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EFFECT OF DOE-LITTER SEPARATION ON MATERNAL BEHAVIOR AND SERUM CORTISOL LEVELS IN RABBITS

(With 7 Tables and 3 Figures)

By

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تأثير انفصال صغار أمهات الأرانب على سلوكيات الأمومة وكذلك على مستوى هرمون الكورتيزول فى سيرم الدم

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تم دراسة عن تأثير بعض عوامل الإجهاد على بعض سلوكيات أمهات الأرانب النيوزيلاندى الأبيض وكذلك تأثيرها على بعض صفات خلفتها وتحديد مدى تأثير هذه العوامل وكذلك تم قياس مستوى هرمون الكورتيزول فى سيرم دم الأمهات الحوامل أثناء الرضاعة. وقد تضمنت هذه الدراسة ثلاث تجارب لدراسة الفصل المؤقت لصغار الأرانب عن أمهاتها لمدد مختلفة ٤ ،٨ ، ٢٤ ساعة. حيث أدى الفصل المؤقت لزيادة شغف الام تجاه صغارها عند الولادة (سلوك حماية الصغار) بينما احدث فصل صغار الأرانب عن أمهاتها مؤقتا لمدة ٢٤ ساعة تأثير واضح على تركيز هرمون الكورتيزول فى سيرم الدم (بعد مرور ٢٤ ساعة من بداية التجربة) وكذلك ظهور بعض الأنماط السلوكية الشاذة لأمهات الأرانب بالإضافة إلي تأثيرها الواضح على موعد اكتمال تفتح أعين الصغار وخروجها من المهد و بداية تناولها العليقة الصلبه. وقد خلصت النتائج إلى أن إنفصال أمهات الأرانب عن صغارها يعتبر من أهم العوامل المسببة للإجهاد والتى لها تأثير مباشر على سلوكيات وإنتاجية هذه الحيوانات.

SUMMARY

The effects of transient doe-litter separation for 4,8 and 24 hours on maternal behaviour and litter traits in female New Zealand White (NZW) rabbits were studied, in order to determine the effect of this factor, on serum cortisol levels,maternal behaviour, some doe-traits and consequently its effectiveness on productive and reproductive performance of lactating does. Diurnal changes in serum levels of cortisol were examined in pregnant, lactating and control does. The

obtained results revealed that; a) diurnal changes of rabbits serum cortisol level were significantly higher at 9 a.m than that recorded at 2 p.m.; b) Serum cortisol level in pregnant and lactating rabbit was high during the last ten days of pregnancy and first fourteen days of lactation. The transient doe-litter separation induced significant increase only in serum cortisol levels in does subjected to 24 hours separation. c) no significant effects of doe-litter separation either for 4, 8 or 24 hours on post-partum behaviours could be achieved; d) abnormal behaviours, such as vigorous burrowing and dugging in the floor of the cage was significantly higher after 24 hours than that after 4 or 8 hours of separation or in control groups; e) complete eye opening and ear opening as well as time of nipple food were significantly different from the control group only after 24 hours of separation.

Key words: *Behavior of rabbit, maternal behavior, meternal separation, stress & behavior in rabbit, serum cortisol in blood's rabbit*

INTRODUCTION

Rabbits are found everywhere and are raised for a variety of reasons such as food supply, high grade wool, furs, miscellaneous products, acts as laboratory and experimental animals, as well as exhibition and as pet animals. Rabbits now realised by many people and in contributing animal protein. Some of these reasons are the fast reproductive rate and quick growth. A doe will produce more meat per unit live weight than any other animal and of excellent quality. Furthermore rabbits need short period to reach their breeding age, high food conversion rate, low energy and the protein requirements in food per unit live weight gain are lower than most animals. Rabbit have high degree of diversity in the gene pool which allows good and speedy adaptation to different environments (Sandford, 1996). In addition, rabbits can be kept in small areas in undeveloped countries where other forms of livestock cannot be found. Numan (1988), reported that maternal behaviour fits into the larger category of parental behavior. Nelson (1995), reported that parental behaviour is any behaviour that contributes directly to the survival of fertilized eggs or off spring that have left the body of the female. If a father performs parental behaviour, then a more specific term, paternal behaviour, is typically used; similarly, parental behaviour performed by mothers is called maternal behaviour. Parental care influences the course of physical and psychological development of offspring, and is also important among many species in the socialization of young Numan (1988). Fleming and Corter (1988), claimed that in all

mammalian species, stimulant from the young elicit maternal responsiveness at both the behavioural and the physiological levels. Numan (1990), said that, maternal care was not only important for the survival of the young, but also influenced the course of physical and psychological development of the offspring. Numan (1988), showed that the survival of the young rabbits depends greatly on maternal behaviour, which was highly sensitive to the hormonal milieu of the mother. Several authors studied the effect short term doe-litter separation for either once or twice times for 24 to 48 hours during early or midlactation period but without information on either serum cortisol levels or maintainance of maternal behavior and litter trait (Hudson and Distel 1982; Calvert *et al.* 1985; Sandford,1996; Alvariño *et al.* (1998) Maertens (1998), Alvariño *et al.* (1999), Bonanno *et al.* (1999a) Bonanno *et al.* (1999b), Szendrõ *et al.* (1999), Theau- Clément and Mercier (1999), Virag *et al.* (1999), Ubilla *et al.* (2000), Bonanno *et al.*, 2002).Therefore the present investigation was designed to study the effect of long-term doe-litter separation for 4hours, 8 hours or 24 hours/week from postnatal day two till day 28 (weaning day) and concomitantly to measure serum cortisol levels and its diurnal changes in experimental does as compared to control groups.

MATERIALS and METHODS

1- Experimental Animals:

White New Zealand breed rabbits were used in this study. Clinically healthy Forty-four does (8 to 10 months in age) and four bucks (about 12 months in age) with average body weight of 2.25 to 3.2 kg were used. Animals were reared in well ventilated house with 16-hours light and 8-hours dark with 25 Lux of light intensity. Three rabbit housing units were used for the experimented animals. Cages and pens were achieved by automatic clean drinking watering system, the hopper was fitted to the outside the cage, with the trough projecting into the cage. The nest box was attached on the outside of the hutch. Constant hygiene and routine maintenance of cages and equipments was adopted. The rabbits were fed ad libitum on pellet ration formulated according to El-Raffa, (1994). Prophylactic measures against rabbit haemorrhagic septicemia using the oil-adjuvant polyvalent rabbit pasteurellosis vaccine as well as treatment and control of both internal and external parasites were adopted. The doe never mated in her own hutch, but placed in the buck's hutch. The doe was lifted with the buck for a considerable period, and mating, may normally be accomplished in a very short time. If mating not

occurred within a few minutes the doe was removed and retried later, or immediately with another buck according to Sandford (1996).

2-Techniques for doe-litter separation.

Lactating does were randomly allocated into three main groups, 10 of each. Each group consisted of control (n=4) and biostimulated (n=6) lactating does. Control lactating does in all groups had free access to nursing, whereas biostimulated does were separated from their litters as follows: Pups from the biostimulated does in group 1, 2 and 3 were separated from their mothers for a period of 4,8 and 24 hours per day, respectively, began from the postnatal day 2, and repeated for three days per week, from 1.00 pm. to 5.p.m.in group 1 and from 1.00 pm to 9 pm in group 2, and from 1.00 pm to 1.00 pm next day in group 3. These experiments were done for four weeks till 28th postnatal day (day of weaning). Litters were removed from their mothers and placed in another room as litter groups each in nest box containing bedding materials similar to that formed by the mother. The pups were kept warm and humid throughout the period of maternal deprivation.

3- Evaluation and interpretation of obtained behavioral response:

The effectiveness of doe-litter separation were observed and recorded concerning the basic following parameters a) postpartum behaviors;b) detection of any abnormal behaviors and c) Litter traits.

a) The post-partum behavior was analysed according to Cross (1957); Denenberg *et al,* (1958); Hafez (1962); Lebas (1969) and Sandford (1996) as follows: the percentage of does delivered inside nest box (D_{DNB}); the percentage of does that cleaned the nest after parturition (D_{Clean}); the percentage of does that groomed theirselves after parturition (D_{Groom}); the percentage of dams that had ability to investigate surrounding including the experimenter after parturition as normal behaviour ($D_{Investigate}$); the percentage of does that deposited few faecal pellets inside nest box (D_{Faecal}).; the percentage of fed young ($K_{sucklins}$); the mean percentage of kits had been licked by the does (K_{Lick}); the mean percentage of kits that doe had devoured their placenta (D_{DP});. One of the doe activities after delivery was eating or devouring the placenta of the newborn offspring (K_{UC}). (K_{Cold}) was the mean percentage of cold young that their temperatures were less than 37.6 ± 0.3°C; D_{Milk} was doe total milk produced from first day-to-day 21 of lactation and was estimated by the total 21 day litter weight per gram.

b) Detection of abnormal behavioral patterns of examined does according to Abdel-Rahman *et al.* (2002); include the followings; percentage of Kits Cannibalized by the doe ($K_{cannibalism}$). The average percentage of Does that made Fur Chewing during Pregnancy (D_{FCP}); the average percentage

of Does that made Fur Chewing during Lactation (D_{FCL}). The average percentage of Does that Throw Food out of feed hoppers during Pregnancy (D_{TFP}) The average percentage of Does that Throw Food out of feed hoppers during Lactation (D_{TFL}). The average percentage of Does that made Another Abnormal Behaviour during Pregnancy (D_{AABP}). The average percentage of Does that made Another Abnormal Behaviour during Lactation (D_{AABL}). Maternal interest and aggression ratings were done on over all scale of five points from zero (no interest) to four (interest in the young to the point of aggressively fighting off any try to approach the nest or manipulate the young, the grades, Denenberg *et al.* (1958)

c) Litter-traits according to Samy (1980); and Hamilton, *et al.* (1997) include the following: 1) Bunny weights which represented by; Bunny weight at birth (BBW). Litter birth weight (Total bunny weight at birth (LBW). Litter weaning weight (Total bunny weight at 28 days of lactation, (LWW). 2) Litter size which include; total litter size born including number of both alive and dead kits (TLSB); Litter size born only alive (LSBA); Litter size or number of kits at 7^{th} ; 14^{th}. 21^{st}. 28^{th} ; 35^{th} and 42^{nd} days, (LS- 1^{st} wk, LS- 2^{nd} wk, LS- 3^{rd} wk, LS- 4^{th} wk, LS- 5^{th} wk, and LS- 6^{th} wk respectively). 3) Mortality rates (M B), including total number of dead born kits (LSBA); number of dead kits at 7^{th} ; 14^{th}. 21^{st}. 28^{th} ; 35^{th} and 42^{nd} days (M- 1^{st} wk, M- 2^{nd} wk, M- 3^{rd} wk, M- 4^{th} wk, M- 5^{th} wk, and M- 6^{th} wk. respectively). The total number dead young during the experimental period ^{TM} (Total Mortality of kits) was counted 4) Survival rates including neonatal survival rate, (NSR) and preweaning survial rate, (PSR) were performed according to Hamilton, *et al.* (1997). Some specific litter traits such as: time of complete fur appearance to all young, (T_{Fur}); time of complete eye opening to all kits (T_{Eye}); time of exit of all kits out of the nest box (T_{Exit}) and time of nipple food by all kits (T_{Nipple}) were done. Also; time of complete ear opening to all young (T_{Ear}) by both observing the ear opening and using a vibrating fork as source of sound if the young response to its sound this was an evidence of ear opening.

4-Techniques for measuring serum cortisol level.

a- Diurnal changes in cortisol levels.

A blood sample of about 3ml was collected from all does (control non-biostimulated does) in groups 1, 2 and 3 (n=12) during pregnancy and lactation periods at 9.00 a.m. and 2 p.m. Similarly another blood samples were obtained from non-pregnant non-lactating does (n=12) in order to determine whether or not there were diurnal changes resulted from pregnancy or lactation. All sera of the collected samples were separated

by the centrifugation at 3000 r.p.m. for 30 minutes and were freezed at -20 °C until analysis, as recommended by Suckow & Douglas (1997) and Afifi & Dachash (1999). Sera were estimated for their levels of cortisol using vitro diagnostic Immulite Cortisol kits with the Immulite analyzer (DPC Immulite ver.411) for the quantitative measurement of cortisol (hydrocortisone, compound F) in serum.

b- Effect of doe-litter separation on serum cortisol levels.

Separation of does from litters was performed after two days of the lactation period at 1.00 p.m. one hour after separation (2.00 p.m.) was considered as the start point for the collection of blood samples in all groups of does. Blood samples (3ml) were collected to determine the profile of serum cortisol in response to DLS. The collected blood samples from both groups were collected from margin ear vein in centrifuge tube then centrifuged at 3000 r.p.m. for 30 minutes. Plasma was stored at -20 °C until analyses.

5- Statistical analysis:

Statistical analysis performed by using SPSS version 11.01 licensed to North Dakota State University.

RESULTS

The results of this investigation were illustrated in Tables (1-7) as well as Figures (1-3).

Table 1: Effect of doe-litter separation on some post-partum behaviors' percentage in lactating does:

Items	D_{DN}	D_{DNB}	D_{Clean}	D_{Groom}	$D_{Investigate}$	D_{Faecal}	$K_{Sucklings}$
Control-1	100%	100%	100%	100%	100%	100%	100%
DLS-4h	100%	100%	100%	100%	100%	100%	100%
DLS-8h	100%	100%	100%	100%	100%	100%	100%
DLS-24h	100%	100%	100%	100%	100%	100%	100%

D_{DN} = the percentage of does delivered normally.
D_{DNB} = the percentage of does delivered inside nest box.
D_{Clean} = the percentage of does that cleaned the nest after parturition.
D_{Groom} = the percentage of does that groomed theirselves after parturition.
$D_{Investigate}$ = the percentage of dams that had ability to investigate surrounding including the experimenter after parturition as normal behaviour.
D_{Faecal} = the percentage of does that deposited few faecal pellets inside nest box.
$K_{sucklins}$ = the percentage of fed young.
DLS = Doe-Litter Separation.

Table 2: Effect of doe- litter separation on statistical mean percentages (or mean) of some post-partum behaviors in experimented does:

Item / Mean±SD	Control - 1	DLS – 4 h	DLS – 8 h	DLS – 24 h
K_{Lick}	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
$K_{Placenta}$	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
K_{UC}	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
K_{DP}	100.00 ± 0.00	94.44 ± 5.56	96.43 ± 3.57	100.00 ± 0.00
K_{Cold}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
D_{Milk}	1338.75 ± 67.88	1513.33 ± 131.79	1740.00 ± 158.44	1812.33 ± 188.82

K_{Lick} = percentage of kits had been licked by the does.
$K_{placenta}$ = percentage of kits that doe had eliminated their placenta.
K_{UC} = percentage of kits that doe had served their umbilical cords.
K_{DP} = the mean percentage of kits that doe had devoured their placenta.
K_{Cold} = percentage of kits that had cold body.
D_{Milk} = doe total milk produced from first day-to-day 21 of lactation.
DLS = Doe-Litter Separation.

Table 3: Mean percentages of abnormal behavioral patterns of does exposed to doe – litter separation:

Mean± SD / Items	Control	DLS - 4 h	DLS - 8 h	DLS- 24 h
$K_{Cannibalism}$	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
D_{FCP}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
D_{FCL}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
D_{TFP}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
D_{TFL}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
D_{AABP}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
D_{AABL}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	83.33 ± 16.67*

Significant at P<0.01

$K_{cannibalism}$ = the average percentage of kits had been cannibalized by their does during lactation.
D_{FCP} = the average percentage of the does that made Fur chewing during pregnancy.
D_{FCL} = the average percentage of the does that made Fur chewing during lactation.
D_{TFP} = the average percentage of the does that threw food out of feed hoppers during pregnancy.
D_{TFL} = the average percentage of the does that threw food out of feed hoppers during lactation.
D_{AABP} = the average percentage of the does that made another abonrrmal behavior during pregnancy.
D_{AABL} = the average percentage of the does that made another abonrrmal behavior during lactation.

Table 4: Statistical mean of the effect of doe-litter separation on some litter – traits in newly born rabbits:

Items / Mean±SD	Control – 1	DLS – 4 h	DLS – 8 h	DLS – 24 h
BBW	55.65 ± 3.54	54.25 ± 2.62	53.00 ± 1.00	61.54 ± 7.78
BWW	455.27 ± 29.79	510.00 ± 42.41	468.12 ± 68.95	376.72 ± 55.07
LBW	347.18 ± 56.11	396.33 ± 43.00	334.75 ± 6.84	480.00 ± 38.07
LWW	2139.00 ± 190.83	2665.00±158.93	2808.75 ± 413.71	2903.67 ± 119.05
TLSB	6.75 ± 1.80	7.33 ± 0.88	6.25 ± 0.25	8.33 ± 1.33
LSBA	6.75 ± 1.80	7.33 ± 0.88	6.25 ± 0.25	8.33 ± 1.33
LS – 1stwk.	5.50 ± 0.65	6.00 ± 0.58	6.00 ± 0.00	8.00 ± 1.00
LS – 2nd wk.	5.50 ± 0.65	5.67 ± 0.88	6.00 ± 0.00	8.00 ± 1.00
LS – 3rd wk.	5.50 ± 0.65	5.67 ± 0.88	6.00 ± 0.00	8.00 ± 1.00
LS – 4th wk.	5.50 ± 0.65	5.33 ± 0.67	6.00 ± 0.00	8.00 ± 1.00
LS – 5th wk.	5.50 ± 0.65	5.33 ± 0.67	6.00 ± 0.00	8.00 ± 1.00
LS – 6th wk.	5.50 ± 0.65	5.33 ± 0.67	6.00 ± 0.00	8.00 ± 1.00
MB	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MR – 1st wk	10.42 ± 0.84	16.67 ± 1.67	0.00 ± 0.00	0.00 ± 0.00
MR – 2nd wk	10.42 ± 0.84	22.22 ± 1.24	3.57 ± 3.57	3.03 ± 0.25
MR – 3rd wk	10.42 ± 0.84	22.22 ± 1.24	3.57 ± 3.57	3.03 ± 0.25
MR – 4th wk	10.42 ± 0.84	26.98 ± 1.00	3.57 ± 3.57	3.03 ± 0.25
MR – 5th wk	10.42 ± 0.84	26.98 ± 1.00	3.57 ± 3.57	3.03 ± 0.25
MR – 6th wk	10.42 ± 0.84	26.98 ± 1.00	3.57 ± 3.57	3.03 ± 0.25
TM	10.42 ± 0.84	26.98 ± 1.00	3.57 ± 3.57	3.03 ± 0.25
NSR	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
PSR	89.58 ± 0.84	73.01 ± 1.00	96.43 ± 3.57	96.97 ± 0.25
T_{Fur}	5.50 ± 0.29	5.25 ± 0.33	5.25 ± 0.25	5.33 ± 0.33
T_{Eye}	9.50 ± 0.29	9.33 ± 0.33	9.25 ± 0.25	13.33 ± 0.33*
T_{Exit}	15.00 ± 0.41	14.67 ± 0.33	14.25 ± 0.25	13.67 ± 0.33*
T_{Nipple}	21.50 ± 0.29	21.33 ± 0.33	21.50 ± 0.29	16.33 ± 0.33*
T_{Ear}	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00

- Significant at P<0.0

BBW = Bunny Birth Weight.
BWW = Bunny Weaning weight.
LBW = Litter Birth Weight.
LWW = Litter Weaning Weight.
TLSB = Total Litter Size Born.
LSBA = Litter Size Born Alive.
LS-1st wk = Litter Size at first week age.
LS-2 nd wk = Litter Size at second week age.
LS-3 rd wk = Litter Size at third week age.

LS-4 th wk = Litter Size at fourth week age.
LS-5 th wk = Litter Size at fifth week age.
LS-6 th wk = Litter Size at sixth week age.
MB = Mortality rate of kits at Birth.
MR-1 st wk. = Mortality Rate at first Week age.
MR-2 nd wk. = Mortality Rate at second Week age.
MR-3 rd wk. = Mortality Rate at third Week age.
MR-4 th wk. = Mortality Rate at fourth Week age.
MR-5 th wk. = Mortality Rate at fifth Week age.
MR-6 th wk. = Mortality Rate at sixth Week age.
TM = Total Mortality of kits.
NSR = Neonatal Survival Rate.
PSR = Preweaning Survival Rate of kits.
T_{Fur} = Time of complete Fur appearance to all kits.
T_{Eye} = Time of complete Eye opening to all kits.
T_{Exit} = Time of Exit of all kits out of nest box.
T_{Nipple} = Time of nipple food by all kits.
T_{Ear} = Time of complete Ear opening to all kits.

Table 5: Statistical mean values of serum cortisol level (µg/dL) in control group of pregnant does:

Items / Means±SD	Control 9 a.m.	Control 2 p.m.
Day before mating (-1)	0.28 ± 0.012^{a}	1.60 ± 0.06^{b}
3rd day of pregnancy	0.13 ± 0.005^{a}	1.50 ± 0.15^{b}
7th day of pregnancy	0.18 ± 0.014^{a}	1.50 ± 0.06^{b}
14th day of pregnancy	0.16 ± 0.010^{a}	1.70 ± 0.12^{b}
21th day of pregnancy	0.33 ± 0.08^{a}	1.90 ± 0.06^{b}
28th day of pregnancy	0.54 ± 0.026^{a}	1.80 ± 0.06^{b}
Day of parturition	2.43 ± 0.07^{a}	3.00 ± 0.10^{b}
3rd PND	0.60 ± 0.08^{a}	2.20 ± 0.21^{b}
7th PND	0.58 ± 0.021^{a}	1.90 ± 0.12^{b}
14th PND	0.37 ± 0.019^{a}	1.70 ± 0.15^{b}
21th PND	0.25 ± 0.009^{a}	1.60 ± 0.21^{b}
28th PND	0.22 ± 0.010^{a}	1.70 ± 0.25^{b}

Means in the same row with different superscripts differs significantly ($P<0.01$)
PND: Post Natal Day.

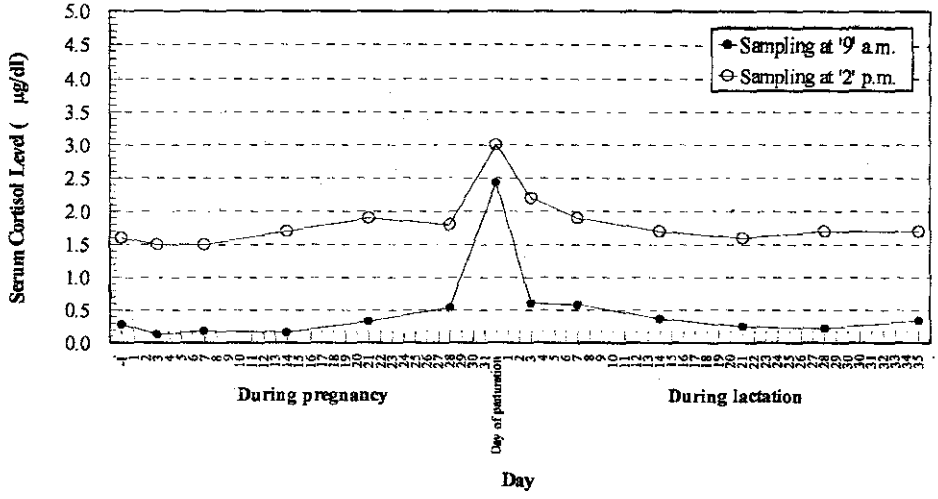

Fig. 1: Serum Cortisol Level in control pregnant and lactating rabbits at ' 9 ' a.m. & ' 2 ' p.m.

Table 6: Diurnal Changes in serum cortisol level (µg/dL) of experimented does:

Items	Time of sampling	Mean ± SD
Control non-pregnant and non-lactating does (NPNL)	9 a.m 2 p.m.	0.44 ± 0.10^{a} 1.72 ± 0.10^{b}
Control pregnant and lactating does (PL)	9 a.m 2 p.m.	0.71 ± 0.07^{a} 1.85 ± 0.03^{b}

Means in the same column with different superscripts differs significantly (P<0.01)

Table 7: Effect of doe-litter separation on mean serum cortisol level (µg/dL) of experimented lactating does:

Items	Mean ± SD
CL	1.78 ± 0.09^{a}
Control NPNL	1.77 ± 0.15^{a}
DLS – 4 h	1.98 ± 0.14^{a}
DLS – 8 h	2.30 ± 0.21^{a}
DLS – 24 h (after 1 h).	2.18 ± 0.24^{a}
DLS – 24 h (after 24 h.)	3.23 ± 0.80^{b}

Means in the same column with different superscripts differs significantly (P<0.01).
CL = Control Lactating doe.
DLS = Doe-Litter Separation.

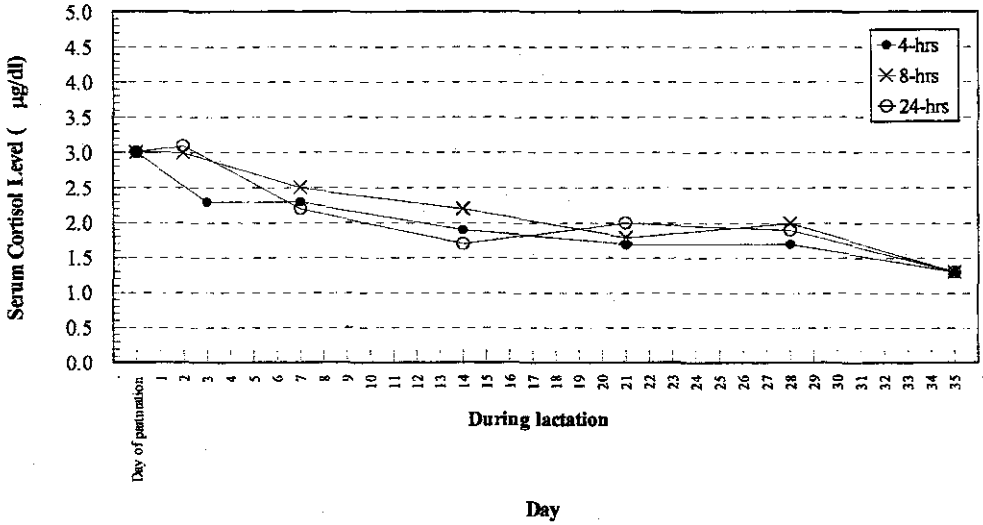

Fig. 2: Serum Cortisol Level in lactating rabbits exposed to doe-litter separationfor 4-hrs, 8-hrs & 24-hrs at ' 2 ' p.m.

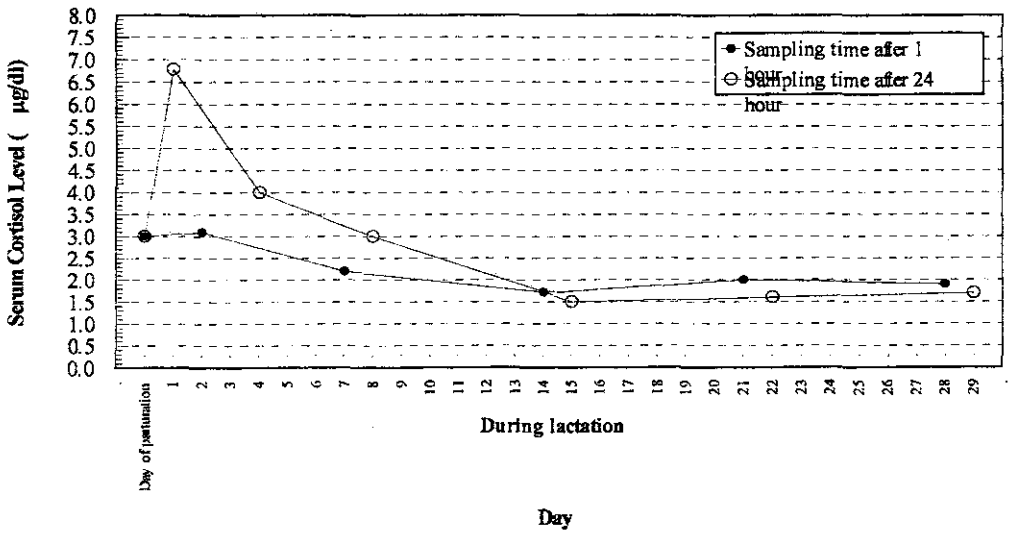

Fig. 3: Serum Cortisol Level in lactating rabbits exposed to doe-litter separation for 24-hrs at ' 2 ' p.m.

DISCUSSION

Knowledge of behavior could lead to better environmental design from the animal's point of view to achieve optimal production and welfare. Ross et al. (1963b) showed that environment exerts an important influence on maternal behavior.

Doe-litter separation and post-partum behaviors:

Table (1) showed the effect of doe-litter separation for different periods (4, 8 and 24 h) on the percentage of post-partum behaviors of lactating does. The incidence of these post-partum behaviors (D_{DN}; D_{DNB}; D_{Clean}; D_{Groom}; $D_{Investigate}$; D_{Faecal}; and $K_{Suckling,}$) was 100% for all and not differ from their corresponding control and indicating that DLS for 4, 8 and 24-h did not induce any effect on these post-partum behaviors. This finding may be attributed to that DLS for these periods did not act as stressors, and serum cortisol level not increased in these cases as showed in Table 7.

Table (2) showed the effect of DLS for 4, 8 and 24-h on the mean of some post-partum behavior in rabbits. These data indicated that, average percentages of young have been either; licked by their does (K_{Lick}); or their placenta eliminated ($K_{Placenta}$); or their umbilical cord severed (K_{uc}); or their placenta devoured (K_{DP}); were insignificantly ($P > 0.05$) affected by separation because these activities occurred immediately after parturition and 2 days before separation. Also, percentage of cold kits (the mean percentage of cold young that their temperatures were less than 37.6 ± 0.3 °C) was similar to control ones, because it has been observed that by approximately an hour before the regular, once-daily arrival of doe to nurse, the kits became active and exposed from this insulating cover. As soon as the doe left the nest the kits simultaneously and vigorously burrowed back under the nest material, fluffing it up and becoming dry again then gradually reassemble in the warmest part of the nest where they remain covered until the next nursing visit where huddling behavior also served the kits' thermoregulatory needs and these achieved observations were in agreement with Hudson & Distel (1982) and Hudson & Distel (1989).

Furthermore, Table (4) also showed insignificant difference in the average milk production after separation (4, 8, 24hr) as compared to control group. The obtained results are agreed with Costantini *et al.* (1986). Moreover, Sandford (1996) and Szendrõ *et al.* (1999), repoted that on the day after the omission of suckling the quantity of milk produced by the does increased by 22% on the three subsequent days milk secretion in these does lagged behind that of the does of control

group. However, Calvert *et al.* (1985); Bonanno *et al.* (1999b); Virag *et al.* (1999) Bonanno *et al.* (2002) and Espinosa *et al.* (2004), found decline in milk secretory rate and involutional changes in milk composition in rabbit does avoided to nurse and separated from their litter for 48 hr.

Doe-litter separations and abnormal behavioral patterns

Table (3) showed the effect of DLS 4, 8 and 24-h on the appearance of abnormal behavioral patters of the experimented does during lactation such as $K_{cannibalism}$; D_{FCP} ; D_{FCL}; D_{TFP} ; D_{TFL}; D_{AABP}; or D_{AABL}; were insignificantly ($P > 0.05$) different from the control group, except that D_{AABL} in DLS 24-h were significantly ($p<0.01$) higher than that in DLS 4 & 8-h and control group. Such phenomenon appeared as soon as the doe left the nest and due to elapsed time of nursing,then does began simultaneously and vigorously burrowed and dug in the floor of her maternal cage, making attempts to clean and close the nest box entrance by pushing scattered material back into the nest, this behaviour not occur in caged rabbits but happen in free reared doe, and this explaning that does tried to make more efforts to protect and hide their kits in trails for preventing their separation.This behavior was not seen in case of DLS 4 & 8-h because such behavior normally occur after nursing time. This result agreed with Hudson and Distel (1982) and Hudson *et al.* (1996a) who observed that after nursing, does jump out of the nest, closing the burrow entrance and leaving the kits alone until the following day.

Doe-litter separation and litter traits:

Data represented in Table (4) showed the effect of DLS for 4, 8 and 24-h on some litter traits.

The obtained data indicated that, the average bunny birth weight (BBW), bunny weaning weight (BWW), litter birth weight (LBW), litter weaning weight (LWW) were insignificantly affected by DLS. These may be explained by repeated DLS 3 times/week made kits only to consume the increased amount of milk secretion which occured on the day after omission of suckling (Szendrõ *et al.* 1999). Moreover, frequency of nursing a day did not influence the weaning weight of kits rabbit (Seitz, 1997). Also, Alvariño *et al.* (1998), Castellini *et al.* (1998) Theau-clement and Mercier (1999) found no changes in growth when kits were separated for up to 24hrs from thier mother began at the age of 2-3 postnatal days. However, Alvariño *et al.* (1999), Szendrõ *et al.* (1999) Bonanno *et al.* (1999a), found that DLS for 36 ,44 or 48 hrs clearly affected the weight of growing and suckling rabbits. The present results indicate that DLS inducing growth reduction in suckling litters

could be attributed to the difference in time of separation, breed, age of kits and frequancey of DLS repetation.

Average percentage of total litter size born (TLSB) and litter size born alive (LSBA), as well as the litter size (LS) at 1st, 2nd, 3rd, 4th, 5th, 6th week age, mortality rate at born (MB), mortality rate (MR) at 1st, 2nd, 3rd, 4th, 5th, 6th week age, total mortality (TM), neonatal survival rate (NSR) and preweaning survival rate (PSR) (Table 4) were insignificantly ($P > 0.05$) different from their controls. This could be explained that, kits obtained sufficient amount of milk for conserving its life and might not differ from those secreted by control does.

These results were agreed with Maertens (1998) and Alvariño *et al.* (1998), who found that DLS did not negatively affect the prewaning survival of young rabbits. Also Alvariño *et al.* (1998) who reported that no information was recorded both the effect of DLS in rabbit on growth and mortality when kits are separated from there mother at the age of 2-3 days and his personal observation indicated that this very young rabbits were able to stand a 48-h separation without apparent modification in mortality rate. In addition, Virag *et al.* (1999) found that DLS 48-h in NZW rabbit have insignificant effect on the size of nursed litter and the lack of milk had no deleterious effect on kits.

Moreover, Alvariño *et al.* (1999) found that DLS at 4 or 11 days old for 0, 24, 36 and 48 hours did not affect litter survival rate. Furthermore, Theau-clement and Mercier (1999) found that DLS-24 h doe-litter separation (nest-boxes closed) did not influence litter size at birth and at weaning. Also, Ubilla *et al.* (2000), found that DLS-48h did not affect litter size or litter survival. Espinosa *et al.* (2004) found no difference in mortality rate at weaning (35 day age) and the changes observed in the development of litters separated from their mothers 48 hours at 9 days of lactation were transient where fasting period of 48 hours at 9 days of age does not compromise the subsequent developmental of young rabbits.

The low mortalities in DLS may be attributed to high interest of the does after litter were returned back to their mothers and this was agreed with Ross *et al.* (1963a), who found that, in rabbit races which showed high interest toward their young, the mortality of the young was low and the mothers were characterized by better nursing and vise versa.

The average of time of complete fur appearance (T_{fur}), complete eye opening (T_{Eye}), complete exist of the young out of the nest box (T_{Exit}), all litter began to nipple food (T_{Nipple}) and complete ear opening (T_{Ear}) after DSL for 4 and 8hrs (Table 4) were insignificantly ($P > 0.05$) differ from the control group.however, after 24hrs of separation, T_{Eye},

T_{Exit} and T_{Nipple} were significantly ($P < 0.01$) different from the control group. This could be explained that DLS for 4 and 8hrs did not interfere with the time of nursing but DLS for 24 hours might interfered with nursing time so litters opened their eyes earlier as an attempt to search for food in the absence of mothers (Sandford,1996). Also, it was found that DLS 4, 8, 24, hours did not significantly affect both time of complete fur appearance and ear opening.

Serum cortisol levels during pregnancy and lactation:

In this experiment maternal serum cortisol (cort) concentration have been measured both at 9 a.m. and 2 p.m. along pregnancy and lactation periods in normal (non-biostimulated) rabbits does. Data represented in Table (5) and assimilated on Figure (1) showed gradual increase in serum level of cortisol from the day 21st of pregnancy (DP21) and reaching its maximum value (2.43 ± 0.07 and 3.000 ± 0.10) at the day of parturition (at 9 a.m. and 2 p.m. respectively). After parturition the cortisol value gradually decreased, reaching its normal value at day 21 of lactation (PND21). These results agreed with Simpson *et al.* (1973); Heather *et al.* (1980) ;Hummelink and Ballard (1986) and Sandford (1996) who found that plasma corticosteroid tends to increase just before the time of parturition and during early lactation, because normal functions such as kindling, pregnancy and lactation impose considerable stress in doe rabbit, (Sandford ,1996).

Diurnal changes in serum cortisol level:

Data represented in Table (6) showed the effect of sampling time on the average serum cortisol level of both non-pregnant and non-lactating (NPNL) or pregnant and lactating does (PL) indicated that, the average serum cortisol levels of samples at 2 p.m. were significantly ($P<0.01$) higher than those taken at 9 a.m.These results indicating a diurnal changes of cortisol in doe rabbits and are in agreement with circadian variation of cortisol that happen normally and reported in domestic animals (Kaneko *et al.*, 1997) .

Secretion of cortisol by the adrenal cortex occurs in response to three identifiable influences: ACTH, a diurnal rhythm, and stress. Secretion of ACTH from the pituitary is stimulated by corticotrophin-releasing hormone (CRH) produced by the hypothalamus. CRH is released in a circadian pattern and in response to physiologic stimuli such as stress and hypoglycemia. The second influence on plasma cortisol levels is diurnal pattern, which, in turn, is due to a circadian pattern of ACTH release. There is a major increase in secretion between 4:00 a.m. and 8:00 a.m., followed by a decrease in ACTH during the rest of the day. The third important influence on cortisol secretion is stress.

Response to stress may be absent or decreased in magnitude in patients to whom large doses of steroids have been administered for some time. Most disorders of cortisol secretion can be classified by patterns of three test results: ACTH, plasma cortisol, and urinary free cortisol. Timing of specimen collection is important because of the circadian variation in ACTH. Mcnatty and Young (1973) reported diurnal changes of serum cortisol level in sheep.

Doe-litter separation and serum cortisol level:

Data represented in Table (7) that assimilated on Figure (2 & 3) showed the effect of doe-litter separations (DLS) for different periods on the mean level of serum cortisol in the examined does during lactation. These data showed no significant difference in cortisol levels in does separated for 4, 8 and 24 h (sampling after 1hr of separation) as compared to control non-separated lactating does (CL) or control non-pregnant non-lactating (NPNL) does.It is well known that doe rabbits nurse their pups only once per day for only 2-5 minutes,usually at night, or in the early morning Cross and Harris (1952); Davis, (1957); Zarrow *et al.* (1965); Hafez, (1970); Lincoln, (1974); Samy, (1980); Distel and Hudson (1984); Sandford, (1996) and Seitz *et al.* (1998). So, the present finding could be explaned that, when cortisol levels were measured at one hour after separation of does from their litter for either 4, 8, and 24 hrs, it stimulated does when returned back to the nest to nurse their kits even if this time of nursing falls outside the period of natural nursing activity, and suggesting that DLS for 4, 8 and 24 hr did not act as stress factor for such periods of separation.

However, when cortisol levels were measured at one hour before returning does that were separated for 24 hrs i.e; means after 23hrs of separation, a significant increase ($P<0.01$) in cortisol levels was recorded as compared to all experimental and control groups, So DLS for 24-h act as stressor as time of nursing passed without nursing litters. This type of experiment when the samples for cortisol levels were taken after 23hrs of separation, which mean 1 hr before returning those mothers to their litters, indicating that such period of separation (24hrs) creating sever stress to the mother does and the biological responses to stressors expressed in a significant increase of cortisol levels when compared to all other experiments of does-litter separation.

CONCLUSION

In conclusion, The effects of transient doe-litter separation for 24 hours on maternal behaviour and litter traits in female New Zealand

White (NZW) rabbits had a significant effect on both mother and new born litter behaviors as well as the mother's serum cortisol level. Therefore, stability of the mother-young bond inside the nest is of great importance for their normal behavior and so, production.

REFERENCES

Abdel-Rahman, M.A.; Ahmed, M.M. and Darwish, M. (2002): Effects of some microclimatic stressors and food deprivation on health status, maternal behaviour and newlyborn development and viability in Baladi; Boscat and California Rabbits. Ass. Univ. Bull. Environ. Res. Vol. (5) No. (1).

Afifi, D.S. and Dachash, H.A. (1999): Reproductive performance of California doe rabbits as affected by feeding freshly Nigella Sativa seeds. Alex. J. Sci. 15 (5): 995 - 1008.

Alvariño, J.M.R.; Del Arco, J.A. and Bueno, A. (1998): Effect of mother-litter separation on reproductive performance of lactating rabbit females inseminated on day 4 or 11 postpartum. World rabbit Science, Vol. 6 (2), 191 – 194.

Alvariño, J.M.R.; Bueno, A.; Santiago, M.E. and Del Arco, J.A. (1999): Effect of doe-litter separation on survival and growth of young rabbits. World Rabbit Science. Vol. 7 (3), 161-163.

Bonanno, A.; Alabiso, M.; Di Grigoli, A. and Alicata, M.L. (1999a): Effect of change of cage and/or 44 h mother-litter separation on prodcutivity of non-receptive lactating rabbit does. Preliminary investigation. Wrold Rabbit Science, Vol. 7 (2), 107 – 111.

Bonanno, A.; Alabiso, M.; Di Grigoli, A.; Alicata, M.L. and Leto, G. (1999b): Effect of a 48h delayed insemination with or without a 48h doe-litter separation on performance of non-receptive rabbit does. World Rabbit Science, Vol. 7 (3): 171 – 175.

Bonanno, A.; Di Grigoli, A.; Alabiso, M. and Boiti, C. (2002): Parity and number of repeated doe-litter separation treatments affect differently the reproductive performances of lactating does. World Rabbit Science, Vol. 10 (2), 63 – 70.

Calvert, D.T.; Knight, C.H. and Peaker, M. (1985): Milk accumulation and secretion in the rabbit. Quarterly j. Exp. Physiol. and Cognate Medical Sci., 70, 357 – 363.

Castellini, C.; Canali, C. and Boiti, C. (1998): Effect of mother-litter separation for 24 hours, by closing the nestbox or change of cage, on rabbit does reproduction performance. Wrold Rabbit Sci., 6 (1), 199 – 203.

Costantini, F.; Panella, F. and Castellini, C. (1986): Management of rabbit breeding. Riv. Coniglicoltura, 23(2), 44 – 46.

Distel, H. and Hudson, R. (1984): Nipple-search performance by rabbits: Changes with age and time of day. Animal Behaviour; 32: 501-507.

Cross, B.A. (1957): The mechanism of labour in the rabbit. J. Ednocrinology. 16: 261 - 276.

Cross, B.A. and Harris, R. (1952): Cited by Zarrow, M.X.; Farook, A.; Denenberg, V.H.; Swain, P.B. and Ross, S. (1963): Maternal behaviour in the rabbit: Endocrine control of maternal nest building. J. Reprod. & Fertility.; 2: 152.; 6: 375 - 383.

Davis, J. (1957): Cited by Zarrow, M.X.; Deneberg, V.K. and Anderson, C.O. (1965): Rabbit frequency of suckling in the pup. Science, Washington, U.S.A.; 150: 1835 - 1836.

Denenberg, V.H.; Sawin, P.B.; Frommer, G.P. and Ross, S. (1958): Genetic, physiological and behavioural background of reproduction in the rabbit:IV. An analysis of maternal behaviour at successive parturitions.

Distel, H. and Hudson, R. (1984): Nipple-search performance by rabbits: Changes with age and time of day. Animal Behaviour; 32: 501-507.

Espinosa, A.; Rebollar, A. and Carabanõ, R. (2004): Impact of doe-litter separation on milk production, feed intake of does and on digestive paramteres of young rabbit's growth. World Rabbit Science 12: 184 – 185.

El-Raffa, A.M.E. (1994): Some factors affecting economical, productive and reproductive traits in rabbits. Ph.D. Thesis, Fac. of Agric., Alexandria. Univ., Egypt.

Fleming, A.S. and Corter, C. (1988): Factors influencing maternal responsiveness in humans: usefulness of an animal model. Psychoneuroendocrinol. 13: 189 - 212.

Hafez, E.S.E (1962): The behaviour of rabbits, the behaviour of domestic animals, London, Bailliere, Tindall & Cox., 3rd Ed.

Hafez, E.S.E. (1970): Reproduction and breeding techniques for laboratory animals. Lee and Febiger. Philadelphia, 2nd ed. U.S.A.

Hamilton, H.H.; Lukefahr, S.D. and MacNitt, J.I. (1997): Maternal nest quality and its influence on litter survival and weaning, performance in commercial rabbits. J. Anim. Sci; 75: 926 - 933.

Heather, A.B.; Lugg, M.A. and Nicholas, T.E. (1980): Cortisone and cortisol in maternal and fetal blood and in amniotic fluid during the final ten days of gestation in the rabbit. Biol. Neonate.; 38: 214 - 220.

Hudson, R. and Distel, H. (1982): The pattern of behaviour of rabbit pups in the nest. Behaviour 79: 255 – 271.

Hudson, R. and Distel, H. (1989): The temporal pattern of suckling in rabbit pups: A model of circadian synchrony between mother and young. Development of circadian Rhythmicity and photoperiodism in Mammals. Ed. S. M. Reppert, Boston, Perinatology Press; 83-102.

Hudson, R.; Bilkó, A. and Altracker, V. (1996a): Nursing, weaning and the development of independent feedingt in the rabbit. Z. Sáugetierkund., 61, 39 – 48.

Hummelink, R. and Ballard, PL. (1986): Endogenous corticoids and lung development in the fetal rabbit, Endocrinology, Vol. 188 (4), 1622 - 1629.

Kaneko, J.J; Harvey, J.W. and Bruss, M.L. (1997): Clinical Biochemistry of Domestic Animals.5th Edition.Academic Press. San Diego and London.

Lebas, F. (1969): Milk feeding and ponderal weight of the rabbit before weaning (In French). An. Zootech. (Paris); 18: 197 - 208.

Lincoln, D.W. (1974): Suckling a time-constant in the nursing behaviour of the rabbit. Physiological Behaviour; 13 (5): 711 – 714.

Maertens, L. (1998): Effect of flushing, mother-litter separation and PMSG on the fertility of lactating does and the performance of their litter. World Rabbit Sci., 6 (1), 185 – 190.

McNatty, K.P. and Young, A. (1973): Diurnal changes of plasma cortisol levels in sheep adapting to a new environment. J. Endocr. 56; 329 - 330.

Nelson, R.J. (1995): An introduction to behavioural endocrinology. The Johns Hopkins University Sinauer Associated, Inc. Publishers, Sunderland, Massachuelts.Copyright by Sinauer Associates, Inc.

Numan, M. (1988): Maternal behaviour. In Knobil E.; Neil J. (Eds.). Physiol. Reprod.; 1569-1645, Raven Press, New York.

Numan, M. (1990): Neural control of maternal behaviour. In: Krasnegro N.A., Bridge R.S. (eds.), Mammalian Parenting, Oxford Uni. Press.; pp. 213-259.

Ross, S.; Sawin, P.B.; Zarrow, M.X. and Denenberg, V.H. (1963a): Maternal care in mammals, Nature; 163 - 44.

Samy, M.S.M. (1980): Rabbit production, Dar El-Fiky El-Arabia, Egypt. (P. 93 - 112).

Sandford, J.C. (1996): The domestic Rabbit, Fifth edition. Alden press limited, Oxford (UK).

Seitz, K. (1997): Untersuchungen zum säugeverhalften von Hauskaninchen-zibben Sowie Zu Milchaufnahme, lebendmassen-twicklung und verlustegeschehen der Jungtiere. Thesis Univ. Gießen.

Seitz, K.; Hoy, S.T. and Lange, K. (1998): Untersuchungen zum Einfluss Verschiedener Faktoren auf das Säugeverhalten Bei Hauskaninchen. Bed. Münch. Tierärzth. Wschr; 111, 48 – 52.

Simpson, A.A.; Simpson, M.H.W.; Sinha, Y.N. and Schmidt, G.H. (1973): Changes in concentration of prolactin and adrenal corticosteroids in rat plasma during pregnancy and lactation. J. Endocr. 85, 675 – 676.

Suckow, M.A. and Douglas, F.A. (1997): The laboratory rabbit. CRC Press, Boca Raton, New York.

Szendrõ, Zs.; Jovánczai Zs.; Theau-Clément, M.; Radnai, I.; Biro-Németh, E. and Milists, G. (1999): The effect of doe-litter separation on reproduction perforamance in rabbit does and their kits. World Rabbit Sceince. Vol. 7(3), 165 – 169.

Theau-Clément, M. and Mercier, P. (1999): Effect of a 24 hour Doe-litter separation on Rabbit Doe Reproductive performance and Growth of the young. World Rabbit Science. Vol. 7 (3), 177–179.

Virag, G.Y.; Kustos, K. and Szabo, L. (1999): Effect of a 24 Hours Doe-litter Separation on Rabbit Does Reproductive Performance and offespring's Growth. World Rabbit Science, Vol.. 7 (3), 155-159.

Ubilla, E.; Rebollar, P.G.; Pazo, D.; Esquifno, A.L. and Alvairño, J.M. (2000): Pituitary and ovarian response to transient doe-litter separation in nursing rabbits. J. Reprod Fertil; 118 (2): 361 - 6.

Ubilla, E.; Rebollar, P.G.; Pazo, D.; Equifino, A. and Alvairño, J.M. (2001): Endocrine profiles during doe –litter separation and the subsequent pregnancy in rabbits. J. Physiol Biochem.; 57 (2): 23 - 9.

Zarrow, M.X.; Deneberg, V.K. and Anderson, C.O. (1965): Rabbit frequency of suckling in the pup. Science, Washington, U.S.A.; 150: 1835 - 1836.