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Determination of the Ovulation Time in the Laboratory Rats (*Rattus norvegicus*)

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Abstract

The rat has been elected as the main animal model in different studies involving reproduction. However, there are scarce and conflicting data related to its estrous cycle. The aim of the experiment was to determine the time of ovulation in the primiparous laboratory rats (*Rattus Norvegicus*) by counting the number of graafian follicles present in the ovary at the time of oestrus, determining the percentage of these follicles that eventually ovulate and determining the 'spread' of ovulation during the oestrus period. Fifty (50) albino laboratory rats were observed in oestrus with the help of males to determine the time the female first stood to be mounted. This time was considered the onset of oestrus and used as a landmark for timing of oestrus period. From this onset of oestrus, the period was divided into 10 x 1-hour intervals into which the female rats were grouped. Each hour interval had five rat members. Ovaries of rats harvested at the end of their group-hour intervals were studied histologically, for functional structures. Observed structures were counted for the calculation of ovulation rates. All oestrus rats had far shorter periods, the ovulation in this study were found to be widespread from the onset to the tenth hour peaking maximally at the sixth and seventh hour of oestrous. Follicular activity was found to be more in the left ovaries while ovulatory activity was more in the right ovaries. It was concluded that rats presented far shorter oestrus periods and all ovulations in the albino rats used in our laboratory occurred at the onset of oestrus and that the mechanism responsible for the first "stance for mounting" is responsible for the ovulation and that ovulation is induced in these rats.

Keywords: Corpora lutea, Histology, Rats, Estrous, Ovulation

Introduction

The rat has been elected as the main animal model in several studies involving reproduction. However, there are scarce and conflicting data related to its estrous cycle. It comprises phases characterized by different cell types in vaginal smears (proestrus, estrus, metestrus and diestrus) (1, 2). Ovulation is the release of matured eggs from the ovaries. It is the culminating effect of the event of the oestrous cycle which is the rupture of the follicle and the shedding of the ovum (2). Since the laboratory rat *(Rattus norvigecus*) is the most used animal in various scientific researches worldwide especially in reproductive analysis, the improvement of its reproductive results and fertility rates seems to be a worthy objective. The basic principles for detection of ovulation in warm climates are similar to those for cool or temperate climates (3).

Rats which have been adapted to tropical conditions are generally considered to have a regular oestrous cycle. Basic information concerning the spread and timing of ovulation and the optimum time of mating is needed to obtain maximum fertility both in natural and in reproductive research purposes. Information relative to such needs are lacking in Nigerian laboratories. Accurate detection of ovulation and its correct timing are, two prerequisites for the successful use of laboratory rats as reproductive research models. Behavioural observations measuring ovulatory manifestations and adequate description of the behavioural characteristics known as oestrous ('heat') should be provided in the studies of ovulation. Studies have shown that in rats as well as other animals, conception will only occur in those animals that are ovulating. Detection of the onset of ovulation, the seasonal patterns of rats' reproduction and breeding behavior are remarkably varied, therefore, it is very important to achieving high conception rates. However, detection of the onset of ovulation is not very easy, particularly in the tropics (4).

Therefore, the goal of this present investigation is to study the ovulation time in primiparous laboratory rats through the counting the number of graafian follicles present in the ovary at the time of oestrus, determining the percentage of these follicles that eventually ovulate, determining the 'spread' of ovulation during the oestrus period.

Materials and Methods

Animals

For this study, 50 albino laboratory female rats weighing between 110g - 185g and some males, bred in Theriogenology laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, were used. Selected female rats must have whelped. Approval for this study was sought and gotten

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from the Ahmadu Bello University Committee for animal use and care. Rats were housed separately and used for the detection of oestrus in the females. All rats were kept in plastic shoes-box cages covered with wire mesh tops, fitted with drinkers. Each cage was bedded with clean wood shavings. The work was carried out in Theriogenology laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.

Feeding

The rats were fed with homemade pellets prepared with commercial chicken feed produced by feed masters company limited, composed of protein (4%), fats (2%) calcium (1%) phosphorus (0.4%) lysine (0.55%) methionine (0.25) with net energy of 2500 cal. /kg, palm oil as a source of vitamin A. The feed components were thoroughly mixed together and made into paste by adding water, and moulded into pellets. The pellets were dried in an electric oven at a temperature of 150 °C. Dried pellets and water were given to the rats ad libitum and continued till the end of study. Drinking water was provided in sipper bottles suspended with the feed on the wire mesh. Other materials used are; recording materials, cotton buds, physiological saline, cotton wool, diethyl ether, slides, microscope, specimen bottles, dissecting kit, masking tape for sticking labels on cages, weighing scale.

Experimental Groups and Behaviour

The female rats were divided into 10 groups (n=5), each group corresponding to one of one to 10 equal intervals of the oestrus period, which is approximately one hour. For all groups, oestrus was detected in each female by the use of males. The first time the female stands to be mounted is the onset of oestrus.

Detection of Oestrus

From personal communication, oestrus is known in albino rats, in the Theriogenology Laboratory where this work was carried to occur between 4:00pm and early hours of the morning. Therefore, male rats were introduced into the female cages shortly before 4:00pm daily and observed for behaviour and mating. Females rats pursued vigorously and standing to be mounted by the males were taken to be in oestrus. The time when females being pursued stood to be mounted was recorded. Groups of females were anaesthetized with diethyl ether inhalation at the end of the groups time interval, which was one hour, two hours, three hours and 10 hours for the respective groups.

Vaginal Cytology

Rats were restrained and cotton buds wet with normal saline was gently inserted into the vagina until it reached the anterior. Then, the bud was rolled once in the vagina for a swab and removed to prevent hyperstimulation which can lead to pseudo-pregnancy. The swab was placed on a clean microscope slide and rolled to achieve a smear. The smear was allowed to dry, dried slides were observed under the microscope for the cell types (5).

Restraints during Handling of Rats

For vaginal smear collection, rats were placed on a table with the palm of the left hand placed over the dorsum, with a slight pressure, the thumb and forefinger were a used to pull the base of the tail backwards, this prevented the rats from struggling and it exposed the vaginal opening for the insertion of the swab.

For Rats Dissection and harvest of the ovaries, the rats were caught in the palm of the left hand with the thumb resting between the forelimbs and under the jaw to prevent bites, then a chunk of cotton wool soaked in ether was placed over the nostrils and mouth to prevent breathing of air until anaesthesia was achieved.

Confirmation of Ovulation

At the end of each group time interval, individuals were anesthetized. The ovaries were removed and pooled together for each time group. The left ovaries were kept separately from the right. Harvested ovaries cleaned of fat and other tissues were Fixed in Bouins solution for twenty four hours and then transferred to 70% alcohol. Ovaries were embedded, sectioned and stained for histological examination. The ovaries were serially sectioned at 5μm thick and five to six sections of each ovary picked at regular intervals in the series was placed, in sequence, on a slide for examination (6).

Analysis of ovulation rate

The graafian follicles and corpora lutea were counted in the sections from each ovary and pooled, for left and right per time group of rats. For each pool, ovulation rate was calculated as**:**

$$\frac{a}{a+b} \times 100$$

where *a* is the total number of corpora lutea counted per group and *b* is the total number of Graafian follicles still present in the same ovaries.

Graphical Representation of Data

Proportionate number of ova released per group of rats or per time was plotted. This showed the relationship between the number of ova released and the time of their release during the oestrus period. The peak of the curve now represented the best time of ovulation. Time from the onset of oestrus corresponding to the peak of the curve will be recommended for use in the determination of ovulation time in albino rats.

Results

Oestrus period or heat was observed in all the fifty rats used in this study as the "female" standing to be mounted by the male. It was observed that the vagina at this time is wide open, pinkish and moist. Prior to standing for mounting, there is usually a period of vigorous running activity during which the male “disturbs” the female and the female resists mating. The period was considered the transition period from proestrus to oestrus in this study. This activity starts at different times for different female individuals but rarely before 1600 hours in the laboratory where this work was done.

From the longer time groups of 5 to 10 hours, the oestrus activity was observed not to last longer than 4 to 5 hours for each individual. From the time the females stands to be mounted for the first time, running activity reduces or stops as she remains to be severally mounted by the same male, or several others until intromission and ejaculation is achieved. There are usually several mountings before intromission and ejaculation is achieved.

Vaginal Cytology

Vaginal smears taken at the time of first intromission presented only cornified superficial cells in all individuals. This cellular constitution did not change in any group of individuals when it was taken again at the end of the group's time period.

Functional Structures of the Ovaries

The data on Graafian follicles (GF) and corpora lutea (CL) counted from the sectioned ovaries of 50 rats are presented in Tables' 1 - 3 and Figure 1. Contrary to expectation, some individuals did not present any functional structures at all on sectioning. Only group one had all the members presenting functional structures with two only on the right ovaries. One member each in group 2, 6, 9 and 10, and two each from 3, 4, 5, 7 and 8, making a total of 14 rats out of 50, did not present any GF or CL in both right ovaries (RO) and left ovaries (LO).

Out of 36 rats with apparent ovarian activity, 11 (30.6 %), 13 (36 %) and 12 (33.3 %) were active only on the RO, LO and both ovaries respectively. However, out of the 36, only 26 (72 %) showed any signs of ovulation in their ovary sections with rates ranging from 33 % to 100 % in different groups: All the members of groups six and seven and a couple of individual members in groups 1, 2, 3, 4, 5, 8 and 10, making 15 individuals presented 100 % ovulations of all GFs developed. On the other hand, the entire number of rats in group nine and another couple of individuals from groups 1, 3, 4 and 5 making a total of nine rats presented zero ovulations of all the GFs observed. Both extremes included all categories of active rats.

A total of 78 GFs (36 RO, 42 LO) with 47 (60 %; 23 RO and 24 LO) becoming CLs were obtained from a pool of all the rats. Ovulation rates pooled for active only on the ROs or LOs were 58 % (11 CLs of 19 GFs) and 54 % (13 CLs of 24 GFs) respectively. Of the 12 rats active on both ovaries, ovulation rates were spread as 0 %, 33 %, 43 % 50 %, 67 %, 80 % and 100 % in 1, 2, 1, 3, 2, 1 and 2 rats respectively. Total average for ovulation rate in this study is 60 %.

Discussion

This study was aimed at pegging the ovulation time for rats in our local laboratory for greater usefulness as reproductive research models. Ovulation occurs within the oestrus period in most mammals (6) and has been reported to occur at 8-10 hours into oestrus in rats with a heat period of 12-15 hours (2, 5). Though no data was collected on the heat period of the laboratory rat in this study, it appeared to be much shorter than 12 hours. This is in contrast with Paccola *et al*., (1) that found out that the ovulation was 12 hours with an average duration of estrous cycle ranges from 3.5 to 5.5 days. This substantiates the need for knowledge of the exact ovulation time in the laboratory rats used in our own environment. From the tables and the figure presented, it is clear that ovulation time is not pegged in the laboratory albino rat, at least in the laboratory where the work was carried out. It is more or less a spread from the onset of oestrus (7). From the high ovulation rate observed in the first hour (72%), it is possible that the rest of the groups could have ovulated in the first hour also.

It is also confusing, that about 33% of the rats that participated in vigorous sexual activity and were confirmed to be in oestrus by vaginal smear examination could neither present CLs nor GFs . Well, Krinke (8) reported two types of cycle in the rats such as a cycle with a luteal phase (induced ovulation) and one without luteal phase (spontaneous ovulation) when the rats receive some certain stimuli. Nalbandov (2, 9) also reported that in spontaneous ovulators, LH is cyclic, independent of copulation but provoked by the interplay of the neuroendocrine system.

Induced ovulation, on the other hand, occurs only when the cervix or part of the vagina is appropriately stimulated (10, 11). In this case, our oestrus rats that did not present functional structures could be grouped under the spontaneous ovulators producing GFs, ovulating but not developing CL (12). But these groups of rats involve LH action; therefore, the inability to produce CL is not quite clear. If the reason for lack of CL development is inappropriate induction, at least, an ovulated follicle could have been observed. There is a need for a better understanding of the physiology of the ovarian cycle in these groups of rats.

About 67% of the obviously active rats showed signs of activity only on one ovary. It is not clear whether it can also be attributed to incomplete induction as they all carried primary and growing follicles but no GFs on the affected ovaries. Furthermore, it is reported that the left ovary is more active in mammals than the right ovary (6), observation in this study cannot quite stand with that bias because while correctly, there were higher follicular, activities on the LOs (42:36), ovulatory activities, on the contrary were higher on the ROs (58 %:54 %).

This work also has no explanations for complete ovulations in one quarter of the active rats.

Conclusion

It can be concluded from this work that what triggers the "stance" for mounting in the albino laboratory' rat is the mechanism behind the ovulation process. It can also be concluded that any follicle that did not ovulate at the time of onset of mounting may not ovulate again. Therefore, we conclude that the ovulation time in the laboratory albino rat in our laboratory, and probably elsewhere, is the beginning of oestrus, peaking maximally at the sixth and seventh hour of oestrous. However, every observation needs validation in wider studies**.**

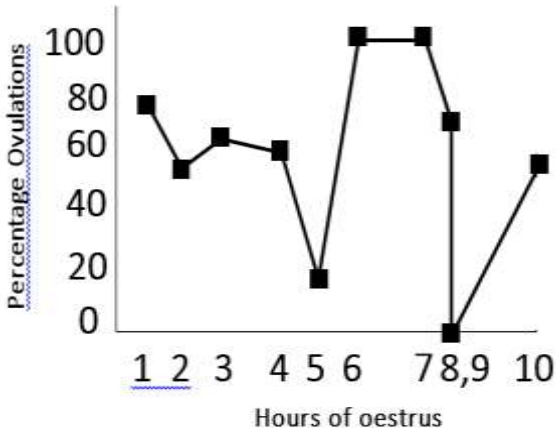

Figure 1. Ovulation rate in different groups of rats corresponding to different hours of oestrus periods

Table 1: Numbers of Graafian Follicles and Corpora Lutea in the Ovary Pairs of Individual Rats, Groups of Rats, their Averages and Percentage Ovulations

Groups	RAT	Left ovaries		Ovulation	Left ovaries		Ovulation
		Corpora Lutea	Graafian follicles	(%)	Corpora Lutea	Graafian follicles	(%)
One	1	1	-	100	3	-	100
	2	2	1	67	2	1	67
	3	3	-	100	0	*0*	0
	4	-	1	0	0	0	0
	5	1	1	50	-	1	0
Group average		1 4	2	70	1	1.4	71.4
Two	1	-	-	0	-	-	0
	2	-	-	0	1	2	33.3
	3	-	-	0	2	-	100
	4	-	-	0	1	-	100
	5	1	1	50	-	1	0
Group average		0.25	0.5	50	1	1.75	57.1
Three	1	-	1	0	-	-	0
	2	1	-	100	-	1	0
	3	1	-	100	3	1	75
	4	-	-	0	-	-	0
	5	-	-	0	-	-	0
Group Average		0.67	1	67	1	1.67	60
Four	1	-	-	0	-	-	0
	2	-	-	0	-	1	0
	3	-	1	0	-	-	0
	4	1	-	100	-	-	0
	5	-	1	0	1	-	0
Group Average		0.33	0.67	50	0.33	0.67	50
Five	1	-	-	0	1	-	100
	2	-	-	0	-	-	0
	3	-	1	0	-	3	0
	4	-	-	0	-	-	0
	5	-	1	0	-	-	0
Group Average		0	0.67	0	0.33	1.33	25
Six	1	-	-	0	1	-	100
	2	2	-	100	-	-	0
	3	-	-	0	-	-	0
	4	1	-	100	-	-	0
	5	2	-	100	-	-	0
Group Average		1.25	1.25	100	0.25	0.25	100
Seven	1	-	-	0	-	-	0
	2	-	-	0	-	-	0
	3	-	-	0	1	-	100
	4	-	-	0	2	-	100
	5	-	-	0	2	-	100
Group Average		0	0	0	1.67	1.67	100
Eight	1	1	-	100	1	1	100
	2	2	-	100	-	-	0
	3	-	-	0	-	-	0
	4	-	-	0	-	-	0
	5	-	1	0	-	-	0
Group Average		1	1.33	75	0.33	0.66	50
Nine	1	-	-	0	-	-	0
	2	-	-	0		1	0
	3	-	-	0	-	1	0
	4	-	-	0	-	1	0
	5	-	-	0	-	1	0
Group Average		0	0	0	0	1	0
Ten	1	-	-	0	-	-	0
	2	1	1	50	-	-	0
	3	1	-	100	1	-	100
	4	-	1	0	1	-	100
Group Average	5	1	2	50	0.75	1.25	0.60
Average of averages		0.59	0.94	62.8	0.67	1.66	0.40

Table 2: Ovulation Success of rats in oestrus

Groups	Number of rats in oestrus	Number of ovulating rats	Proportion of ovulating rats	Percentage ovulation
One	5	4	80	72
Two	5	4	80	53
Three	5	3	60	64
Four	5	2	40	50
Five	5	1	20	12.5
Six	5	4	80	100
Seven	5	3	60	100
Eight	5	3	60	63
Nine	5	0	0	0
Ten	5	4	80	55

Table 3: Ovulation rates of rat per time group

Groups	Corpora Lutea	Graafian follicles	Ovulation(%)
One	12	5	72
Two	5	4	56
Three	5	3	63
Four	2	2	50
Five	1	5	17*
Six	6	0	100*
Seven	5	0	100*
Eight	4	2	67
Nine	0	4	0*
Ten	7	6	54
Total	47	31	60

Conflicts of interest

The authors declare that they have no competing interests.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Approval for this study was sought and gotten from the Ahmadu Bello University Committee for animal use and care.

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