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Module 2, Video 11: Cell and Tissue Considerations for SABV

In 2001, the US Institute of Medicine declared that every cell has a sex [1], referring to the cell's XX or XY makeup. But what does it mean to consider sex in in vitro research? In this video, we will provide guidance for incorporating both male- and female-derived cells or tissues into in vitro research.

Incorporating females into in vitro research has faced greater resistance than in other preclinical studies. Although cells may respond differently once removed from the body [3, 6], sexually dimorphic differences between male- and female-derived cells have been found in many cell populations [7], suggesting that sex as a biological variable is indeed relevant for in vitro research. But many researchers argue that the availability of tissues or cells is a huge limiting factor. In vitro experiments use either primary cells harvested directly from the tissues of humans or animals or commercially available immortalized cell lines. Both show inherent advantages and challenges, particularly in the context of studying sex differences.

Limited donor cell and tissue availability is particularly relevant in humans. In some cases, there might be a natural bias toward one sex. For example, adipocytes are typically derived from discarded fat cells obtained from cosmetic surgery. Thus, significantly more of these cells come from females versus males. Immortalized cell lines are always derived from a single donor source of a single sex. When a similar cell line derived in both sexes can be found, the cells may have different demographic profiles. If you can find male and female cell lines that are demographically similar, you still should not claim a sex difference. This is because the two lines were established from a single male and female, which is the equivalent of an N of one [2, 4]. Cell lines also demonstrate chromosomal instability so authentication of sex chromosome configuration is necessary [2]. Primary cells also face challenges of demographic variability but it IS possible to recruit male and female donors with similar demographic profiles. However, establishing human cell lines is labor, skill and time intensive and additional donors would be necessary for multiple experiments as primary cell lines from humans can only divide a defined number of times.

In rodents, cells for primary cultures are derived from neonates or embryos. In both cases, acquiring male and female samples may be easier due to greater availability, but it is still time and labor intensive [5]. For embryonic cultures, cells can be derived from single pups and sexed after plating. But these cultures are typically less robust and have a limited number of cells compared with mixed-sex cultures, reducing the number of treatments and outcomes that can be evaluated. Neonatal cultures can be sexed and grouped by sex prior to plating.

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How should you design in vitro experiments to consider sex as a biological variable? First, you should perform a literature search with adequate search terms for "sex" and "gender" to fully assess previously documented sex differences in your area of research. This literature search should then form the basis for your research questions and hypotheses. Next, you must determine IF and HOW you will incorporate sex into your research. While documented sex differences or a skew in disease prevalence to a single sex clearly provide a rationale for studying sex, the absence of sex differences does not justify the use of a single sex. Sex differences should ALWAYS be investigated before they can be ruled out. In terms of the HOW, there are 3 options: Mixed sex cultures without sex-disaggregated data collection refers to culturing cells of both sexes together in the same dish. This approach should only be used if you can definitely state there are no sex differences in response to the intervention AND you know that the culture conditions affect the cells of both sexes equally. Certain factors such as the cell media, growth factors, apoptotic agents and even some plastics used in culture dishes have been shown to exert estrogen-like actions [2]. Mixed sex cultures with sex disaggregated data collection are ideal, especially if a sex difference is expected based on the literature search or unknown. However, the tissues and cells should be matched according to non-sex characteristics that might influence the results, or the results should be adjusted statistically to account for these variables. Single-sex cultures are less ideal for obvious reasons. However, they may be used, for example, to fill gaps in the research, such as exploring an effect in females when you have an established effect in males. In this case, a validation cohort should be used as with animal studies. Single sex studies can also be used to investigate female or male only interventions or diseases, investigate differences within cell types of one sex, or study how cells differ according to different factors.

In all cases, data should be interpreted carefully. Care should be taken to avoid assuming that findings in one sex apply to the other, especially with single-sex cultures. Confounding variables related to culture conditions or inherent differences in the cells based on sex should also be statistically factored. Finally, findings should be transparently reported and without overinterpretation of the effects of the sex of the cells or tissue [2]. The lack of sex differences should also be reported to guide future research [5].

As has been demonstrated in this video, incorporating sex as a biological variable in in vitro research is possible, potentially very informative, and one step further toward improving the reproducibility and translational relevance of preclinical research.

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