



## **Determination of Commencement of Gestation from the Vaginal Cytology in the Wistar Rats**

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### **ABSTRACT**

The process of confirmation of pregnancy and commencement of gestational dating in the laboratory animals is a fundamental and vital procedural step particularly necessary in the course of reproductive or developmental research; but most of the commonly employed methods for this exercise had been proving vague and disappointing both to the young and the experienced researchers. The changes in the vaginal epithelia cytology during the estrus cycle appear to be more accurate approach to monitor the ovarian activities, and hence, more reliable, and this is presented here and discussed.

**Keyword:** Vaginal Cytology, Estrus Cycle, Gestation, Rats

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Vaginal cytology evaluation means the use of wet vaginal cell smears to determine the phase of a test animal's estrous cycle and in the rat, common cell types found in the smears correlate well with various stages of the estrous cycle and to changes occurring in the reproductive tract.

Confirmation of pregnancy and commencement of gestation in the laboratory animals is usually of great importance as it is one of the fundamental experimental processes particularly in reproductive or developmental biology; and this is, to some novice and even the experienced workers, at times, cumbersome to do. Some of the procedures commonly employed include among others, smear of vaginal swab to observe presence of sperm, gradual increase in weight, protrusion or reddening of breast teats and occasional vaginal bleeding days after mating. However, the reliance on these observations had often proven to be more of speculative and not error-free even to the keenest observers and well experienced workers, and as such, and accurate or more reliable method for confirmation of conception remains a lingering matter of concern.

In non-seasonal or continuous breeders such as rats, sexual cycles are repeated throughout the year; and moreover, such non-primates permit copulation only at definite period within the cycle, called 'heat' or estrus

phase, and the rest phases between commencements of one estrus to the commencement of the next makes an estrus cycle. During the other phases, male advances are repelled by the females. Heat or estrus phase coincides with the peak of ovarian follicle development and hence, ovulation. In mammalian females such as rat, spontaneous ovulation is a cyclically repeated event, which normally occurs at regular intervals except during pregnancy; this differs from induced ovulation which normally occurs in animals such as cats, rabbits, following penile stimulation of the cervix. The records of physical activities during estrus cycle, which though had been well documented in the rodents plus a keen monitor of the changes in the vaginal epithelia cytology without surgery make it possible to correlate with the concurrent ovarian events, which appears to be a better reference procedure for the formation of pregnancy and this is here simplified and presented in the wistar rat, a common laboratory animal (Papanicolau 1954, Long and Evens 1922, Brambell 1956, Nalbandov 1954, Eddy and Walter 1969, Prakkal 1973, Ofordile and Ngokere 2003, Blandau 1978).

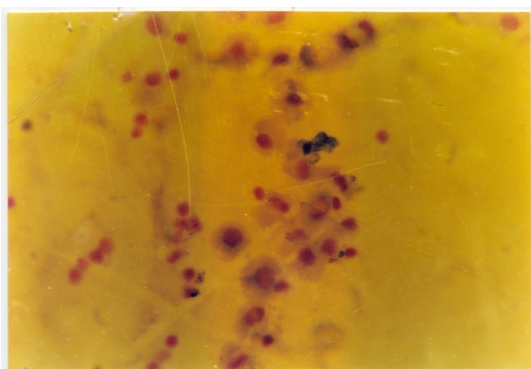
### **MATERIALS AND METHODS**

Twenty five mature wistar rats weighting between 200g and 250g comprising five males and fifteen females were procured and caged in

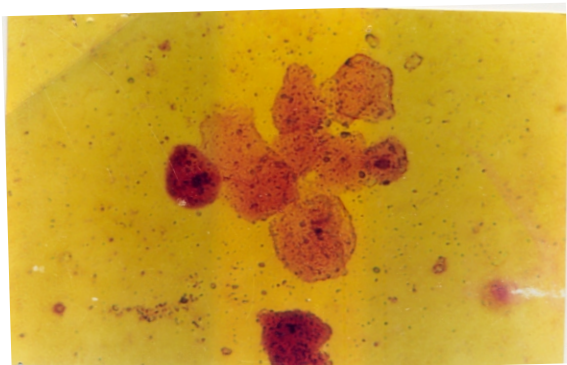
fives (4 females + 1 males) overnight for mating and all the behaviours normally associated with the oestrus cycle were keenly noted (Fraser, 1980, Adebisi 1995). To assess animals at which phase in the cycle, early the next morning the vaginal epithelia were carefully swabbed with a small stick wand with cotton at one end, and used to obtain specimen of the cells. This was smeared on clean slides, air-dried and processed for staining according to the methods of Asling (1960) and the experiment was continued in the animals till each attained the estrus phase.

## RESULT

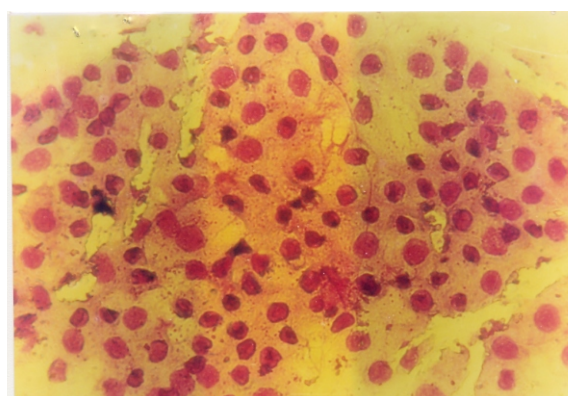
Fig 1-3 show the respective cytology of the vaginal epithelia at the 3 main phases of estrus periods in the rat: proestrus, estrus and diestrus. In those undergoing estrus, increased physical activity, body temperature plus restlessness, but receptiveness to males were observed. The characteristic appearance of the cells coupled with the typical cyclic behaviours at each stage is indicative or betrays an ongoing process in the animal.



**Fig 1: Cytology of vaginal epithelia: Proestrus period**



**Fig 2: cytology of vaginal epithelia: Estrus period**



**Fig 3: Cytology of vaginal epithelia: diestrus period**

**Table 1: Temperature range and the estrus periods in the rats.**

| No. of rats | Temperature Range(°C) | Estrus Period (Hrs) |
|-------------|-----------------------|---------------------|
| 3           | 35.91                 | 24                  |
| 5           | 36.03                 | 18                  |
| 4           | 37.52                 | 12                  |
| 5           | 36.82                 | 24                  |
| 3           | 37.03                 | 18                  |

## DISCUSSION

The phenomenon of estrus is one of the very crucial bio-physiological event during the reproductive process in the female rat in particular and non-primate in general as ovulation marks the peak of activity, while copulation within hours of this period leads to possible conception. Rats typically have rapid cycle times of 4 to 5 days. Although they ovulate spontaneously, they do not develop a fully functioning corpus luteum unless they receive coital stimulation. Fertile mating leads to pregnancy in this way, but infertile mating leads to a state of pseudo-pregnancy which lasts about 10 days. Mice and hamsters have similar behaviour (Marc 1994). The events of the cycle are strongly influenced by lighting periodicity (Heap 1900). As set of follicles start to develop near the end of pro-estrus and grow at a nearly constant rate until the beginning of the subsequent estrus when the growth rates accelerate eightfold. They then ovulate about 109 hours after starting growth. Oestrogen peaks at about 11am on the day of pro-estrus. Between then and midnight there is a surge in

progesterone, LH and FSH, and ovulation occurs at about 4am on the next, estrus day. The following day, metestrus, is called early diestrus or diestrus 1 by some authors. During this day the corpus lutea grow to a maximal volume, achieved within 24 hours of ovulation. They remain at that size for 3 days, halve in size before the metestrus of the next cycle and then shrink abruptly before estrus of the cycle after that, thus the ovaries of cycling rats contain three different sets of corpora lutea at different phases of development (Toshinaga 1973).

The concurrent alterations in vaginal epithelia cytology monitored during the cycle is thus a useful indicator to first, identify animals undergoing estrus; and secondly to ascertain in particular, the release to ovum and confirms pregnancy if copulation occurs within the 6-8 hours that it lasts in the rat. This marks the zero hour/day and plate 3 indicates the ovulatory stage with non-nucleated scale-like epithelia; and a marked spontaneous rise in temperature and physical restlessness which gradually receded<sup>4</sup>. It was observed that 11 out of the 20 female rats were in estrus the first night and the rest by the second night as shown in their vaginal cytology, and all were confirmed pregnant and littered 21 or 22 days later: affirming the reliability of the method, and thus prescribing its preference over the others which are rather presumptive. The accuracy of this method compared to the other methods that most times ended in disappointments, had also been attested to by other workers in our and other laboratories while recalling their experiences over the years (Adebisi 2002, 2003, 2006). More so, it is anticipated that this rather more scientific approach will receive better and wider acceptance as a reference laboratory procedure in confirming pregnancy in the rodents, and rat in particular. The characteristic disposition of the vagina cells during estrus cycle in this species is a replica of such cytological changes in the uterus during menstrual cycle in the primates, which are in response to hormonal control, mainly estrogen and progesterone. The mechanism of ovulation which is the ultimate event in the ovary is

though not yet well understood, but it is known to be an oozing, and not an explosive process initiated by the total amount of circulating leutenizing hormone, (LH) which decreases as follicle matures, causing constriction of the capillaries with a drastic decrease blood flow through the follicle. Within hours, a small tear appears in a corner of this apparent ischemic follicle and the ovum bulges though it, and a times copulation results in a possible conception. Those ovarian events can be diagnosed by simple non-surgical techniques since it has been observed that vaginal epithelial does not remain constant during estrus cycle as it is cyclically torn down and rebuilt, fluctuating between stratified squamous and cuboidal cell type. These changes are monitored by the vaginal smear technique in which the debris accumulated in vaginal lumen are stained and examined under the microscope. The types of cell predominating in the smear at any point in time indicate which phase of estrus is the animal. The technique is most useful particularly with animals like rat having short estrus cycles and in them, the vaginal cytology reflects ovarian events most accurately (MacDonald 1980, Asdell 1946, Eckstein and Zuckerman 1956). The estrus cycle comprises the recurring physiologic changes that are induced by reproductive hormones in most mammalian females. Humans undergo a menstrual cycle instead. Estrous cycles start after puberty in sexually mature females and are interrupted by anestrous phases. Typically estrous cycles continue until death. Some animals may display bloody vaginal discharge, often mistaken for menstruation also called a "period". Mammals shares the same reproductive system, including the regulatory hypothalamic system that release ganadotropin releasing hormone in pulses, the pituitary that secretes follicle stimulating hormone and luteinizing hormone, and the ovary itself releases sex hormones including estrgens and progesterone. However, species vary significantly in the detailed functioning. One difference is that animals that have estrous cycles reabsorb the endometrium if conception

does not occur during that cycle. Animals that have menstrual cycles shed the endometrium through menstruation instead. Another difference is sexually activity. In species with estrous cycles, females are generally only sexually active during the estrus phase of their cycle. This is also referred to as being "in heat". In contrast, females of species with menstrual cycles can be sexually active at any time in their cycle, even when they are not about to ovulate. Humans, unlike some other species, do not have any obvious external signs to signal estral receptivity at ovulation concealed ovulation. Recent research suggests, however, that women tend to have more sexual thoughts and are far more prone to sexual activity right before ovulation (Geoffrey 2007).

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