OPEN ACCESS
Journal of Animal Health and Production

Research Article

Effects of Postpartum Separation of Rabbit Offspring from their Mothers on Pregnancy Rate, Milk Production, Behavioral Indicators, Blood Biomarkers of Oxidative Stress, Hormonal Levels, and Performance of Young Rabbits from Birth to Weaning

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Abstract | The goal of the study was to determine how the 22-hour daily separation of newborn bunnies from their mothers affected the health and ability of doe rabbits to reproduce as well as the survival of the young from birth to weaning. In this study, twenty female New Zealand White (NZW) rabbits were employed, immediately following first parity. The young rabbits were left with their mothers for the first two days after kindling so they could nurse on colostrum. By adding or removing the kits on the third day, the quantity of each litter for each mother was consistent with six, ensuring an equal number of litters at the beginning of the experiment. One of the two groups of animals received a random distribution of female rabbits. Ten does in group one acted as controls and remained with their kits in a group during free lactation (doe-litter together, DLT). In group two, ten does were isolated for 22 hours from their litter (doe-litter separated, DLS). The findings revealed that mothers in the DLS group had a higher ($P<0.01$) conception rate (28.6%), significantly lower ($P<0.05$) feed intake, and milk output than mothers in the DLT group. At all experimental ages, the body weight of the separated offspring from their mothers was considerably lower than that of the DLT group ($P<0.05$). Concentrations of blood biochemical and hormonal levels were affected ($P<0.05$) due to separation. On day 7, the antioxidant enzyme activities were considerably lower ($P<0.05$) in the DLS group of rabbits than in the DLT group of animals. It can be inferred that the separation of the kits from their mothers has an impact on the mothers at the beginning of separation while the isolation approach raises the level of the hormone estradiol, which improves the rate of conception.

Keywords | Behavioral parameters, Conception rate, Milk yield, Rabbits, Separation.

Received | June 12 2022; **Accepted** | September 01, 2022; **Published** | February 05, 2023
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Citation | Habeeb AA, Kamal AM, Atta MAA, Sharaf AK (2023). Effects of postpartum separation of rabbit offspring from their mothers on pregnancy rate, milk production, behavioral indicators, blood biomarkers of oxidative stress, hormonal levels, and performance of young rabbits from birth to weaning J. Anim. Health Prod. 11(1): 34-44.
DOI | http://dx.doi.org/10.17582/journal.jahp/2023/11.1.34.44
ISSN | 2308-2801

INTRODUCTION

The fact that a lactating rabbit nurses its young once or twice a day, for around five minutes each, in its natural environment is well accepted (Ubilla et al., 2001). On the other side, breastfeeding rabbits that miss the suckling phase experience an increase in milk in the mammary glands alveoli, which impairs the mammary gland's ability to produce milk and severely impacts the growth and wellbeing of the young rabbits (Ubilla et al., 2000a). Blood levels of prolactin and oestradiol during lactation may be antagonistic hormonally, which could lower rates of receptivity and fertility (Fortun & Bolet, 1995; Ubilla et al., 2000b). In comparison to non-responsive rabbits,

the receptive rabbits do exhibit higher plasma oestradiol concentrations and lower prolactin concentrations (Theau-Clement & Roustan, 1992). Doe litter separation boosts the proportion of oestrus symbols seen and affects fertility (Castellini et al., 1998). The separation carried out before to artificial insemination to boost conception was acknowledged for its lack of sucking incidences and greater oestradiol and lower prolactin levels (Ubilla et al., 2000b). The majority of studies focused on the doe-separation procedure in breastfeeding rabbits before artificial insemination (AI), which was used to enhance fertility and reproductive success by enhancing ovarian activity and elevating the blood oestradiol concentration in female rabbits (Cano et al., 2005a, b; Rebollar et al., 2004). Maertens (1998) discovered that after process separation in breastfeeding rabbits for 40 hours, receptivity and fertility were (78.8 and 78.0 percent) considerably greater than the non-separated does (40.8 and 66.9 percent). Furthermore, right after the regulated suckling, receptivity and conception rate increased by around 30 percent and 10 percent, respectively. In contrast to does that had free nursing, Bonanno et al. (2004) discovered that doe-litter separation for two days before to AI increased the fertility of doe rabbits. The authors came to the conclusion that the doe-litter separation group's productivity increased by 35.4–44.2 percent. By altering the secretion of FSH and LH to be connected with the increased impulse of the ovulatory response to mating or by causing the AI process in does to separate from their offspring, Cano et al. (2005a) discovered that litter separation is an active strategy to encourage ovarian activity before AI. Young rabbits being taken away from their mothers may put the does under stress and cause a lot of reactive oxygen species to be produced (ROS). It may lessen antioxidant enzymatic activity, which could start health problems and have a negative impact on animal performance (Aschbacher et al., 2013). There isn't much evidence, however, on how separation affects the welfare of the does, particularly behavioral markers of does after separation from their litter, blood biochemical components, hormone levels, and antioxidant enzyme activities in the separated doe. The aim of this study was to determine the effects of restricted breastfeeding, 22-hour separation, and two-hour milking vs. free lactation on the welfare and reproductive performance of doe rabbits as well as the vitality of the offspring from birth to weaning.

MATERIALS AND METHODS

Experimental site

During Egypt's winter (March, April ,and May of 2021)., the practical work was carried out at the Rabbit Farm project of the Radioisotopes Application Division of the Nuclear Research Center of the Atomic Energy Authority in the Inshas area of Egypt (latitude 31o 12' N to 22o 2' N, longitude 25o 53' E to 35o 53' E).

Experimental ethics

The Egyptian Atomic Energy Authority's Animal Care and Welfare Committee approved this investigation. Essential data on the endeavor to lessen animal suffering and adherence to the strictest veterinary care standards are included in experimental ethics.

Experimental design

In this investigation, twenty female New Zealand White (NZW) rabbits were used each one just after first parity. The young female rabbits were left with their mothers for the first two days after kindling so they could nurse on colostrum. By adding or removing the kits on the third day, the quantity of each litter for each mother was constant with six, ensuring a comparable equal number of litters at the beginning of the experiment. To one of the two animal groups, random distribution of female rabbits was made. Ten does in group one acted as the control and were allowed free access to suckle their young during the whole lactation period (doe-litter together, DLT). Except on the day of the estimate of milk production, does in the control group were not allowed to nurse their young. From day 3 to day 28 post-partum, ten mothers in group two underwent regulated lactation (every 22 h Plus 2 h milking) while being kept apart from their litter for 22 hours. The second day, at 9:00 a.m., the nest boxes of the doe-litter separation group were opened to enable for the nursing of the young. The metallic screen was taken off, and the kits were then given two hours to nurse from their mothers before being taken apart. From the time the kits were weaned at 11 a.m. until the following morning at 9 a.m., the nest boxes of the separated does were sealed for a total of 22 hours.

Feeding, housing, and management of experimental does

The commercially available pelleted diets were fed to the two experimental groups of lactating female rabbits for the course of the trial. Forty percent clover hay, 25 percent wheat bran, 15 percent yellow maize, 10 percent soybeans, 5 percent molasses, 2 percent bone meal, 1 percent calcium carbonate, 1 percent sodium chloride, 0.5 percent vitamins & minerals premix, and 0.5 percent DL-methionine are the ingredients of the marketable food. Commercially available pelleted diets as a percentage of dry matter (DM) contain 18.5% crude protein, 12.5 % crude fiber, 3.5 % ether extract, 56 % nitrogen-free extract, and 9.5 % ash, according to biochemical analysis. 2600 kcal/kg DM are available for digestion. The experimental rabbit had unlimited access to the pelleted diets that were available on the market and to fresh water. Every morning, feces and urine were cleaned up from the floor and cages. Throughout the entire experimental period, which ran from the beginning

of March to the end of May 2021, the experimental groups were maintained under natural light-dark cycles (16 hours of light and 8 hours of darkness) and natural environmental temperature ranges (minimum 14°C at night and maximum 20°C during the day). The relative humidity ranged from 60-70 percent during the entire experiment. Both doe groups were housed in separate metal cages that were each 70×70x48 cm high and had a food and nest box outside. An over-under pressure ventilation system with warm air has been used to maintain the interior temperature in the windowless experimental stable throughout the winter.

Estimation of doe weekly milk yield and feed intake

Daily milk yield (DMY, g) per animal was calculated from the weight of each doe progeny before and after the end of suckling on days 7, 14, 21, and 28 of the suckling period. The weight of each doe litter before and after the completion of the suckling at midday of the second day of the isolation, as stated by Szendro et al. (1999) was used to estimate the DMY per animal in the group of DLT. On days 7, 14, 21, and 28, each doe's daily feed intake (g/day) from kindling was noted. By removing the leftover food from the food that was provided the day before, the amount of food consumed was recorded.

Estimation of the conception rate

In order to create a natural mating on day seven from kindling, both groups of does were randomized and introduced for two days to the good and healthy four NZW bucks. All the animals were willing to mate. The males that were used for mating were kept in a different section of the same building and belonged to a heavier male line of the same strain. Ten days after mating, does were palpated by hand to check for pregnancy. The lower abdomen of the animal was felt with the thumb and forefinger. Regardless of the influence of bucks, the conception rate was recorded for each group after the pregnancy was confirmed (CR = No. of pregnant rabbits delivered/No. of rabbits joined to the males x100).

Estimation of daily gain and vitality of young rabbits

Estimates were made for the quantity, weight, and death rate of the kits. To determine the average daily increase and vitality of the kits in the two experimental groups, the weight of the kits was calculated starting on day 1 and ending on day 28. Vitality percent from birth to day 28 was also estimated, along with mortality number and percentage from birth to day 28.

Observation of the doe-behavior parameters

Two co-authors were able to observe some behavioral changes that occurred in both groups of rabbits during the trial period. Each co-author is seated in a steady position while the other observes each co-behavioral author's scores once each week from 10.0 to 12.0 a.m., three times a day and three various behaviors were recorded. The behavioral indications of moving, scratching, difficulty remaining alert, and an increase in stirring were used to identify anxiety. When the female rabbits appeared to be isolated in their cage, the withdrawal was scored. The length of feed consumption per hour in response to the food offer was scored during the delay interval. Doe-behavior parameter scores were recorded every day and evaluated for explanation (Rafel et al., 2012; Mutwedu et al., 2021). According to Ngoula et al. (2017) each behavior was given a score between 0 and +4 based on its severity and frequency (0 being none, +1 being extremely weak, +2 being weak, +3 being moderately weak, and +4 being very weak).

Estimation of blood components, hormonal levels, and antioxidant enzyme activities

On the mornings of days 7, 14, 21, and 28 after kidding, single blood samples (4 ml) from the marginal ear vein were collected from vacationer tubes without anticoagulant material from the two experimental groups. Following collection, blood samples were immediately centrifuged at 1000g for 15 min. Before analysis, the serum was stored at a temperature of –20°C. Utilizing commercial chemical reagent kits mass-produced by Diamond Diagnostic Company, Egypt, the amounts of total protein, globulin, γ-globulin, LDL, and glucose were estimated. Using the Radioimmunoassay (RIA) approach, commercial kits measured the serum quantities of oestradiol-17beta and cortisol (Diagnostic Product Corporation, Los Angeles, USA). The tubes with the antibody coating were ^{125}I-labeled. Following incubation, the liquid in the tubes is aspirated, and the radioactivity is measured using a Computerized Gamma Counter in accordance with the manufacturer's instructions. Colorimetric analysis was used to measure the antioxidants. According to Rotruck et al. (1973), Misra and Fridovich (1972) and Goth (1991), Glutathione peroxidase (GSH), Superoxide dismutase (SOD), and catalase (CAT) as antioxidant enzyme activities were measured in the serum using chemical reagent kits (SIGMA-ALDRICH, Inc. St. Louis, MO., USA).

Statistical analysis

Data were statistically analyzed using SPSS (2012) procedures in accordance with the following model: Yij = + Ti + eij, where eij = residual error, = overall mean, Ti = fixed effect of treatments (1 = DLT, 2 = DLS), and = overall mean. Duncan's novel multiple ranges test was used to compare the significant differences between means (Duncan, 1955). The Chi-square test was used to assess statistical differences between the two treatments for the likelihood of conception rate and kit vitality and significant

results were further quantified using multiple Z-tests to compare parallel proportions.

RESULTS

Influence of separating rabbit's offspring from their mothers on doe feed intake

When compared to free-nursing does, the DLS group's rabbits significantly (P<0.05) consumed less feed from the concentrated diet (DLT). Feed intake was higher in DLT than in DLS on days 7, 14, 21, and 28 of the lactation period by 15.2, 12.2, 11.9, and 12.73 %, respectively (P<0.05). The overall mean feed intake reduced from 195.75 g/day to 170.5 g/day (13.0 %) when a doe was separated from its litter at 22 hours, as shown in Table (1).

Influence of separating rabbit's offspring from their mothers on daily milk yield

Does the DLS group produce significantly (P<0.05) less milk per day than the free-nursing group (DLT). Daily milk supply was 27.1, 16.2, 10.2, and 8.6 % lower in DLT than in DLS on days 7, 14, 21, and 28 of the lactation period (P< 0.05). When a doe was separated from her litter at 22 hours, the overall milk production fell from 181.7 g/day to 159.5 g/day, or 12.3%, and the total milk production for the course of the experiment fell from 710.9 g in DLT to 599.2 g in DLS, or 13.2%. (111.7g). Due to nursing day, the average milk supply was greatly impacted. The lowest values were on days 7 and 28, whereas the highest values occurred on day 14. Day 21's milk yield was lower (P<0.05) than Day 14's milk yield and Lower (P<0.05) than Day 28's milk output (Table 2).

Influence of separating rabbit's offspring from their mothers on doe weight

The doe's weight was unaffected by the separation treatment on any of the experiment's days. According to data in Table 3, there was no discernible change in the weight of does over the course of the trial between the DLT and DLS groups. The doe's average weight is of little consequence because, according to numerous reports, intestine weight fluctuations, among other potential causes, have little effect on body weight during lactation. The same Table-3, however, makes it abundantly evident that a doe's weight naturally increased dramatically as her pregnancy progressed. The larger weights in both experimental groups were on day 28, while the lower weights were on the day of mating. The weight of the litter and the fetus' growth are the causes of the increases in doe weight with advanced pregnancy (Table 3).

Influence of separating rabbit's offspring from their mothers on conception rate

From 7.0 rabbits in the DLT group to 9.0 rabbits in the DLS group, there is a noticeable rise in the number of pregnant animals. Female rabbits in the DLS group had a conception rate (CR %) that was 28.6% greater than the female rabbits in the DLT group (P<0.01) (Table 4).

Effect of doe-litter separation on kit's daily gain and vitality

Ten out of 60 kits (16.67%) and 15 out of 60 kits (25.00%), respectively, died during the trial days in the DLT and DLS groups. Separation of the litter from their dams has a major impact on the survival of the litter. In both groups, the first week contained the majority of the dyed kits. In the DLT and DLS groups, the vitality rates were 83.33 and 75.00%, respectively. The weight growth of the young who were not with their mothers was considerably lower (15.21 percent, P<0.05) than that of the DLT group. Throughout the lactation period, these lighter weights were maintained even longer. The young in the DLS group weighed 88.0 g less than the young in the DLT group (Table 5).

Influence of separating rabbit's offspring from their mothers on behavioral indicators

Weekly behavioral measurements in rabbits from both the DLT and DLS groups revealed anxiety and withdrawal to a corner of the cage. The findings demonstrate that during the first week, anxiety and withdrawal behaviors altered from being nonexistent in the DLT group to being extremely weak in the DLS group (+1). In the second, third, and fourth weeks, there were no behavioral factors that changed, and there were scored none (0). Since this observation changed from none in the DLT group to very weak in the DLS group on day 7, it continued to follow the same pattern of prior behavior parameters (scoring +1), and there were no changes in their behavioral parameters in the following weeks (scored none (0), as indicated in Table (6).

Influence of separating rabbit's offspring on blood biochemistry in doe rabbits

Total protein, globulin, and γ-globulin concentrations appear to have been 12.09, 14.37, and 16.77 % (P<0.05) lower in the DLS group than in the DLT group on day 7 of the trial, but there was no discernible difference between the two experimental groups after the first week. During the first two weeks, the DLS group's glucose levels were higher (P<0.05) than the DLT group's. On days 7 and 14, the DLS group experienced a rise in percentage of 26.45 and 21.70, respectively. Between the two experimental groups, the glucose concentration did not significantly differ. On days 7 and 14, LDL concentrations were 22.87 and 23.33% higher (P<0.05) in the DLS group than in the DLT group. LDL concentrations remain unchanged after doe-litter treatment for the remaining trial days (Table 7).

Table 1: Effect of doe-litter separation on doe daily feed intake (g/doe).

Daily feed intake (g/doe)	Doe –litter Together (DLT)	Doe-litter Separation (DLS)	Change due to separation %
1st week (day 07)	165.0[a] ± 2.4	140.0[b] ± 2.2	-15.2 (P<0.05)
2nd week (day 14)	188.0[a] ± 3.1	165.0[b] ± 2.5	-12.2 (P<0.05)
3rd week (day 21)	210.0[a] ± 4.5	185.0[b] ± 3.8	-11.90 (P<0.05)
4th week (day 28)	220.0[a] ± 3.6	192.0[b] ± 3.9	-12.73 (P<0.05)
Overall mean	195.75[a] ± 12.24	170.50[b] ± 11.67	-13.00 (P<0.05)

a, b…Means in the same row having different superscripts differ significantly at P<0.05.

Table 2: Effect of doe-litter separation on doe daily milk yield (g/doe).

Days of duration	Daily milk yield (g/day/doe).			
	Doe –litter together (DLT)	Doe-litter separation (DLS)	Change in milk yield (g) due to separation	Overall mean (g)
Day 07	165.70[a]±3.5	120.80[b]±3.5	-27.1 (P<0.01)	143.25[C]±22.5
Day 14	222.40[a]±4.3	186.40[b]±4.2	-16.2 (P<0.05)	204.4[A]±18.0
Day 21	184.50[a]±4.1	165.60[b]±4.1	-10.2 (P<0.05)	175.05[B]±9.5
Day 28	138.30[a]±3.0	126.35[b]±2.6	-8.6 (P<0.05)	132.33[D]±6.0
Average ±SE	181.73[a]±17.66	159.45[b]±15.76	-12.3 (P<0.05)	
Total milk, g	710.90	599.15	-111.75	

a, b…Means in the same row having different superscripts differ significantly at P<0.05.
A ,B…Means in the same column having different superscripts differ significantly at P<0.05.

Table 3: Effect of doe-litter separation on doe live body weight from mating till day 28

Experimental days	Doe weight, g		
	Doe –litter Together (DLT)	Doe-litter Separation (DLS)	Average doe weight, g
Doe weight at mating, g	2815±24	2810±33	2813[d]±2.50
Doe weight (g) at day 07 after mating	2850±30	2859±97	2855[d] ±4.50
Doe weight (g) at day 14 after mating	3065±48	3110±77	3088[c]±22.5
Doe weight (g) at day 21 after mating	3252±68	3294±92	3273[b]±21.0
Doe weight (g) at day 28 after mating	3453±72	3411±105	3432[a]±21.0

a, b…Means in the same column having different superscripts differ significantly at P<0.05.

Table 4:Effect of doe-litter separation on conception rate of doe rabbits

Conception rate	Doe –litter Together (DLT)	Doe-litter Separation (DLS)	Change in fertility rate due to separation
Doe number at mating	10.0	10.0	-
No of pregnant doe	7.0	9.0	+2
Conception rate %	70	90	+20
Increase% in conception rate	-	28.6 (P<0.01)	

Table 5: Effect of doe-litter separation on kits daily gain and vitality of suckling kits

Vitality of kits	Kits of the doe litter together (DLT)	Kits of the doe litter separation (DLS)
Number of does rabbit	10	10
Total kits at the 1st day of the experiment	60.0	60.0
Total kits at day 28 of parturition	50.0	45.0
Mortality No from birthing to d 28	10 .0	15.0

Mortality % from birthing to d 28	16.67[b]	25.00[a]
Vitality % from birthing to d 28	83.33[a]	75.00[b]
Average kit weight (g) at day 28 of parturition	578[a]±3.23	490[b]±1.66
Body weight gain, g/day (day 1 to day 28)	20.64[a]±0.16	17.50[b]±0.22

a, b…Means in the same row having different superscripts differ significantly at $P<0.05$.

Table 6: Effect of doe-litter separation on doe-behavior parameters of rabbits.

Doe-behavior	Experimental days	Doe –litter Together (DLT)	Doe-litter Separation (DLS)	Change due to separation
Anxiety	Day 07	0	+1	Change from none to very weak
	Day 14	0	0	No change
	Day 21	0	0	No change
	Day 28	0	0	No change
Withdrawal	Day 07	0	+1	Change from none to weak
	Day 14	0	0	No change
	Day 21	0	0	No change
	Day 28	0	0	No change
Feed intake duration/hour	Day 07	0	+1	Change from none to weak
	Day 14	0	0	No change
	Day 21	0	0	No change
	Day 28	0	0	No change
	Day 14	0	0	No change
	Day 21	0	0	No change
	Day 28	0	0	No change

Table 7: Effect of doe-litter separation on blood biochemical levels in doe rabbits

Immune response in does	Experimental days	Doe –litter Together (DLT)	Doe-litter Separation (DLS)	Change due to separation
Total protein (g/dl)	Day 07	7.28[a]±0.26	6.40[b]±0.25	-12.09($P<0.05$)
	Day 14	6.75±0.15	6.43±0.20	-4.74 [NS]
	Day 21	6.55±0.19	6.45±0.21	-1.53 [NS]
	Day 28	6.60±0.06	6.45±0.10	-2.27 [NS]
Globulin (g/dl)	Day 07	3.48[a]±0.09	2.98[b]±0.09	-14.37($P<0.05$)
	Day 14	3.35±0.06	3.25±0.06	-2.98 [NS]
	Day 21	3.43±0.13	3.35±0.18	-2.33 [NS]
	Day 28	3.41±0.11	3.39±0.07	-0.59 [NS]
γ- globulin (g/dl)	Day 07	1.32[a] ± 0.01	1.10[b] ± 0.01	-16.77($P<0.05$)
	Day 14	1.30 ±0.03	1.28±0.03	-1.54[NS]
	Day 21	1.32± 0.02	1.23 ±0.03	-6.82[NS]
	Day 28	1.25 ± 0.01	1.22±0.01	-2.40[NS]
Glucose (mg/dl)	Day 07	46.50[b]±2.1	58.80[a]±3.2	+26.45($P<0.01$)
	Day 14	49.40[b]±2.8	60.12[a]±2.4	+21.70($P<0.01$)
	Day 21	55.12[b]±2.2	67.21[a]±2.9	+21.93($P<0.01$)
	Day 28	59.12[b]±2.7	70.12[a]±3.1	+18.61($P<0.05$)
LDL cholesterol (mg/dl)	Day 07	56.4[b] ± 3.6	69.3 [a]±4.5	+22.87($P<0.01$)
	Day 14	60.0[b] ± 4.8	74.0[a] ± 5.1	+23.33($P<0.01$)
	Day 21	45.6±2.3	48.2±3.5	+5.70[NS]

	Day 28	44.5±1.9	46.8±2.8	+5.17^{NS}

a, b…Means in the same row having different superscripts differ significantly at $P<0.05$ or $P<0.05$.
NS=not significant.

Table 8: Effect of doe-litter separation on hormonal profile in doe rabbits

Blood hormonal levels	Experimental days	Doe –litter Together (DLT)	Doe-litter Separation (DLS)	Change due to separation	Overall mean
Estradiol-17beta (pg/ml)	Day 07	12.8^{b}±0.26	17.0^{a}±0.28	+32.8 ($P<0.01$)	14.9^{D}
	Day 14	17.8^{b}±0.23	22.7^{a}±0.23	+27.5 ($P<0.01$)	20.3^{C}
	Day 21	21.3^{b}±0.22	27.3^{a}±0.20	+28.2 ($P<0.01$)	24.3^{B}
	Day 28	24.0^{b}± 0.28	31.5^{a}±0.33	+31.3 ($P<0.01$)	27.8^{A}
Cortisol (ng/ml)	Day 07	4.78^{b}±0.01	6.06^{a}±0.02	+26.8 ($P<0.01$)	5.42^{A}
	Day 14	1.27±0.01	1.35±0.01	6.30^{NS}	1.31^{B}
	Day 21	1.08±0.01	1.15±0.01	6.50^{NS}	1.12^{B}
	Day 28	1.38±0.01	1.46±0.02	5.80^{NS}	1.42^{B}

a, b…Means in the same row having different superscripts differ significantly at $P<0.01$.
A, B ..Means in the same column within each item having different superscripts differ significantly at $P<0.05$.
NS=not significant.

Table 9: Effect of doe-litter separation on antioxidant enzymes activities.

Serum antioxidant enzymes	Experimental days	Doe –litter Together (DLT)	Doe-litter Separation (DLS)	Change due to separation
Glutathione peroxidase (GSH), U/ml	Day 07	26.40^{a}±0.86	22.60^{b}±0.74	-14.4 ($P<0.05$)
	Day 14	16.55±1.1	15.63±1.0	-5.60^{NS}
	Day 21	14.80±0.69	14.50±0.70	-2.03^{NS}
	Day 28	14.88±0.77	13.95±0.88	-6.25^{NS}
Superoxide dismutase (SOD), U/dl	Day 07	15.82^{a}±0.03	12.06^{b}±0.05	-23.77 ($P<0.01$)
	Day 14	12.50±0.01	12.20±0.02	-2.40^{NS}
	Day 21	12.90±0.02	12.40±0.01	-3.88^{NS}
	Day 28	12.90±0.02	12.20±0.03	-5.43^{NS}
Catalase (CAT), U/dl	Day 07	51.23^{a}±2.2	45.13^{b}±1.3	-11.9 ($P<0.05$)
	Day 14	38.80±2.8	37.18±1.5	-4.17^{NS}
	Day 21	31.90±1.2	31.05±1.4	-2.66^{NS}
	Day 28	30.88±1.1	30.22±1.3	-2.14^{NS}

a, b…Means in the same row having different superscripts differ significantly at $P<0.05$ or $P<0.01$. NS=not significant.

Influence of separating rabbit's offspring on doe hormonal profile

Estradiol-17beta hormone levels were higher (P<0.01) in the DLS group than the DLT one. Estradiol-17beta levels were elevated by the DLS treatment on all trial days, with an increasing percentage ranging from 26.8 to 32.8. The amount of the hormone estradiol-17beta also rose (P<0.5) with the number of experimental days, going from 14.9 pg/ml on day 7 to 27.8 pg/ml on day 28 (Table 8). On day 7 of the experiment, it appears that the cortisol concentration in the DLS group was greater (6.06 ng/ml) than in the DLT group (4.78 ng/ml) by 26.8% (P<0.01). In addition, there was no detectable change in cortisol levels between the two experimental groups after the first week (Table 8).

Influence of separating rabbit's offspring on antioxidants enzymes activities

On day 7, the activity of the enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were all significantly lower in animals from the DLS group than in rabbits from the DLT group. On day 7, GSH activity dropped (P<0.05) by 14.4% from 26.4 U/ml in the DLT group to 22.6 U/ml in the DLS group. Day 7 saw a drop in SOD enzyme activity of 23.77% (P<0.01), from 15.82 U/dl in the DLT group to 12.06 U/dl in the DLS group. On day 7, CAT enzyme activity dropped (11.9%) from 51.23 U/dl in the DLT group to 45.13 U/dl in the DLS group (P<0.05). During the remaining trial days, the GSH-Px, SOD, and CAT enzyme activity were not significantly impacted by separation. On day 7, the ac-

tivity of the enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were all significantly lower in animals from the DLS group than in rabbits from the DLT group. On day 7, GSH activity dropped (P<0.05) by 14.4% from 26.4 U/ml in the DLT group to 22.6 U/ml in the DLS group. Day 7 saw a drop in SOD enzyme activity of 23.77% (P<0.01), from 15.82 U/dl in the DLT group to 12.06 U/dl in the DLS group. On day 7, CAT enzyme activity dropped (11.9%) from 51.23 U/dl in the DLT group to 45.13 U/dl in the DLS group (P<0.05). During the remaining trial days, the GSH-Px, SOD, and CAT enzyme activity were not significantly impacted by separation (Table 9).

DISCUSSION

Influence of separating rabbit's offspring on milk yield and feed intake of doe

The overall mean daily milk output in the DLS group was considerably lower than that in the DLT group by 12.3% due to the mothers' separation from their offspring. The total milk output during the trial days fell by 111.7 g less in the DLS group than in the DLT group. In comparison to the DLT group, the DLS group's overall mean feed intake was 13.0 % lower on average. According to Rebollar et al. (2004), open access rabbits produced more milk than transitory doe-litter separation rabbits (P<0.05) (5090 g vs. 4593g). The DLS group's decreased feed consumption may be related to their decreased milk production, and it's possible that their energy balance was better than that of the free nursing rabbits (DLT) (Rebollar et al., 2004). Without a discernible difference, the DLT group's feed intake was 7961 g, while the DLS group's intake was 7834 g (Rebollar et al., 2004). The entire mammary gland in the DLS and the reduced feed intake seen in this study could have a negative impact on milk synthesis and, in turn, milk production in lactating rabbits. Absence of nursing in suckling rabbits increased milk yield in the mammary alveoli, which inhibited gland activity and adversely affected the doe-litter separation rabbits' ability to produce milk, as well as the growth and welfare of the kits and the mother's capability to consume food (Rebollar et al., 2004).

Influence of separating rabbit's offspring on doe conception rate

Female rabbits in the DLS group had conception rates that were 28.6 % higher than those in the DLT group, which was a statistically significant difference. From 7.0 does in the DLT group to 9.0 does in the DLS group, the number of pregnant animal dramatically rose. Receptivity and fertility were significantly greater (78.8 and 78.0 %, respectively) than in non-separated does (40.8 and 66.9 %), according to Maertens (1998). Receptivity and conception rate rose by roughly 30 % and 10 %, respectively, after the controlled suckling. Since productivity increased by 35.4-44.2 % in the DLS, Bonanno et al. (2004) observed that doe-litter isolation for two days before to AI improved the fertility of doe rabbits compared to does with free access to the nest box. The percentage of rabbits displaying oestrus signals may increase in the DLS constituted of greater oestradiol and lower prolactin concentrations due to the absence of suckling episodes, hence increasing the conception rate (Rebollar et al., 2004). Short-term litter separation increased the doe's sexual responsiveness and fertility because it caused an increase in FSH and LH. LH and FSH are activated by mating or artificial insemination in animal that have experienced brief litter separation (Cano et al., 2005).

Influence of separating rabbit's offspring on daily gain and vitality of suckling rabbits

Separating the kits from their mothers in this study had a major impact on the vitality of the kits. In the DLS and DLT groups, the vitality rates were 75.00 and 83.33 %, respectively. According to the study's findings, kits that were raised apart from their mothers (DLS) had considerably lower body weight and daily gains than the DLT group, and these decreases persisted throughout the lactation period. The 22 h/day separations' detrimental impact on the weight of the newborn bunnies can be explained by the reduced milk intake. About 15% of the young's living weight is made up of the daily milk intake. These mothers' milk output considerably dropped throughout nursing, which contributed to the young people's lighter weight. The DLT group had a daily gain that was much higher than the DLS group. The fact that doe-litter together contributes to greater development performance may be because the young bunnies are kept with their mothers in cages longer and consume more milk after extra feedings than their DLS group counterparts, leading to superior nutrition (Zhang et al., 2018). The weight of the kits decreased 9.0 g (9.6%) after a separation lasting 24 hours, and the loss increased as the separation duration was extended (Alvarino et al., 1999). Weight of the litter was decreased as a result of doe-litter separation (Ubilla et al., 2000b). However, compared to control animals, DLS rabbits had bigger litter sizes at weaning and experienced decreased rabbit losses, according to Bonanno et al. (2004). The weight of the rabbits at weaning was not significantly affected by the 24-hour mother-litter separation, according to Castellini et al. (1998). Separation of the litter from its mother had no impact on the survival rate of the litter (Alvarino et al., 1999). Ubilla et al. (2000b).showed that there were no appreciable variations in the number of dead kits per litter or the number of dead kits from birth to weaning in the successive litters of separation between the separated and non-separated does. The latter authors noted that the weaning litter size went from 7.1 in non-separated does to 7.4 in separat-

ed animals, and that the kindling rate increased from 83.3 in the non-separated group to 100% in the separate group. The litter weights were 217.6 and 302 g after separation of 48 hours before to artificial insemination, and on day 21 following parturition, they were 2402.1 and 2234.3 g in separated and non-separated does, respectively.

Influence of separating rabbit's offspring on behavioral indicators of doe

Doe behavioral indicators are impacted when babies are separated from their moms, particularly at the start of the experiment. The mothers' anxiety and withdrawal, as well as their feed intake duration/hour behaviors shift from none (0) in the DLT group to very weak (+1) in the DLS group over the first week of the experiment. All previous behavior parameters are scored as none during the residual phase (0). These findings support Ngoula et al. (2017)'s methodology and suggest that the first week of offspring separation from their mother may be particularly stressful. After this time, the doe rabbits' behavior, though, returned to normal. According to Schepers et al. (2009), the conditions in which pet rabbits are kept frequently harm their welfare. The study by Zuotian et al. (2021) demonstrates that immunological challenge-induced behavioral impairments and postpartum separation of nursing mice's mothers and offspring during lactation promote anxiety and suppress neuroinflammation.

Influence of separating rabbit's offspring on doe blood and hormonal levels

The DLS group had significantly lower levels of total protein, globulin, and -globulin on day 7 than the DLT group. During the first two weeks, the DLS group's glucose and LDL values were considerably higher than those of the DLT group. Total protein, globulin and γ-globulin, glucose, and LDL concentrations are unaffected by the doe-litter treatment's separation during the remaining trial days. The metabolism of protein and energy is impacted by the separation of young bunnies from their mothers, particularly early in the experiment. The drop in blood proteins in the first week may be related to the DLS's reduced feed intake. Separation may also accelerate glycogenesis, which elevates glucose and ALD levels in the DLS group, particularly in the first two weeks of the experiment.

Estradiol-17beta levels rose between 25.0 and 35.0% throughout each day of the experiment when neonates were separated from their mothers compared to the hormonal level in the DLS group. DLS had higher levels of estradiol-17beta than DLT, according to Ubilla et al. (2001). Nursing does are affected by a drop in prolactin levels, which may promote the progression of follicular waves and strong steroidogenesis activity, resulting in an increase in the concentration of estradiol-17beta (Ubilla et al., 2000a). By regulating the release of FSH and LH to the increased reaction of the ovulatory response to mating, the litter separation is a proactive strategy to promote ovarian activity (Cano et al., 2005a). The lack of suckling incidents in doe-litter separation may be due to the stimulating effects of ovarian activity, which raises the blood level of estradiol-17beta in does to improve receptivity and fertility. On day 7 of the experiment, the DLS group's cortisol concentration dramatically rose by 36.8% compared to the DLT group. In the first week of the experiment, separating offspring from their mothers considerably raised the cortisol level. Animals' cortisol levels have been utilized extensively as an indicator of stress. The first week of the separation may have exposed the doe rabbits to some stress, which may have contributed to the separation of the young from their mothers (DLS). The doe rabbits returned to normal, though, after the first week, and the cortisol levels also stabilized. The hypothalamus-pituitary-adrenal axis may be stimulated by removing kits from their mothers. This may result in a rise in adrenocorticotropic hormone (ACTH) released by the pituitary gland, which will subsequently activate the production and release of cortisol. Since stress may be present in the first week of the experiment for rabbits, the degree of oxidative damage has a considerable impact on cortisol levels in the blood (Bonanno et al., 1999; Pompella et al., 2003).

Influence of separating rabbit's offspring on antioxidants activities in doe

Catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) are the three main endogenous antioxidant enzymes. In the initial week of the trial, antioxidant biomarker levels were lowered by separating the offspring from their mothers. Animals in the DLS group had significantly lower serum GSH-Px, SOD, and CAT antioxidant activity than those rabbits in the DLT group. Separation had very little impact on the antioxidant activities for the remaining trial days. Reduced antioxidant activity levels in the DLS group during the first week of separation suggest that does in the separation of doe-litter (DLS) may experience oxidative stress during this time, which is supported by an increase in cortisol levels and some unfavorable behaviors seen during this time Pompella et al. (2003) and Jimoh and Abubakar, 2019). According to the findings of this study, the doe rabbits were not subjected to any stress after the first weak. Oxidative stress occurs when the production of potentially harmful reactive oxygen species (ROS) surpasses the animal body's built-in antioxidant defenses. This damages biological macromolecules and impairs physiological functions and metabolic activity (Habeeb, 2018a). When ROS and their metabolites are catalytically converted into stable, non-toxic compounds, antioxidant enzymes play a key role. By oxidizing reduced glutathione (GSH) into glutathione disulfide, glu-

tathione peroxidase (GSH) catalysis the reduction of H_2O_2 and lipid peroxides to H_2O and their corresponding lipid alcohols (GSSG). The superoxide anion is changed into H_2O_2 and O by the enzyme superoxide dismutase (SOD). H_2O_2 is converted to H_2O and O by the enzyme catalase. Antioxidant enzymes are therefore the most important defense against oxidative stress, which promotes cell death. Doe rabbits who have been separated may exhibit increased oxidative stress, particularly in the first week after separation. Oxidative stress raises the possibility of natural abortion, affects the health of the animals, and lowers milk yield and some reproductive efficiency (Habeeb, 2018b).

CONCLUSION

The separation of the kits from their mothers has an effect on the mothers that exhibited in the form of a behavioral indicator disruption and results in a reduction in the duration of feeding, the volume of milk produced, and the immunity function at the beginning of separation while the isolation approach increased the concentration of the hormonal level of estradiol, which improved the rate of conception.

ACKNOWLEDGMENTS

The Egyptian Atomic Energy Authority, the Biological Application Department, the Radioisotopes Applications Division, and the Rabbits Farm project all provided support for this work.

All authors gave their consent to take part in finishing this work. All of the writers of this research report actively contributed to the design, implementation, and analysis of the study. The submitted final version has been read by all authors and received their approval.

FUNDING

According to the consensus of all authors, no funding was provided to finish this publication.

CONFLICTS OF INTEREST

None of the authors disclosed any potential conflicts of interest.

DECLARATIONS AND DISCLOSURES

The Egyptian Atomic Energy Authority's appropriate ethical committee for research involving animals approved our study specifically, and a declaration on the welfare of animals and our work submitted for publication have no implications on the common welfare or health of the community.

NOVELTY STATEMENT

The quantity of milk produced and the immunity function at the beginning of the separation are both impacted by the kits being taken away from their mothers, and the concentration of the hormone estradiol has grown, increasing the rate of conception.

AUTHORS CONTRIBUTION

Alsaied Alnaimy Habeeb was responsible for the idea and design of the study's protocol as well as the final approval of the version to be presented.
Amaal M. Kamal critically revised the article and looked for significant intellectual material.
Mostafa Abbas A. Atta performed the data interpretation and article drafting.
Ahmed K. Sharaf collected the data and performed a statistical analysis of the data.

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