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# BEHAVIOURAL AND PHYSIOLOGICAL RESPONCES OF BALADI RED AND NEW ZEALAND WHITE RABBIT TO NATURAL OESTRUS INDUCTION METHODS

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## ABSTRACT

This experiment was designed to study the behavioural and physiological responses of two breeds of rabbits to natural oestrus induction methods in non-receptive female rabbits to mating. One hundred multiparous female rabbits from Baladi Red (BR) and New Zealand White (NZW) breeds (50 from each breed) and twenty mature bucks from two breeds (10 from each breed) were used in the study. The following two methods were used: (1) doe-litter separation in the suckling females, or (2) presence of a doe beside buck cage in the non-suckling females. Basic behavioural (time of standing, walking and sitting %) and sexual behavioural activities (frequency of male circling around female, female circling around male, male mounting female and actual mating) were recorded for each male and female rabbits. Receptivity and conception rates were calculated in each treatment group. Also, serum concentrations of estradiol-17β hormone were determined in does under investigation. The results of this experiment indicated that animals after application of natural inducing oestrus treatments are more active than before treatments. Time of standing was significantly higher than time of sitting in both breeds after treatments compared with those before treatments. Moreover, animals after application of treatments showed significantly higher frequency of female circling around male, male mounting female and actual mating, and insignificantly higher frequency of male circling around female, than those values recorded in animals before application of treatments. Oestrogen levels significantly increased after presence doe beside buck cage and insignificantly increased after doe-litter separation in both breeds. Both treatments showed pronounced improvement in terms of receptivity and conception rates, irrespective of breed. NZW does were significantly superior over BR does in most studied traits. Highly significant positive correlations were found between both sexual behaviour and oestrogen level with receptivity and conception rates. Generally, natural methods used to induce oestrus led to a positive change in the basic and sexual behaviour as well as improvement in the physiological performance of non-receptive female rabbits for mating.

**Key words:** oestrus induction; behaviour; doe-litter separation; male effect; rabbit does

## INTRODUCTION

Recently, bio-stimulation methods to synchronize estrus in rabbits does were used to induce receptivity rather than hormonal treatments. The natural methods used such as female beside male cage (Enas Abd El Wahed, 2017), feed restriction (Mehaisen and Abbas, 2014), mother-litter separation (Ilès *et al.*, 2013), flushing and changes of female cages before mating (Manal, 2010) or lighting regime (McNitt, 1992).

All these studies showed a significant improvement in the physiological, reproductive and productive performance of rabbit does. On the other hand, mammalian reproduction starts with mating behavior and terminates when the young are weaned. Between these two events, there are complex chain of behavioural phenomena, which are critical for the survival of the youngs (Marai and Rashwan, 2003). Rabbits are induced ovulators with no regular oestrous cycle (Maertens *et al.*, 1995).

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The stimulus of mating initiates the ovulation process, due to the surge of GnRH from hypothalamus after mating by physical stimulation of genital areas causing the LH peak, which initiates the ovulatory process (McNitt, 1992). In nursing rabbits, sexual receptivity and fertility is depressed during the period of lactation, presumably through a hormonal antagonism between prolactin and gonadotropin release. These endocrine changes would explain the activation of ovarian function (Gonzalez-Mariscal, 2001).

Nevertheless, there are few studies on the behavioural changes of female rabbits during accepted and refused matting. Therefore, the objectives of the present study were to record and investigate the behavioural responses of two breed of rabbits under two natural methods to induce oestrus in non-receptive doe rabbits. In addition, determination of some reproductive parameters was done during the study.

## MATERIAL AND METHODS

This experiment was carried out at a Rabbitry of the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Two bio-stimulation methods were used to induce oestrus in normally healthy and non-receptive rabbit females. These methods were: 1) doe-litter separation in suckling females (n = 61) for 24 h, and 2) presence of doe beside male cages in non-suckling females (n = 39) for 24 h. Each method was chosen according to the female status (suckling or non-suckling).

### Animals, husbandry and experimental design

One hundred multiparous female rabbits from Baladi Red (BR) and New Zealand White (NZW) breeds (50 from each breed) and twenty mature bucks form two breeds (10 from each breed) were used in the study. Animals were free of any external parasites or skin diseases and were not treated hormonally before this experiment. Age of animals ranged from 10 – 12 months.

Animals were housed individually in galvanized wired cages (40 x 40 x 30 cm), where feed and water were provided *ad libitum*. Animals were fed on basal pellet ration contained yellow corn, soybean meal, corn gluten, minerals and vitamins premix, bone and molasses. The calculated chemical components of the diet were: 17 % crude protein, 2.8 % fat, 10 % crude fibre and 2600 kcal digestible energy/kg diet. Lighting system was 16 h light/8 h dark in the rabbitry during the experimental period. Does were transferred to the rabbit buck cages for natural mating process and kept under examination until natural mating was successfully completed. The experiment lasted seven months, from September to April. At the start of the experiment, all females were non-pregnant. All females were naturally mated. Bio-stimulation methods were applied on females, who rejected mating once at any time during the experiment.

All procedures were reviewed and approved by the Faculty of Agriculture, Suez Canal University and complied with the Institutional Animal Care and Use Committee of Suez Canal University.

### Studied traits

#### Behavioural activities

Basic and sexual behavioural activities of males and females of the two breeds were studied during the experimental period. The behavioural traits of animals were recorded after and before application of treatments in both breeds by using a video camera for one hour during the morning from 9 to12 am. Each doe in each treatment was transferred into a buck cage of the same breed. From the video tapes, the basic behavioural activities (percentage of bucks and does standing, sitting or walking) were recorded at 3 min interval (sampling time) and scored according to Khalil *et al.* (2014). In addition, sexual behavioural patterns of both sexes, such as frequency of male circling around female, female circling around male, male mounting female and actual mating (Hassan *et al.*, 2015) were recorded during sexual desire and mating.

### Receptivity and conception rates

Receptivity rate was determined in rabbit doe breeds after application of the selected bio-stimulation method in suckling or non-suckling females. Receptivity was determined by the willingness of the doe to mate combined with signs of oestrus, such as swelling of the vulva, vulva colour, exposition of the rear quarters and lordosis. Doe rabbits were transferred to male cages to check receptivity for 5 min and, if refused, the treatment was repeated again on the next day.

Litter separation method was applied on the next day after delivery. Conception rate was diagnosed in rabbit does after 12 – 15 days of successful mating. Abdomen palpation was applied on assumed pregnant does by the same technician. Data on reception and conception rates were calculated as a percentage from non-responsive does.

**Blood sampling and oestrogen hormone determination**

Blood samples were collected from the ear vein of females, which rejected mating; after applying the bio-stimulation method from females that accepted mating, samples were taken after one hour following treatment. The blood was left for coagulation at the fridge temperature (at 4 °C) before centrifugation at 4000 rpm for 20 min to separate serum. Serum samples were frozen at -20 °C until hormonal analyses. Oestrogen hormone was determined by using VIDAS Estradiol II kits from bioMerieux SA, France. The lower detection limit was 9 $pg.mL^{-1}$, the intra- and inter-assay CV were 7.5 % and 9.5 %, resp.

**Statistical Analyses**

Data were analysed by using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 2004). Differences (LSD) between means were tested using a Duncan's multiple range test (Duncan, 1955). Correlation coefficients among traits were estimated.

**Behavioural activities and hormonal profile**

Factorial design was applied for the evaluation of the experiment. Three-way analysis of variance (ANOVA-test) with three-way interactions was carried out using the following model:

$Y_{ijkl} = \mu + B_i + D_j + T_k + B*D_{ij} + B*T_{ik} + D*T_{jk} + B*D*T_{ijk} + e_{ijkl}$,

where:

$Y_{ijkl}$ = the observation on the $k^{th}$ individual from the $i^{th}$ breed in $j^{th}$ doe status.

$\mu$ = the overall mean.

$B_i$ = the fixed effect of the $i^{th}$ breed (i = Baladi and NZW).

$D_j$ = the fixed effect of the $j^{th}$ doe status (presence beside male and litter separation).

$T_k$ = the fixed effect of the $k^{th}$ time (before and after treatment).

$B*D_{ij}$ = the interaction among $i^{th}$ breed and $j^{th}$ doe status.

$B*T_{ik}$ = the interaction among $i^{th}$ breed and $k^{th}$ time.

$D*T_{ik}$ = the interaction among $j^{th}$ doe status and $k^{th}$ time.

$B*D*T_{ijk}$ = the interaction among $i^{th}$ breed, $j^{th}$ doe status and $k^{th}$ time.

$e_{ijkl}$ = the random error associated with the individual ijkl.

**Receptivity and conception rates**

Factorial design was applied for the evaluation of the experiment. Two-way analysis of variance (ANOVA-test) with two-way interactions was carried out using the following model:

$Y_{ijk} = \mu + B_i + D_j + B*D_{ij} + e_{ijk}$

Where:

$Y_{ijk}$ = the observation on the $k^{th}$ individual from the $i^{th}$ breed in $j^{th}$ doe status.

$\mu$ = the overall mean.

$B_i$ = the fixed effect of the $i^{th}$ breed (i = Baladi and NZW).

$D_j$ = the fixed effect of the $j^{th}$ doe status (presence beside male and litter separation).

$B*D_{lij}$ = the interaction among $i^{th}$ Breed and $j^{th}$ Doe status.

$E_{ijk}$ = the random error associated with the individual ijk.

## RESULTS

**Basic behavioural activities**

Data on basic behavioural activities of BR and NZW rabbit breeds are presented in Tables 1 and 2. Significant differences ($P \leq 0.05$) in standing and walking behavioural activities between two breeds were found. NZW does had significantly ($P \leq 0.05$) lower time of standing (28.17 %) and higher ($P \leq 0.05$) time of walking (23.41 %) than those recorded in BR does (35.71 and 16.66 %, respectively). However, no significant differences were found between two treatments in basic behavioural activities. Moreover, after application of natural inducing oestrus treatments on rabbit does, which refused mating, standing behavioural activity was significantly ($P \leq 0.05$) increased but sitting behavioural activity was decreased ($P \leq 0.01$), compared with those before treatments in both breeds (Table 1).

In addition, significant ($P \leq 0.01$) differences were found among the treatment effect interactions in all basic behavioural activities. The highest ($P \leq 0.01$) standing behaviour was recorded in BR does treated with beside males (47.61 %), but the lowest ($P \leq 0.01$) value was recorded in NZW does before treated with doe-litter separation (17.46 %). However, the lowest time of walking behaviour was observed in BR does after treated with beside males (9.52 %),

**Table 1. Basic behavioural activities (%) in NZW and BR rabbit does after inducing oestrus to females (mean ± SE)**

| | | Standing (%) | Walking (%) | Sitting (%) |
|---|---|---|---|---|
| Breed | NZW | 28.17 ± 2.37[b] | 23.41 ± 1.48[a] | 48.41 ± 2.97 |
| | BR | 35.71 ± 2.58[a] | 16.66 ± 2.23[b] | 47.61 ± 1.85 |
| | P-value | 0.043 | 0.020 | 0.823 |
| Type of treatment | Doe-Litter Separation (DLS) | 28.57 ± 2.55 | 20.23 ± 1.95 | 51.19 ± 2.87 |
| | Doe Beside Male (DBM) | 35.31 ± 2.51 | 19.84 ± 2.33 | 44.84 ± 1.48 |
| | P-value | 0.07 | 0.897 | 0.063 |
| Time | Before (control) | 28.17 ± 2.44[b] | 19.44 ± 1.80 | 52.38 ± 2.48[a] |
| | After (treated) | 35.71 ± 2.52[a] | 20.63 ± 2.44 | 43.65 ± 1.64[b] |
| | P-value | 0.043 | 0.699 | 0.008 |
| Interactions | | | | |
| Breed*Treatment*Time | NZW*DLS*Before | 17.46 ± 1.58[c] | 19.04 ± 2.74[abc] | 63.49 ± 3.17[a] |
| | NZW*DLS*After | 30.15 ± 4.19[b] | 28.57 ± 2.74[a] | 41.26 ± 1.58[b] |
| | NZW*DBM*Before | 33.15 ± 2.74[b] | 20.63 ± 1.58[ab] | 46.03 ± 4.19[b] |
| | NZW*DBM*After | 31.74 ± 4.19[b] | 25.39 ± 1.58[a] | 42.85 ± 2.74[b] |
| | BR*DLS*Before | 33.33 ± 5.49[b] | 14.28 ± 2.74[bc] | 52.38 ± 2.74[b] |
| | BR*DLS*After | 33.33 ± 2.74[b] | 19.04 ± 2.74[abc] | 47.61 ± 5.49[b] |
| | BR*DBM*Before | 28.58 ± 2.74[b] | 23.80 ± 5.49[ab] | 47.61 ± 2.74[b] |
| | BR*DBM*After | 47.61 ± 0.0[a] | 9.52 ± 2.74[c] | 42.85 ± 2.74[b] |
| P-value | | 0.001 | 0.009 | 0.006 |

[a,b,c] Means within column not sharing a common superscript differed significantly.

but the highest value was recorded in NZW does after treated with doe-litter separation (28.57 %). NZW does before treated with doe-litter separation had significantly ($P \leq 0.01$) increased time of sitting behaviour compared to all treated groups.

In relation to males, no significant differences were found between bio-stimulation methods and breeds in basic behaviour activities, but significant ($P \leq 0.05$) differences were found in their interactions in time of standing and sitting behaviour activities. The highest ($P \leq 0.05$) standing behaviour was recorded in BR breed before treated with beside males (66.66 %), but the lowest value was observed in BR after treated with doe-litter separation (49.20 %). Moreover, sitting behaviour was significantly ($P \leq 0.01$) increased in bucks of BR breed after treated with doe-litter separation compared to other treatment groups.

**Sexual behavioural activities**

Frequency of male circling around female (MCAF), female circling around male (FCAM), male mounting female (MMF) and actual mating (AM), as affected by treatments and their interactions, are presented in Table 3. The results show that NZW breed had significantly ($P \leq 0.05$) increased frequency of AM, but insignificantly higher frequency of MCAF, FCAM and MMF, than those values recorded in the BR breed.

Significant ($P \leq 0.01$) differences in all sexual behaviour traits, except AM, were found due to the oestrus bio-stimulation methods. The group treated with beside males had significantly ($P \leq 0.01$) higher frequency of MCAF, FCAM and insignificantly higher frequency of AM, but significantly lower frequency of MMF than those recorded in the group treated with doe-litter separation.

Analysis of variance showed significant ($P \leq 0.01$) differences between the control and

**Table 2. Basic behavioural activities (%) in NZW and BR rabbit bucks after inducing oestrus to females (mean ± SE)**

| | | Standing (%) | Walking (%) | Sitting (%) |
|---|---|---|---|---|
| Breed | NZW | 59.12 ± 1.48 | 25 ± 0.85 | 15.87 ± 1.22 |
| | BR | 59.12 ± 2.89 | 22.22 ± 1.07 | 18.65 ± 2.58 |
| | P-value | 0.999 | 0.055 | 0.342 |
| Type of treatment | Doe-Litter Separation (DLS) | 56.74 ± 2.15 | 22.61 ± 1.03 | 20.63 ± 2.14[a] |
| | Doe Beside Male (DBM) | 61.50 ± 2.22 | 24.60 ± 0.98 | 13.88 ± 1.36[b] |
| | P-value | 0.138 | 0.179 | 0.014 |
| Time | Before (control) | 60.31 ± 1.88 | 23.41 ± 1.09 | 16.26 ± 1.48 |
| | After (treated) | 57.93 ± 2.61 | 23.80 ± 1.01 | 18.25 ± 2.47 |
| | P-value | 0.467 | 0.792 | 0.499 |
| Interactions | | | | |
| Breed*Treatment*Time | NZW*DLS*Before | 58.73 ± 1.58[ab] | 22.22 ± 1.58 | 19.04 ± 2.74[b] |
| | NZW*DLS*After | 60.31 ± 4.19[ab] | 25.39 ± 1.58 | 14.28 ± 2.74[b] |
| | NZW*DBM*Before | 57.14 ± 2.74[ab] | 26.98 ± 1.58 | 15.87 ± 1.58[b] |
| | NZW*DBM*After | 60.31 ± 4.19[ab] | 25.39 ± 1.58 | 14.28 ± 2.74[b] |
| | BR*DLS*Before | 58.73 ± 3.17[ab] | 22.22 ± 3.17 | 19.04 ± 0.00[b] |
| | BR*DLS*After | 49.20 ± 5.72[b] | 20.63 ± 1.58 | 30.15 ± 4.19[a] |
| | BR*DBM*Before | 66.66 ± 5.49[a] | 22.22 ± 1.58 | 11.11 ± 4.19[b] |
| | BR*DBM*After | 61.90 ± 5.49[ab] | 23.80 ± 2.74 | 14.28 ± 2.74[b] |
| P-value | | 0.050 | 0.392 | 0.010 |

[a,b,c] Means within column not sharing a common superscript differed significantly.

treated animals in all sexual behaviour traits except MCAF. Treated animals after application of treatments had significantly ($P \leq 0.01$) higher frequency of FCAM, MMF and AM, and insignificantly ($P = 0.06$) higher frequency of MCAF, than those values recorded in the control animals before application of treatments.

Significant ($P \leq 0.01$) differences were found among the treatment effect interactions in all basic behavioural activities. The highest frequencies of MAF and FAM were recorded in BR does treated with beside males, but the lowest values were recorded in BR does before treated with doe-litter separation. The lowest frequency of MMF was observed in BR does before treated with beside males, but the highest value was recorded in NZW does after treated with doe-litter separation. Moreover, AM frequency was significantly increased ($P \leq 0.01$) in all treated groups compared to the control animals before application of treatments.

**Oestrogen hormonal profile**

Table 3 shows levels of estradiol-17β ($E_2$) in NZW and BR breeds before and after application of treatments. No significant differences were found between both breeds in $E_2$ levels. However, NZW breeds showed slightly higher $E_2$ level than that in BR breed. Overall effects of natural inducing oestrus methods showed significant ($P \leq 0.01$) differences in $E_2$ level. Females treated with presence beside male had significantly ($P \leq 0.01$) higher $E_2$ (53.4 pg.mL$^{-1}$) than those measured in females treated with doe-litter separation method (47.5 pg.mL$^{-1}$). The overall effect of time (before and after treatment) on $E_2$ levels was significant ($P \leq 0.00$). Concentration of $E_2$ after application of treatments was significantly ($P \leq 0.01$) higher (54.3 pg.mL$^{-1}$) than those recorded before application of the treatment (46.6 pg.mL$^{-1}$). The interaction effects among treatments showed significant ($P \leq 0.01$) differences in serum $E_2$ level. The highest ($P \leq 0.01$) $E_2$ level was recorded in NZW

**Table 3. Frequency (number of movements per hour) of some sexual behavioural activities traits of NZW and BR rabbits and estradiol-17β ($E_2$) as affected by treatments (mean ± SE)**

| Breed | Male circling around female (No) | Female circling around male (No) | Male mounting female (No) | Actual mating (No) | $E_2$ (pg.mL$^{-1}$) |
|---|---|---|---|---|---|
| NZW | 111.7 ± 9.2 | 105.5 ± 10.8 | 53.1 ± 3.3$^{a}$ | 4.7 ± 1.44 | 51.0 ± 1.9 |
| BR | 95.2 ± 15.2 | 86.0 ± 16.8 | 44.0 ± 1.9$^{b}$ | 4.2 ± 1.33 | 49.8 ± 1.1 |
| P-value | 0.365 | 0.340 | 0.028 | 0.802 | 0.601 |
| Type of treatment | | | | | |
| Doe-Litter Separation (DLS) | 68.7 ± 8.2$^{b}$ | 70.0 ± 10.9$^{b}$ | 54 ± 3.1$^{a}$ | 4.2 ± 1.33 | 47.5 ± 0.7$^{b}$ |
| Doe Beside Male (DBM) | 138.2 ± 6.1$^{a}$ | 121.8 ± 13.2$^{a}$ | 43.1 ± 1.9$^{b}$ | 4.7 ± 1.44 | 53.4 ± 1.8$^{a}$ |
| P-value | 0.000 | 0.000 | 0.008 | 0.802 | 0.006 |
| Time | | | | | |
| Before (control) | 87.2 ± 9.6 | 60.9 ± 7.8$^{b}$ | 43.2 ± 2.2$^{b}$ | 0.00$^{b}$ | 46.6 ± 0.6$^{b}$ |
| After (treated) | 119.5 ± 13.7 | 130.5 ± 11.7$^{a}$ | 53.9 ± 2.9$^{a}$ | 4.5 ± 0.4$^{a}$ | 54.3 ± 1.6$^{a}$ |
| P-value | 0.066 | 0.000 | 0.009 | 0.000 | 0.000 |
| Interactions | | | | | |
| Breed*Treatment*Time | | | | | |
| NZW*DLS*Before | 69.0 ± 1.1$^{e}$ | 70.0 ± 1.1$^{e}$ | 55 .0 ± 1.7$^{b}$ | 0.0$^{c}$ | 45.6 ± 1.2$^{c}$ |
| NZW*DLS*After | 113.0 ± 1.3$^{d}$ | 120.0 ± 2.8$^{c}$ | 70.0 ± 1.1$^{a}$ | 4.8 ± 0.3$^{a}$ | 48.5 ± 1.7$^{c}$ |
| NZW*DBM*Before | 110.0 ± 2.8$^{d}$ | 74.6 ± 1.4$^{e}$ | 40 .0 ± 1.1$^{e}$ | 0.0$^{c}$ | 47.0 ± 1.7$^{c}$ |
| NZW*DBM*After | 155.0 ± 2.8$^{b}$ | 157.7 ± 1.5$^{b}$ | 40.0 ± 1.4$^{cd}$ | 4.5 ± 0.4$^{a}$ | 62.7 ± 2.6$^{a}$ |
| BR*DLS*Before | 45.0 ± 1.1$^{f}$ | 18.0 ± 1.1$^{f}$ | 43 .0 ± 1.1$^{de}$ | 0.0$^{c}$ | 46.3 ± 1.2$^{c}$ |
| BR*DLS*After | 48.0 ± 1.1$^{f}$ | 72.3 ± 1.4$^{e}$ | 48.0 ± 1.1$^{c}$ | 3.5 ± 0.5$^{b}$ | 49.7 ± 1.5$^{c}$ |
| BR*DBM*Before | 125.0 ± 2.8$^{c}$ | 81.0 ± 1.1$^{d}$ | 35.0 ± 1.1$^{f}$ | 0.0$^{c}$ | 47.2 ± 1.7$^{c}$ |
| BR*DBM*After | 163.0 ± 1.3$^{a}$ | 173.0 ± 1.3$^{a}$ | 50.0 ± 2.8$^{c}$ | 4.8 ± 0.3$^{a}$ | 56.2 ± 1.1$^{b}$ |
| P-value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

[a,b,c,d,e,f] Means within column not sharing a common superscript differed significantly.

does after treated with beside males, but the lowest values were recorded in NZW does before treated with doe-litter separation. These results indicated different mode of action of both treatments on the inducing oestrus in BR and NZW breeds.

**Receptivity and conception rates**

Table 4 shows receptivity and conception rates in NZW and BR does, as affected by treatments. Bio-stimulation methods significantly ($P \leq 0.05$) increased receptivity percentage in females that rejected mating, in both breeds, compared with the control animals. This increment was 15 % in both breeds (Table 3). However, females treated with doe-litter separation had insignificantly ($P = 0.14$) increased receptivity percentage (6.26 %) compared with females treated with beside males, irrespective of breeds. On the other hand, the interaction effects of treatment and rabbit breeds showed insignificant ($P = 0.31$) differences between treatments within the breeds (Table 4). A significant difference was found between two bio-stimulation methods in the conception rate. Females treated with beside females had significantly higher ($P \leq 0.00$) conception rate (72.29 %) than those in females treated with doe-litter separation (55.21 %). Moreover, no significant differences were detected between the breeds ($P = 0.74$) and the interaction effects ($P = 0.06$) in the conception rate (Table 4). NZW does had insignificantly higher conception rate

**Table 4. Receptivity and conception rates (%) in NZW and BR rabbits after inducing oestrus (mean ± SE)**

| | | Receptivity % | Conception rate |
|---|---|---|---|
| Before treatment (Control group) | | | |
| Breed | NZW | 54.80 ± 3.54 | 69.36 ± 5.81 |
| | BR | 60.83 ± 5.15 | 62.68 ± 4.94 |
| | Overall | 57.81 ± 3.26$^{B}$ | 65.56 ± 4.45 |
| | P-value | 0.354 | 0.425 |
| After treatment (Treated group) | | | |
| Breed | NZW | 70.04 ±3.13 | 65.72 ±5.01 |
| | BR | 75.20 ± 2.86 | 63.60 ± 4.13 |
| | Overall | 72.84 ± 3.86$^{A}$ | 64.48 ± 4.18 |
| | P-value | 0.227 | 0.743 |
| Treatment | Doe-Litter Separation (DLS) | 76.32 ± 3.13 | 55.21 ± 4.98$^{b}$ |
| | Doe Beside Male (DBM) | 70.06 ± 2.85 | 72.29 ± 3.95$^{a}$ |
| | P-value | 0.142 | 0.007 |
| Interactions | NZW*DLS | 72.79 ± 4.93 | 55.45 ± 8.04 |
| | NZW*DBM | 67.95 ± 4.07 | 73.55 ± 6.14 |
| | BR*DLS | 78.95 ± 4.06 | 55.03 ± 6.36 |
| | BR*DBM | 71.89 ± 4.00 | 71.20 ± 5.17 |
| | P-value | 0.314 | 0.065 |

$^{a,b}$ Means within column not sharing a common superscript differed significantly.
$^{A, B}$ Means within column not sharing a common superscript differed significantly.

(65.72 %) than those recorded in BR does (63.60 %). Also, the highest conception rate was obtained in NZW does treated with beside males (73.55 %) than those recorded in BR does (55.03 %).

**Correlation coefficient among some studied traits**

Results in Table 5 demonstrate prevalent moderate-to-high significant positive correlation between both $E_2$ levels with each of some basic and sexual behavioural activities, such as standing females, walking males, males around females, females around males and actual mating. In addition, positive correlation was observed between actual mating with each of male circling around females, females circling around male, male mounting females, receptivity and conception rates. Also, moderate-to-high positive correlation was determined between receptivity and standing females, male around females, actual mating and $E_2$. On the other hand, low-to-moderate negative correlation was observed between $E_2$ levels and both sitting males and females, and with males mounting females.

## DISCUSSION

Results of the present study showed clearly that both bio-stimulation methods significantly affected and markedly improved most behavioural activities and physiological traits. After treatments, animals had significantly decreased time of sitting (flattening on the floor cage) and increased the time of standing, while insignificantly increased the time of walking behavioural activities compared with those recorded before treatments. Moreover, frequency of female circling around male, male mounting female and actual mating after application of natural inducing oestrus was also increased compared with before treatments.

Preparation of a receptive doe for mating takes few seconds; the sexually mature doe in heat accepts the male after 2 or 3 initial attempts. The libido in the male is so vigorous that he becomes tired if 4 or 5 attempts to mate failed. A successful mating is recorded from the characteristic wooing sound of the male followed by his falling off the female either in a backward or in a sideway direction

**Table 5. Correlation coefficient among some behavioural traits and oestrogen hormone**

| | Wlk F | Sit F | St M | Wlk M | Sit M | Male circling around female | Female circling around male | Male mount. Female | Actual mate. | $E_2$ | Rec. | Con. Rate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| St F | -0.513 | -0.587 | -0.095 | -0.016 | -0.072 | 0.457 | 0.429 | -0.261 | 0.443 | 0.492 | 0.476 | 0.723 |
| Wlk F | 1 | -0.234 | 0.122 | 0.499 | -0.257 | -0.024 | 0.101 | 0.289 | 0.124 | 0.031 | 0.181 | 0.141 |
| Sit F | | 1 | -0.135 | -0.519 | 0.297 | -0.597 | -0.678 | -0.008 | -0.653 | -0.643 | -0.567 | -0.969 |
| St M | | | 1 | 0.331 | -0.932 | 0.299 | 0.303 | -0.247 | -0.127 | 0.136 | -0.398 | 0.154 |
| Wlk M | | | | 1 | -0.637 | 0.668 | 0.478 | 0.075 | 0.195 | 0.407 | -0.211 | 0.548 |
| Sit M | | | | | 1 | -0.489 | -0.408 | 0.186 | 0.059 | -0.234 | 0.412 | -0.349 |
| Male circling around female | | | | | | 1 | 0.830 | 0.101 | 0.581 | 0.747 | 0.030 | 0.662 |
| Female circling around male | | | | | | | 1 | 0.227 | 0.808 | 0.857 | 0.181 | 0.731 |
| Male mount. Female | | | | | | | | 1 | 0.524 | -0.044 | 0.482 | 0.114 |
| Actual mate. | | | | | | | | | 1 | 0.743 | 0.683 | 0.740 |
| $E_2$ | | | | | | | | | | 1 | 0.561 | 0.601 |
| Rec. | | | | | | | | | | | 1 | 0.496 |
| Con. Rate | | | | | | | | | | | | 1 |

Wlk F = Walking females, Sit F = Sitting females, St M = Standing males, Wlk M = Walking Males, Sit M = Sitting males, $E_2$ = Estradiol-17β (pg.mL$^{-1}$), Rec. = Receptivity (%), Con. Rate = Conception rate (%).

(Lebas *et al.*, 1986; Pfaus *et al.* (2015). In contrast, a doe in low receptivity refuses to accept the buck. She runs or keeps her head up in the air, she flattening on the floor of the cage, circling, circling and flattening, running away quickly from the male, trying to mount the male and occasionally showed an aggressive behaviour (Forcada and Abecia, 1990; McCroskey, 2000).

In this context, serum concentration of $E_2$ increased significantly after application of natural inducing oestrus compared with before treatments. Also, receptivity percentage and conception rate improved after treatments by 72.84 and 64.48 %, respectively. Moreover, highly positive correlations were found between $E_2$ concentration and both sexual and basic behavioural activity, such as frequency of female circling around male, male circling around female, actual mating and time of female standing behaviour (r = 0.857, 0.747, 0.743 and 0.492 respectively). These results confirmed that, oestrogen hormone is very responsible for display doe sexual behaviour and acceptance of the male (Ilès *et al.*, 2013). Several studies have demonstrated that separation of the doe from its litter for short periods of time is very effective in stimulating ovarian activity of the doe. This, in turn, leads to raise the level of oestrogen in the blood (Manal, 2010; Ilès *et al.*, 2013).

Our results are similar with the results obtained by Virag *et al.* (1999), who found that using doe-litter separation method for one day or more on day 9 after suckling increased fertility by 20 %. Also, Rebollar *et al.* (2008) and Ilès *et al.* (2013) recorded that using doe-litter separation method for 48 h improved sexual receptivity and, consequently, fertility of the lactating does. However, McNitt (1992) and Rebollar *et al.* (1992) attributed the reduction of receptivity percentage and failure of mating during suckling period to the rise in prolactin and low secretion of oestrogen by the ovaries, which has a negative effect on receptivity percentage and sexual behaviour. Also, these results are nearly consistent with Rebollar *et al.* (2009) and Marongiu and Dimauro (2013), who reported that sexual behaviour was very poor in primiparous lactating rabbits.

Moreover, our results indicated that both bio-stimulation methods were positive and effective in all studied traits, but presence of a doe beside buck cage method was superior over the doe-litter separation method in most studied traits. Using the doe beside buck cage method had significantly higher frequency of male circling around female, female circling around male than those values obtained by doe-litter separation method. $E_2$ level and conception rate were significantly higher using this method than doe-litter separation method. This improvement may be due to higher serum concentrations of $E_2$ in female near buck cage method than in doe-litter separation method. McNitt (1992) and Berepubo *et al.* (1993) reported that presence of female beside buck is very important for the continuous contacts of visual, auditory, olfactory and tactile between them. This, in turn, activates the hypothalamus-pituitary-gonads axis to release GnRH and sexual hormones, which have a strong influence on rabbit sexual behaviour (Pau *et al.*, 1986; Bakker and Baum, 2000; Melo and Gonzalez-Mariscal (2010). On the other hand, prolactin hormone is increasing in suckling females compared to non-suckling females. McNitt (1992) reported that reduction of sexual behaviour during suckling period returned to the rise in prolactin, which has a negative effect on receptivity percentage. Moreover, highly positive correlations were found between $E_2$ concentration and sexual behavioural activities and physiological traits (Table 5). This may explain the lower response in most traits in suckling doe group (doe-litter separation treatment) compared to the non-suckling one (existence beside male cage treatment) in both breeds in our study.

Finally, our results clearly confirmed that significant differences, found in behavioural activities and physiological traits, are due to breeds of rabbits, irrespective of treatments. NZW does had significantly higher time of walking and lower time of standing behaviour than those recorded in BR does. Also, NZW breed had significantly increased frequency of actual mating, but insignificantly higher frequency of male circling around female, female circling around male and male mounting female, than those values recorded in the BR breed. Moreover, NZW does had insignificantly higher $E_2$ level and conception rate than BR does. Our results are in agreement with those obtained by Khalil *et al.* (2014), who reported that NZW breed had more active and higher reproductive efficiency than the BR breed, under the same managerial conditions. These differences between the two breeds may be due to genetic variation between exotic and local breeds.

## CONCLUSION

Bio-stimulation methods resulted in positive changes in the basic and sexual behaviour, as well as improvement in the reproductive parameters of non-receptive female rabbits for mating. Therefore, these methods are strongly suggested to induce oestrus in rabbits instead of hormonal treatments. These methods are easy and cheap to apply, very consistent with animal welfare and well adapted to cyclic rabbit production systems at commercial intensive bases, as recommended by many rabbit European association for rabbit production and welfare.

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