

## Theme III

## Animal welfare and refinement for high-quality science

107180

## Refinement of administration of injectable hormonal contraceptive in Wistar rats

**Couto, BB**

Fluminense Federal  
University, Niteroi, RJ,  
Brazil  
E-mail: [mptmags@yahoo.com.br](mailto:mptmags@yahoo.com.br)

Subcutaneous (sc) or intramuscular (im) injections may be used for pharmaceuticals and for materials designed to be used as implants or prostheses. Hormonal contraceptive (Mesigyna®, Schering) is im injected monthly in women. In rats, dose and frequency of administration are different from human, due to differences in metabolism, which is faster in rats. The aim of this study is to define the best route to apply injectable hormonal contraceptive (HC) in rats, taking into account animal welfare. The experimental protocol was approved by the Ethics Committee on Animal Research PROPPi/UFF (0010/2010). Thirty rats, weighing  $167.8 \pm 2.6$ g were randomly divided in four groups (n=5-10). Group 1 received one im injection/week (0.02ml) of Mesigyna® (norethisterone enanthate and estradiol valerate). Group 2 received one im injection/week (0.02ml) of sterile water. Groups 3 and 4 were similar to groups 1 and 2 respectively, but injections were sc. All animals were euthanized on day 28. Body weight and food consumption were controlled as parameters of animal welfare. Periodic vaginal cytology was performed to assess the hormonal influence. Data were compared using Kruskal-Wallis or Friedman test and Dunns post-hoc, with significant level at  $P < 0.05$ . Food intake and weight gain in im HC group was lower than other groups, and significant lower ( $P < 0.05$ ) when compared to sc control group. Hormonal stimulus by HC was similar over time by both injections route. Subcutaneous injection is better than intramuscular for HC injection in rats due to less stress, better welfare and constant hormonal effect in the animals.

**KEYWORDS:** *refinement; injection; hormonal contraceptives; rats*

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## Simple and effective non-invasive methodology to assess the effects of sex steroid hormones in Wistar rats

**Couto, BB**

Fluminense Federal  
University, Niteroi, RJ,  
Brazil  
E-mail: [mptmags@yahoo.com.br](mailto:mptmags@yahoo.com.br)

The well-known uterotrophic bioassay is an end-point test based on the increase of uterine weight in response to estrogens. Invasive procedure (blood collection) would be necessary for periodic testing of hormonal levels. The aim of this study is to validate vaginal cytology and uterine weight as a simple and effective method to monitor the hormonal effect of an injectable contraceptive in Wistar rats. The experimental protocol was approved by the Ethics Committee on Animal Research PROPPi/UFF (0010/2010). Twenty rats weighing  $174.1 \pm 3.2$ g were randomly divided in four groups (n=5). Rats were im or sc injected once a week with 0.02ml of Mesigyna (estradiol valerate and norethindrone enanthate) or placebo. Periodic vaginal cytology and final uterine weight were used as complementary and indirect parameters to assess the hormonal effect induced by steroid sex hormones. Data were compared using Mann-Whitney test, with significant level at  $P < 0.05$ . Treatment with Mesigyna showed hormonal stimulus (HS) in the vaginal cytology by im and sc injection, lasting until the end of the experiment, five and four weeks respectively. HS was characterized by vaginal smears with a mixed pattern of metestrus and proestrus. The cytological pattern we observed is consistent with the effect of Mesigyna, which is an estrogen (determining the proestrus phase) combined to a progestogen (determining the metestrus phase). Uterine weight showed significant increase ( $P < 0.05$ ) by im (7.6%) and sc (4.8%) Mesigyna treatment compared to the respective control. Uterine weight and vaginal smears morphology are sensitive non-invasive parameters to monitor the hormonal effects *in vivo*.

**KEYWORDS:** *refinement; injection; hormonal contraceptives; rats*