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Do Frequent Lactation Periods in Laboratory Rat Colonies Have a Negative Effect on Mothers' Care and Feeding of Themselves and Their Offspring?

Laboratuvar Sıçan Kolonilerinde Sık Laktasyon Dönemleri Annelerin Kendilerine ve Yavrularına Bakım ve Beslenmesi Üzerinde Olumsuz Etkisi Olur mu?

ABSTRACT

Laboratory rats are the second most preferred mammal species in experimental research. Do the frequent lactation periods of the female rats used in production in this study negatively affect the care of the offspring and themselves? For this purpose, the care of mother rats from a conventional outbred Wistar Albino rat colony for themselves and their offspring was monitored in two consecutive lactation periods. Mother rats successfully raised all their offspring in two lactation periods, mothers spent 85% of their daily life in the early phase of the two lactation periods (Post Natal-PN 1-8 days), 60% of their daily life in the mid-lactation phase (PN 9-18 days) and 60% of their daily life in the late lactation phase (PN 19-23 days) have been found to spend 30% of their daily lives with their offspring. While there was a significant difference in terms of the times that mother rats were with their babies in the early, middle, and late stages of the lactation period, the time they were separated from their babies and the frequency of separation ($p < .001$), no significant difference was found when the same stages of the two lactation periods were compared with each other ($p > .05$). Live weight change, feed consumption, and water consumption changes of mother rats in two lactation periods were compared and no significant difference was found. It was concluded that there was no negative effect on the care of mother rats and their offspring during frequent lactation periods.

Keywords: Dry period, Frequent lactation period, Laboratory mother rat, Offspring care, Self-care of mothers.

ÖZ

Laboratuvar sıçanları deneysel hayvan araştırmalarında en çok tercih edilen ikinci memeli türüdür. Bu araştırma, "laboratuvarda üretimde kullanılan dişi sıçanların emzirme dönemlerinin sık olması yavruların ve kendilerinin öz bakımını olumsuz etkiler mi?" sorusuna cevaplandırmak için yapılmıştır. Bu amaçla, geleneksel bir Wistar Albino sıçan kolonisinden gelen anne sıçanların kendileri ve yavruları için bakımı, birbirini takip eden iki emzirme dönemi bu çalışma kapsamında izlendi. Çalışmaya dahil edilen anne sıçanlar doğurdukları tüm yavrularını iki laktasyon döneminde başarıyla büyütmüşlerdir. Anne sıçanların iki laktasyon döneminde de erken laktasyon evresinde (PostNatal-PN1-8 gün) günlük yaşamlarının %85'ini yavruları ile birlikte geçirdiği tespit edilmiştir. Laktasyon döneminin ikinci ve orta evresinde (PN9-18 günleri arasında) günlük yaşamlarının %60'ını, laktasyonun geç ve son evresinde (PN19-23 günleri arasında) günlük yaşamlarının %30'unu yavrularıyla birlikte geçirdikleri tespit edilmiştir. Anne sıçanların laktasyon döneminin erken, orta ve geç dönemlerinde yavrularıyla birlikte kalma süreleri, yavrularından ayrılma süreleri ve ayrılma sıklıkları açısından anlamlı farklılık bulunurken ($p < .001$), iki laktasyon döneminin aynı dönemleri birbiriyle karşılaştırıldığında anlamlı bir fark bulunamadı ($p > .05$). Anne sıçanların iki laktasyon dönemindeki canlı ağırlık değişimi, yem tüketimi ve su tüketimi değişimleri karşılaştırıldığında önemli bir fark bulunamadı. Anne sıçanların ve yavrularının sık laktasyon dönemlerinde bakımında herhangi bir olumsuz etkinin olmadığı sonucuna varıldı.

Anahtar kelimeler: Annenin öz bakımı, Kuru dönem, Laboratuvar anne sıçanı, Sık emzirme dönemi, Yavru bakımı.

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Introduction

Breast milk is the best nutrition for infant growth and development, and is also rich in antibodies that provide the first source of adaptive immunity in a newborn's intestinal tract (Ballard & Morrow, 2013; Bautista et al 2019; Li et al, 2019; Wu et al, 2018). Experimental animal research is an indispensable and irreplaceable research method of translational medicine. The animals most used in experimental animal research are rodents, following rats. Rats give birth prematurely to many offspring (polyparous) in one litter. Mammal mothers perform high efforts to ensure that their babies hold on to life, from the first period of pregnancy until they are weaned. Mothers' struggle against all environmental challenges is vital for the development of their offspring. There is ample evidence for in utero effects before birth on adult physiology and disease risk in mammals, based on information obtained from early epidemiological study in a series of experimental studies in laboratory animals (Barker, 1990; Forsdahl, 1977). Breastfeeding is a critical period for newborn nutrition and baby development. Breast milk is a complex metabolic fluid that supports newborn mammals grow and develop. (Martin, Ling, and Blackburn 2016). Since milk is the most basic food consumed by young in all mammals for a while after birth, it contains all the essential nutrients necessary for early growth and development. In mammals, the act of breastfeeding during lactation is a period in which the mother provides more than just nutrition to the offspring and vital activities that have lifelong effects are carried out (Power & Schulkin, 2013).

Breast milk contains bioactive molecules that contribute to organ development and healthy microbial colonization (Ruiz et al, 2017). The composition of milk changes throughout the day and throughout the lactation period, even if the content of the feed consumed by the mother does not change. During breastfeeding, important stages of development, maturation and differentiation occur in many organs (Bauman & Bruce Currie, 1980; Bautista et al, 2008). Studies have been conducted examining the strong bonds between mother and offspring during the lactation period of rat mothers (Gunderson et al, 2018; Victora et al, 2016) The developmental programming effects of maternal obesity are now well accepted based on results from compelling human epidemiological and animal studies (Zambrano & Nathanielsz, 2013). The extant animal literature shows a clear and convincing role for the effects of bioactive compounds in breast milk on growth and body

composition in offspring. Elegantly designed cross-fostering animal experiments have demonstrated the effects of lactational programming as well as the effects of breastmilk leptin and insulin (Fields vd., 2016).

While mouse pups weaned early from their mothers have low rates of play-fighting and exploratory behavior and increased anxiety in adulthood, mice weaned early display altered social interactions as well as aggression in their juvenile years (Bartlett D, Piper MC, 1993). Under typical laboratory conditions, rodent pups are weaned approximately 21 days after birth. Despite established differences in the development of systems across species, animal models allow the investigation of underlying mechanisms that are not possible in human studies. In addition, comparative animal physiology creates opportunities for better understanding by allowing the investigation of similarities and differences (Sinclair vd., 2016). Since the first 8 days of the lactation period of laboratory rats involve intensive pup care, mother rats are with their pups for most of the day. It has been shown that in the later middle and late periods of lactation, the time the puppies spend with their mothers gradually decreases (Yılmaz et al 2023). In light of the above information, it is understood that the breastfeeding period in mammals is of vital importance for the optimal development of the offspring.

Hundreds of rat colonies are established and routine breeding is carried out in thousands of laboratory animal facilities around the world. The female rats used in production in these rat colonies experience frequent pregnancy and lactation periods throughout the year. We wanted to find an answer to the question of whether these frequent pregnancy and lactation periods have a negative impact on their own self-care and the care of their offspring. In addition, it was conducted to find answers to the questions of how the frequently repeated lactation periods of mother rats create boredom in mothers and affect the process that mothers spend with their offspring, whether there are offspring losses, and whether there is a change in the mother's own nutrition, food consumption and live weight under the intense workload of feeding and caring for a large number of offspring. This study will contribute to a better understanding of the vital lactation period of mother rats, weaning stress models, research covering the lactation period, the design and planning of better pediatric models, and a better interpretation of experimental results.

Materials and Methods

Although there was no procedure that would harm the welfare of the mother rats followed in this study, the study approval was received from the DEU Animal Experiments Local Ethics Committee with protocol approval number 38/2022. The study was conducted on mother rats in the Wistar Albino rat colony where conventional outbred breeding was carried out in the Multidisciplinary Experimental Animal Laboratory of Dokuz Eylül University Faculty of Medicine. Seven pregnant mother rats from a Wistar Albino rat colony routinely bred in the experimental animal laboratory were included in the study. Mother rats were monitored for two consecutive lactations, the dry period between lactation periods, and the pregnancy period. Each mother and her offspring were housed in standard rat cages at 22-25°C with a 12-hour light/12-hour dark cycle, 50-60% humidity. All mothers were fed ad libitum with standard rodent pellet food. Cage bedding was changed twice each week. The lactation period of the mothers included in the study was completed in 23 days. In order to better analyze the 23-day lactation period of mother rats, the first eight days after birth (Post Natal (PN) days 1-8) are considered as the early lactation phase, the next nine days (PN days 9-18) are considered as the mid-lactation period and more. We also examined the next period (Post Natal- between days 19-23) by dividing it into stages as the late lactation period (Figure 1).

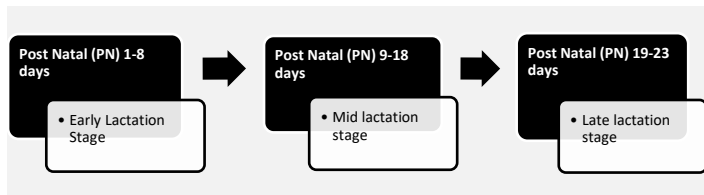


Figure 1. Sub-Periods of the lactation period in mother rats

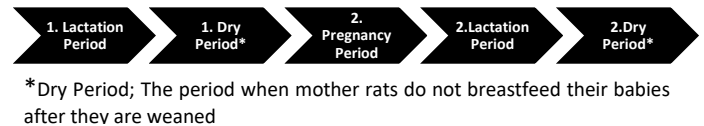
Şekil 1. Anne sıçanlarda laktasyon döneminin alt dönemleri

The main reason why we examined three separate sub-periods during the lactation period in rats is that the first eight days after birth (PN1-8) are the premature period of the offspring, and the later middle and late lactation stages of lactation are better analyzed and compared between these periods.

The period in which mother rats are not breastfed for approximately 3 weeks after being separated from their offspring after their lactation period is referred to as the dry period. After the first drying period, the rat mothers were mated one to one again and the second pregnancy

period was started. After the second pregnancy period, the mother rats were followed during the second lactation and second drying periods. Mother rats included in the study were followed for 121 days. Both lactation periods of the mother rats were continuously recorded with a night vision video camera. Camera recordings were taken in 24-hour periods (09:00-09:00) without disturbing the mother rats. Feed and water consumption and body weight of each rats were measured once a day at 09:00 in the morning. During the lactation period of mother rats, the duration of a day is 1440 minutes (24 hours x 60 minutes = 1440 minutes). The recordings taken in one day (1440 minutes) were watched again and the time the mothers were with their babies, the time the mothers were separated from the babies, the number of times the mother was separated from their babies, and how much time the mothers devoted to themselves were calculated in minutes.

Within the scope of this study, seven Wistar albino mother rats were followed for a total of 121 days, including the first lactation period of 23 days, the first drying period of 26 days, the second pregnancy period of 21 days, the second lactation period of 23 days and the second drying period of 26 days. Figure 2 shows the follow-up periods of mother rats.



*Dry Period; The period when mother rats do not breastfeed their babies after they are weaned

Figure 2. Monitored periods of mother rats

Şekil 2. Anne sıçanların izlendiği dönemler

Statistical Analysis

For continuous numerical data, the normal distribution of the residuals of the parametric model in repeated measurements was analyzed by the Shapiro-Wilk test. The difference between repeated measures when it complied with normal distribution was analyzed with repeated measure ANOVA F test. If the F test was significant, multiple pairwise comparisons were made with the posthoc Bonferroni correction test. $p = .05$ was accepted as the cut-off value for statistical significance for the F test and posthoc Bonferroni test. Data were analyzed with SPSS 24.0 statistical program. The changes in measurements for each variable over the days were shown graphically.

Results

Number of Offspring of Mother Rats: The average number of offspring in the first birth of the seven mothers followed in the study was 10.8 ± 1.2 , and the average number of offspring in the second birth was 11.1 ± 1.06 . When mothers were compared in terms of the number of offspring in their first and second births, no statistically significant difference was found ($p > .05$).

Evaluation of Mother Rats in Two Lactation Periods: The time the mother rats followed in the study were with their pups during the two lactation periods, the time the mothers were separated from their pups, and the frequency of separation of mothers from their pups were evaluated. Table 1 shows the averages and standard deviations of the first and second lactation periods. Both lactation periods of mother rats were analyzed by dividing them into early lactation phase (PN 1-8 days), mid-lactation phase (PN 9-18 days) and late lactation phase (PN 19-23 days).

Table 1. Evaluation of lactation periods of mother rats

Tablo 1. Anne sıçanların laktasyon sürelerinin değerlendirilmesi

Lactation Stages	First Lactation Period			Second Lactation Period		
	Early	Middle	Late	Early	Middle	Late
Lactation Days	PN* 1-8	PN 9-18	PN 19-23	PN 1-8	PN 9-18	PN 19-23
Mean time the mother was with her offspring \pm SD (min.)	1170 \pm 40	905 \pm 60	441 \pm 27	1147 \pm 45	875 \pm 35	418 \pm 45
Mean time mothers were separated from their babies \pm SD (min.)	232 \pm 20	524 \pm 35	1078 \pm 27	278 \pm 35	575 \pm 22	1095 \pm 32
Frequency of mothers being separated from their offspring mean \pm SD (minutes)	17 \pm 5	25 \pm 7	36 \pm 3	15 \pm 4	27 \pm 3	38 \pm 7

*PN: Post Natal

When the average times that mothers were with their babies in the early, middle and late stages of both lactation periods were compared with each other, a statistically significant difference was found between the three periods ($p < .001$ and all pairwise post-hoc Bonferroni < 0.001). Table 1 shows that mothers spend more time with their babies in the early stages of both lactation periods than in the middle and late lactation stages. While mother rats spend 85% of the day (1170 ± 40 minutes of 24 hours (1440 minutes)) with their offspring in the early phase of the lactation period, they spend 65% of the day with their offspring in the middle phase and 30% in the late phase. It has been determined that the time mothers spend with their babies after 19 days after birth decreases significantly, but they are not completely separated from their babies. When the same lactation substages of the mothers' two lactation periods were compared with each other, no statistically significant difference was found ($p > .05$). In other words, it was determined that mothers were with their babies for a similar period of time in the early, middle and late stages of the first lactation and in the early, middle and late stages of the second lactation.

In Table 1, when the times that mothers stay away from their babies during the first and second lactation periods are compared, it has been determined that while it is very short in the early stages of both lactation periods, the times that mothers stay away from their babies gradually increase in the middle and late lactation stages. A statistically significant difference was found when the times that mother rats remained away from their offspring in the early, middle and late lactation stages of the first and second lactation periods were compared with each other ($p < .001$ and all double post-hoc Bonferroni < 0.001). When the same substages of the mothers' first and second lactation periods were compared with each other, no statistically significant difference was found ($p > .05$). Mother rats are separated from their babies 15% of the day in the early stage of the lactation period, 35% in the middle stage, and 70% in the late stage. During the late lactation period, the time during the day that mother rats spend with their offspring gradually decreases.

Table 1 shows the frequency of separation of mothers from their offspring in the first and second lactation periods. While mothers leave their offspring alone less in the early stage of lactation than in the middle and late stages of lactation, it is seen that they leave them alone more in the

middle and late stages. When the frequency of separation of mother rats from their offspring was compared in the early, middle and late stages of the first and second lactation periods, a significant difference was found in terms of the frequency of separation from the mother ($p < .001$ and all binary post-hoc Bonferroni < 0.001). There was no significant difference in the frequency of separation of mother rats in the first and second lactation periods when the same periods were compared ($p > .05$).

Figures 3 and 4 show the time the mothers were together and apart from their offspring during the first and second lactation periods of the mother rats. As can be seen from both graphs, it is seen that rat mothers devote a lot of time to their offspring between days PN1-8 in the early stages of lactation, and this time gradually decreases in the second and third stages of lactation. As seen in Figures 3 and 4 mother rats spend half of the day with their babies on days 18-19 of both lactation periods, while keeping the other half for themselves. The amount of time that mother rats spend with their offspring gradually decreases in the following lactation days. As mother rats raise their pups during the lactation period and their need for the mother decreases, the time they spend with their pups decreases and the time they spend away from their pups increases. It should not be overlooked that the fact that rat pups start feeding on their own in addition to mother's milk from the age of 16 onwards and gradually switch to solid foods has a significant effect. As mother rats raise their offspring and the needs of the offspring decrease, the frequency and duration of mothers' separation from their offspring gradually increases. Separating the mothers from the cubs gives the cubs the opportunity to be on their own. It is possible to say that repeated lactation periods of rat mothers do not cause weakness in offspring care.

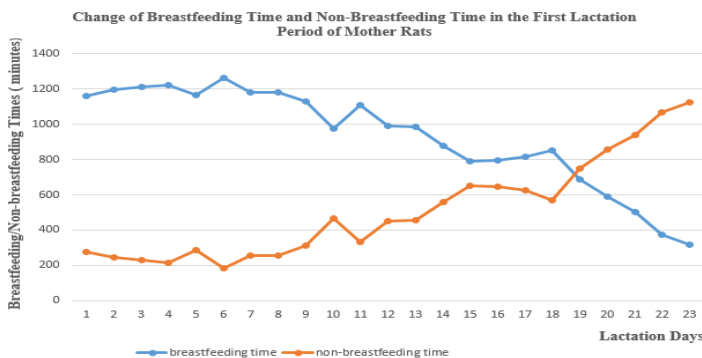


Figure 3. Periods During the First Lactation Period When Mothers Breastfed / Non Breastfeed

Şekil 3. İlk Laktasyon Döneminde Annelerin Emzirdiği/Emzirmediği Dönemler

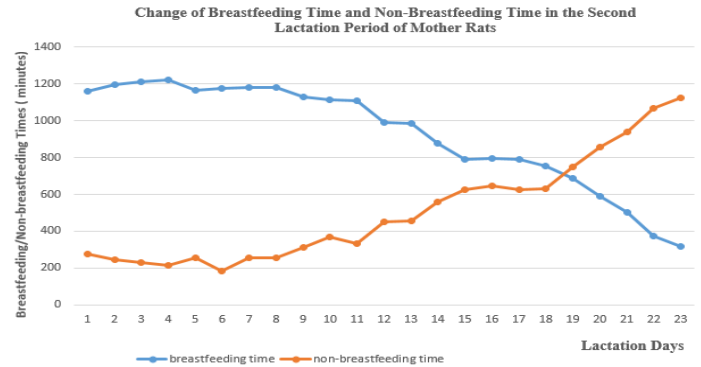


Figure 4. Times in which mothers breastfed and did not breastfeed their babies during the second lactation periods.

Şekil 4. Annelerin ikinci emzirme dönemlerinde bebeklerini emzirdikleri ve emzirmedikleri zamanlar.

Body weight change of mother rats: Body weight averages, standard deviations and statistical comparison of mother rats during successive lactation and dry periods are given in Table 2.

Table 2. Average Body Weights of Mother Rats During Lactation and Drying Periods and Statistical Comparison Results

Tablo 2. Anne Sıçanların Laktasyon ve Kurutma Dönemlerindeki Ortalama Vücut Ağırlıkları ve İstatistiksel Karşılaştırma Sonuçları

Follow-up Periods of Mother Rats	Average Body Weight of Mother Rats (g)	Std. Deviation	p
1.Lactation Period	321.0	23.9	.022
1.Dry Period	347.2	5.8	
2.Lactation Period	314.2	12.8	.026
2.Dry Period	338.7	8.4	
1.Lactation Period	321.0	23.9	.380
2.Lactation Period	314.2	12.8	
1.Dry Period	347.2	5.8	.100
2.Dry Period	338.7	8.4	

A statistically significant difference was found when the average body weight of mother rats was compared between the first lactation period and the first dry period, and the second lactation period and the second dry period ($p < .05$). It is observed that the body weight of mother rats decreases slightly during the lactation period and regains its previous weight during the subsequent drying period. When the average body weight of mother rats in the first and second lactation periods was compared, no statistically significant difference was found ($p > .05$). As seen in Table 2, no statistically significant difference was found when the average body weights of mother rats were compared during the first and second drying periods ($p > .05$). Apart from this, it can be said that mother rats maintain their body weight well in different periods such as lactation, drying and pregnancy.

Changes in Feed Consumption of Mother Rats: Changes in feed consumption of mother rats during successive lactation, drying and pregnancy periods were followed, and feed consumption averages, standard deviations and statistical comparison results of mother rats are given in Table 3. A statistically significant difference was found when the feed consumption of mother rats during the lactation period was compared with the feed consumption during the dry period ($p < .001$). When the average feed consumption amounts of mother rats in the first and second lactation periods were compared, no statistically significant difference was found ($p > .05$). When the average feed consumption amounts of mother rats in the first and second drying periods are compared in Table 3, no statistically significant difference was found ($p > .05$). A gradual increase in the amount of feed consumption was detected, especially during the lactation period. During the first and second lactation periods, the puppies start to switch to solid food after the 16th day of lactation, so it should not be ignored that some of the feed consumed in the cages originates from the puppies. The average feed consumption amounts of mother rats in the early, middle and late stages of lactation were measured as 72 ± 9 g, 120 ± 18 g and 142 ± 48 g, respectively. When these three periods were compared with each other, a statistical difference was found in the amount of feed consumption between the stages ($p < .05$ and all double post-hoc Bonferroni 0.05).

Table 3. Average Feed Consumption Amounts of Mother Rats in Different Periods and Statistical Comparison Results

Tablo 3. Anne Sıçanların Farklı Dönemlerdeki Ortalama Yem Tüketim Miktarları ve İstatistiksel Karşılaştırma Sonuçları

Follow-up Periods of Mother Rats	Average Feed Consumption of Mothers (g)	Std. Deviation	<i>p</i>
1.Lactation Period	170	26.1	.001
1.Dry Period	40.8	3.9	
2.Lactation Period	170.8	45.4	.001
2.Dry Period	40	3.6	
1.Lactation Period	170	26.1	.946
2.Lactation Period	170.8	45.4	
1.Dry Period	40.8	3.9	.671
2.Dry Period	40	3.6	

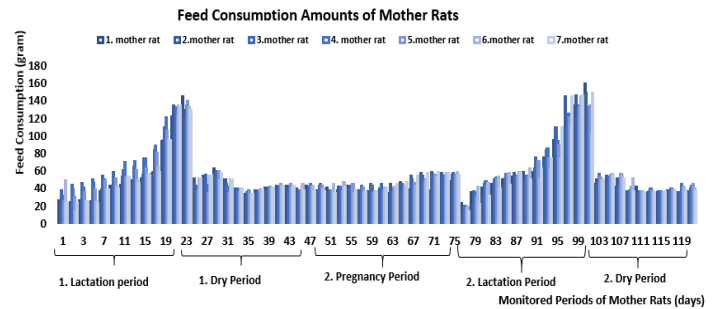


Figure 5. Changes in Average Feed Consumption Amount of Mother Rats in Different Periods

Şekil 5. Farklı Dönemlerde Anne Sıçanların Farklı Dönemlerdeki Ortalama Yem Tüketim Miktarlarındaki Değişimler

As seen in Figure 5, it shows the change in feed consumption during lactation periods, dry periods and pregnancy periods. Figure 5 shows that the feed consumption of mother rats increases significantly, especially during lactation periods, compared to other periods.

Changes in Water Consumption of Mother Rats: Changes in water consumption of mother rats during successive lactation, dryness and pregnancy periods were followed, and the water consumption averages, standard deviations and statistical comparison results of mother rats are given

in Table 3. A statistically significant difference was found when the water consumption of mother rats during the lactation period was compared with the water consumption during the dry period ($p < .001$). When the average water consumption amounts of mother rats in the first and second lactation periods were compared with each other, no statistically significant difference was found ($p > .05$). When the average amount of water consumption of mother rats was compared during their dry period, no statistically significant difference was found ($p > .05$). A gradual increase in the amount of water consumption was detected, especially during the lactation period. The average water consumption of mother rats in the early, middle and late stages of lactation was measured as 91 ± 9 ml, 123 ± 12 ml, 173 ± 25 ml, respectively. When the water consumption amounts of the lactation stages were compared with each other, a statistical difference was found ($p < .05$ and all double post-hoc Bonferroni < 0.05). It should not be ignored that during the first and second lactation periods, the puppies start to switch to solid food after the 16th day of lactation, and therefore some of the water consumption in the cages is due to the puppies.

Table 4. Average Water Consumption Amounts of Mother Rats During Lactation and Drying Periods and Statistical Comparison Results

Tablo 4. Anne Sıçanların Laktasyon ve Kurutma Dönemlerinde Ortalama Su Tüketim Miktarları ve İstatistiksel Karşılaştırma Sonuçları

Follow-up Periods of Mother Rats	Average Water Consumption of Mothers (ml)	Std. Deviation	<i>p</i>
1.Lactation Period	283.8	21.9	>.001
1.Dry Period	76.3	8.5	
2.Lactation Period	283.3	35.5	>.001
2.Dry Period	81.3	6.3	
1.Lactation Period	283.8	21.9	.975
2.Lactation Period	283.3	35.5	
1.Dry Period	76.3	8.5	.116
2.Dry Period	81.3	6.3	

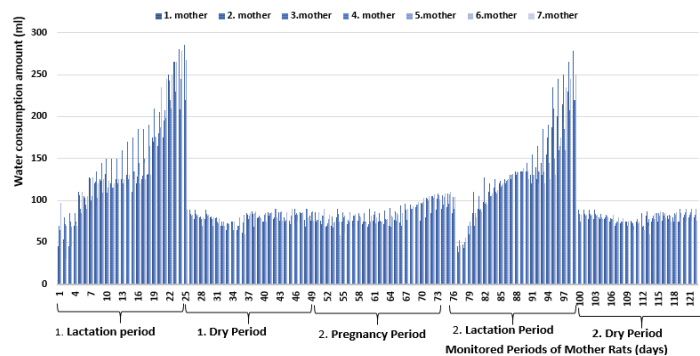


Figure 6. Changes in the average water consumption amount of mother rats in different periods

Şekil 6. Anne sıçanların farklı dönemlerdeki ortalama su tüketim miktarlarındaki değişimler

As seen in Figure 6, it shows the change in water consumption of mother rats during lactation periods, dry periods and pregnancy periods. Figure 6 shows that the water consumption of mother rats increases significantly, especially during the lactation period, compared to other periods.

Discussion

Female rats used in the production of rat colonies in thousands of laboratory animal facilities experience frequent pregnancy and lactation periods. Mother rats give birth to many premature babies and perform their maternal duties with great devotion, ensuring the survival of their babies. Mother rats are in a frequently repeated cycle for the future of their colony. We set out with the hypothesis that frequent pregnancy and lactation periods of mother rats would negatively affect their own self-care and the care and nutrition of their offspring.

Rat mothers carry out an intense maternal duty to ensure the survival of their prematurely born offspring (eyes closed, ears closed, hairless, toothless, unable to regulate temperature) in the first 8 days after birth (PN 1-8 days). Considering that the medical team carries out a very vital process in the intensive care unit of a premature human baby with serious equipment support to ensure the baby's survival, rat mothers provide the same intensive care process to many of their babies without any support and ensure that their babies hold on to life. In the early lactation phase (between PN1-8 days) of the mother rats included in the study, in both lactation periods followed, each mother's daily life is spent 85% of her daily life working intensively to provide regular care, feeding,

heating and meeting all needs, and the remaining 15% of the day is her own life. It has been determined that he separates his care and nutrition. This result is evaluated as rat mothers providing an extraordinary intensive care service. For this reason, the early lactation phase of mother rats is not just about breastfeeding, it is the period when they struggle very hard to keep their babies alive. The early lactation period of mother rats is a vital period in terms of feeding, warming, protecting their babies, keeping them in the nest because they do not move, and most importantly, helping them hold on to life. Between PN9 and 18 days, which is the middle phase of the two lactation periods examined and followed in mother rats, the eyes and ears of their babies are opened, they can move and they can thermoregulate themselves with the growth of their hair, so there is a partial change in their maternal roles and the time the mothers spend with their babies decreases. It was determined that between days PN19 and 23, which is the late phase of the lactation period of mother rats, the babies' teeth came out, they fed themselves, albeit very little, their fur was completed, they moved on their own, and since maternal dependency partially decreased, the time spent by mothers with their babies decreased considerably in this phase. Mother rats are separated from their offspring more frequently in the last period of lactation, encouraging them to move more independently.

When the frequency of separation of mother rats from their offspring during the day was compared in the early, middle and late stages of the two lactation periods followed, a significant difference was found between the lactation stages. However, when the same stages of both lactation periods were compared in terms of separation frequency, no significant difference was found. In the first phase of both lactation periods (PN days 1-8), mother rats are least frequently separated to ensure the survival of their offspring. It has been observed that in the early stages of lactation, mother rats leave their babies and nests less during the day than in the middle and late lactation stages. It has been determined that as mother rats raise their offspring and the needs of the offspring decrease, they are less likely to be with their offspring and abandon their offspring more frequently.

It has been determined that the mother rats lose live weight during the lactation period, especially in the first stage, because they neglect their own nutrition under intense workload. It has been measured that mother rats consume more feed and water than normal in order to produce intensive milk to feed a large number of their babies during the lactation period. Mother rats consume

more water and feed during lactation due to the high energy needs associated with milk production. Since the most basic component of milk is water, the main reason for the increase in water consumption of mother rats during the lactation period from birth is related to the increased milk production to feed a large number of offspring. However, it should be taken into account that after the 16th day of breastfeeding, the puppies begin to consume food and water as a result of the emergence of their teeth.

During the 121-day follow-up of the mother rats, when their live weight, feed consumption and water consumption were evaluated during two lactations, two dry periods and one pregnancy period, it was determined that all mothers included in the study showed similar characteristics. It is the most important result that confirms that studies involving lactation, dryness and pregnancy periods in rats should be carried out using the least number of animals. The fact that each mother has similar biological characteristics to the other supports the ethical principle of using at least one animal. If biological variability such as regular estrous cycles, mating, pregnancy, lactation and drying periods are minimized in production colonies under optimal laboratory conditions, it will reduce the variations in research and contribute to achieving reproducible results.

In our study, the fact that rat mothers were with their offspring 85% of the day in the early stages of lactation (PN1-8) is an indicator of the close relationship between mother and offspring. It is observed that the time mothers spend with their babies gradually decreases in the middle and late stages of lactation. Researchers studying the separation stress model from the mother will increase the model's effectiveness if they apply this stress model in the first 8 days. In this respect, especially if pediatric experimental models cover the lactation period, the details of the lactation period must be known for a successful model design. When designing studies that include the breastfeeding period in the evaluation of neonatal experimental animal models, the natural course and stages of the breastfeeding period must be taken into account.

In terms of laboratory animal science, this research is valuable in that it sheds light on the care of the breeding mothers used in rat colonies during the successive lactation and drying periods of the mothers for their offspring and for themselves. Mother rats showed high performance in the care of their offspring during two lactation periods and raised all their offspring without any loss, demonstrating that laboratory rats are mammals that show selflessness and high effort. Although mother rats have very little time

for themselves in the early lactation phase, they use the time well and try to feed themselves and their babies with more frequent meals. When the feed and water consumption of mother rats during lactation, drying and pregnancy periods were compared, it was determined that there was a significant increase in the lactation period compared to other periods. It is possible to say that the mother rats' frequent lactation periods do not cause any change in their interest in their offspring and that they make the same effort to ensure the survival of all their offspring.

Conclusion

As a result, this study found that rat mothers were different in terms of the time they were with their babies, the time they were not together, and the frequency of separation in the early, middle and late stages of the lactation period. It has been revealed that as rat mothers raise their offspring, their dependency on them decreases, the time they spend together decreases, and they are separated from their offspring more frequently. It was concluded that in the rat breeding colony, the burden of frequent pregnancy and lactation periods on mother rats did not have a negative impact on their care and attention to their offspring and that they did not neglect their offspring. It has also been shown that mother rats can provide adequate nutrition and care despite the negative impact of repeated lactation periods on their self-care under an intense workload. During the dry period after the lactation period, they recover very quickly and become pregnant again. Therefore, the results of this research have provided valuable results in terms of laboratory animal science and translational medicine. A more detailed examination of the lactation period of laboratory rats will make significant contributions to laboratory animal science and experimental animal research.

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