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IMPACT OF NATURAL ESTRUS INDUCTION METHODS ON RECEPTIVITY, CONCEPTION RATE AND SEXUAL HORMONAL LEVELS IN BALADI RED AND NEW ZEALAND WHITE RABBIT DOES

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SUMMARY

The present study was carried out at the rabbitry of the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Natural estrus induction methods were used in non receptive female rabbits for mating. Methods used were litter separation in the suckling females or presence of a female near buck cage in the non-suckling females of two differnt breeds, Baladi Red (BR) and New Zealand White (NZW). Treatments showed pronounced improvement in both breeds in terms of receptivity to males and conception rate. The NZW rabbits doe showed improvment in receptivity up to 68% in non-suckling and 73% in suckling females, while the percentages in Baladi rabbits were 72% and 79% in non- suckling and suckling ones, respectively. In addition, natrual methods tended to enhance conception rate in NZW rabbits to 74% in non-suckling and 56% in suckling females, while the corrseponding values of Baladi does were 71% and 55%, respectively. Significant decrease was observed in prolactin hormone level in response to litter separation from 42 to 6 ng/ml in NZW and from 15 to 2 ng/ml for Baladi does. However, progesterone was increased from 0.9 to 2.2 ng/ml in litter separation method and from 1.5 to 11.5 ng/ml in presence beside male cage in NZW rabbits. In Baladi does, values of progesterone hormone were increased from 0.8 to 6.7 ng/ml in litter separation and from 3.8 to 12.1 ng/ml in presence beside male cage. Estrogen levels of NZW does showed insignificant increase after litter separation and significant increase after presence beside male cage (48 to 63 pg/ml). In addition, Baladi rabbits had insignificant increase after litter separation. However, significant increase in estrogen values (from 47 to 56 pg/ml) after treatment were observed by presence beside male cage. Generally, the natural methods used to induce estrus in NZW and Baladi rabbits were successful and applicable. They can be widely used in rabbit farms instead of hormonal methods.

Keywords: Estrus induction, Litter separation, Presence beside male, rabbit Does

INTRODUCTION

Rabbits are considered to be induced ovulators, with no regular estrous cycle (Maertens *et al*., 1995). 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). El-Kelawy (1997) found that ovulation occurs 10-13 h after copulation, whereas, 20 to 25% fail to ovulate. Females rabbits are usually mated soon after parturition and sustain both lactation and next pregnancy at the same time. With the fall in the level of circulating progesterone, the first wave of follicle maturation begins in late pregnancy, normally on day 29-30 (Diaz *et al*., 1987). In nursing rabbits, sexual receptivity and fertility achieved after artificial insemination, is depressed during the period of lactation, presumably through a hormonal antagonism between prolactin and gonadotropin release, with endocrine changes that may explain the activation of ovarian function. The daily nursing visit of the doe to kids is extremely regular, with some individuals showing a day-to-day variability of only a few minutes. Estrogen, androgen, progesterone and prolactin promote the onset of this behavior in does (Gonzalez-Mariscal, 2001) while its maintenance relies on stimuli from the litter (i.e., maternal responsiveness is altered or abolished by prevention of mother/young contact at parturition or during early lactation). Therefore, a female rabbit is considered to be in heat when the doe accepts service.

Bio-stimulation methods to synchronize estrus in rabbits are used to induce receptivity rather than hormonal treatments. The natural methods used such as supplementary lighting affecting changes in gonadotropin releasing hormone (GnRH) release (McNitt, 1992), male presence, and mother-litter separation.

The “buck presence” is intended for the continuous contacts of visual, auditory, olfactory and tactile between the male and female (McNitt, 1992 and Berepubo *et al*., 1993). 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.

In rabbit breeding, the most common method to induce estrus is the administration of pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG). However, the widespread use of hormones has a reduced effect after several uses besides increasing cost (Marai and Rashwan, 2003).

The interaction between re-mating interval and the reproductive performance has a significant effect on

reproductive rate of rabbits without negative effects on litter and doe performance (Smith and Somade, 1994). Therefore, the objective of the present study was to test the effectiveness of using two natural methods to induce estrus in non-receptive doe rabbits in order to improve rabbit breeding systems depending on does status as follows:
Litter separation in suckling does, and presence beside male cages in non- suckling does.

MATERIALS AND METHODS

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

Animals and Husbandry:

One hundred multiparous female rabbits from Baladi Red (BR) and New Zealand White (NZW) breeds were used (50 from each breed). Animals were healthy and free of any external parasites or skin diseases. Age of animals ranged from 10 -12 months. Animals were individually housed in galvanized wired cages, 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 fiber and 2600 kcal digestible energy/kg diet. Lighting system was 16 hrs light/8 hrs dark in the rabbitry during the experimental period. Does were transferred to the rabbit bucks cages for natural mating process and kept under examination until natural mating was successfully completed.

Experimental design:

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 experiment.

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 estrus such as; swelling of the vulva, vulva color, exposition of the rear quarters and lordosis. Doe rabbits were transferred to male cages to check receptivity for five min and if refused, the treatment was repeated for another time on next day.

The litter separation method was carried out on the next day after delivery. Conception rate was diagnosed in rabbit does after 12-15 days of successful mating. Abdomen palpation was used on assumed pregnant does by the same individual. Data on reception and conception rates were calculated as percentage from non-responsive ones.

Blood sampling:

Blood samples were obtained from the ear vein of females that rejected mating and after applying the bio-stimulation method from females that accepted mating. Blood was left for coagulation at refrigerator temperature (4^0C) before centrifugation at 4000 rpm for 20 min to separate serum. Serum samples were frozen at -20^{o}C until hormonal analyses.

Hormones determination:

Prolactin was determined through immunoradiometric assay by using commercial kits (RIAKEY Prolactin IRMA Tube II kits) obtained from Shin Jin Medics Inc., Republic of KOREA. The lower limit of detection was 0.022 ng/mL, and the intra- and interassay CV were 1.34% and 1.73%, respectively.

Progesterone was determined through radioimmunoassay method by using commercial kit obtained from DIA source PROC-RIA-CT kit, Belgium. The lower limit of detection was 1.27 ng/mL, and the intra- and interassay CV were 5.2% and 8.6%, respectively.

Estrogen was determined by using VIDAS Estradiol II kits obtained from bioMerieux SA, France. The lower limit of detection was 9 pg/mL, and the intra- and interassay CV were 7.5% and 9.5%, respectively.

Statistical Analyses:

Data were analyzed by using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 2004). Differences (LSD) between means were tested according to Duncan's multiple range test (Duncan, 1955).

For receptivity and conception rates:

Factorial design was applied on the experiment. Two-way analysis of variance (ANOVA-test) with two ways 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 kth individual from the ith breed in jth doe status.

μ = the overall mean.

B_i = the fixed effect of the ith breed (i= Baladi and NZW).

D_j = the fixed effect of the jth doe status (presence beside male and litter separation).

$B*Dl_{ij}$ = the interaction among ith Breed and jth Doe status.

E_{ijk} = the random error associated with the individual i^{jk}.

For hormonal profile:

Factorial design was applied on experiment. Three-way analysis of variance (ANOVA-test) with three ways 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.

μ = 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.

RESULTS AND DISCUSSION

Receptivity and conception rates:

Receptivity was determined by the willingness of the doe to mate. Roca (1986) reported that high receptivity rabbit does were significantly associated with red coloration of the vulva. Our personal observation in the present study agrees that the red coloration of doe vulva is associated with accepting mating. Results presented in Table (1) show receptivity and conception rates in NZW and BR does after natural application of estrus induction methods to females that rejected mating. At the beginning of the experiment, natural mating was applied to all does. Results were 54.8 and 60.8% of females accepted mating in NZW and BR rabbits, respectively. Bio-stimulation methods were applied to induce estrus in female that rejected mating and resulted in 70.0 and 75.2% accepted mating in NZW and BR, respectively. The interaction effects of treatments and rabbit breeds showed insignificant differences between treatments within breeds (Table 1). This indicates the effectiveness of both treatments in inducing natural estrus in rabbit does. Theau-Clément (2007) reported that, the percentage of does that accept a male is very high on the day of parturition, decreases at day four postpartum, increased at day 11 postpartum, and returned to the highest level after weaning. Challis *et al*. (1974) and Nicosia *et al*. (1975) reported that the highest levels of sexual activity are noticed on days 1, 2 and 5 post-partum in primparous as on days 1 and 9 post-partum in multiparous does. Other authors indicate that doe sexual heat is greater on the first day than on the tenth day post-partum (Perrier *et al*., 1982; Surdeau *et al*., 1982). Cudnovskii (1957) and Berepubo *et al*. (1993) claimed that the natural receptivity cycle of a doe is 5 to 7 days, with duration as short as 6 to 7 hours during some times of the year. Our study agrees with the previous results because we used doe on the next day after delivery.

Table 1. Receptivity and conception rates (%) in New Zealand White (NZW) and Baladi Red (BR) rabbits after inducing estrus (mean± S.E)

		Receptivity	Conception rate
Control Groups (before treat.)			
Breed	NZW	54.80 ± 3.54	69.36 ± 5.81
	BR	60.83 ± 5.15	62.68 ± 4.94
	P. Value	0.354	0.425
Treated Groups (after treat)			
Breed	NZW	70.04 ±3.13	65.72 ±5.01
	BR	75.20 ±2.86	63.60 ±4.13
	P. Value	0.227	0.743
Treatment			
	Litter Separation (LS)	76.32 ± 3.13	55.21 ±4.98 b
	Beside Male (BM)	70.06 ±2.85	72.29 ±3.95 a
	P. Value	0.142	0.007
Interactions			
	NZW*LS	72.79 ±4.93	55.45 ±8.04
	NZW*BM	67.95 ±4.07	73.55 ±6.14
	BR*LS	78.95 ±4.06	55.03 ±6.36
	BR*BM	71.89 ±4.00	71.20 ±5.17
	P. Value	0.314	0.065

[a,b]Denote differences between means in the same category column at less than the corresponding P value.

No significant differences were found between breeds in conception rate (Table 1). In control groups, conception rate values were 69.4 and 62.7% in NZW and BR breeds, respectively. Application of estrus induction methods to non-receptive females stimulated receptivity and conception in both breeds. In treated groups, conception rates were 65.7 and 63.6% for NZW and BR, respectively. However, the effect of treatment (litter separation vs. presence beside male) on conception rate showed significant (P<0.01) difference (Table 1). Presence beside male treatment (72.3%) was higher (P<0.01) than that in litter separation (55.2%) treatment in conception rate. Regarding the interaction effects of doe breed and treatment, the effect of presence beside male treatment tended to be higher than that in litter separation in both breeds. Results in Table (1) show slight increase but insignificant in receptivity in litter separation treatment than that in presence beside male and on the other hand, higher insignificant conception rate in presence beside male treatment than that in litter separation.

Mating was not followed by ovulation in some does, both suckling and non-suckling, in the current study. However, Pau *et al*. (1986) reported that in the primiparous does this lack of ovulation occurred only in the suckling group, which may indicate different effects exerted by suckling upon the hypothalamic centers regulating sexual receptivity and the release of luteinizing hormone. This may explain in our study the lower response in conception rate in suckling doe group (litter separation treatment) compared with the non-suckling one (presence beside male cage treatment) in both breeds.

In addition, Marongiu *et al*. (2013) reported that in primiparous lactating rabbits performed very poor mating result on day 21 postpartum, the day of maximum milk production and lowest live body weight for primiparous rabbits as reported by Rebollar *et al*. (2009). In other words, the energy deficit during this time could have an effect on rabbits sexual receptivity and consequently on their conception rate.

Sexual hormones profile

Table (2) shows levels of prolactin, progesterone and estradiol-17β in NZW and BR breeds before and after application of treatments (third level interaction). No significant differences were found in control animals between both breeds in sexual hormone levels (Table 2). In general, NZW breeds showed slightly higher values in hormone levels (except progesterone) than that in BR breed. Overall effects of natural inducing estrus methods showed significant ($P<0.05$) differences in progesterone and estrogen levels. Litter separation method caused significant decrease in progesterone and estrogen hormones and insignificant decrease in prolactin hormone than presence beside male treatment (Table 2). The overall effect of time (before and after treatment) on hormone levels was significant ($P<0.001$) in progesterone and estrogen but insignificant in prolactin hormone.

Table 2. Sexual hormonal changes in New Zealand White (NZW) and Baladi Red (BR) rabbits after inducing estrus (mean± S.E)

		Prolactin ng/mL	Progesterone ng/mL	Estradiol-17β pg/mL
Breed	NZW	20.96±4.72	4.03 ± 1.33	51.08 ± 1.93
	BR	17.26 ± 4.70	5.87 ± 1.52	49.89 ± 1.18
	P. Value	0.583	0.374	0.601
Treatment	Litter Separation (LS)	16.51 ± 4.69	2.64 ± 0.80 b	47.54 ± 0.77 b
	Beside Male (BM)	21.71 ± 4.68	7.27 ± 1.69 a	53.43 ± 1.85 a
	P. Value	0.440	0.020	0.006
Time	Before	18.63 ± 4.46	1.76 ± 0.45 b	46.66 ± 0.63 b
	After	19.59 ± 4.99	8.14 ± 1.64 a	54.31 ± 1.68 a
	P. Value	0.886	0.001	0.000
Interactions				
	NZW*LS*Before	42.41 ± 9.72 a	0.86 ± 0.26 b	45.60 ± 1.25 c
	NZW*LS*After	5.58 ± 2.09 c	2.17 ± 0.47 b	48.50 ± 1.71 c
	NZW*EBM*Before	8.82 ± 3.31 c	1.58 ± 0.63 b	47.50 ± 1.19 c
	NZW*EBM*After	27.03 ± 6.51 ab	11.54 ± 3.13 a	62.75 ± 2.60 a
	BR*LS*Before	15.83 ± 2.97 bc	0.82 ± 0.15 b	46.30 ± 1.20 c
	BR*LS*After	2.23 ± 0.96 c	6.71 ± 2.21 ab	49.76 ± 1.50 c
	BR*EBM*Before	7.46 ± 4.39 c	3.81 ± 1.14 b	47.25 ± 1.70 c
	BR*EBM*After	43.52 ± 8.71 a	12.14 ± 4.15 a	56.25 ± 1.10 b
P. Value		0.000	0.001	0.000

[a,b,c]Denote differences between means in the same category column at less than the corresponding P value

The third level interaction effects between breed, treatment and time of blood sampling showed variable results. In the present study, reduced ($P<0.001$) values in prolactin hormone were found in suckling does of both breeds after treatment (litter separation) than that before treatment. However, the opposite trend was true for progesterone and estrogen hormones, where significant ($P<0.001$) increases were found after treatment in both breeds (Table 2). Presence beside male treatment showed significant ($P<0.001$) increase in prolactin, progesterone and estrogen hormones after treatment in both breeds (Table 2). This indicates different mode of action of both treatments on inducing estrus in BR and NZW breeds. In ovariectomized rabbits, as in many other species, estradiol induces sexual behavior while progesterone inhibits this behavior (Beyer and McDonald, 1973). Nevertheless, hares and rabbits may display estrous behavior even when circulating progesterone concentrations are high. This may explain the receptivity of does after applying estrus induction methods in spite of higher levels of progesterone in the present study (Table 2). Stoufflet and Caillol (1988) found a relationship between circulating progesterone concentrations and sexual behavior. They reported that in late pregnancy the number of receptive females was higher when progesterone values were decreased. However, they noticed that rabbit does remained sexually receptive even when progesterone concentrations were high but non-receptive females exhibited consistently higher values than did receptive ones.

Kermabon *et al*. (1994) stated that the high concentration of prolactin during lactation is noticed to coincide with a reduction in gonadotrophin secretion. In the present study, prolactin level was decreased due to doe-litter separation treatment and estrus (receptivity) of does was observed. On the other hand, presence beside male treatment did not reduce prolactin level, and no difference in reception rate was obvious, in spite of high ($P<0.001$) result in conception rate in both breeds.

Among the possible stimulation methods, the so called “buck effect” has not been widely investigated. The buck effect, understood as the visual, auditory, olfactory and tactile contact between female and male, following a period of separation from the male, is known to be effective in ewes and sows in stimulating and synchronizing estrus (Marai and Rashwan, 2003). The existence of stimulus of an olfactory nature has also been established in rabbits. In fact, the pheromones secreted by the sebaceous glands in males have been shown to induce sexual maturity in young female rabbits (Marai and Rashwan, 2003).

CONCLUSION

The bio-stimulation methods are strongly needed to induce estrus in rabbits instead of hormonal treatments. These methods are easy and cheap to apply, very consistent with animal welfare, and well adapted to cycled rabbit production systems at commercial intensive bases.

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تأثير طرق طبيعية لإستحداث الشياع على القابلية للتزاوج ونسبة الحمل ومستويات الهرمونات الجنسية فى الأرانب البلدى الأحمر والنيوزيلاندى الأبيض

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أجريت هذه الدراسة فى عنبر الأرانب التابع للمزرعة التجريبية لكلية الزراعة ، جامعة قناة السويس ، الإسماعيلية ، مصر. أستخدمت طرق طبيعية لإستحداث الشياع فى إناث الأرانب الرافضة للتلقيح. كانت الطرق المستخدمة هى فصل الخلفة عن الأمهات المرضعة أو تواجد الإناث غير المرضعة بجوار قفص الذكر فى السلالتين المستخدمة (البلدى الأحمر و النيوزيلندى الأبيض). أظهرت المعاملات تحسن واضح فى كل من السلالتين بالنسبة لقبول الأنثى للتلقيح و فى نسبة حدوث الحمل. أظهرت سلالة النيوزيلندى الأبيض تحسنا فى قبول الذكر حتى ٦٨% فى الإناث غير المرضعة و ٧٣% فى الإناث المرضعة ، بينما كانت النسب فى البلدى الأحمر ٧٢ فى غير المرضعة ، ٧٩% فى المرضعة على التوالى.
كما حسنت (P<0.06) هذه الطرق الطبيعية من نسبة الحمل فى الأرانب النيوزيلاندى الأبيض إلى ٧٤% فى الإناث غير المرضعة ، ٥٦% فى المرضعة، بينما كانت الأرقام المقابلة لها فى البلدى الأحمر هى ٧١% ، ٥٥% على التوالى.. وجد أنخفاض معنوى (P<0.0001) في مستوى البرولاكتين نتيجة فصل الخلفة من ٤٢ إلىng/ml ٥ في النيوزيلاندى الأبيض ، ومن ١٦ إلىng/ml ٢.٢ في البلدى الأحمر. بينما زاد معنويا (P<0.001) مستوى هرمون البروجستيرون من ٠.٨ إلىng/ml ٢ نتيجة فصل الخلفة ، ومن ١.٦ إلى ng/ml ١١.٥ في طريقة التواجد بجوار الذكر في الأرانب النيوزيلاندى الأبيض. بالنسبة لأرانب البلدى الأحمر قيم البروجستيرون زادت من ٠.٨ إلى ng/ml ٣.٧ فى طريقة فصل الخلفة ، ومن ٣.٨ إلىng/ml ١٢ فى طريقة التواجد بجوار الذكر. أظهرت قيم هرمون الإستروجين زيادة غير معنوية فى الأرانب النيوزيلاندى الأبيض بعد فصل الخلفة و معنوية بعد طريقة التواجد بجوار الذكر (٤٧ إلىpg/ml ٦٢). بينما الزيادة فى الإستروجين لم تكن معنوية فى الأرانب البلدى الأحمر بعد فصل الخلفة لكن كانت معنوية (P<0.01) بعد المعاملة بالتواجد بجوار الذكر (٤٧ إلىpg/ml ٥٦). بصورة عامة كانت الطرق الطبييعية لإحداث الشياع ناجحة فى الأرانب النيوزيلاندى الأبيض و البلدى الأحمر و يمكن تطبيقها فى مزارع الأرانب على نطاق عريض بدلا من أستخدام الطرق الهرمونية.