

## Ethological observations from gestation to adulthood in Albino Rats: A case study

Eshita Pandey \* and Zeba Afroz

*Department of Zoology, Dayanand Girls PG College, Kanpur, UP, India.*

World Journal of Biology Pharmacy and Health Sciences, 2024, 20(03), 184–192

Publication history: Received on 25 October 2024; revised on 02 December 2024; accepted on 05 December 2024

Article DOI: <https://doi.org/10.30574/wjbphs.2024.20.3.0979>

### Abstract

This case study was done to explore and understand the ethological behavior patterns of female albino rats within a controlled laboratory environment. Their behavior was observed to help us provide insights into the underlying mechanisms governing reproductive behavior. The study encompasses behavior and morphological observations of female albino rats from the time they conceive, give birth, raise their young ones till they are weaned and are self-sufficient. This study involves behaviors like territoriality, reproductive behavior, nesting behavior, maternal care, foraging, weaning etc. This case study provides a detailed examination and empirical evidence of the behavioral observations of female albino rats and thus leading to better understanding and cognition of animal behavior.

**Keywords:** Ethology; Animal behavior; Female albino rats; Social interactions; Reproductive behaviors; Exploratory behaviors; Environmental stimuli; Case study

### 1 Introduction

Pregnancy plays a very important phase in the life cycle of any organism. It is marked by significant physiological and behavioral alterations which can be observed both preceding and following conception (Kepley *et al.*, 2023). These changes can have serious effect both at pre and postpartum followed by disturbance in metabolic equilibrium (Skalkidou, *et al.*, 2012). Subsequently, pregnancy plays a vital role in the well-being and sustainability of populations.

Since the sixteenth century scientific research on laboratory animals are being conducted. Many rodents are used in laboratory for research and many scientific studies are done for the welfare of humans and the ecosystem. As the rats are closer to the humans so they are considered a model for human studies as well. Many scientific studies are done for research purpose based on them (Russow and Theran, 2003).

Rats go through many phases while undergoing maturation and attaining puberty. This cycle helps them to reproduce and raise new progeny necessary for race survival and evolution.

Mostly rats take 15 to 20 days to reach puberty. Verging opening in female rats and balanopreputial separation in male rat are the signs of sexual maturity. Hormonal fluctuations also indicate sexual maturity. High luteinizing hormone (LH) level in rats is the sign of entering into puberty which normally occurs after 25 to 30 days after birth. Releasing of LH causes maturation of ovary. Eight days prior to the first pro-estrus, the LH concentration changes and the period is deemed anestrous (Ojeda and Skinner, 2006).

Ovulatory Phase in rats includes ovulation which occurs every four to five days throughout the year in the young adult laboratory rats. Unlike humans' rats have an estrus cycle. As menstrual cycle plays an important role in human's life so far estrus cycle has the same importance in rodents emphasizing in rats (Caligioni, 2009). Estrus cycle is necessary for the sexual maturation and shows reproductively active phase in rodents.

\* Corresponding author: Eshita Pandey

Although Rats do not shed off their endometrium when they are sexually active and their estrus cycle is not similar as menstrual cycle. Estrus cycle is categorized into four phases which can be analyzed by vaginal smear, namely proestrus, estrus, metestrus and diestrus (Marcondes *et.al.*, 2002).

### 1.1 Pro-Estrus Phase

The duration of pro-estrus phase varies from species to species with a minimum of twelve hours to one day duration. The days of phases vary according to the age of the rats. This phase defines the sexually immature female rat. As the influence of estrogen increases, several follicles start to grow in ovaries including endometrium lining of uterus (Parkening *et. al.*, 1982).

### 1.2 Estrus Phase

A sexually active phase of rat where the female behaves receptively. This phase exists for about 8 -16 hrs. and is characterized by cornified squamous epithelial cells found in clusters. It is devoid of nucleus and cytoplasmic granules and has an irregular shape. Level of E2 is found on its peak in morning and lower at afternoon (Walmer *et.al.*, 1992).

### 1.3 Metestrus Phase

This phase is also known as diestrus phase I. Characterized by the presence of leukocytes cells, mucus secretion and nucleated epithelial cells. This phase exists from 6 to 9 hours. This duration is for vulva tumefaction in the vagina. This phase is responsible for the pregnancy in the female rats due to high progesterone level. Elevated progesterone level is responsible for maintenance of pregnancy (Antunes *et.al.*, 2016).

### 1.4 Diestrus Phase

This phase is also known as the diestrus Phase II. The changes observed in this phase are high level of leukocytes and mucus in the vaginal smear. It is the longest phase of estrus cycle as this sustains for two to three days. The progesterone hormone is responsible for the successful pregnancy the actual action is seen in the diestrus phase II (Lohmiller, 2006).

---

## 2 Methodology

### 2.1 Material

3 pairs of albino rat including male and female albino rat (*Rattus norvegicus domestica*) were utilized for the study. They were kept in a well-ventilated cage and provided food and water *ad libitum*. They were physically examined for any unusual activity, wound or discomfort. After a week of acclimatization, they were kept in separate cages in pairs. The cages used for the study were 20 inches\* in width 18 inches\* in length.

The rats were fed normal diet including grains, green vegetables, fruits etc. They were also provided nesting material like cotton cloth inside the cages. Visual observations were done physically as well as using audio-visual aids like camera.

### 2.2 Methodology

The rats were kept in pairs in cages. They were given food and water and were observed for their behavior. The observations were based on understanding the physical changes and confirming the establishment of pregnancy. Followed by other observable evidences like changes in weight, physical appearance, gestation period, parental care by mother rat and maturation of pups to adults.

Along with this a keen periodic undisturbed physical observation at regular intervals was also done to compare and finalize the results. Ethical use of animals under study was considered and obtained.

### 3 Result

#### 3.1 Observations during pregnancy

##### 3.1.1 Observation Of Physical Changes

The female rats were checked for mucus secretion from their vaginal opening every day in the morning to confirm signs of copulation. The physical changes observed included appearance of teats in females during pregnancy. Slight loss of hair was observed in females post pregnancy. Most of the hair loss was observed behind ears and on neck area.

##### 3.1.2 Observation Of Weight Difference

Once the signs of pregnancy were visible the rats were measured for weight frequently, to check an important signal of confirmed pregnancy. Table I represents the initial and final weight observed for all the three pairs of rats following intercourse.

**Table 1** Weight of rats

Weight	Rat pair I		Rat pair II		Rat pair III	
	Male	Female	Male	Female	Male	Female
Before intercourse	250 gm	240 gm	260 gm	265 gm	285 gm	280 gm
After intercourse	255 gm	295 gm	262 gm	310 gm	290 gm	325 gm

##### 3.1.3 Observation Of Gestation Period

The male rats were separated after the confirmation of pregnancy so that the female behavior during gestation period could be observed. The pregnant rat's abdominal portion became broader, rounder and more defined. As pregnancy progressed the pregnant rats showed lethargy and slept during most of the day time. They also had increasingly difficult time moving around the cage. With the gestation coming to termination and delivery day coming near the rats moved around more in their cage and rarely stayed still. The normal time for the gestation lasts for 20 to 26 days. Rat I gave birth after 21 days, Rat II after 23 days and Rat III after 26 days of being pregnant.

##### 3.1.4 Observation Of Child Birth

As soon as the delivery day came near the rats started to move around more frequently in their cage and rarely stood still for long periods. Increased restlessness was observed in them. The time period for pup delivery of the litter was approximately one and a half hour but exact duration was different for every rat. After completion of 21 (Rat I), 23 (Rat II) and 26 days (Rat III) three rats the litter was delivered. Each pregnant albino rat took a different time span to deliver all the pups. The clutch size was different for all the three females.

**Table 2** Clutch size

	RAT I	RAT II	RAT III
Clutch size	06	09	08
Delivery time for the clutch	40 minutes	85 minutes	77 minutes

#### 3.2 Observation of parental care

##### 3.2.1 Physical Appearance of Neonates

The pups born were blind, their eyes were covered by membrane, were deaf, ears were not visible and had no hair when born. The color of their skin was pinkish. All the three females were extremely protective of their litter and did not allow anyone to approach them. They utilized the cloth provided to them as nesting material and covered up their pups in a manner where they were completely hidden.

One of the female rats (Rat II) showed a deviation in parental behavior. Initially it took care of its pups as others but after completion of a couple of days (7 days precisely) it stopped feeding them slowly and fed irregularly. It killed its pups

on day 12 and ate up all of the litter (09 pups) in 24 hrs. The pups were devoured partially and rest of the body was left unattended. This happened due to postpartum effect and maybe a feeling of overcrowding in its cage.

### 3.2.2 Nest construction/ Nesting Behavior

The female mother rat made a single, central nest site to care for its young. Nest construction is done by carrying nest material, in this case the cloth provided, with the mouth, or pushing it with the snout or paws, to form a potential nest site. The female created a tightly bound mass of material with a depression in the center to interact with the pups. She kept the nest clean and kept her pups covered by nesting material for the first few days approximately for the first one week till the pups were 7-8 days old.

### 3.2.3 Retrieval Of Pups

The females regularly retrieved their displaced or scattered pups. They did this by first orienting themselves towards their pups and moving towards them. Then they sniffed them before gently picking it up with the incisors. They then carry it back to the nest site. Most of the times the mother orally repositioned the pups within or around the nest.

### 3.2.4 Aggressive Behavior

The mothers did not allow any external touching of their pups. They behaved aggressively towards any external stimuli. They were observed to try biting, scratching and attacking the external source in order to protect their pups from potential dangers.

### 3.2.5 Lethargy Behavior

Post delivery there was general lethargy among the mother rats as observed although Rat II was more lethargic as compared to the Rat I and III. Their diets were also reduced as compared to the diets before pregnancy. This behavior was observed from the fourth day of delivery of their respective litters.

### 3.2.6 Licking Of The Pups

The mothers spent a long time licking their young while they were awake. This behavior was done to clean the pups. This also increased the pup's activity. Almost two thirds of waking time was spent on licking and cleaning the pups.

### 3.2.7 Actively Hovering Over Pups

The mother rats were observed to position themselves actively over some or all of the pups in the nest most of the times. They performed activities like licking the pups, self-grooming, or moving nest material while hovering.

### 3.2.8 Nursing Of the Pups

The lactating mothers nursed their young regularly for prolonged periods of time. A number of nursing postures were observed in the mothers like an upright crouch, lying flat, on top of the litter etc. Although feeding and nursing the young ones is a natural process yet the three mother rats behaved quite distinctly. Two mothers (Rat I, III) kept on feeding and grooming their pups timely. The third mother (Rat II) stopped nursing her pups after 7 days. She fed them quite irregularly and after 9 days she stopped feeding them at all. On day 12 day she ate all her pups.

### 3.2.9 Maturation Of Pups

Appearance Of Hair- The appearance of complete hair on the pups took 12 days at an average for the Rat I litter, 12 days at an average for the Rat II litter and 14 days at an average for the Rat III litter. Although no pups were alive for the Rat II after 12 days as she ate all of them on day 12.

**Table 3** Appearance of hair

	LITTER		
	RAT I	RAT II	RAT III
Clutch size	06	09	08
Mean number of days for the appearance of hair	12	14	12

Initiation Of Walking- The pups started to walk after 15 days at an average for the Rat I litter and 17 days at an average for the Rat III litter. The were observed to move in small hops by 21 days on an average and as the days passed, they were able to run around the cage quite actively by day 26.

**Table 4** Initiation of walking

	LITTER		
	RAT I	RAT II	RAT III
Clutch size	06	09	08
Mean number of days for pups to start walking	15	17	NA

**3.2.10 Weaning Of The Pups**

The pups slowly shifted from suckling of milk to eating solid food. They started feeding on solid food as a symbol of weaning and attaining adulthood. This took about 25 days at an average for the first litter (Rat I) and 23 days at an average for the Rat III litter. The pups preferred eating crunchy vegetables, fruits, solid grains, and cooked food too like bread, chapattis etc.

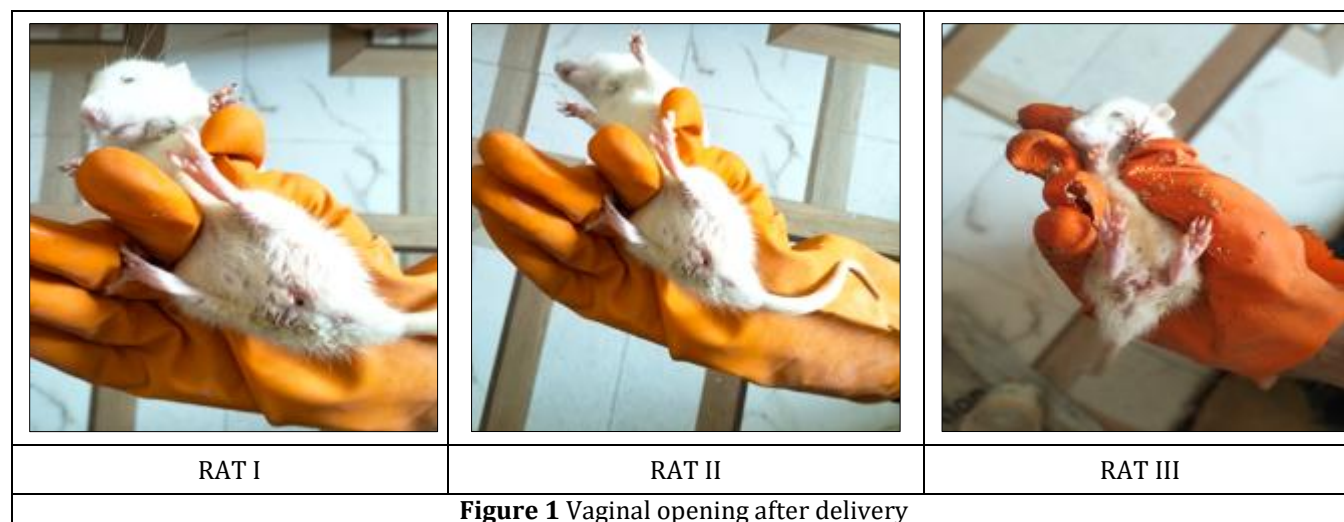
**3.2.11 Play Behavior Of Pups**

After completion of 12 to 16 days the pups displayed noticeable playing activities. These activities included nipping, biting, and wrestling with each other. The playful activities of pups were harmless even if they were wrestling and biting. They did not perform any aggressive behavior while playing.

During play they made noises such as chirps and other vocalizations. They were observed to be running around in the cage and jumping. They were observed to be rolling on each other playfully. At times they were observed to engage in playful activities with their mother. They developed social behavior while growing up.

**3.2.12 Miscellaneous Behaviors Observed**

Pregnant rats exhibited variations in behavior. Rat II became more irritable or aggressive as gestation progressed, while Rat III and Rat I were calmer and more docile as compared to Rat II. Rat I and Rat III seemed to seek extra attention.





**Figure 2 Clutch of female rats**



**Figure 3 Full development of hair in a grown pup**



**Figure 4 An overcrowded litter**



**Figure 5 Rat II which ate all her pups**

#### 4 Discussion

Rats and many rodents are the essential model organisms of laboratory experiments in the field of biological science therefore their handling and behavioral study is important (Klein and Bayne, 2007; Hubrecht, 2011). At times it becomes difficult and challenging to differentiate between normal fat rat and pregnant rat as both may appear bulky so it becomes important to know the difference (Modlinska and Pisula, 2020). If rat's body appears bulky from the spinal region and from the shoulder, then this kind of rat would be considered as a fatty rat. On the other hand, if the rat's body appears bulky from the abdominal region or its stomach appears bulky then such a rat may be considered as a pregnant rat (Ypsilantis *et al.*, 2009). A pregnant rat's abdominal portion will become broader, rounder and more defined and the belly would appear swollen. This swelling should not be confused with a recent meal or eating a lot of food (Zahra, 2022). Although these symptoms alone are not sufficient to clarify that the rat is fatty or pregnant so few more symptoms along with this should be examined.

One more symptom which can be observed clearly is the appearance of darker teats. The pregnant rats have more noticeable teats as they increase in size (Freund, 2021). Another noticeable symptom is that the female rats eat less and decrease their movement as their due date approaches as was also observed in Rat I and III.

As their gestation period starts to end their nest building activities and food collecting activities increase (Janina *et al.*, 1980; Garcia, 2024). Nest building behavior can be noticed in pregnant rats as observed in this case also by all the rats under study and it is done with the objective to protect their young from predators (Black, 2021).

By observing the parental care and the changes in the pup's appearance the conclusion is that parental care needs to be done for 18 to 21 days. After the completion of 21 days the pup turns into adult and they can now eat, play and live independently as observed in the study.

Play behavior is crucial for the development of the pups they start showing playing activities around 15-16 days of their age and it continues as they grow. Playing behaviour is essential for their cognitive and social development (Thor and Holloway, 1984; van den Berg *et al.*, 1999). As mammals shows sexual dimorphism in case of rats it is helpful to recognize the difference as male pups showed more active playful behaviour as compare to female pups (Auger and Olesen 2009).

The rat pups under observation showed rough-and-tumble play which included activities like nipping, biting, and wrestling etc. Pups performed these activities with each other and/or with their mother too. These types of activities increase social bonding among them as also observed by VanRyzin *et al.*, 2020).

The pups showed non aggressive behavior during play and did not hurt each other inspite of nipping or biting. They did not make any harm nor made any cuts. They also learn social hierarchy as they grow up as observed by Vmd, 2023.

---

#### 5 Conclusion

Albino rats display various ethological and physiological changes from gestation till adulthood in a highly coordinated reproductive cycle. In gestation period the rats undergo many behavioural and physiological adaptations for their foetal development such as appearance of teats and weight gain. After giving birth to the pups the female rats adopt maternal ethological changes which help pups to gain adulthood such as grooming behaviour, nesting behaviour, aggressive behaviour towards any external object for the protection of pups, caring of pups and nursing. This period also includes initiation of lactation. The ability of maternal changes in rats to successfully transition from the gestation to postpartum care is essential for the survival and health of offspring. External factor including environmental condition such as overcrowding of pups, optimal nutrition can influence the both maternal ethological and physiological behaviour.

In summary, the period of gestation to adults in female albino rats is a dynamic and complex phenomenon involving intricate hormonal regulation and behavioural adaptation these changes ensure the survival and development of offsprings; considering the maternal health. This period is a key aspect of reproductive success which provides us an idea to understand the mammalian reproduction and relationship between mother and offspring interaction.



---

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

All protocols were approved by the Institutional Ethical Committee of the Dayanand Girls P.G. College, Kanpur, UP.

---

## References

- [1] Antunes, I. B., Silva, A., Kawakami, R., & Andersen, M. L. (2016). The female rat. In Springer eBooks (pp. 95–109).[https://doi.org/10.1007/978-3-319-11578-8\\_8](https://doi.org/10.1007/978-3-319-11578-8_8)
- [2] Auger, A. P., & Olesen, K. M. (2009). Brain sex differences and the organisation of juvenile social play behaviour. *Journal of Neuroendocrinology*, 21(6), 519–525. <https://doi.org/10.1111/j.1365-2826.2009.01871.x>
- [3] Black, D. (2021, January 20). Is your rat pregnant or just fat? How to tell. *Animal Knowhow*.
- [4] <https://animalknowhow.com/is-your-rat-pregnant-or-just-fat-how-to-tell/>
- [5] Caligioni, C. S. (2009). Assessing Reproductive Status/Stages in mice. *Current Protocols in Neuroscience*, 48(1). <https://doi.org/10.1002/0471142301.nsa04is48>
- [6] Freund, W. M. (2021, November 16). 12 Clear signs for how to tell if a rat is pregnant – Rat Universe. *Rat Universe*.<https://ratuniverse.com/how-to-tell-if-a-rat-is-pregnant/>
- [7] Garcia, A. (2024, April 9). How to Tell if a Rat is Pregnant? Signs and Symptoms. *The Rat World*. [https://theratworld.com/how-to-tell-if-a-rat-is-pregnant/#elementor-toc\\_heading-anchor-7](https://theratworld.com/how-to-tell-if-a-rat-is-pregnant/#elementor-toc_heading-anchor-7)
- [8] Hubrecht, R. (2011). *Guide for the Care and Use of Laboratory Animals, Eighth Edition 2011* The Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). Published by the National Research Council of the National Academies, Washington DC, USA. 219 pp Paperback (ISBN 0-309-15400-6). Price US\$19.95. *Animal Welfare*, 20(3), 455–456. <https://doi.org/10.1017/s0962728600003067>
- [9] Janina R. G., Gary N. Z., A. Leah N., Hamish N. M., 1980, Marginal Protein Deficiency in Pregnant Rats. II. Impaired Behavior during Pregnancy, *The Journal of Nutrition*, Volume 110, Issue 7, Pages 1298-1302, ISSN 0022-3166,
- [10] <https://doi.org/10.1093/jn/110.7.1298>.<https://www.sciencedirect.com/science/article/pii/S0022316623281274>
- [11] Kepley, J. M., Bates, K., & Mohiuddin, S. S. (2023, March 12). Physiology, maternal changes. *StatPearls - NCBI Bookshelf*. <https://www.ncbi.nlm.nih.gov/books/NBK539766/>
- [12] Klein, H. J. and Bayne, K. (2007). Establishing a culture of care, conscience, and responsibility: Addressing the improvement of scientific discovery and animal welfare through science-based performance standards. *Ilar Journal*, 48(1), 3–11. <https://doi.org/10.1093/ilar.48.1.3>
- [13] Lohmiller J, Swing SP (2006). *Reproduction and Breeding*. In M. A. Suckow, S. H. Weisbroth and C. L. Franklin. *The Laboratory Rat*. 2nd ed. (pp. 147-164). Elsevier Academic Press. <https://shop.elsevier.com/books/the-laboratory-rat/suckow/978-0-12-074903-4>
- [14] Lonstein, J. S., & Fleming, A. S. (2002). Parental Behaviors in Rats and Mice. *Current Protocols in Neuroscience*. doi:10.1002/0471142301.ns0815s17  
Doi:10.1002/0471142301.ns0815s17.[https://www.researchgate.net/publication/5423454\\_Parental\\_Behaviors\\_in\\_Rats\\_and\\_Mice](https://www.researchgate.net/publication/5423454_Parental_Behaviors_in_Rats_and_Mice) [accessed Mar 22 2024]
- [15] Marcondes, F. K., Bianchi, F., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian Journal of Biology*, 62(4a), 609–614. <https://doi.org/10.1590/s1519-69842002000400008>
- [16] Klaudia Modlinska, Wojciech Pisula, (2020)The Natural History of Model Organisms: The Norway rat, from an obnoxious pest to a laboratory pet *eLife* 9:e50651. <https://doi.org/10.7554/eLife.50651>
- [17] Ojeda, S. R., & Skinner, M. K. (2006). Puberty in the rat. In Elsevier eBooks (pp. 2061–2126). <https://doi.org/10.1016/b978-012515400-0/50043-9>



- [18] Parkening, T. A., Collins, T. J., & Smith, E. (1982). Plasma and pituitary concentrations of LH, FSH, and Prolactin in aging C57BL/6 mice at various times of the estrous cycle. *Neurobiology of Aging*, 3(1), 31–35. [https://doi.org/10.1016/0197-4580\(82\)90058-6](https://doi.org/10.1016/0197-4580(82)90058-6)
- [19] Quesenberry, K. E., & Boschert, K. R. (2020, April 9). Breeding and reproduction of rats. *MSD Veterinary Manual*. <https://www.msddvetmanual.com/all-other-pets/rats/breeding-and-reproduction-of-rats>
- [20] Russow, L. M., & Theran, P. (2003). Ethical issues concerning animal research outside the laboratory. *Ilar Journal*, 44(3), 187–190. <https://doi.org/10.1093/ilar.44.3.187>
- [21] Skalkidou, A., Hellgren, C., Comasco, E., Sylvén, S., & Poromaa, I. S. (2012). Biological aspects of postpartum depression. *Women S Health*, 8(6), 659–672. <https://doi.org/10.2217/whe.12.55>
- [22] Thor, D. H., & Holloway, W. R. (1984). Developmental analyses of social play behavior in juvenile rats. *Bulletin of the Psychonomic Society*, 22(6), 587–590. <https://doi.org/10.3758/bf03333916>
- [23] van den Berg, C. L., Hol, T., Van Ree, J. M., Spruijt, B. M., Everts, H., & Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social challenges in the rat. *Developmental psychobiology*, 34(2), 129–138. <https://pubmed.ncbi.nlm.nih.gov/10086231/>
- [24] VanRyzin JW, Marquardt AE, McCarthy MM. Assessing Rough-and-tumble Play Behavior in Juvenile Rats. *Bio Protoc.* 2020 Jan 5;10(1):e3481. doi: 10.21769/BioProtoc.3481. PMID: 33654714; PMCID: PMC7842561. <https://pmc.ncbi.nlm.nih.gov/articles/PMC7842561/>
- [25] Vmd, L. J. (2023, September 8). 10 Pet rat behaviors and what they Mean. *PetMD*. <https://www.petmd.com/exotic/10-pet-rat-behaviors-and-what-they-mean>
- [26] Walmer, D., Wrona, M., Hughes, C. L., & Nelson, K. (1992). Lactoferrin expression in the mouse reproductive tract during the natural estrous cycle: correlation with circulating estradiol and progesterone. *Endocrinology*, 131(3), 1458–1466. <https://doi.org/10.1210/endo.131.3.1505477>
- [27] Ypsilantis, P., Deftereos, S., Prassopoulos, P., & Simopoulos, C. (2009). Ultrasonographic diagnosis of pregnancy in rats. *Journal of the American Association for Laboratory Animal Science*, JAALAS, 48(6), 734–739. <https://pmc.ncbi.nlm.nih.gov/articles/PMC2786927/#:~:text=In%20practice%2C%20pregnancy%20in%20rats,and%20is%20confirmed%20by%20birth.>
- [28] Zahra, M. (2022, May 20). How to tell if a rat is pregnant: Signs and symptoms! *Ratwhisperer*. <https://www.ratwhisperer.net/blogs/blog/how-to-tell-if-a-rat-is-pregnant-signs-and-symptoms>