A Fit-for-Purpose Animal Model for Endocrine Studies in Female Rats

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1 Introduction

Ovariectomized females implanted with silastic capsules containing estradiol have been widely used in mechanistic endocrine studies in rats. With the elimination of endogenous estradiol, and supplementation with exogenous estradiol, this model allows for evaluation of treatment-related effects in females that are synchronized in proestrus. As reproductive hormones are also responsive to stress, handling of animals during the study is critical.

This study was designed to evaluate the use of Vascular Access Buttons™ (VAB) in lieu of traditional methods of blood collection and to determine if measurable levels of estradiol were maintained for up to 10 days post-surgery.

2 Materials and Methods

- 16 naïve Crl:CD(SD) females, surgically implanted with Vascular Access Buttons™ (Charles River Laboratories, Raleigh, NC).
- Animals were single housed, and maintained under standard environmental/food/water conditions with the exception that a 14-hour light/10-hour dark cycle was maintained.
- VABs were flushed with saline and locked with heparinized saline (5 IU/mL). Animals were periodically checked for catheter patency throughout the study period.
- Estradiol benzoate (BCBS6446V, Sigma Aldrich) was prepared at 4 mg/mL by stirring on low heat [50°C-60°C] in sesame oil. Then was added to the capsule using a plastic or glass syringe fitted with a 25 gauge 1.5 inch needle.

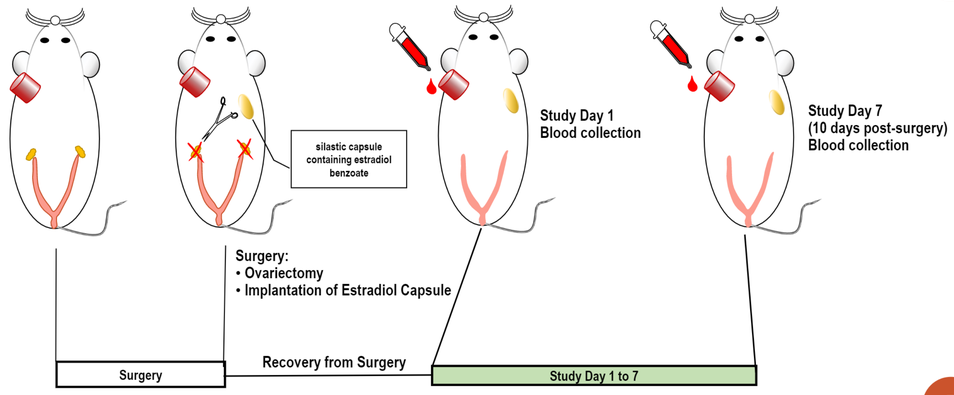
2 Materials and Methods (cont'd)

- Capsules were made from sections of silastic tubing (12-14 mm in length; Scientific Products medical grade silicon 0.062" ID x 0.125" OD) and sections of white Teflon® solid piping (2 to 4 mm in length). The solid white Teflon® piping was inserted into the ends of the silastic tubing, glued with silicone adhesive (7128 RTV Adhesive Glue, World Precision Instruments) and allowed to dry overnight.
- Prior to placing the silastic estradiol capsule into the animals, the capsules were wiped with isopropyl alcohol.
- Serum estradiol level were analyzed by Cobas e411 multichannel analyzer using a qualified electrochemiluminescence (ECL) method.

3 Establishment of the Animal Model

- A bilateral ovariectomy (resulting in the elimination of endogenous estradiol) was performed on each female. On the day of surgery, each female was anesthetized with isoflurane the hair was clipped from the dorsal back region and left and right lateral flanks. A small midline dorsal incision was made approximately halfway between the midline of the back and the base of the tail, and the ovaries were removed from the peritoneal cavity.
- A 12–14 mm long silastic capsule containing estradiol benzoate (4 mg/mL in sesame oil) was implanted subcutaneously in the right flank region. The estradiol silastic capsule was implanted to maintain serum estradiol at levels comparable to those seen in intact females and to produce a daily afternoon surge in serum prolactin levels.
- Beginning 3 days following surgery, all animals were orally administered with 0.25 mL of deionized water on a daily basis for 7 consecutive days (Study Days 1-7).
- Blood samples for serum estradiol concentration measurements were collected via the VAB. 5 blood samples per animal were collected on Study Days 1 and 7.
- The first serum sample collected on Study Day 1 for each animal and the last sample collected on Study Day 7 for each animal were analyzed for serum estradiol concentration.

Figure 1. Illustration of establishment of animal model and study design



- The following parameters were evaluated:
  - **Clinical Observations**: Daily
  - **Body Weights:** Daily
  - **Estradiol Level:** Serum samples were collected and analyzed on Study Day 1 and 7
  - **Scheduled Necropsy:** Study Day 7

4 Results

- Fifteen VAB female survived to scheduled necropsy and retained catheter patency for the duration of the study. One female died due to surgery complications.
- There were no remarkable clinical observations, body weight gains.
- On Study Day 1, individual serum estradiol concentrations ranged between 154.9 to 768.7 pg/mL with a coefficient of variation (% CV) of 59.9%.
- On Study Day 7, individual serum estradiol concentrations ranged between 49.0 and 165.5 pg/mL, with a %CV of 46.7%.
- At 10 days post-surgery, the individual serum estradiol levels were still above functional physiological level in rats (~30 pg/mL).

Table 1. Individual Serum Estradiol Levels (pg/mL)

Animal	Study Day 1	Study Day 7
5893	313.3	81.1
5895	192.5	58.9
5896	642.3	144.9
5897	266.7	53.7
5899	283.4	76.4
5901	382.7	123
5902	154.9	72.2
5903	224.3	51.4
5904	ND	64.4
8332	240	79
8333	768.7	165.5
8335	161.5	81
8336	173	51.1
8337	177.7	49
8338	533.6	163
Mean	30.6	30.2
S.D.	2.1	2.1
%CV	59.9%	46.7%
N	14	15

ND = None Detected

5 Conclusions

- This model allows for minimal handling of surgically modified animals which is important when evaluating stress responsive hormones on mechanistic endocrine and reproductive toxicity studies.