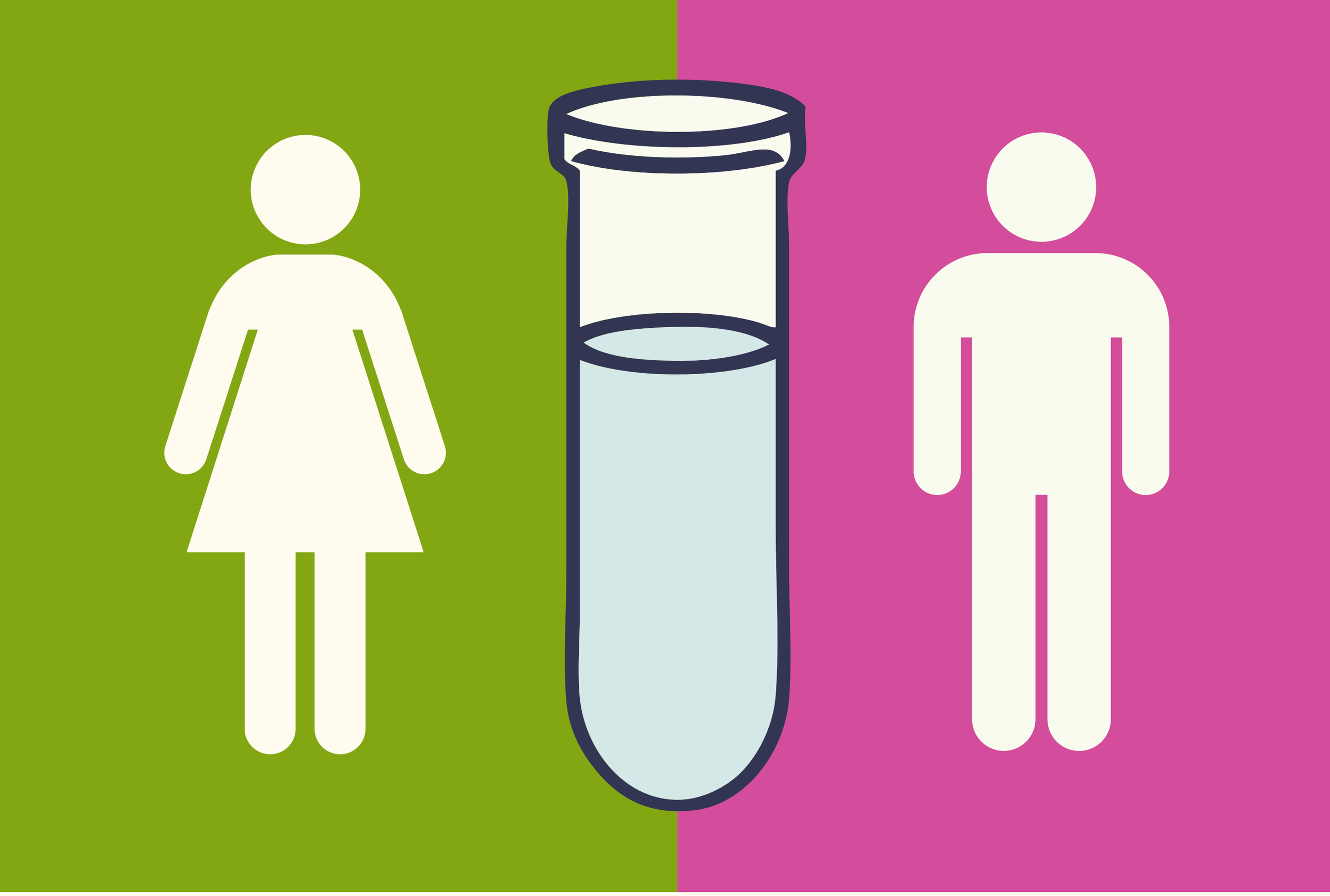

THE SEX AND GENDER
DIMENSION OF RESEARCH

Case studies

EXEMPLARS AND EXPERT OPINIONS
FROM BASIC BIOMEDICAL RESEARCH

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By the LIBRA consortium

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http://www.eu-libra.eu/work-packages/integrate-sex-gender-dimension-research

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TABLE OF CONTENTS

Rationale

Recently, a growing movement in science is advocating for the so called "Sex and Gender Dimension of Research" (**SGR**). Indeed, despite the prevalence of sex differences and sexual dimorphism in mammalian (and other animals) phenotypic traits, sex and gender have often been overlooked in biomedical studies. This may result in research outcomes that are suboptimal with regards to human health.

The Sex and Gender Dimension of Research is relevant when it[1]:

- Involves human subjects
- Uses human cells or tissues and/or animal cells or tissues as models for human physiology or disease
- May have an impact on diagnosis or treatment
- Will bring about products for human use

A number of funding agencies (e.g. NIH, European Commission, Irish Research Council) is, moreover, requiring to include consideration of sex and gender in their funding applications.

Methodology

The research some of the LIBRA partner institutes published is already considering such aspects. Therefore, we interviewed our but also external scientists to further our understanding of the issue and to inspire other researchers to consider sex and/or gender in their research.

1. Would you please describe briefly your research and also explain which parts of it are relevant to SGR as described above?
2. In your research, you show some sex and/or gender specific differences. Did you plan to look at sex and/or gender differences or they arose during the project?
3. Are you planning to follow up on SGR in your future research?
4. In what ways can your research contribute to gendered innovations (i.e. sex and/or gender specific improvements in medical and biological knowledge, diagnostic tools or therapy)?
5. How did you reach this awareness of SGR (for example: attendance to specific meetings, seminars or other dedicated events; personal scientific interest; etc.). Please specify.
6. In research involving model organisms, do you think that the default option should be to use animals of both sexes? Please explain also by describing your personal experience. [Please do not answer if you never worked with model organisms]
7. Do you think the same should apply also with regards to cell lines/primary cells? Please explain also by describing your personal experience. [Please do not answer if you never worked with cell lines/primary cells].
8. In your funding applications, have you ever addressed SGR specifically? If yes, for which funding agency?

[1] L. Nieuwenhoven, M. Bertens and Ineke Klinge 2007. *Gender Awakening Tool. Bibliography: Sex & Gender in Biomedical and Health Research*. Maastricht University, Centre for Gender and Diversity.

In the following set of questions, we asked the researcher for their opinion about general issues related to SGR.

1. One possible critique to the default use of both sexes in experiments is that this would double the cost of the research. What is your opinion on it?
2. When there is no obvious reason to discriminate, do you think scientific journals should require authors to include SGR in their papers?
3. Do you think that funding agencies are correctly approaching the issue? If not, what would you suggest to improve on the current situation?
4. How would you evaluate the general knowledge of SGR among your colleagues and collaborators?
5. What is the one thing that you would mostly recommend to improve scientists´ awareness of SGR?

LIBRA Case Study 1

Epigenetic mechanisms in development and ageing

Scientist and Affiliation:

Prof. Wolf Reik, The Babraham Institute, Cambridge (UK)

Relevant publication:

Milagre et al 2017. Gender differences in global but not targeted demethylation in iPSC reprogramming. *Cell Rep* 18: 1079-1089 (DOI: 10.1016/j.celrep.2017.01.008)

Link to institutional page:

https://www.babraham.ac.uk/our-research/epigenetics/wolf-reik

1. **Would you please describe briefly your research and also explain which parts of it are relevant to SGR as described above?**

The Reik lab explores epigenetic mechanisms in development and ageing. This programme of research mainly uses mouse as a model organism, but also uses established human cell lines. With mice, typically whole litters are used so samples are 50/50 from each of the sexes and the sex is routinely noted. If using mouse embryos, the sex is identified through sequencing. In ageing studies, male mice have been used. In dietary restriction studies, female mice have been used as the males are aggressive. With previous work on imprinting, whether the gene has come from the mother or the father has always been noted. Work on X-inactivation makes use of the sex differences to explore X-chromosome dosage effects.

2. **In your research, you show some sex and/or gender specific differences. Did you plan to look at sex and/or gender differences or did they arise during the project? In case this was not planned, how did you become aware of such differences?**

This particular piece of research looks at induced pluripotent stem cell (iPSC) re-programming. Initial work started using female mouse fibroblasts. However, during iPSC reprogramming, the inactivated X-chromosome is re-activated which influences methylation patterns. Therefore, it was essential to look at this in male iPSCs to control for X-inactivation. A sex difference was observed which interestingly, was not replicated in human cells, as they failed to reactivate the X-chromosome after reprogramming.

3. **Are you planning to follow up on SGR in your future research? If not, why?**

Not currently as there is little interest in this aspect from current post-docs and students. However, it is recognised that the lab needs to be mindful of possible sex differences in epigenetic re-programming when looking this mechanism during pluripotency and ageing.

4. **What was the direct benefit of addressing SGR to your science?**

Discovery of new knowledge, and insights into mechanisms that differ when reprogramming female or male fibroblasts.

5. **In what ways can your research contribute to sex and/or gender specific improvements in medical and biological knowledge, diagnostic tools or therapy (i.e. gendered innovations)?**

It adds to the body of evidence that there are sex differences and, given the potential of iPSCs, alerts those at the more clinical stage of research to possible issues that requires their consideration.

6. **How did you reach this awareness of SGR (for example: attendance at specific meetings, seminars or other dedicated events; personal scientific interest; etc.). Please specify.**

Our initial results were obtained using female fibroblasts and we were curious to understand if these results were the same when using male fibroblasts. When reviewers requested that the experiments be carried out in male as well as female cells we had already decided to investigate what happens when reprogramming male fibroblasts.

7. **In research involving sexed organisms, do you think that the default option should be to use animals of both sexes? Please explain also by describing your personal experience.**

Yes, sex and gender should be considered by default unless there are circumstances making it impossible.

8. **Do you think the same should apply also with regards to cell lines/primary cells? Please explain by describing your personal experience.**

Yes, availability permitting.

9. **In your funding applications have you ever addressed SGR specifically? If yes, for which funding agency?**

Yes, for an application to the ERC. While sex is considered implicitly for much of this research area, it has not been considered explicitly in other grant applications.

10. **One possible critique of using both sexes in experiments is that this would double the cost of the research. What is your opinion on it?**

The use of all litter mates prevents increased mouse costs, however the analysis costs will double due to the doubling of sample numbers.

11. **The use of both sexes in experiments may also be problematic if we take into account the 3Rs (Replacement, Reduction, and Refinement) requirements for animal research. What is your opinion on it?**

Making use of whole litters should prevent any increase in numbers of animals used.

12. **When appropriate, do you think scientific journals should require authors to consider SGR in their papers? Please explain.**

Rather than impose regulation on manuscript submissions, editors and peer reviewers should routinely consider this issue.

13. **Do you think that funding agencies are correctly approaching the issue? If not, what would you suggest to improve on the current situation?**

Funding agencies should encourage researchers to think about the science appropriately – that may well include consideration of sex and/or gender. Imposition of rules to enforce its consideration would not be appropriate.

14. **How would you evaluate the general knowledge of SGR among your colleagues and collaborators?**

Knowledge and awareness is area-dependent. In imprinting research it is considered implicitly. In other areas the consideration it is given is questionable.

15. **What is the one thing that you would recommend to improve awareness of SGR?**

Increase the awareness of researchers by providing examples.

LIBRA Case Study 2

Translational control of dosage compensation in drosophila RNA binding proteins and cancer progression

Scientist and Affiliation:

Fátima Gebauer, Centre for Genomic Regulation (CRG), Barcelona (Spain)

Relevant publication:

M. Mihailovic et al 2012. Widespread generation of alternative UTRs contributes to sex-specific RNA binding by UNR. *RNA* 18(1): 53-64 (DOI: 10.1261/rna.029603.111)

Link to institutional page:

http://www.crg.eu/en/fatima_gebauer

1. **Would you please describe briefly your research and also explain which parts of it are relevant to SGR as described above?**

I am working in the field of RNA regulation. I am particularly interested in RNA binding proteins, which regulate the metabolism of mRNA molecules, and their role in embryonic development and disease, specifically cancer. For the case of embryonic development, we are using Drosophila as a model organism, and here we started to find sex-specific dimensions in our research results. What we found was basically an RNA binding protein (termed UNR*) that is present more or less equally in males and females, but displays different molecular roles in both sexes in the process of X chromosome dosage compensation, and perhaps also in other non-sex related processes. What is X chromosome dosage compensation? Organisms that depend on a different number of X-chromosomes for sex determination need to equalize the expression of X-linked genes. Actually, many genes on the X chromosome are not related to the determination of sex but have normal housekeeping functions which are equally needed in males and females. We found that the protein* UNR *has opposite molecular functions in male and females. In males,* UNR *promotes the dosage compensation process, while in females it represses this process. This means that depending on the sex of the cell, the protein behaves on the molecular level very differently. In females, it associates with a female-specific RNA binding protein and thus regulates the translation of an mRNA that encodes a rate-limiting component of the dosage compensation complex. However, in males the protein´s function has nothing to do with translation. It works as an RNA chaperone modifying the structure of a long-noncoding RNA that is also part of the dosage compensation complex, and finally promoting the assembly of that complex on the X-chromosome.*

We observed the sex-specific role of UNR *in the process of dosage compensation, which per se relates to sex specificity. We were wondering if its sex-specific role could extend to other processes unrelated to dosage compensation. Indeed, we could identify hundreds of mRNAs that are present in both sexes, but which are targeted by the* UNR *protein only in one sex. We*

found that the molecular reason of this sex-specificity is not a matter of different expression levels of the RNA in both sexes, but of alternative processing of the RNAs that still encode for the same proteins. That means a protein which is present in male and females is translated from RNAs which are not identical in both sexes and thus provides the capacity for sex-specific regulation at the stage of protein translation. Coming back to UNR*, this sex-dependent alternative processing of target mRNAs creates* UNR *binding sites in one gender but not the other. So, males and females are super-different. Even if we express the same genes, the regulation of their expression can be very different. Now, we just started to study the protein* UNR *in human cell lines and tissues, investigating its capacity to regulate stress and to promote metastasis. In this study, we will definitely look again at sex-specific differences.*

2. **In your research, you show some sex and/or gender specific differences. Did you plan to look at sex and/or gender differences or did they arise during the project? In case this was not planned, how did you become aware of such differences?**

As we wanted to study how UNR represses dosage compensation, we originally planned to study only female unr mutants. However, by chance we observed that male UNR mutant flies died at a similar rate that females did, which we did not expect. Due to that observation, we decided to include male and female unr mutant flies in our study, and search for the reasons of the male lethality. Researchers who investigate dosage compensation are in general sensitive to the sex aspect in research and often look for sex-specific differences when studying mutant organisms.

3. **Are you planning to follow up on SGR in your future research? If not, why?**

Yes. I will always have it in mind for research in our lab. The new projects focus not anymore on dosage compensation, but cancer progression where I also see the strong relevance for sex-specific analysis.

4. **In what ways can your research contribute to sex and/or gender specific improvements in medical and biological knowledge, diagnostic tools or therapy (i.e. gendered innovations)?**

Our studies about the role of RNA binding proteins in certain kind of cancers will lead to a better knowledge of the diseases in both sexes and might help to identify diagnostic tools or even therapies adapted to each sex.

5. **How did you reach this awareness of SGR (for example: attendance at specific meetings, seminars or other dedicated events; personal scientific interest; etc.)?**

Through the research on dosage compensation, I got more sensitive for the importance of SGR in research in general.

6. **In research involving sexed organisms, do you think that the default option should be to use animals of both sexes? Please explain also by describing your personal experience.**

I think this should be a must. It is true that it seems to collide with the 3Rs requirements for animal research. But I think that wherever possible one could save animals by using in-vitro

methods first, and then use animals for well-thought, essential experiments. If animals are used, both sexes should be included in equal numbers, at least for the most critical conclusions.

7. **Do you think the same should apply also with regards to cell lines/primary cells? Please explain by describing your personal experience.**

In case of cell lines it is more difficult since often they are not even categorised by sex, unless you generate them yourself. Due to the frequent number of passages cell lines often adopt massive genomic alterations, including multiplications of chromosomes. So, the question is how valid an initial sex characterisation would be after a few years… For primary cell lines, I think it would be a good idea to use both sexes as default.

8. **In your funding applications have you ever addressed SGR specifically? If yes, for which funding agency?**

In all grant applications, we usually write: "We will take the sex dimension into account. We will do test our conclusions using animals of both sexes."
We recently applied to World Wide Cancer Research (WCR) in the US and national funding agencies, where they did not specifically ask the researchers to comment on SGR. But we always do.

9. **One possible critique of using both sexes in experiments is that this would double the cost of the research. This is especially problematic if we take into account the 3Rs requirements for animal research. What is your opinion on it?**

As I commented before, one should be careful and use only the required animals for experimentation, trying to minimize the number of experiments by using alternative approaches. But it is also important to generate useful data including both sexes. If only one sex is used, it is important to state that the conclusions are only valid for the one studied sex, and still needs to be shown for the other sex.

10. **When appropriate, do you think scientific journals should require authors to consider SGR in their papers?**

No, as far as the authors clearly acknowledge that the experiments have been done in only one sex.

11. **Do you think that funding agencies are correctly approaching the issue? If not, what would you suggest to improve on the current situation?**

So far, I did not feel any pressure from funding agencies, but I heard that some funders start to request the information about SGR in grant applications. Since funders are giving the money for research, I think they should be the ones pushing for a change and ask applicants to acknowledge sex as a biological variable in their research if relevant.

12. **How would you evaluate the general knowledge of SGR among your colleagues and collaborators?**

People working in the dosage compensation field are very aware. Outside of that field, I do not think they are. On meetings where I present first my Drosophila work and highlight the sex-specific translation regulation, and then introduce my new projects on cancer progression

in human cells, people actually ask if I observe sex-specific differences. If I had not presented the Drosophila story first, people would not ask these questions about my cancer research.

13. What is the one thing that you would recommend to improve awareness of SGR?

I think having examples of projects that have been affected by SGR. Or even inviting medical doctors to report that it is not the same to treat a woman or a man, that symptoms for the same disease or event (even the so common cardiac failure!) can be different in men and women, and that SGR needs to be considered in biomedical research. The format could be a scientific seminar. But nothing will change, if the funders don't enforce it.

LIBRA Case Study 3

Involvement of miR10 in the development of intestinal adenomas

Scientist and Affiliation:

Pr. Anders H. Lund, Biotech Research & Innovation Centre (BRIC), Faculty of Health and medical Sciences, University of Copenhagen (Denmark)

Relevant publication:

G. Stadthagen et al 2013. Loss of miR-10a Activates Lpo and Collaborates with Activated Wnt Signaling in Inducing Intestinal Neoplasia in Female Mice. *PLoS Genetics* 9(10):e1003913 (DOI: 10.1371/journal.pgen.1003913)

Link to institutional page:

https://www.bric.ku.dk/Research/Lund_Group/

1. **Would you please describe briefly your research and also explain which parts of it are relevant to SGR as described above?**

We had generated a knockout mouse strain for a particular microRNA. To investigate a possible impact on cancer development, we crossed the mice to a tumour-prone strain. We detected that female mice developed more intestinal adenomas than male mice. Follow-up experiments showed that loss of the microRNA resulted in a potent upregulation of an enzyme capable of converting oestrogen to DNA-damaging adducts.

2. **In your research, you show some sex and/or gender specific differences. Did you plan to look at sex and/or gender differences or did they arise during the project? In case this was not planned, how did you become aware of such differences?**

This was not planned. We had included mice of both sexes and followed the data.

3. **Are you planning to follow up on SGR in your future research? If not, why?**

Our focus is on basic cancer mechanisms. If sex-specific data arise, we will explore but we do not address these from the point of the initial hypotheses.

4. **What was the direct benefit of addressing SGR to your science?**

We gained an understanding of the molecular mechanism.

5. **In what ways can your research contribute to sex and/or gender specific improvements in medical and biological knowledge, diagnostic tools or therapy?**

I don't know.

6. **How did you reach this awareness of SGR (for example: attendance at specific meetings, seminars or other dedicated events; personal scientific interest; etc.).**

We had included mice of both sexes and followed the data.

7. **In research involving sexed organisms, do you think that the default option should be to use animals of both sexes? Please explain also by describing your personal experience.**

Yes. We use mice of both sexes in our experiments.

8. **Do you think the same should apply also with regards to cell lines/primary cells? Please explain by describing your personal experience.**

This is trickier as suitable cell lines may not always exist from both sexes. Also, drugs are (hopefully) not developed based on data from cell culture models alone and it may be better to inspect sex-specific effects in pre-clinical models.

9. **In your funding applications have you ever addressed SGR specifically? If yes, for which funding agency?**

No.

10. **One possible critique of using both sexes in experiments is that this would double the cost of the research. What is your opinion on it?**

True. Quality costs money. A 3-wheeled car with no airbags would be cheaper, but…

11. **The use of both sexes in experiments may also be problematic if we take into account the 3Rs (Replacement, Reduction, and Refinement) requirements for animal research. What is your opinion on it?**

True. Quality costs money. Running under-powered experiments is a waste of mice and money. Granting agencies could require both sexes and research institutes could subsidize.

12. **When appropriate, do you think scientific journals should require authors to consider SGR in their papers?**

In general, I do not think commercial journals should be put in charge of policing research. This should be done by research institutions.

13. **Do you think that funding agencies are correctly approaching the issue? If not, what would you suggest to improve on the current situation?**

I don't know.

14. How would you evaluate the general knowledge of SGR among your colleagues and collaborators?

There was a recent questionnaire on this – look at the data rather than asking my subjective opinion ;)

15. What is the one thing that you would recommend to improve awareness of SGR?

Information. To start with information on how the majority of drugs are tested mainly on young healthy males as these are the only ones willing to take an unknown pill for money.

LIBRA Case Study 4

Pathogenesis and molecular features of bladder cancer

Scientist and Affiliation:

Prof. Margaret A. Knowles, Leeds Institute of Cancer & Pathology, Leeds (UK)

Relevant publication(s):

CD Hurst et al 2017. Genomic Subtypes of Non-invasive Bladder Cancer with Distinct Metabolic Profile and Female Gender Bias in KDM6A Mutation Frequency. *Cancer Cell* 13;32(5): 701-715.e7. (DOI: 10.1016/j.ccell.2017.08.005)

Link to institutional page:

https://medhealth.leeds.ac.uk/profile/900/854/professor_maggie_knowles

1. **Would you please describe briefly your research and also explain which parts of it are relevant to SGR as described above?**

We work on the molecular biology of bladder cancer. This includes whole exome sequencing and whole transcriptome analysis. Bladder cancer is 3x more common in men. The explanation for this is not understood but may have a hormonal basis. However, bladder cancer in females is often more aggressive. Again, this is not understood.

2. **In your research, you show some sex and/or gender specific differences. Did you plan to look at sex and/or gender differences or did they arise during the project? In case this was not planned, how did you become aware of such differences?**

We unexpectedly found a difference in mutation frequency of a single gene in female tumours. The mutations caused inactivation of the gene and as this gene is on the X chromosome this funding is counterintuitive. The group of tumours in which we found this are the least aggressive ones – so this is not related to the female gender-related poor prognosis in advanced disease.

3. **Are you planning to follow up on SGR in your future research? If not, why?**

We are currently working to confirm the observation in a larger series of samples which include equal numbers of male and female samples and a full distribution of grades and stages. As the gene in question is a chromatin modifier we wonder whether there is a fundamental difference in the epigenome of male and female bladder epithelium. We may possibly pursue this.

4. **What was the direct benefit of addressing SGR to your science?**

It was not our objective to address this.

5. **In what ways can your research contribute to sex and/or gender specific improvements in medical and biological knowledge, diagnostic tools or therapy (i.e. gendered innovations)?**

It is possible that our findings may in future provide give clues to the gender-related incidence of bladder cancer.

6. **In research involving sexed organisms, do you think that the default option should be to use animals of both sexes? Please explain also by describing your personal experience.**

If there is a hint of a gender-related difference then both should be used. Otherwise I don't think it should be the default. Final confirmation that data holds for both sexes would be ideal.

7. **Do you think the same should apply also with regards to cell lines/primary cells? Please explain by describing your personal experience.**

If there is a hint of a gender-related difference then both should be used.

8. **In your funding applications have you ever addressed SGR specifically? If yes, for which funding agency?**

No.

9. **One possible critique of using both sexes in experiments is that this would double the cost of the research. What is your opinion on it?**

I don't think it should be the default.

10. **The use of both sexes in experiments may also be problematic if we take into account the 3Rs (Replacement, Reduction, and Refinement) requirements for animal research. What is your opinion on it?**

Both should only be used if there is good reason.

11. **When appropriate, do you think scientific journals should require authors to consider SGR in their papers? Please explain.**

No.

12. **Do you think that funding agencies are correctly approaching the issue? If not, what would you suggest to improve on the current situation?**

I am not aware of the approaches of funding agencies.

13. **How would you evaluate the general knowledge of SGR among your colleagues and collaborators?**

Fairly good in my field.

14. **What is the one thing that you would recommend to improve awareness of SGR?**

High impact publications would have most impact in my field.

LIBRA Case Study 5

Sex differences in the reinstatement of methamphetamine seeking behaviour in rats

Scientist and Affiliation:

Jana Rudá-Kučerová and Alexandra Šulcová, Department of Pharmacology, Masaryk University, Brno (Czech Republic)

Relevant publication:

J. Rudá-Kučerová 2015. Sex differences in the reinstatement of methamphetamine seeking after forced abstinence in Sprague-Dawley rats. *Frontiers in Psychiatry*, 06 July (DOI: 10.3389/fpsyt.2015.00091)

Link to institutional page:

https://www.muni.cz/en/people/169493-jana-ruda/projects
https://www.muni.cz/en/people/1937-alexandra-sulcova

1. **Would you please describe briefly your research and also explain which parts of it are relevant to SGR as described above?**

I mostly study addictive behaviours in a model of depression induced by olfactory bulbectomy in rats. The ultimate aim is to provide a model in which we may test drugs designed specifically for addiction treatment in depressed individuals. Despite the majority of my research is conducted using male animals, after finding a drug effective in our model in males, we definitely need to test the same paradigm in females. Otherwise the validity of such finding would not be adequate and this would eventually decrease the IF potential of the research.

2. **In your research, you show some sex and/or gender specific differences. Did you plan to look at sex and/or gender differences or did they arise during the project? In case this was not planned, how did you become aware of such differences?**

This particular paper was a lucky coincidence but I have studied sex-differences earlier: https://www.ncbi.nlm.nih.gov/pubmed/20035259.

3. **Are you planning to follow up on SGR in your future research? If not, why?**

Yes, as a standard extension of our pilot studies and in agreement with especially US Journals which insist on testing both sexes.

4. **What was the direct benefit of addressing SGR to your science?**

Papers. I have two papers related to the SGR research per se and another is in preparation.

5. **In what ways can your research contribute to sex and/or gender specific improvements in medical and biological knowledge, diagnostic tools or therapy (i.e. gendered innovations)?**

We may find out that particular medication is more/less/not effective in one of the sexes. However, our usual conduct – testing males first and in case of positive results follow-up with females rules out the possibility of revealing a mechanism effective in females only.

6. **How did you reach this awareness of SGR (for example: attendance at specific meetings, seminars or other dedicated events; personal scientific interest; etc.).**

Sex differences were part of my PhD project which I applied for.

7. **In research involving sexed organisms, do you think that the default option should be to use animals of both sexes? Please explain also by describing your personal experience.**

Generally, yes. But it largely depends on what you want to see. In drug development definitely.

8. **In your funding applications have you ever addressed SGR specifically? If yes, for which funding agency?**

Yes, but mostly not as the main topic – GAČR, IGA MZ, L´Oreal for women in science, GAMU.

9. **One possible critique of using both sexes in experiments is that this would double the cost of the research. What is your opinion on it?**

Unfortunately, in some cases we have to pay it. Otherwise it compromises the validity of the findings.

10. **The use of both sexes in experiments may also be problematic if we take into account the 3Rs (Replacement, Reduction, and Refinement) requirements for animal research. What is your opinion on it?**

Depends on what we study. However, in most cases I believe it is necessary. Also, approximately the same numbers of male and female animals are born. So shall we just kill the females? My answer to this ethical struggle is to use as much as we can from each animal born in the lab. Only that way they do not die in vain.

11. **When appropriate, do you think scientific journals should require authors to consider SGR in their papers?**

They already do. Exp Clin Psychopharmacol made us change the title from "rats" to "male rats" pointing out that "rats" mean generalization and we cannot generalize because we did not test females.

12. **Do you think that funding agencies are correctly approaching the issue? If not, what would you suggest to improve on the current situation?**

I probably do not have enough experience but to my knowledge EU grant proposals are strongly encouraged to test females as well.

13. **How would you evaluate the general knowledge of SGR among your colleagues and collaborators?**

Everybody knows that and nobody wants to pay for it.

14. **What is the one thing that you would recommend to improve awareness of SGR?**

On which level? Big grant agencies already support this. Some Journals demand it almost ridiculously, others do not care at all but this situation is hardly going to change. Maybe small Institutional grants intended for re-testing females as an extension of already performed experiments with males could make some difference. I believe a nice project proposal could be put together by animal researchers from Masaryk University and the Central Animal Facility, with the aim to validate all procedures for SGR. The outcome could be a research and breeding guideline for successful testing in females. I would be thrilled to be a part of such project.