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MEET THE METHODS SERIES: MEASURING AND MANIPULATING SEX HORMONES IN LABORATORY ANIMALS

In basic science, sex hormones can be measured and manipulated in mice and rats using a variety of techniques. The CIHR Institute of Gender and Health asked Margaret McCarthy, Ph.D., Professor of Pharmacology at the University of Maryland, about her views on how to best integrate these methods in her research. Margaret McCarthy's research focuses on the influence of sex hormones on the developing brain, with a special emphasis on understanding the cellular mechanisms that establish sex differences. Here are Margaret McCarthy's recommendations:

When should studies with female mice or rats be staging the estrous cycle?

Start by overlooking the estrous cycle. Look at your data and see if there is variability in your male or female experimental groups. Variability in males may be due to group housing, which can alter steroid levels in mice and rats. **Learn more**[1].

If there is low variability in males, but high variability in females, then it is advisable to stage the estrous cycle to see if this is a source of variability. To do this, determine and monitor the stage of the estrous cycle by visual observation or vaginal cytology over 2 weeks to uncover how changes in estrogen and progesterone levels may be important for your experimental endpoints. **Learn more**[2].

TIP: Estrous cycle dynamics can vary between different strains of mice and rats.

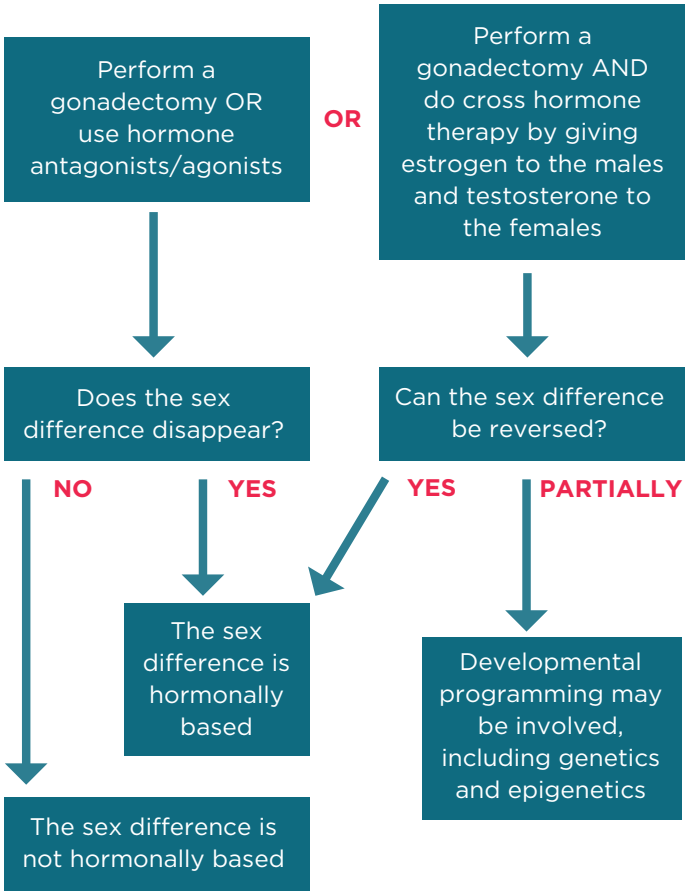
What assays do you recommend to quantify sex hormone levels in mice and rats?

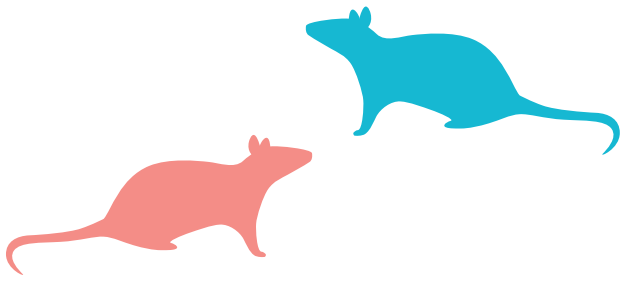
There are several assays that can be used to measure androgens, estrogens and progesterone. Consider the advantages and limitations of each and decide which method is most suitable to your research.

Method	Advantages	Limitations
ELISA	• Highly sensitive and specific • Widely available • No specialized training required • Inexpensive equipment	• Because it is antibody based, cross-reactivity is common and requires strict standardization • Only measures circulating hormone levels, not reliable in some tissues where steroids are concentrated, such as the brain
RIA	• More sensitive and specific than ELISA • Can measure hormones in blood and tissues	• Potential radiation hazards due to use of radioisotopes • Requires specialized licensing and training • Can have some cross reactivity due to being antibody-based
GC-MS	• The most precise with no cross reactivity • Although less sensitive than ELISA and RIA, sensitivity is improving • Can measure hormones in blood and tissues	• Requires the use of a core facility with specialized training • Often more expensive than other approaches

What methods do you recommend to manipulate sex hormones in male and female mice and rats?

Once a sex difference is known, there are several methods to determine if the effect is driven by sex hormones:





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What protocol do you recommend to recapitulate the estrous cycle in rats or mice following gonadectomy?

Wait one week after gonadectomy before administering exogenous sex hormones. Circulating hormones will be gone within 24 hours, but there should be enough time for hormones to be removed from the tissues. Don't wait too long or the receptors will be disturbed! The specific protocol depends on whether you are using mice or rats, and which phase of the estrous cycle you are interested in replicating.

TIP: Antagonists to gonadotrophin releasing hormone (eg. Acyline) or estrogen (eg. Tamoxifen) are suitable alternatives to gonadectomy!

RATS

For rats, subcutaneous injections can be given over a couple of days to reasonably recapitulate the estrous cycle. To measure diestrus, inject 0.3 µg of estradiol benzoate a few hours before the experimental endpoint[3]. To measure proestrus, inject between 1-10 µg of estradiol benzoate a few hours before the experimental endpoint[3,4]. If you want to maintain a simulation of the estrus cycle for an extended period, administer 2 µg estradiol benzoate every four days. To measure sexual stimulation or behaviour, this requires 10 µg estradiol benzoate, two days in a row. Then skip a day and give one injection of 1 mg progesterone and test four hours later, making sure to test in the afternoon of the dark phase of the cycle[5].

MICE

With mice, it is not possible to use injections to mimic the estrous cycle. They must be implanted with a capsule subcutaneously that will release estradiol continuously for a week or two to get chronically high estradiol levels before they become sexually receptive. This would indicate if the effect is mediated by estrogen. If it's not, then add a progesterone injection on top of the chronically high estrogen level to see if the effect is mediated by progesterone. The progesterone will have an effect within four to six hours.

What do you recommend as the best method of delivery for administering exogenous hormones to mice and rats?

There are several approaches that can be used. Consider the advantages and limitations of each and decide which method is most suitable to your research.

Method	Advantages	Limitations
Injection	• Quicker and easier to administer than pellets and silastic implants • Ideal for short-term studies	• Time-consuming approach for long-term studies • Can cause distress to animals if injected daily
Pellet	• Ideal for short-term experiments • Can be purchased commercially	• Not as reliable as injections and silastic implants • Need to do an ELISA to validate if desired hormone levels were achieved • Requires anaesthesia and surgery to administer
Silastic Implant	• Ideal for long-term studies • Reliable (delivers consistent hormone concentrations) • Have been well characterised and optimised in the literature	• Cannot be ordered commercially and must be handmade, which requires handling hazardous steroid powders • Requires anaesthesia and surgery to administer

What other advice can you provide basic scientists?

Keep in mind that good old-fashioned bioassays can be used as surrogate markers of sex hormone levels.

For **males**, seminal vesicle weight can be a good indicator of androgen levels. The higher the weight, the higher the androgen levels.

For **females**, uterine weight can be used as an indicator of estrogens in non-pregnant mice or rats. The higher the weight, the higher the estrogen levels.

The views expressed in this document are those of Margaret McCarthy and do not necessarily reflect those of the CIHR Institute of Gender of Health or the Government of Canada.

References

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