ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.5152/JLASP.2023.23008

Highlights for the Research Involving the Lactation Period in Laboratory Rats

Laboratuvar Sıçanlarında Laktasyon Dönemini İçeren Araştırmalarda Öne Çıkanlar

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ABSTRACT

Lactation is an important period in newborn nutrition since nutritional factors in the early stages of development have life-long impacts. Lactation provides various important long-lasting health benefits to the offspring. The lactation period, however, provides much more than just nutrients. The composition and quantity of nutrients in breast milk are not the only factors that can influence offsprings during breastfeeding. Maternal behaviors to nourish and protect her litters during lactation are also important in programing. The current study attempted to focus on specific characteristics of the breastfeeding period, such as changes in food consumption, mother's weight, and the time dams spend lactating with or without pups. A deeper understanding of this critical period will allow for designing better pediatric models including maternal separation, artificial rearing, and studies covering maternal manipulations.

Keywords: Lactation period, maternal care, milk, mother rats, newborn

ÖZ

Emzirme, yenidoğan beslenmesinde önemli bir dönemdir çünkü gelişimin erken dönemlerindeki beslenme faktörleri uzun vadeli bir etkiye sahiptir. Emzirme, yavruların sağlığına uzun dönemde etkileri olan faydalar sağlar. Bununla beraber laktasyon dönemi yavrulara, besinlerden çok daha fazlasını sağlar. Emzirme sırasında yavruları etkileyebilecek tek faktör anne sütündeki besinlerin bileşimi ve miktarı değildir. Aynı zamanda laktasyon döneminde yavrularını beslemek ve korumak için gösterdiği annelik davranışları da programlamada önemlidir. Bu çalışmada, emzirme döneminde anne sıçanların yem ve su tüketimindeki değişiklikler, anne ağırlığı, annelerin yavrularını emzirmek için harcadığı zaman, annelerin yavrularından ayrı kaldığı süre gibi belirli özelliklerine odaklanmaya çalışıldı. Bu kritik dönemin daha iyi ve derinlemesine anlaşılması, anneden ayrılma stresi modelleri, suni yetiştirme ve anne manipülasyonlarını kapsayan araştırmalar da dâhil olmak üzere daha iyi pediatrik modellerin tasarlanmasına olanak sağlayacaktır.

Anahtar Kelimeler: Laktasyon dönemi, anne bakımı, süt, sıçan anneler, yeni doğan

Received/Geliş Tarihi: 26.04.2023
Accepted/Kabul Tarihi: 19.07.2023
Publication Date/Yayın Tarihi: 20.08.2023

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Cite this article as: Yılmaz, C., Engür, D., Keskinoğlu, P., Kumral, A., & Yılmaz, O. (2023). Highlights for the research involving the lactation period in laboratory rats. *Journal of Laboratory Animal Science and Practices* 2023;3(2):45-50.

Introduction

Mammalian mothers are signaling biochemically to their offspring from the moment of implantation until weaning. The maternal response to environmental challenges modulates her signaling to her offspring, which in turn modulates offspring development. The evidence for in utero effects on adult physiology and disease risk in mammals is substantial, from the early epidemiological work of Barker (1990) and Forsdahl (1977) to a host of experimental studies on laboratory animals. Lactation is a critical phase in newborn nutrition. Maternal milk is a complicated metabolic fluid that helps newborn mammals grow and thrive (Martin et al., 2016). Since milk is the only food consumed by all mammals for a while after birth, it must contain all the essential nutrients required for early growth and development. However, lactation offers considerably more than just nutrition to the offspring; it has numerous additional lifelong health effects (Power & Schulkin, 2013).

Milk contains bioactive molecules that contribute to organ development and healthy microbial colonization (Ruiz et al., 2017). The composition of milk varies at different stages of the same feed, during the day and throughout the lactation period (Ballard & Morrow, 2013). Breastfeeding also has long-term beneficial effects for the mother, such as a reduced risk of breast and ovarian cancer and a reduced incidence of type 2 diabetes (Gunderson et al., 2018; Victora et al., 2016). The phenotype of the offspring is determined by the interaction between genes and the environment, especially in the early stages of development (Ramos & Olden, 2008; Suzuki, 2018; Zambrano & Nathanielsz, 2013). Experimental studies are mostly carried out to observe the effects of problems experienced during pregnancy (Li et al., 2019; Vieau, 2011; Wang et al., 2019). Less attention has been paid to developmental challenges during breastfeeding (Bautista et al., 2019). It is known that many factors such as breastfeeding stage, mother's genetic factors, infant health status, baby's gender, mother's socioeconomic status, maternal age, environmental factors, and maternal health status affect the nutritional composition of milk (Wu et al., 2018). Studies show that proper breastfeeding reduces the risk of obesity in later childhood (de Waard et al., 2017; Fields et al., 2016; Qasim et al., 2018). During lactation, key stages of development, maturation, and differentiation occur in many organs (Bauman & Bruce Currie 1980; Bautista et al. 2008). Despite these limitations caused by differences in the timing of organ development between species, animal studies provide an opportunity to further explore the underlying mechanisms and control for external confounding factors in ways not possible in human studies. In addition, comparative animal physiology provides an opportunity to observe and learn from the differences and similarities between species (Sinclair et al. 2016).

Lactation is vital to optimal development and provides a window of opportunity for developmental programing. The content and amount of nutrients in breast milk are not the only elements that may program the offspring during lactation. The relationship formed between the mother and the offspring during the suckling stage and the maternal behavior to nourish and protect her litters during early development is also significant in programing. Rodent pups are born quite immature, i.e. unable to see, hear, or regulate body temperature, and with limited motor coordination. Maternal stimulation through licking and grooming is a major source of sensory, social, and hormonal cues for nervous system maturation. In rodents and nonhuman primates, as well as humans, maternal attitudes toward growing neonates influence the development and structure of neurological and behavioral systems. The current study aimed to focus on specific aspects of the lactation period, such as changes in food consumption, mother weight, and time spent lactating with or without pups. A better understanding of this vital phase will allow for the development of better pediatric models including maternal separation, artificial rearing, and studies covering maternal manipulations.

Methods

This experimental study was carried out on female Wistar albino rats from Multidisciplinary Experimental Animal Laboratory at Dokuz Eylul University, Faculty of Medicine. It was carried out after obtaining the approval of the Animal Experiments Local Ethics Committee of Dokuz Eylul University with the protocol date: July 8, 2022 approval number 09/2022. In this study, female rats from the Wistar Albino colony routinely produced in the experimental animal laboratory were used. The mothers used in the study were selected among the young mothers who became pregnant for the first time and their breastfeeding periods were followed. The animals were maintained on a 12-h light–dark cycle, with lights on at 6:00 a.m. The temperature was maintained at 22–25°C with 50–60% humidity. In an attempt to examine the nature of the lactation period, data of eight healthy lactating animals with pups consisting of eight to ten young were used.

Each dam and her respective pups were housed in a separate acrylic cage (40 × 33 × 16 cm) with free access to food and water. Animals received commercially available standard pelleted rodent diet. Every week, fresh wood shavings were added to the nest. The food container was positioned 9 cm above the cage floor to stop the pups from consuming the dams' diet during the first stage of lactation. Animals were monitored through a camera with night vision features starting from the day of birth until postnatal day (PN) 26. The breastfeeding durations of the mothers were taken as the 24-hour periods from 09:00 to 09:00 the next day. The equivalent of these 24-hour periods in minutes is 1440 minutes. Recordings taken in 1440 minutes were monitored again, and the duration of breastfeeding, the time that dams did not breastfeed, and the frequency of separation from the pups were determined from the records. During the lactation period, the feed and water consumption of the mothers and the changes in the live weights of the mothers were measured once a day at 09:00.

Statistical Analysis

Statistical analysis, for continuous numerical data, the normal distribution of residuals of the parametric model in repeated measurements was analyzed by the Shapiro–Wilk test. Repeated measures analysis of variance *F*-test results were used for the difference between the repeated measurements when conforming to the normal distribution. If the *F*-test was significant, multiple paired comparisons were performed with the post hoc Bonferroni correction test. For the *F*-test and the post hoc Bonferroni test, $p < .05$ was accepted as the cutoff value of statistical significance. Data were analyzed with Statistical Package for the Social Sciences (SPSS) 24.0 software statistics program. For each variable, the variation of the measures according to the days was presented with graphs.

Results

Characteristics of the Lactation Period

Since female rats give birth to their pups prematurely, they have to devote most of their daily time to their pups in the first days of the lactation period. The mean time spent by dams together with their pups was observed as 1198 ± 40 min/day between PN1 and PN8. The time gradually seems to decrease after PN9 yielding to a mean of 931 ± 60 min/day between PN9 and PN18 (Figure 1). After PN19, the time significantly decreases, however, continues until the end of the lactation period and has a mean of 434 ± 37 minutes between PN19 and PN 21. The difference between these three time periods was significant ($p_F < .001$ and all pairwise post hoc $p_{bonferroni} < .001$). Dams spend 82% of the day with their pups between PN1 and PN8 and spend 65% of the day with their pups between PN9 and PN18. After PN19, dams still spend approximately one-third of the day with their pups. The mean time that dams stayed alone was 243 ± 40 min/day between PN1 and PN8, 506 ± 64 min/day between PN9 and PN18, 1007 ± 37 min/day between PN19 and 26. These three periods are significantly different among each other ($p_F < .001$ and all pairwise post hoc $p_{bonferroni} < .001$).

Dams are separated from pups 18 ± 2 times/day between PN1 and PN8, 23 ± 3 times/day between PN9 and PN18, 32 ± 2 times/day between PN19 and PN26. These three periods differ significantly from one another ($p_F < .001$ and all pairwaise post hoc $p_{bonferroni} < .001$).

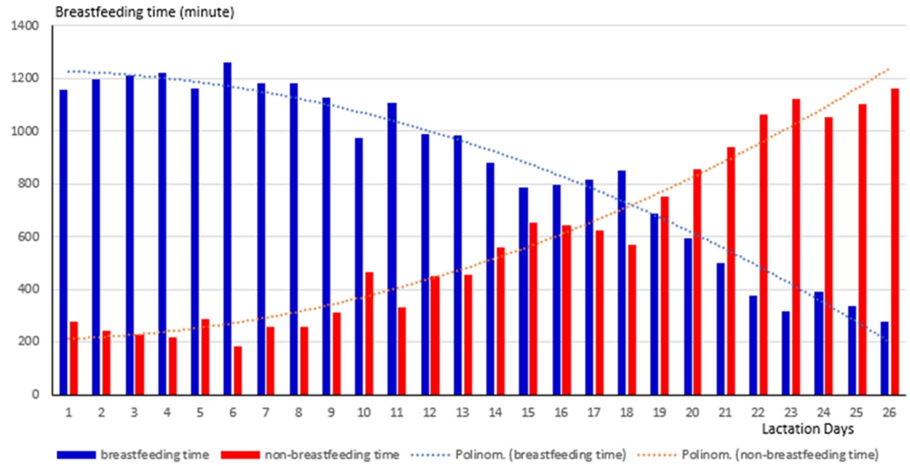

Figure 1.
Breastfeeding/Non-Lactating Periods of Mother Rats During the Lactation Period.

Body Weight Change of Mother Rats During the Lactation Period

During the lactation period of mother Wistar Albino rats, the body weights of the mothers were followed. Maternal weight during the lactation period was monitored (Figure 2). Daily measurements were performed once at 9–10.00 a.m. Mean body weight was 327 ± 9 g, 339 ± 13 g, and 328 ± 12 g during PN1–8, PN9–18, and PN19–26, respectively. Although maternal weight tends to increase between PN9 and PN–18, when compared to the first and third periods, this difference was not statistically different ($p_F > .05$).

Food Consumption of Mother Rats During the Lactation Period

Maternal food consumption during the lactation period was monitored. Daily measurements were obtained once at 9–10.00 a.m. There was a gradual increase in the amount of food consumed during lactation period (Figure 3). The daily maternal food consumption was 72 ± 9 g, 120 ± 18 g, and 142 ± 48 g during PN1–8, PN9–18, and PN19–26, respectively, and the difference among groups was significant ($p_F < .05$ and all pairwise post hoc $p_{bonferroni} < .05$).

Water Consumption of Mother Rats During the Lactation Period

Maternal water consumption during the lactation period was monitored. Daily measurements were obtained once at 9–10.00 a.m. There was a gradual increase in the amount of water consumed during lactation period (Figure 4). Daily maternal water consumption was 98 ± 10 mL, 129 ± 19 mL, and 183 ± 47 mL during PN1–8, PN9–18, and PN19–26, respectively, and the difference among groups was significant ($p_F < .05$ and all pairwise post hoc $p_{bonferroni} < .05$).

Discussion

The phases of lactation in rats are not well described most likely due to difficulties in analyzing the composition of milk during the early stages of lactation. Generally, lactation has been split into three stages in the laboratory experiments: early, mid, and late lactation. The early phase lasts from birth to day 6, the mid period lasts from day 7 to day 14 and is regarded as the period of peak or maximal milk production, and the late period lasts from day 15 until the end of lactation when milk supply falls. Although rat pups can consume solid food and maintain body temperature by themselves starting from PN13, lactation period is defined to last until PN21. We followed pups together with their dams until the cessation of lactation and lactation persisted until PN26. After PN21, dams still spend nearly one-third of the day with their pups while pups continued to consume milk until PN26.

Pups constitute a powerful reinforcing stimulus for their mother. Dams display a strong drive to seek and keep in touch with their

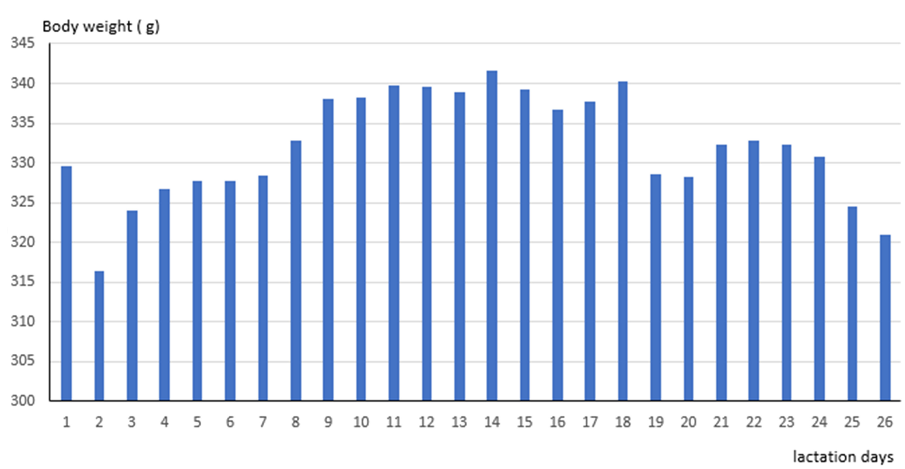

Figure 2.
Body Weight Change of Mother Rats During the Lactation Period.

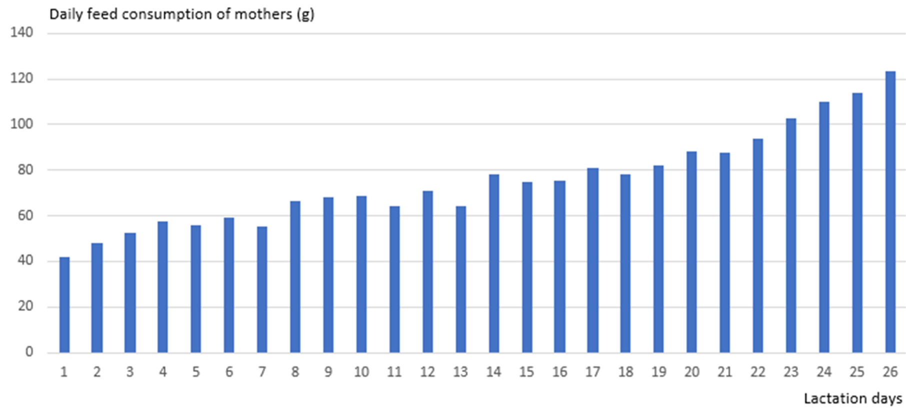

Figure 3.
Feed Consumption of Mother Rats During the Lactation Period.

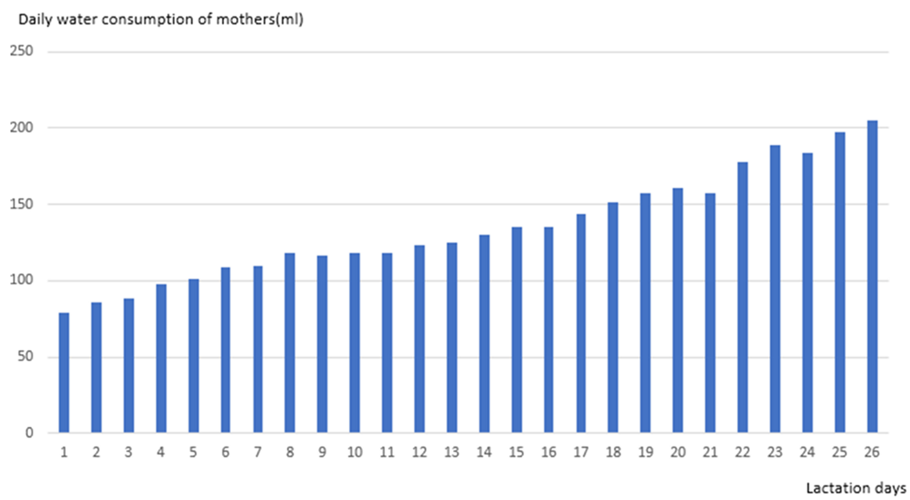

Figure 4.
Water Consumption of Mother Rats During the Lactation Period.

young if they are separated from them. Mother rats will traverse electrified water, learn to navigate mazes, and even bar press hundreds of times over long periods of time to get closer to their offspring. They also exhibit a conditioned location preference for a region previously connected with pup interaction, emphasizing the offspring's reinforcing features to their mother (Moss, 1924; Simons, 1924; Wilsoncroft, 1968). Within the first 8 days, dams even prefer their offspring to cocaine (Mattson, Williams, Rosenblatt, & Morrell, 2001).

Pediatric experimental models frequently cover the lactation period. In order to create a successful model, the lactation period should be further investigated in detail. With a closer look into this complex period, we aimed to provide an insight for the researchers in this field to understand better and evaluate the factors that influence the experimental design. Creation of a better experimental plan would allow researchers to obtain the maximum amount of information specific to their objectives and to carry out their research in an objective and controlled fashion and precision will be maximized. Maternal separation models, ischemia–reperfusion models, and neonatal pain and stress models are some of the many models that cover the lactation period.

Rats weaned early have a low rate of play-fighting and exploratory behavior and increased anxiety in adulthood, while mice weaned early exhibit altered social interactions as juveniles, along with aggressiveness (Bartlett and Piper 1993). Under typical laboratory conditions, pups are ready to be weaned approximately 21 days after birth. However, it was reported that nursing can continue up until PN70 (Pfister, Cramer, & Blass, 1986).

The mean duration of the lactation session was highest in the early stages of lactation. Significant weight change is not observed despite intense milk production and breed care. The increased food intake that occurs during a typical lactation period in rats is brought on by the high energy needs associated with the production of milk. In the second and third postpartum weeks, there was an increase in the number of meals consumed during the day, which indicates that there is an exceptionally high rate of energy expenditure. The most basic component of milk is water. The main reason for the increase in water consumption of mother rats during lactation from birth can be attributed to the increased milk production to feed a large number of offspring. However, after the 16th day of lactation, it should be taken into account that the puppies start to consume food and water as a result of their teeth coming out.

Besides feeding, this early period includes an intense care. Mothers spend extensive efforts to provide thermoregulation, establishing a feeding order between offsprings, keeping the pups in the nest, and grooming them. Since pups lack protective fur and visual and auditory abilities, they are highly dependent on this intensive maternal care. In our study, during PN1-8, the time that dams spent together with their pups constituted 80–90% of the day. The time period that dams spent with their pups lowered gradually afterward.

Lactation models are frequently utilized models among pediatric experimental models. The natural course of the lactation period should be considered when designing studies including lactation period, such as neonatal disease models, maternal separation stress models, and repeated noxious stimuli models. In order to model prematurity or to study the effects of early neonatal exposure, the nature of the lactation period should be taken into account. For example, the success rate of the maternal separation model will be different in different phases of lactation. The time period dams spend with their pups is highest in the first phase and gradually lowers but still high until the cessation of lactation.

Growth and development of the pups and the organ systems occur during the lactation phase (Bauman & Bruce Currie, 1980; Bautista et al., 2008, 2019). Despite the established differences regarding the development of systems among species, animal models provide an investigation of underlying mechanisms which could not be possible in human studies. Additionally, comparative animal physiology allows investigation of similarities and diversities creating opportunities for better understanding (Sinclair et al., 2016).

Conclusion and Recommendations

In conclusion, this study aims to better delineate the characteristics of the lactation in rats especially focusing on the amount of time that the dam spends nursing her young during different phases of the lactation period. Rat dams start to spend less time with their pups after the first phase and the duration of nursing bouts displays a steady decline through the lactation starting from the first day.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dokuz Eylul University Local Ethics Committee For Experimental Animals (Date: July 8, 2022, Number: 09/2022).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – C.Y.; Design – C.Y.; Supervision – D.E.; Resources – O.Y.; Materials – O.Y.; Data Collection and/or Processing – C.Y.; Analysis and/or Interpretation – P.K.; Literature Search – C.Y.; Writing Manuscript – C.Y., O.Y.; Critical Review – A.K.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Dokuz Eylül Üniversitesi, Deney Hayvanları Etik Kurulu'ndan (Tarih: 8 Temmuz 2022, Sayı: 09/2022) alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir– C.Y.; Tasarım – C.Y.; Denetleme – D.E.; Kaynaklar – O.Y.; Malzemeler – O.Y.; Veri Toplanması ve/veya İşlemesi – C.Y.; Analiz ve/veya Yorum – P.K.; Literatür Taraması - C.Y.; Yazıyı Yazan – C.Y., O.Y.; Eleştirel İnceleme – A.K.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

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