A NEW AND SIMPLE METHOD OF CONFIRMATORY DETECTION OF MATING IN ALBINO RATS (*Rattus norvegicus*)

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ABSTRACT

A new and simple method of detecting mated female albino rats was developed and tested for precision and accuracy. The method involved the gross observation of grey to yellowish protein coagulates (remnants of the copulatory plug) on vaginal smears of mated females made on clean glass slides. Results of test of the new method showed that the mean length of time between observation of the protein coagulates on vaginal smears and delivery of the mated pregnant females was 21.39 ± 0.11 days (range = 20 – 23 days, n = 58), which concurred with the normal 21-day gestation length of rats. The coefficient of variation (CV) of imprecision of the new method was found to be 3.74 %. This new method is simple, easy to apply and does not interfere with fertilization and pregnancy, and also does not involve either the use of specially designed rat cages or microscopy of vaginal smears, which were the constraints of the former methods of confirming mating in rats.

Keywords: Albino rats, Detection of mating, New method, Vaginal smears

INTRODUCTION

Laboratory animal experimentation is an important tool for the investigation and understanding of various biological principles and study of human and animal disease mechanisms. It forms the backbone of biomedical, bio-agricultural and bio-industrial researches that enable the development of new medical and veterinary pharmaceuticals, vaccines, surgical materials and diagnostic techniques, and also the investigation of experimental diseases as models of most human and animal diseases (CCAC, 1984; NIH, 1999a; Gallagher, 2003). The rat is the most widely studied experimental animal because the rat model possesses enormous strengths and versatility of application that have made it the most appropriate and almost indispensable animal model for the study of human biological mechanisms and diseases (NIH, 1999b; Gallagher, 2003). Presently rats comprise more than 28 % of laboratory animals and provide important animal models for almost every aspect of biomedical and behavioural research, including reproductive physiology and behaviour, repro-toxicology and reproductive diseases (CCAC, 1984; NIH, 1999b).

Rats are the most preferred experimental animals for reproductive studies because of numerous reasons, which include their short gestation length (20 – 22 days), short oestrous cycle (4 – 5 days), litter size of about 7 – 9, weaning age of about 21 days and a relative short period/age (7 – 8 weeks) of sexual maturity (Hafez, 1970; Baker *et al.,* 1980). In addition, rat pregnancies are more size consistent (compared to mouse), rat cycling is relatively non-pheromonal (similar to humans), rats can be bred quickly after parturition, and rat brains show early sexual dimorphism (NIH, 1999b).

In reproductive studies, it is usually necessary to precisely and accurately date and time the sex act in order to estimate various pregnancy and birth expectations and also to know the number of matings that occur before pregnancy results. Mating is not always easy to judge, and it is not always practical to observe the copulatory act, which usually may be nocturnal (Hafez, 1970; Inglis, 1980). Routine confirmation of mating in the rat is therefore usually made by checking for the presence of spermatozoa in a vaginal lavage or by visualization of the copulatory plug (Bennett and Vickery, 1970; Berthelot, 1981). Checking of spermatozoa in vaginal lavage is a technical and time consuming procedure involving microscopy, while visualization of the copulatory plug can only be carried out using specially designed single-rat cages that permit the copulatory plug to fall through the floor mesh on to a tray beneath – in some cases the plug may for a variety of reasons not be found (Bennett and Vickery, 1970; Mathews and Adler, 1978; Inglis, 1980; Berthelot, 1981). In the present study, we describe a method of confirming mating in rats by grossly visualizing remnants of the copulatory plug on vaginal smears made on glass slides, without using specially designed cages or microscopy procedures. The development of this new method was based on the

fact that the copulatory plug is a coagulated mass of proteins (Mathews and Adler, 1978; Voss, 1979; Seitz and Aumuller, 1990; Carballada and Esponda, 1993), and that even the smallest remains can be picked up by a vaginal swab and be grossly visualised on clean glass slides.

MATERIALS AND METHODS

The experimental rats used for the study were the out-bred strain of the Sprague-Dawley (SD) albino rats. Eighty two (82) matured SD rats comprising of 70 females of 12 - 14 weeks of age and 12 males of 14 - 16 weeks of age, procured from and maintained in the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The rats weighed 150 – 170 g at the time of commencement of the study. They were kept in standard clean rat cages, fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited, Sapele) containing 16 % crude protein, and supplied with clean drinking water all through the study.

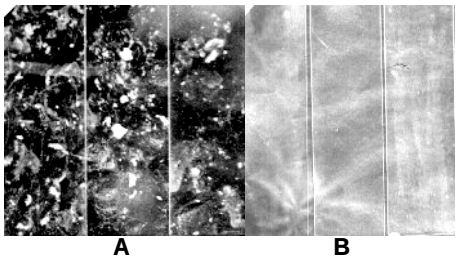
The seventy (70) female rats were randomly distributed into 14 cages such that each cage contained 5 female rats. Each of the rats was identified with an indelible marker. After the distribution and identification of the female rats, a vaginal smear of each of them was made on a labelled clean glass slide. The smear was collected by carefully inserting a cotton-tipped swab moistened with normal saline into the vaginal cavity of the rats. The swabs used measured about 7.5 cm in length with the cotton-tipped end of 1.5 cm circumference, almost the same dimensions as the typical “cotton buds’ used in cleaning the inside of the ear in humans. The swabs were applied gently against the vaginal wall and rolled around carefully before being withdrawn. Immediately after withdrawal from the vaginal cavity, the moist swab was rolled / smeared onto a labelled clean glass slide. The smear was observed grossly to check for the presence of protein coagulates (remnants of the copulatory plug).

After the initial smears were collected from the female rats, the 12 male rats were randomly distributed into 12 out of the 14 cages, such that the male:female ratio in these 12 cages was 1:5. No males were introduced into the two remaining cages that contained 10 females that served as control. After the introduction of males into the 12 mating cages, vaginal smears were made as described above for each of the females in all the 14 cages twice a day (6.30 am in the morning and 6.30 pm in the evening), and the smeared slides were observed grossly for protein coagulates. The observation of grossly visible protein coagulates on the vaginal smear of each female was recorded as evidence of mating. Pregnancy in the mated females was followed up individually in the cages such that already mated females could be re-mated if the initial mating did not result in pregnancy. Once the protein coagulates were observed on the vaginal smear of each rat, the rat was thereafter weighed at four-day intervals to check the progress of the pregnancy. Obviously pregnant ones were removed from the mating cages at the last trimester of pregnancy when their abdomen was found to be conspicuously enlarged; they were kept in single-rat nursing cages for delivery. Pregnant females kept in nursing cages were observed twice daily to record the date of delivery. For each rat, the day that the protein coagulates were found on their vaginal smear (day of mating) was regarded as day 1 of pregnancy and the day of delivery was taken as the last day of gestation. The number of matings that resulted in pregnancy was also recorded for each female rat.

Data generated from the study were presented as frequency tables along with means with standard deviations. The mean body weight of the mated-pregnant rats was compared with that of the unmated-controls using students t - test. The precision and accuracy of the procedures used was determined by computing the coefficient of variation (CV) of imprecision around the mean gestation period.

RESULTS

Gross observation of the initial vaginal smears made before introducing the males into the mating cages showed no protein coagulates, but smears made from females in the mating cages showed the presence of grey to yellowish protein coagulates (Figure 1) in about 18 out of the 60 females exposed to males within the first 24 hours of introducing males into the mating cages.



A **B**

Figure 1: Protein coagulates observed on slides on which vaginal smears of mated rats were made (3 slides on the left labelled A); compare with the clear slides with no protein coagulates on the right (3 slides labelled B) on which vaginal smears of unmated control rats that were not exposed to males were made

By the second day of exposure to males, 15 more females showed protein coagulates on their vaginal smears. The observation of protein coagulates on the vaginal smears of females exposed to males continued, and by day 5 of exposure to males, protein coagulates had been observed in the vaginal smear of all the 60 females that were exposed to males. All through the study period, the observation of these protein coagulates in vaginal smears made from females exposed to males was found to be consistent, and this was never observed in the

vaginal smears of the 10 control rats which were not exposed to males. The number of times these protein coagulates were observed on the vaginal smears of individual female rats exposed to males (number of mating before pregnancy) ranged from once to three times with a mean of 1.21 ± 0.45 times (Table 1).

Table 1: Frequency table showing the number of times protein coagulates were observed on the vaginal smear of female rats exposed to males (number of mating before pregnancy resulted)

No. of times that protein coagulates were observed on vaginal smears	Number of females
1	47
2	10
3	1

Mean = 1.21; Standard deviation = 0.45; Mode = 1; Median = 1; n = 58

Out of the 60 female rats exposed to males, 58 (96.7 %) became pregnant and delivered, while 2 (3.3 %) had pseudo-pregnancy. None of the control rats that were not exposed to males showed any signs of pregnancy, pseudo-pregnancy or delivery. The time period between the last observation of protein coagulates on the vaginal smears of those that became pregnant and the day of delivery (gestation period) ranged from 20 – 23 days with a mean of 21.39 ± 0.80 days (Table 2). The mode and median of the gestation lengths were both 21 days respectively.

Table 2: Frequency table showing the period of time between grossly observing protein coagulates on vaginal smears and the day of delivery (gestation period) of the female rats exposed to males

Time period (days)	No. of females
20	6
21	29
22	18
23	5

Mean = 21.39 days; Standard deviation = 0.80; Mode = 21 days; Median = 21; n = 58

In assessing the precision and accuracy of this method of detecting mating, the CV of imprecision computed around the mean gestation period was calculated to be 3.74 % for the 58 females that got pregnant and delivered.

The mean body weights of the mated-pregnant female rats were found to be significantly higher ($P < 0.01$) than that of the unmated controls from day 8 post-observation of protein coagulates on vaginal smear (post-mating) and was consistently significantly higher ($P < 0.01$) until delivery (Figure 2). No significant difference ($P > 0.05$) was observed between the mean body weights of the mated and unmated controls during the first four days post-observation of protein coagulates.

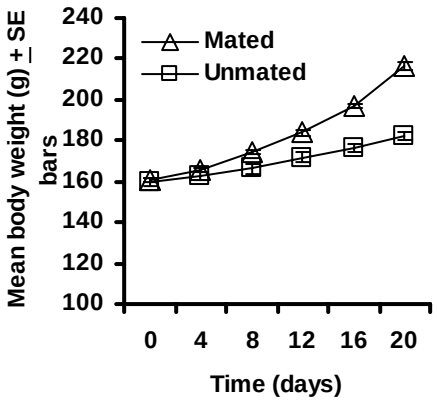


Figure 2: Comparison of the body weights of the mated and unnmated rats

DISCUSSION

The copulatory plug, also known as the vaginal plug, is a white or grey to yellowish waxy coagulated mass of proteins which is usually deposited by males in the female reproductive tract at mating in some mammalian species including rats; it is formed by the secretions of the male accessory sex gland (Bennett and Vickery, 1970; Seitz and Aumuller, 1990; Carballada and Esponda, 1993; Ramm *et al.,* 2005). The plug had been reported to function in the "enforcement of chastity" (Voss, 1979) and stimulation of sperm transport to the uterus (Matthew and Adler, 1978; Carballada and Esponda, 1992). The plug usually fills the vagina from the vulva to the cervix, but soon after deposition shrinks and falls out and can be observed when rats are kept in specially designed cages that allow the plug to fall off without being soiled by faeces (Bennett and Vickery, 1970; Inglis, 1980; Berthelot, 1981). The present study had shown that remnants of this copulatory plug could be grossly observed on smears made from vaginal swabs of mated female rats. The mean time period of 21.39 days (range of 20 - 23 days) between the observation of the protein coagulates on vaginal smears and delivery of the pregnant rats concurred with the normal 21-day gestation period (range of 20 – 22 days) of albino rats (Hafez 1970; Inglis, 1980). The observation of the protein coagulates on vaginal smears of mated female rats in this study is a confirmatory indicator of mating. This method of detecting mating could also be said to be precise and accurate with the CV of imprecision being 3.74 % (less than 5 %).

The collection of vaginal swabs for making the smears did not interfere with fertilization and pregnancy as indicated by the fact that 96.7 % (58 out of 60) of the mated females from whom swabs were collected became pregnant and delivered their offspring.

Two out of the 60 mated females (3.3 %) exhibited pseudo-pregnancy – a post-mating state in which female rats exhibit signs and endocrine

changes associated with pregnancy when in the actual sense they are not pregnant (Kovacic, 1970; Inglis, 1980). Pseudo-pregnancy in rats is caused by infertile mating following insufficient intromissions that prolong the life / action of the corpus luteum and thus the diestrus period up to 13 days (Wilson *et al.,* 1965; Bennett and Vickery, 1970; Renfree, 1994).

The finding in this study of a significant increase in the body weight of the mated-pregnant rats when compared to the unmated controls from day 8 post-mating validates earlier claims that monitoring body weight changes could be used to detect pregnancy in these species (Hendricks and Houston, 1970; Inglis, 1980; Grant, 2006).

In conclusion, it can be stated that this new method of detecting mating by observing protein coagulates on vaginal smears made on glass slides is simple, precise and accurate. It overcomes the constraints of the former methods of confirming mating as it neither involves the use of specially designed cages nor does it require microscopy of vaginal smears.

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