Endocrinol 57*, 169-178. doi:10.1016/0303-7207(88)90072-X

Wesierska-Gadek, J., Schreiner, T., Maurer, M. et al. (2007). Phenol red in the culture medium strongly affects the susceptibility of human MCF-7 cells to roscovitine. *Cell Mol Biol Lett 12*, 280-293. doi:10.2478/s11658-007-0002-5

Conflict of interest

The authors declare that they have no conflicts of interest.