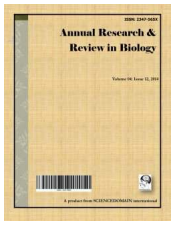

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Modified Vaginal Sampling Technique Reduces Interference on Estrous Cycle's Phases of Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: Two sampling techniques are used in preparing rodent vaginal smear. These techniques include vaginal lavage with sterile fluid using an eye dropper or a pipette and swab with moistened cotton bud with sterile fluid. The current study aims to investigate the influence of the different techniques of vaginal sampling on estrous cycle (EC) regularity.
Study Design: Four groups of adult female rats, each consisting of 10 animals, were selected for the study.
Methodology: Vaginal samples were obtained using different techniques including cotton bud, eye dropper, pipette, and modified pipette for four animal groups during six ECs.
Results: The modified pipette technique showed less influence on EC regularity in rats compared with the other techniques. The modified pipette animal group showed a significantly ($p < 0.05$) higher percentage of animals with regular ECs (98±4.1) compared with the cotton bud animal group (75.0±13.8).
Conclusion: The modified pipette technique has less interference on the EC phases of rats than the other techniques, and it does not require assistance compared with the pipette technique.

Keywords: Vaginal sampling technique; estrous cycle; rodent.

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1. INTRODUCTION

The estrous cycle (EC) in rodents has three main phases. The EC phases and length in rats have been detailed since 1918 [1]. The duration of the individual phase of EC based on the vaginal smear (VS) pattern for rats with a four-day EC was as follows: proestrus, 12 h to 14 h; estrus, 25 h to 27 h; metestrus, 6 h to 8 h; and diestrus, 55 h to 57 h. Rats with a five-day EC generally show either an extra day of estrus or an extra day of diestrus [2]. EC is regulated by sex hormones [3] and reflects the physiological functions of the reproductive system in rats. Hormonal fluctuations during EC are also similar in humans and rats [3,4]. Rat is a spontaneous ovulator with gonadotropins triggering comparable follicular and oocytic maturational changes within the ovaries. Furthermore, rats with polycystic ovaries mimic those of humans with irregular ovarian histology and menstrual cycle [5]. The EC of rats has been used to evaluate the toxicity of substances on the female reproductive system [6]. Several techniques of vaginal sampling have been commonly used in toxicological studies, and they can be classified into old and recent vaginal sampling techniques. The old techniques involve the use of an eye dropper and a cotton bud, whereas the recent technique involves the use of a pipette [7]. The current study aims to investigate the influence of the different techniques of VS on EC regularity.

2. METHODS AND MATERIALS

Sexually mature, virgin female Sprague-Dawley (SD) rats (180 g to 220 g, 11 to 12 weeks old) were obtained from the Animal Research and Service Center, Universiti Sains Malaysia (USM). The rats were housed in groups of three to four animals in each cage under ambient temperature (23℃±2℃) with a 12:12 h light–dark cycle at the Pharmacology Department of the School of Pharmaceutical Sciences, USM. The rats were fed with commercial rat diet (Gold Coin Sdn. Bhd.) and water *ad libitum*. The animal care and experimental protocols were approved by the Animal Ethics Committee of the university under reference number: USM/PPSF/50(092)Jld.2. VS was obtained daily between 9:00 am and 11:00 am [8] to monitor microscopically the cytological changes following the different phases of the cycle in rats from all groups [1,3]. After a rat had two consecutive regular 4 d to 5 d ECs, it was randomly assigned to be tested.

Experiment 1: A total of three sexually mature, virgin female SD rats (180 g to 220 g, 11 to 12 weeks old) were sacrificed. Hall reproductive system was excised, and vaginal length was established by taking the length between the vaginal orifice and the cervix of the sacrificed adult female rat. Experiment 2: A total of 40 sexually mature, virgin female SD rats (180 g to 230 g, 11 to 12 weeks old) were divided into four groups of 10 animals. The four groups of animals were assigned to different vaginal sampling techniques. Groups 1, 2, 3, and 4 were assigned to cotton bud [3], eye dropper [3], pipette [7], and modified pipette techniques, respectively. The VS of each group was observed and recorded. The stages of EC were checked and recorded daily, and the mean percentage of animals with regular EC during six ECs was calculated for each animal in each group.

The mean percentage of animals with regular EC during six ECs was obtained and analyzed by one-way ANOVA followed by Tukey's test. The level of significant difference was determined at 0.05. SPSS® ver. 15 software was used for all statistical analyses.

3. RESULTS

The length of the vagina of female rat, The distance between the vaginal orifice and the cervix was approximately 1.1 cm (see Plate 1).

Techniques of vaginal sampling, VS for regular EC female rats were taken for six ECs using four different techniques: modified pipette (T1), eye drop (T2), pipette (T3), and cotton bud (T4) (Plate 2).

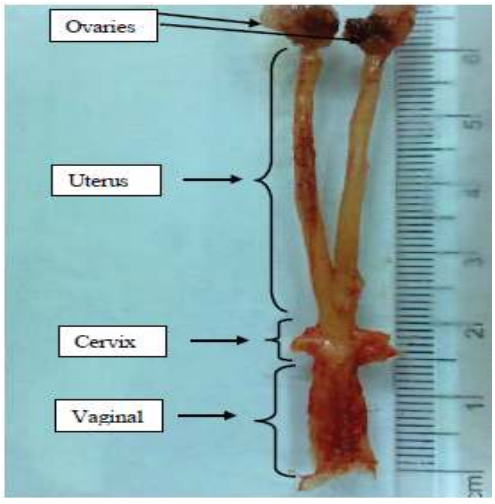

Plate 1. Photograph of reproductive tract adult female rats

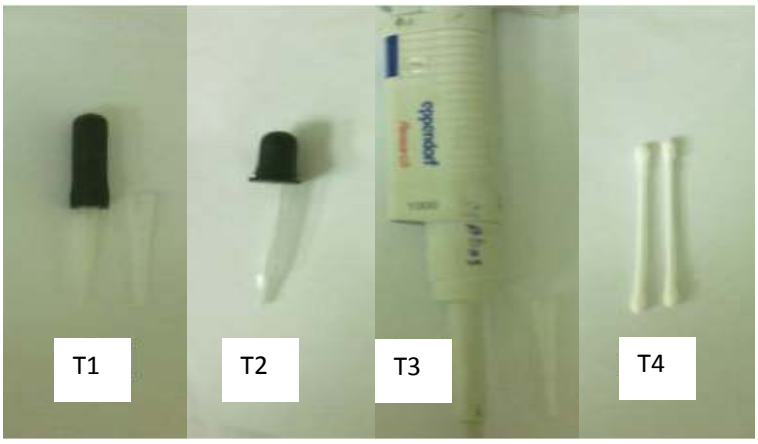

Plate 2. Photograph of techniques of vaginal smear
T1= modified a pipette; T2= eye dropper; T3= a pipette; T4= cotton bud

Vaginal sampling techniques effect on EC regularity: The mean percentage of animals with regular EC in the T1 technique animal group was significantly ($P < 0.05$) different (98.3±4.1) compared with the T4 technique animal group (75.0±13.8). The mean percentage of animals with regular EC in the T1 technique animal group did not show significance difference when compared with the other techniques (T2 81.7±16.0, T3 95.0±5.5) (Fig. 1).

4. DISCUSSION

EC represents an important tool in the study of reproductive and pharmacological aspects [2,9-11]. Assessment strategies for assigning the EC stage of rats rely on the relatively non-invasive sampling techniques to obtain VS at 24 h intervals. The vaginal sampling techniques may influence the quality of VS and can induce stress to the tested animals. The interpretation of VS becomes difficult and the stress can be affected by the EC regularity [12], which can affect the outcome. Several techniques of vaginal sampling have been reported, with each technique having its own advantages and disadvantages. Studies on the compression between different vaginal sampling techniques on EC regularity are lacking. The present study on vaginal sampling techniques was conducted by comparing modified pipette technique (T1) with three vaginal sampling techniques including eye dropper (T2), a pipette (T3), and cotton bud (T1) (Plate 2). In the first experiment, the vaginal length of the tested animals, which is the space between the vaginal orifice and the cervix, was determined to be approximately 1.1 cm (Plate 1). The animal handling procedure was followed accordingly [7] to reduce stress manipulation. A modification in the T3 technique was performed by replacing the micropipette with a rubber bulb (Plate 2). As shown in previous reports [7,11], our results confirmed that the mean duration of the EC of the rat is 4 d. In the second experiment, all animals in different groups display regular EC except group 4, in which VS was collected by the T4 technique. The repetition of the T4 technique may induce stress to the tested animals and affect the quality of VS. Sometimes, the obtained VS is unclear because the cell becomes overlapping in smear or appears broken under the microscope. The T2 technique did not affect the quality of VS nor induced stress to the tested animal. However, this technique is more time consuming than the other techniques because the dropper needs to be adequately washed between smearing to prevent inappropriate sample collection. In the T3 technique, inappropriate sample collection can be avoided and less time is required, but assistance may be required in terms of the length of the micropipette especially when the animal size is quite large and vaginal injury or cervix stimulation can occur. The T1 technique did not require assistance because of the replacement of the micropipette with a rubber bulb. The T1 technique showed less interference on the EC phases of rats when compared with the other techniques. The mean percentage of animals with regular EC is less in groups T4 (75.0±13.8), T2 (81.7±16.0), and T3 (95.0±5.5) when compared with the T1 group (98.3±4.1) but significantly ($P < 0.05$) different from the T4 group. The T1 technique is slightly aggressive and free of unnecessary stress factors.

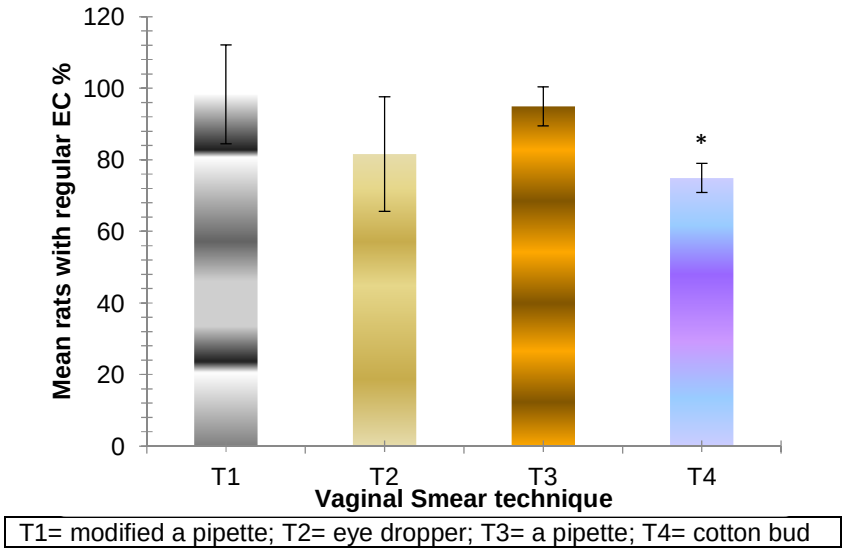

Fig. 1. Effects of different vaginal sampling techniques on estrous cycle regularity of female rats

The mean percentage of rats with regular estrous cycles (mean ± SD; each group of animal n=10) using different sampling vaginal techniques. T1= modified a pipette; T2= eye dropper; T3= a pipette; T4= cotton bud. Bars with different superscripts are significantly different at P<0.05

5. CONCLUSION

The T1 technique is a simpler and faster way of determining the reproductive status of a female subject in different experimental protocols. This technique is useful in long protocols in which the determination of EC phases is made for experiments that last for hours or all day.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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