

## Cytological Determination Of The Estrus Cycle In Guinea Pigs

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### ABSTRACT

A cytologic study was carried out to determine the Estrus cycle in 5 female guinea pigs of reproductive age, for a period of 28days in the Animal House of the College of Medicine, University of Nigeria, Enugu Campus, Enugu. Vaginal smears were collected from the animals and made on clean grease-free slides, fixed in 95% Ethanol and stained by Papanicolauo technique. The slides were microscopically examined and the 140 specimens graded accordingly. The morphological, cytological and clinical manifestations observed are presented in the results. The study suggests that the ovulation period in female guinea pigs lasts between 19-21days, usually accompanied with an increase in body temperature within the range of 36.51-37.01°C.

**KEYWORDS:** Guinea Pig, Estrus cycle, Ovulation Period, cytology

Estrus refers to the systemic sexual behavioural pattern that develops in female animals after puberty (Arthur, 1987). The combination of all the physiological events that begin at one estrus period and end at the next is termed estrus cycle with "day zero" being the first day of estrus. Based on the ovarian changes, which can be categorized according to the effect of copulation in ovulation, three types of estrus cycle are commonly observed. Non seasonal breeders such as cows, sows and wares, or seasonal breeders such as ewes, during their breeding seasons in these species, there is a spontaneous ovulation of mature follicles with corpus luteum automatically forming and existing for a definite period of time.

The cyclical changes of vaginal epithelium during the estrus and menstrual cycles have been studied extensively by light and transmission electron microscopy, which according to Long and Evans (1922) have clearly shown alternating patterns of keratinization and mucification during the collicular and luteal phases of the cycle. According to Eddy and Walker (1969) and Parakkal (1970), the same phase can be artificially produced in ovariectomised animals by estradiol and progesterone combined.

Kanagawa et al (1972) noted that despite the advent of scanning electron microscopy, studies of the vaginal surface have remained cursory and have to take into account different phases of the cycle. Regulation of cyclical activity in the female still remains a complex process. With the development of new techniques, particularly those of hormone assays, there is a continual advance in the knowledge and understanding of the mechanisms involved. Although much of the work was done on laboratory animals notably the rat and the rabbit, not so much has been done on guinea pigs particularly in this part of the country, hence the current investigation.

### MATERIALS AND METHODS

About 140 specimens of vaginal smears collected from five mature female guinea pigs weighing between 200-270g were used for this study. The animals which belonged to the same breed were all purchased from Veterinary Research Institute, Vom, Jos and moved into the Animal house of the College of Medicine, University of Nigeria, Enugu Campus, Enugu and further allowed 2 weeks acclimatization. They were also tagged and kept in a cabinet cage with air outlets, maintained in a temperature and light controlled cage, (i.e. 25°C,

12hrs of light and 12hrs of darkness) and were provided with food and water *ad libitum*.

The procedure for the detection of estrus in this study involved clinical observations, which included morphological, behavioural and cytological changes in females during the estrus period. Vaginal smears were collected from the animals using cotton wool tipped applicator inserted through the vulva, until it reached the upper third of the lateral walls of the vagina, where it was gently swabbed. On withdrawal, the cellular materials were smeared onto previously labeled clean grease free glass slides. The slide smears were fixed in 95% alcohol in a jar to avoid drying for a whole day. The slides were then stained by Papanicolaou technique (Papanicolaou, 1954) and microscopically examined to observe the various types of epithelia at different phases of the estrus cycle.

## RESULTS

Clinical observations were recorded in five different columns, labeled guinea pigs 1 – 5. The behavioural signs observed during the Estrus included:

- Nervousness and running about in the cage
- General loss of appetite
- Frequent urination
- Increase in body temperature
- Vaginal and cervical secretion which initially was slimy, later became mucoid.

The morphological changes in the genital tract of the female guinea pigs during the estrus were:

- Enlargement of the vulva lip with accompanying congestion of the vulva.
- Sometimes, copious mucus discharge from the vulva
- Tumefied and soft cervix
- A distinct crystalline aborisation pattern show in the mucous smear during Estrus.

The cytological changes in the vaginal epithelium during the various stages of Estrus showed the following features.

- The pre-estrus phase epithelium consisted of squamous cells, which were flat and discrete and stained blue-green.

- The Estrus phase epithelium consisted of large keratinized squamous cells with square borders, and small homogeneous pyknotic nuclei. The cytoplasm appeared eosinophilic, staining pinkish.
- The post-estrus phase epithelial cells were polygonal in shape, having nuclei similar to those of basal cells. The cells were either seen in singles or in clumps showing flattened or folded appearance. The cytoplasm stained blue-green.

Table 1 displays the period of ovulation (in hours) of the female guinea pigs during the Estrus cycle. The range and the mean were 84-72 hours and 57.6 hours respectively. Table II shows the morphological changes in the genital tract at various phases of Estrus cycle for the five animals.

Table IV displays the body temperature distribution pattern among the female guinea pigs during Estrus phase. The temperature range and the mean were 36.51-37.01°C and 36.72 ± 0.13°C respectively.

Table V shows the temperature and mean temperature value of the 5 guinea pigs from day one to day 28. The table also shows the highest mean temperature value to be 36 ± 72 ± 0.13°C which was recorded on day 20 of the Estrus phase. The day with the highest mean temperature value represents the day of ovulation in the guinea pig.

Figures 1 and 2 show the daily mean temperatures and highest mean temperature value of the animals respectively while figures 3,4 and 5 represent epithelial cells for the various stages of estrus in the female animals.

**Table 1: Period of ovulation of the female guinea pigs during the Estrus cycle**

Guinea Pig No.	Period of ovulation (in hours)
1	72 hours
2	48 hours
3	72 hours
4	48 hours
5	48 hours
Range:	48-72hours
Mean:	57.6 hours

**Table 2: Morphological changes in the genital tract at various Phases of the Estrus cycle for the 5 animals**

Genital tract	Pre-Estrus phase	Estrus phase	Post Estrus phase
Vulva	Slight Enlargement of the Lips Slightly congested Characteristically wet, But little or no Secretion	Maximum enlargement of the lips Markedly congested copious mucous secretion	Shrinking of the lips Pale completely dry
Cervix	Conically Enlarged and firm	Tumefied and soft	Hard and flattened
Vaginal secretion	Secretion scanty And slimy Crystalline Pattern absent	Mucoid Crystalline pattern present	Scanty and sticky Crystalline pattern absent

**Table 3: Estrus period per female guinea pig**

Guinea pig No.	Estrus period (in Days)
1	21
2	20
3	19
4	20
5	20
Range:	19-20 days
Mean:	19.8days

**Table IV: Body temperature distribution among female guinea pigs during Estrus phase**

Guinea pigs No.	Body temperature (°C)
1.	37.01
2.	36.68
3.	36.89
4.	36.51
5.	36.81
Range:	36.51-37.01
Mean:	36.72 ± 0.13

**Table V: The daily body temperatures and the mean value**

Temperature of the 5 guinea pigs from day one to day 28 (in °c).

Day	1	2	3	4	5	Mean temp.
1	35.40	35.55	35.57	35.90	35.80	35.64±0.20
2	35.51	35.46	35.27	35.56	35.50	35.46±0.11
3	35.55	35.73	35.42	35.65	35.41	35.55±0.14
4	35.76	35.29	35.55	35.46	35.41	35.50±0.17
5	35.66	35.18	35.79	35.31	35.52	35.49±0.25
6	35.93	35.20	35.43	35.58	35.55	35.54± 0.27
7	35.41	35.60	35.59	35.70	35.61	35.58± 0.11
8	35.32	35.55	35.72	35.74	35.63	35.59± 0.17
9	35.57	35.78	35.75	35.61	35.67	35.68± 0.09
10	35.66	35.35	35.89	35.75	35.71	35.67± 0.20
11	35.32	35.36	35.90	35.82	35.72	35.62± 0.27
12	35.18	35.38	35.94	35.95	35.80	35.65± 0.35
13	35.32	35.09	35.97	36.10	35.98	35.48± 0.46
14	35.38	35.35	36.26	36.14	36.10	35.85± 0.44
15	35.74	35.67	36.43	36.18	36.20	36.04± 0.33
16	35.94	35.70	36.45	36.20	36.39	36.14± 0.31
17	36.31	35.94	36.59	36.40	36.41	36.33± 0.24
18	36.45	36.28	36.61	36.43	36.56	36.47±0.13
19	36.76	36.34	36.89	36.48	36.79	36.65±0.23
20	36.85	36.68	36.75	36.51	36.81	36.72±0.13
21	37.01	36.58	36.42	36.44	36.80	36.65±0.25
22	36.98	36.42	36.33	36.39	36.40	36.50±0.27
23	36.84	36.37	36.29	36.37	36.25	36.37±0.24
24	36.78	36.25	36.27	36.30	36.23	36.37±0.23
25	36.63	36.23	36.26	36.25	36.22	36.37±0.18
26	36.62	36.17	36.30	36.21	36.29	36.32±0.18
27	36.41	36.20	36.46	36.22	36.25	36.33±0.11
28	36.20	36.23	36.47	36.20	36.42	36.30±0.13

### Estrus Cycle in Guinea Pigs

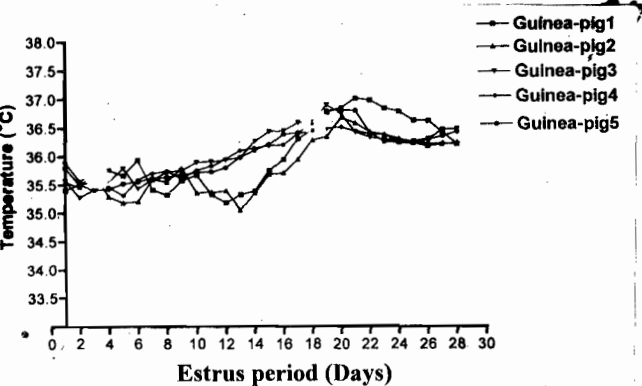


Fig. 1: X-Y plot of the estrus period and the temperature in 5 Guinea-pigs. The ranges of the maximum temperatures for the 5 animals and the corresponding estrus period were found to be 36.57-37.01°C and 19-21 days respectively.

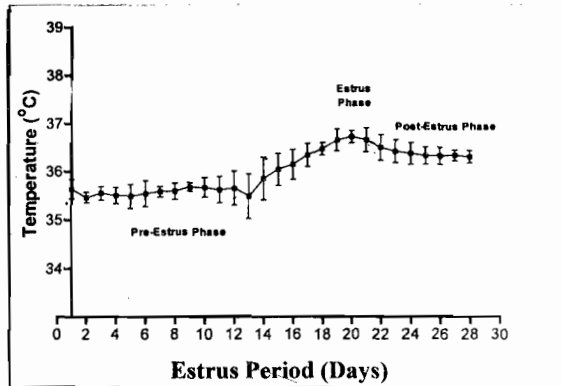


Fig 2 X-Y Coordinate Graph showing the relationship between the Estrus cycle and the mean body temperatures SD of the Guinea pig (n = 5). A maximum temperature of  $36.72 \pm 0.130C$  was determined on day 20 of the estrus phase.  $36.72 \pm 0.13C$ .

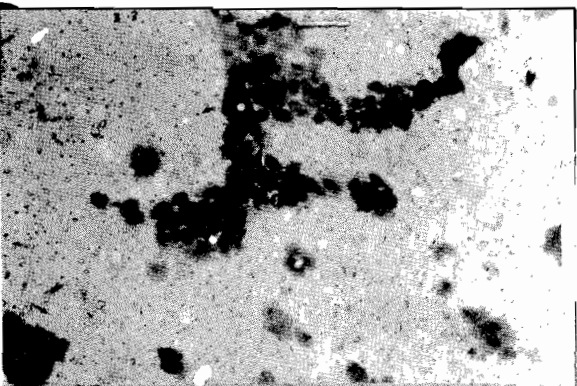


Fig 3 Represents the pre-estrus phase. The squamous epithelial cells lie flat, clustered and occasionally discrete, and stained blue-green stained by pap technique. X 400.

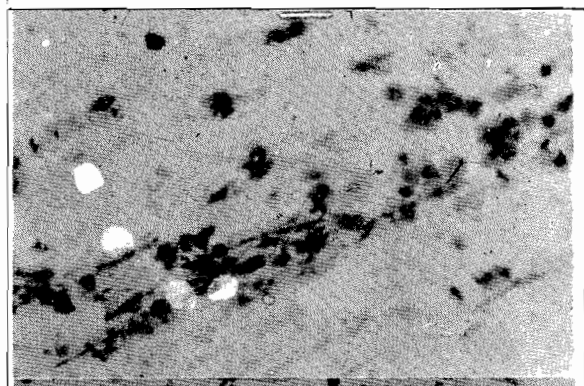


Fig 4 Represents ovulation phase. The superficial squamous cells are large and keratinized with square borders, and square and small homogeneous pyknotic nuclei. The cytoplasm appears eosinophilic and transparent. Stained by Pap technique. X 4

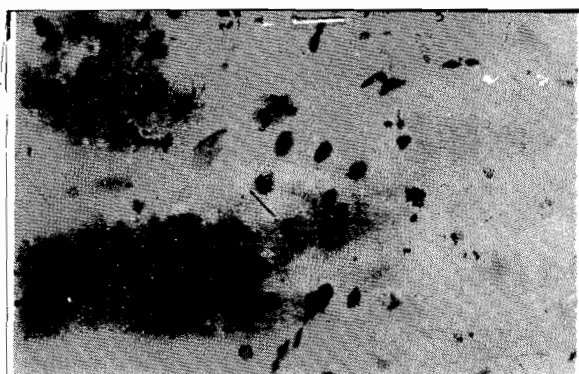


Fig 5. The post estrus phase epithelial cells are polygonal in shape, having nuclei similar to those of the basal cells. The cells lie singly or in clusters having flattened or folded appearance. The cytoplasm appears blue-green. Stained by Pap technique. X 400

## DISCUSSION

The current study has shown some of the more obvious behavioural and morphological signs that occur in certain animals during estrus. These included nervousness, frequent urination, swollen and reddish appearance of the vulva and mucus discharge from the vulva and vagina. This finding is in conformity with those of Fraser (1980) and Mac Donald (1980). However, the degree at which each of these behavioural and morphological signs was exhibited varied from one female guinea pig to another. For instance, in this series, the guinea pigs showed the largest quantity of cervical and vaginal secretion.

The duration of the estrus phase as observed in our case ranged between 48-72 hours, which was accompanied by a spontaneous rise in temperature in the animals. In this study also, a crystalline aborisation pattern of the mucous smear was noted under the microscope. The morphological changes observed in our series such as tumefied, soft cervix and dryeremic vulva have been reported in cows by Arthur (1979). All these changes have been ascribed to the estrogen concentration.

In the current investigation the estrus cycle in female guinea pigs was determined, following the clinical observations made on the female animals, which included behavioural and morphological changes. Ovulation period in the female guinea pigs usually associated with an increase in body temperature has been determined in the current investigation. The study has been able to establish

the ovulation period in these animals during which the day with the highest mean temperature of  $36.72 \pm 0.13^{\circ}\text{C}$  was on day 20 of the estrus cycle. This work therefore suggests that guinea pigs have a specific patter of estrus cycle that can be determined cytologically.

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