Video Article

Ovariectomy and 17β-estradiol Replacement in Rats and Mice: A Visual Demonstration

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Abstract

Estrogens are a family of female sexual hormones with an exceptionally wide spectrum of effects. When rats and mice are used in estrogen research they are commonly ovariectomized in order to ablate the rapidly cycling hormone production, replacing the 17β-estradiol exogenously. There is, however, lack of consensus regarding how the hormone should be administered to obtain physiological serum concentrations. This is crucial since the 17β-estradiol level/administration method profoundly influences the experimental results[1-3]. We have in a series of studies characterized the different modes of 17β-estradiol administration, finding that subcutaneous silastic capsules and per-oral nut-cream Nutella are superior to commercially available slow-release pellets (produced by the company Innovative Research of America) and daily injections in terms of producing physiological serum concentrations of 17β-estradiol[4-6]. Amongst the advantages of the nut-cream method, that previously has been used for buprenorphine administration[7], is that when used for estrogen administration it resembles peroral hormone replacement therapy and is non-invasive. The subcutaneous silastic capsules are convenient and produce the most stable serum concentrations. This video article contains step-by-step demonstrations of ovariectomy and 17β-estradiol hormone replacement by silastic capsules and peroral Nutella in rats and mice, followed by a discussion of important aspects of the administration procedures.

Video Link

The video component of this article can be found at https://www.jove.com/video/4013/

Protocol

1. Ovariectomy via the Dorsal Route

1. Anesthetize the animal, for example using 1.5% isoflurane (4% for induction) in an oxygen/nitrous oxide 30%/70% mixture. Place the animal in prone position on a heating plate connected to a rectal thermometer. It is important to have a thin layer of isolation between the animal and the plate, and that the thermometer feed-back system keeps the body temperature within 37 ± 0.5 °C. Apply gel for eye protection and administer 5 mg Rimadyl/kg bodyweight diluted in saline subcutaneously on the lateral aspect of the animal's neck for postoperative pain relief.
2. Shave a 4*4 cm (3*3 cm for mice) area cephally from the iliac crest. Thoroughly wash the shaved area with for example Jodopax before covering the animal with a 4*4 cm aperture surgery sheet.
3. Make a 2-3 cm midline incision and bluntly dissect the skin from the underlying fascia. One cm lateral of the midline, make another incision through the fascia. Bluntly dissect laterally under this fascia as superficially as possible until reaching the abdominal cavity.
4. Using tweezers, grab the adipose tissue that surrounds the ovary in the abdominal cavity and gently pull it out. Identify the ovary and ligate the uterine horns and vessels 0.5-1 cm proximally of this structure. Cut the ovary and ligated adipose tissue and put the remaining tissue back into the abdominal cavity. Make another incision in the contralateral fascia and repeat the procedure.
5. Close the wounds, preferably using a monofilament suture, and let the animal awake from anesthesia. Place the animal in a heated cage (25-27 °C) during at least 2 hours after surgery. The animals should be housed separately the first days postoperatively. It is pivotal that the cage is frequently cleaned/replaced during the recovery phase. Twenty-four hours after surgery, inject the animals subcutaneously with another 5 mg Rimadyl/kg bodyweight diluted in saline.

2. Subcutaneous Administration of 17β-estradiol through Silastic Capsules

1. Thoroughly mix the desired amount of 17β-estradiol in sesame oil. For physiological concentrations in rats and mice, we recommend 180 µg/mL and 18-36 µg/mL respectively. Placebo capsules are filled with pure sesame oil.
2. Cut 3 cm (2 cm for mice) lengths of silastic tubing and 5 mm (3 mm for mice) lengths of wooden applicator sticks for plugging. Using forceps, squeeze one wooden plug into the silastic capsule before injecting the 17β-estradiol/sesame oil solution to fill the entire length. Gently cap the silastic capsule in the open end with another wooden plug (the plugs are supposed to remain permanently in the capsule). Incubate the capsules in the remaining 17β-estradiol solution overnight before implantation.

3. Implantation of the capsule can be performed during the anesthesia used at ovariectomy in rats. For mice, however, a 14-day recovery period after ovariectomy is strongly recommended to minimize the risk of the capsule protruding through the fragile skin.
4. After surgical preparations as in 1.1, or after closing the ovariectomy incision, shave and wash a 2*2 cm area on the dorsal aspect of the animal's neck. Make a 1 cm incision and bluntly dissect a subcutaneous pocket caudolaterally, with ample space for the capsule. Wipe the silastic capsule (that until this time-point has been incubated in the 17β-estradiol solution) and gently install it through the incision, which is subsequently closed by a suture. It is pivotal to make sure that the capsule strains the skin as little as possible, particularly in the area close to the wound. From production of capsules to the implantation, it is important that contamination is avoided.

3. Peroral Administration of 17β-estradiol via the Nut-cream Nutella

1. Thoroughly dissolve 17β-estradiol in sesame oil, and then mix the sesame oil with the Nutella. We recommend that each daily portion contains 28 µg 17β-estradiol, 5 µL sesame oil and 1 g Nutella per kilogram body weight for rats and 1.12 µg 17β-estradiol, 0.312 µL sesame oil and 60 mg Nutella for each 30-g mouse. Placebo nut-cream is prepared identically except without the 17β-estradiol.
2. Train the animals to eat hormone-free nut-cream for five days prior to the experiment. The first three days of training, the rats should optimally be trained in groups (approximately 0.5 g Nutella to 2-5 animals), in their home cages. On the fourth and fifth days of training, the animals are put in separate cages before receiving the daily portion to resemble the experimental situation.
3. During the experiment, the animals are put in separate cages for every feeding occasion, after which the Nutella portion is served on small ceramic tiles. If training has been performed as above, our experience is that more than 95% of rats and mice accept the nut-cream, and once fully habituated, most of the animals consume it within seconds.

4. Representative Results

Physiological serum 17β-estradiol concentrations, which the current methods aim to reproduce, are approximately 5-140 pg/mL for rats and 5-35 pg/mL for mice[4,6]. The subcutaneous silastic capsules according to the instructions above, when administered to 300 g female Sprague-Dawley rats, produce slowly decreasing 17β-estradiol serum concentrations from around 40 pg/mL day 2 to 10 pg/mL day 35. However, immediately after administration, a serum concentration peak around 1000 pg/mL can be expected, falling to the physiological range within 8 hours (**Figure 1A**)[4,5]. The silastic capsules for mice described above with 36 µg 17β-estradiol/mL, when administered to 25 g female C57BL/6 mice, render serum concentrations that slowly decrease from approximately 90 pg/mL day 2 to 25 pg/mL day 35. This is probably preceded by a similar peak as was found in the rats, although this has not been investigated (**Figure 1B**)[6].

The peroral Nutella method with the dosages described above, when administered to 300 g female Sprague-Dawley rats, renders physiological levels of 17β-estradiol with daily fluctuations between 10-70 pg/mL (**Figure 2A**)[4]. For mice using the above dosage, the daily levels fluctuate between approximately 20 and 120 pg/mL (**Figure 2B**)[6].

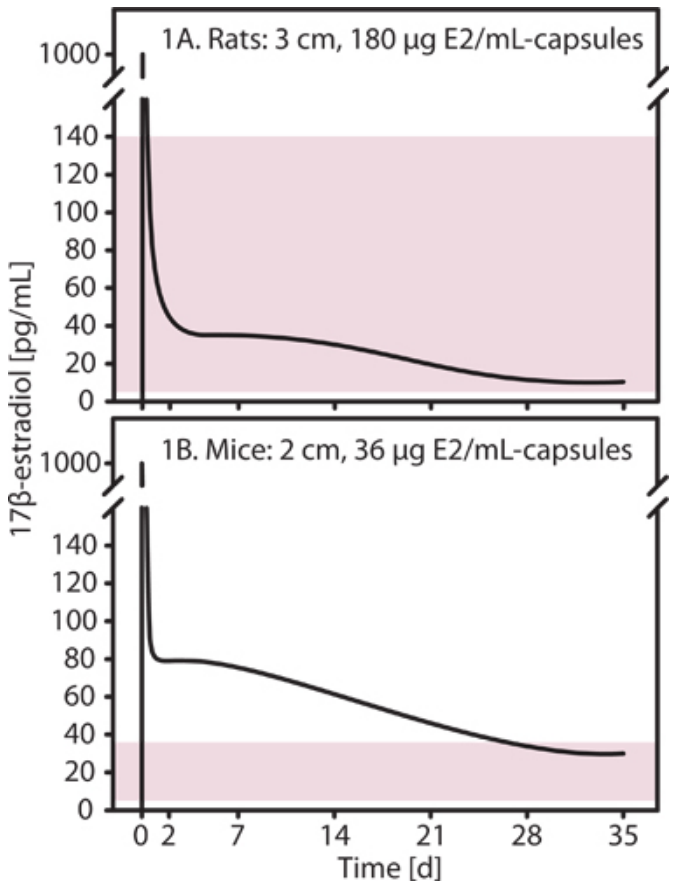

Figure 1. Stylized graphs presenting 17β-estradiol concentrations in rats (A; n=10 in time-points up to 24 h, n=15 in later time-points)[4,5] and mice (B; n=10)[6] after capsule implantation. The silastic capsules produce a short initial peak of serum concentrations up to 1000 pg/mL, but decrease within hours to levels below 100 pg/mL. For the coming 4-5 weeks, the capsules yield stable, although steadily decreasing, serum concentrations. The shaded areas correspond to physiological 17β-estradiol serum concentrations in intact female rats and mice respectively. Even though not visually demonstrated in the graphs, there is inter-individual variability in the 17β-estradiol concentrations, rendering coefficients of variation from 27 to 183 % in rats and from 28 to 115 % in mice with the silastic capsule method (very low mean concentrations often generate high coefficients of variation).

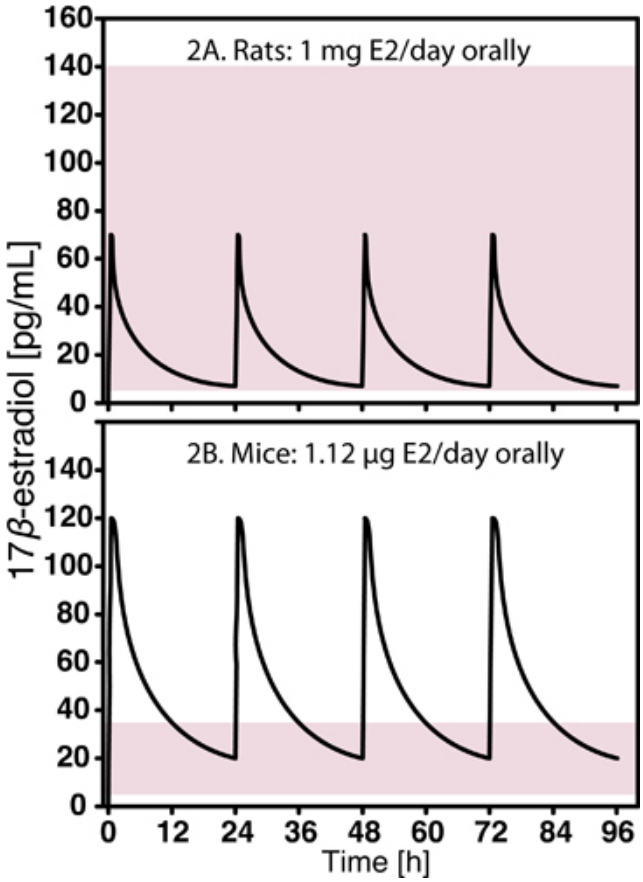

Figure 2. Stylized graphs presenting 17β-estradiol concentrations in rats (A; n=15)[4] and mice (B; n=10)[6] as a consequence of daily hormone administration in Nutella. The peroral Nutella method renders daily peaks, however not by far as high as produced by subcutaneous or intravenous methods. This mimics the pharmacokinetics of human hormone replacement therapy, and the peroral treatment can proceed for as long as needed, even if the example above only present an initial 96 hours. The shaded areas correspond to physiological 17β-estradiol serum concentrations in intact female rats and mice respectively. Even though not visually demonstrated in the graphs, there is inter-individual variability in the 17β-estradiol concentrations, rendering coefficients of variation from 40 to 132 % in rats and from 13 to 219 % in mice with the Nutella method (very low mean concentrations often generate high coefficients of variation).

Discussion

The administration modes presented above have in earlier studies proved superior to commercial slow-release pellets and injections in terms of producing physiological serum concentrations of 17β-estradiol. While the slow-release pellets produce very high and prolonged serum concentration peaks, the injections cause extreme, daily hormone fluctuations and require repeated stressful handling of the animals [4-6]. The importance of choosing adequate administration modes is highlighted by recent studies indicating that while low-dose 17β-estradiol administration regimens (such as the techniques presented in the current article) protect against cerebral ischemia [8-10], the 17β-estradiol concentrations produced by the commercial slow-release pellets may actually increase the cerebral damage [1,2,11-14]. Drinking water administration and oral gavage are two other, less frequent, methods that have been tested. Administering the hormone via drinking water has its greatest benefit in being extremely non-invasive, since almost no animal handling at all is required. However, 17β-estradiol is not soluble in water without an emulsifier, the individual water intake is difficult to control, and if the aim is to reflect the consumption of a pill, another disadvantage is that the mice drink the water during the entire 24 hours. Oral gavage has its greatest drawback in that it is stressful for the animal and may cause esophageal injury, which may affect feeding behavior.

The methods described here - silastic capsules and peroral Nutella - are both suitable for short term estrogen administration and for longer periods of up to 5-6 weeks. If higher doses are used than described above, irrespective of type of administration medium, long-term estrogen administration can cause severe side-effects such as urethral occlusion due to mucosal proliferation[15].

The methods presented here can easily be modified regarding dosage, capsule length etc. For example, it may be warranted to lower both the 17β-estradiol doses in both capsules and Nutella in the mouse regimens to more steadily achieve physiological serum concentrations[6]. However, if any modifications are made, or if the techniques are used in other strains of animals than already investigated, it is crucial to validate the methods anew by measuring 17β-estradiol concentrations in serum samples. These serum samples must be obtained at several time-points to reflect the entire dose-response relation/curve, and not just a short glimpse. Uterine weights have been used by some authors instead of serum 17β-estradiol concentrations as a measure of the biological effects of 17β-estradiol. However, evidently uterine weights reflect the specific effects of estrogens on the endometrium and do not necessarily reflect the effects of estrogens on other systems . Further, uterine weight is only weakly correlated to serum estrogen levels [4,6].

Daily consumption of large quantities of energy-rich Nutella could cause weight gain. However, the small amounts described above only correspond to less than 5% of the animals' daily energy intake and excessive weight gain has not been observed in our studies using Nutella.

The crucial difference in working with rats and mice merits special emphasis. Even though strict hygiene is pivotal regardless of animal species, mice sensitivity to infections calls for scrutiny at even higher level than when working with rats. For example, careful disinfection of the skin prior to incision, frequent change of medical gloves and the use of sterile surgical tools must not be compromised. Implants are always hotbeds for infections. The animals must therefore be regularly examined for signs of infection after capsule implantation. If an abscess is formed around the implant, we recommend that the animal is euthanized or that the capsule is removed, the wound meticulously cleaned, and another capsule reinstated through a new incision. Further, the skin on mice's backs is much more fragile than in rats, warranting two weeks of convalescence after ovariectomy before capsule administration.

The methods presented above can also be used for other substances than 17β-estradiol. However, it is important that the pharmacokinetics and dosages of each regime is carefully characterized, preferably by consecutive serum measurements of the administered substance.

Disclosures

The authors have nothing to disclose.

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