The Relationship Between Vulva Status and Some Physiological, Behavioural, Reproductive and Productive Traits in White New Zealand Rabbit Does

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Abstract: One hundred and fourteen White New Zealand (WNZ) rabbit does were used to study the relationship between rabbit doe's vulva coloration and status at the time of natural mating and some physiological, behavioral, reproductive and productive traits of does in order to determine the proper time of natural mating to achieve a maximum productive and reproductive performance. Animals were divided into four groups according to their vulva coloration and status: pale (P), pale red (PR), rose (R) and turgid dark red (TDR). Estradiol-17β (E2), calcium and inorganic phosphate and iron ion were determined in does blood serum. Also, some behavioral activities of does were recorded before natural mating. In addition, sexual receptivity, conception rate, litter size and weight at birth were determined. Results showed significant differences ($P<0.05$) among the four groups in most traits. Most studied traits tended to increase and improve with increasing vulva coloration. Physiological body reactions (respiration rate and pulse rate) were significantly ($P<0.05$) higher in R and TDR groups than in P and PR groups. The highest levels of E2, calcium ion and inorganic phosphate ion were observed in blood serum of TDR group compared with the other groups. Significant differences ($P<0.05$) were found in some behavioral traits, in which TDR group had the lowest percentage of standing, head moving and scrubbing, and the highest percentage of sitting than the other groups of rabbit does. Results showed that reproductive and productive traits were affected by vulva coloration. The TDR group had superior significantly ($P<0.05$) in receptivity %, conception rate and litter size at birth compared with the other groups. Positive correlations were found among level of E2, vulva-status and sexual receptivity and most productive and reproductive traits. According to the present study, it could be concluded that, the optimal or proper time for natural mating in order to achieve maximum productivety of NZW rabbit does when vulva color was rose or turgid dark red.

Keywords: Rabbit doe, natural mating, vulva coloration, estradiol-17β, behavior, receptivity, conception rate.

INTRODUCTION

Rabbits are classified as induced ovulators (Sawyer and Markee, 1950). The doe rabbit vulva is closely related to doe mating rejection during the mounting and is correlated with the negative influence of low sexual receptivity (Gosalvez, 1986). Diaz *et al.* (1987) studied the relationship between the color of the vulva on the day of mating, ovulation rate and level of fertility. In this study the ovaries and uterine horns of does mated were examined by laparoscopy 10 days after mating. Most of the does that were mated had red (54.1%) or rosy (39.6%) vulva. The ovulation rate was higher in does with red vulva (84.6%) than in those with rosy coloration (68.4%). Each female was assigned to a high or low sexual receptivity level according to Gosalvez (1986) using the color of the vulva as a predictor of sexual behavior. Does with pale or violet non turgid vulva were considered as low sexual receptivity and does with red, rosy and violet turgid vulva were high sexual receptivity. Stoufflet and Caillol (1988) observed that the vulva color was white in early pregnancy and after parturition. The present work aimed (i) to study the relationship between the vulva coloration at time of natural mating and some physiological, behavioral, reproductive and productive traits and (ii) to determine the proper time of natural mating to achieve a maximum productive and reproductive performance in White New Zealand rabbit does.

MATERIALS AND METHODS

Rabbit Does Management:

The present study was carried out at the rabbitry of the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. One hundred and fourteen (114) nulperious mature female New Zealand White (NZW) rabbit does were used in the present experiment. Animals were distributed into four groups based on the color of the vulva at the time of natural mating. The color varied from turgid dark red (n=33), rosy (n=40), red pale (n=25) and pale (n=16). All does were healthy and clinically free of diseases. Rabbit does were individually housed in galvanized wired cages, where feed and water were offered *ad libitum*. Does 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 which agree with NRC (1984). Lighting system was 16 hr light/8 hr dark in the rabbitry. Does were transferred to the rabbit buck cages for natural mating progression and kept under examination until natural mating was completed successfully.

Physiological Parameters:

Physiological Body Reactions:

The following parameters rectal temperature (RT), respiratory rate (RR) and pulse rate (PR) were recorded before natural mating for each doe. Rectal temperature was measured for each doe using clinical thermometer. Respiratory rate (RR/min) was measured by counting the movements of the chest fleece. Pulse rate (PR/min) was measured by counting pulses in the femoral artery with a hand finger.

Blood Sampling and Analysis:

2 ml of blood samples were collected before mating from the marginal ear vein. Blood samples were centrifuged at 3000 rpm for 20 min. Serum then

separated and stored at -20°C until it was analyzed. Estradiol-17β (E2) was assayed using RIA technique utilizing coated tubes kits purchased from Diagnostic Production Corporation, Los Angeles, USA. In addition, serum constituents such as calcium, inorganic phosphorus and iron ion were measured calorimetrically using commercial kits from ELITech Company, France.

Behavioral Parameters

The behavioral traits for 10 females from each group were studied during the experimental period using a video camera. The behavioral traits were recorded for two hours before natural mating. From the video tapes, at 5 min interval (time sampling) the basic activities (percentage of does standing, sitting or walking) and the additional activities (percentage of does feeding, drinking and head moving or scrubbing) were recorded according to Gerken, 1991; Kishk *et al.* (2008). These data were used to calculate the percentages of does observed for each trait.

Reproductive and Productive Parameters

The following reproductive and productive traits were recorded for each doe: doe receptivity percent, conception rate, litter size, litter weight and bunny weight at birth.

Statistical Analysis:

Data were analyzed by using general linear model (GLM) procedures of the statistical analysis system (SAS, 1998). Differences among means were detected using Duncan's new multiple test (Duncan, 1955). Correlation coefficients among traits were estimated on the whole data using Proc CORR (SAS, 1998). The linear mathematical model used for the statistical analysis of the data was as follows:

$$Y_{ij} = \mu_i + v_i + e_{ij}$$

Where:

Y_{ij} = The observation on the j^{th} individual from the i^{th} vulva color.

μ_i = The overall mean.

v_i = The fixed effect of the i^{th} vulva color.

e_{ij} = The random error associated with the individual ij.

RESULTS

Physiological Parameters

Physiological body reaction was significantly ($P<0.05$) affected by doe's vulva status as shown in Table 1. Respiration rate (RR) and pulse rate (PR) were significantly ($P<0.05$) higher in both R and TDR groups than in P and PR groups. However, there were no significant effects of vulva status on rectal temperatures as shown in Table 1. In relation to blood serum constituents, significant differences were recorded among the four groups. TDR group had the highest levels of E2 (15.79), calcium (8.56) and inorganic phosphor (4.85), but the lowest values were detected in P vulva group (9.75, 5.05 and 2.97, respectively).

Behavioral Activities

Effects of vulva status on behavioral activities of does are presented in Table 2. Significant differences ($P\leq0.05$) were found among groups in basic and additional behavioral activities. The group TDR had significantly ($P\leq0.05$) higher percentage of does sitting and lower percentage of does standing, head moving and scrubbing than the other groups. On the other hand, there were no significant differences among groups on walking, feeding and drinking behavior.

Reproductive and productive parameters

Data presented in Table 3 showed significant differences ($P\leq0.05$) among the four groups in reproductive and productive traits. Females with TDR had the highest receptivity % (75.05), conception rate (91.27) and litter size at birth (6.87), but the lowest values were obtained in P vulva group (17.72, 10.24 and 5.42, respectively). On the other hand, litter weight at birth and bunny weight at birth were significantly lower ($P\leq0.05$) in R group compared with other female groups.

Correlation coefficients

Data presented in Table 4 showed the correlation coefficients among traits of the present study. The results showed significant ($P<0.001$) positive correlation between vulva color and all the traits such as, blood serum concentration of E2, calcium, behavior traits, receptivety, conception rate and litter size. There were also significant ($P<0.001$) positive correlations between E2 concentration with the same traits as shown in Table 4.

Table (1): Physiological parameters of WNZ does correlated to different vaginal vulva status (mean±SE)

Traits	Vulva Status			
	Pale	Pale red	Rose	Turgid dark red
Physiological body reactions				
Rectal temperature (°C)	39.46±0.56	39.33±0.65	39.56±0.78	39.68±0.75
Respiration rate (r/m)	134.33±1.44 ^{b}	131.66±1.67 ^{b}	148.23±1.54 ^{a}	150.24±1.35 ^{a}
Pulse rate (p/m)	120.54±2.88 ^{b}	127.29±2.59 ^{b}	137.52±3.05 ^{a}	142.32±3.21 ^{a}
Blood measurements				
Estradiol-17β (pg/µl)	9.75±0.35 ^{d}	11.58±0.54 ^{c}	13.44±0.81 ^{b}	15.79±0.72 ^{a}
Calcium (mg/dl)	5.05±0.28 ^{d}	6.12±0.25 ^{c}	7.35±0.37 ^{b}	8.56±0.30 ^{a}
Inorganic phosphor (mg/dl)	2.94±0.21 ^{c}	3.48±0.19 ^{b}	3.88±0.37 ^{b}	4.85±0.28 ^{a}
Iron (ug/dl)	130.12±11.21	141.64±11.34	149.41±12.12	142.67±10.67

^{a,b} Means in the same row with different superscript differed significantly ($P \leq 0.05$).

Table (2): Behavioral activities (%) of WNZ does with respect to different vulva status (mean±SE)

Traits	Vulva Status			
	Pale	Pale red	Rose	Turgid dark red
Basic behavioral (%)				
Standing (%)	71.15±2.88 [a]	70.83±2.45 [a]	63.76±2.64 [a]	40.32±2.19 [b]
Walking (%)	15.25±1.45	14.62±1.38	15.37±1.29	11.16±1.46
Sitting (%)	13.29±2.09 [c]	14.55±2.17 [c]	20.96±2.64 [b]	48.52±2.48 [a]
Additional behavioral (%)				
Head moving (%)	27.31±1.75 [a]	27.29±1.51 [a]	22.72±1.29 [a]	13.63±1.45 [b]
Scrubbing (%)	27.26±1.16 [a]	22.72±1.09 [b]	15.21±1.26 [c]	4.52±1.34 [d]
Feeding (%)	12.72±1.15	13.64±1.11	15.26±1.25	13.64±1.18
Drinking (%)	4.54±0.45	4.54±0.57	4.53±0.61	4.51±0.62

[a,b] Means in the same row with different superscript differed significantly ($P \leq 0.05$).

Table (3): Reproductive and productive traits of WNZ does with respect to different vulva status (mean±SE)

Traits	Vulva Status			
	Pale	Pale red	Rose	Turgid dark red
Receptivity %	17.72±2.54 [d]	31.25±2.68 [c]	63.28±3.47 [b]	75.05±3.47 [a]
Conception rate (%)	10.24±2.05 [d]	33.18±2.16 [c]	84.36±3.15 [b]	91.27±3.45 [a]
Litter size at birth (no)	5.42±0.28 [c]	5.91±0.35 [b]	6.23±0.32 [b]	6.87±0.31 [a]
Litter weight at birth (g)	392.26±14.12 [a]	412.64±13.48 [a]	323.23±13.67 [b]	381.27±14.17 [a]
Bunny weight at birth (g)	72.66±0.77 [a]	69.33±0.75 [b]	52.12±0.78 [d]	55.33±0.74 [c]

[a,b] Means in the same row with different superscript differed significantly ($P \leq 0.05$).

Table (4): Correlation coefficients among some physiological, behavioral, reproductive and productive traits

	VC	E2	Ca	SI	RR	CR	LS
VC	1.000	0.964***	0.894***	0.910***	0.945***	0.816***	0.816***
E2		1.000	0.847***	0.757**	0.947***	0.809***	0.909***
Ca			1.000	0.849***	0.909***	0.764**	0.886***
SI				1.000	0.971***	0.787**	0.654**
RR					1.000	0.785**	0.746***
CR						1.000	0.812***
LS							1.000

VC= vulva coloration, E2= Estradiol-17β, Ca= Calcium, SI= sitting behavior, RR= receptivity %, CR= conception rate, LS= Litter size, ns = not significant, * significant at ($P \leq 0.05$),
** significant at ($P \leq 0.01$) and *** significant at ($P \leq 0.001$).

DISCUSSION

Female rabbit does are considered as induced ovulator animals (Sawyer and Markee, 1950), they exhibit a distinct heat period and will mate and conceive at almost any time when exposed to a fertile male (Arrington and Kelly, 1967). Depending on species differences, the main problem in breeding rabbits is to determine the proper time of natural mating to achieve the maximum productive and reproductive performance. Although females do not exhibit a definite heat period, they often do exhibit a period of activity which indicates a time of female receptivity as pointed out by many authors (Marai and Rashwan, 2003; Kishk *et al.*, 2006 & 2008).

The results of this experiment showed clearly that, there are three ways to determine the optimal time of natural mating: 1) examination of reproductive status of the doe especially external genitalia, 2) testify of physiological status, 3) observation of behavioral status of rabbit does.

The 1st method is the examination of reproductive status of the doe, especially external genitalia (vaginal vulva coloration). Our results showed significant (P<0.001) positive correlations among vulva coloration and doe receptivity, productive and reproductive traits (Table 4). The highest sexual receptivity, conception rate, litter size at birth were obtained in females with R and TDR vulva color. Our results confirmed the same situation in which the genital characteristics i.e. color and turgidity are strongly related to sexual behavior and subjected to hormonal control. Moreover, Diaz *et al.* (1987) found that higher ovulation and pregnancy rate were recorded in the group of does with R vulva. On the other hand, white color of vaginal-vulva is associated with a high percent of does rejecting the male (Marai and Rashwan, 2003).

In addition, these results are in aggrement with those obtained by Kishk *et al.* (2006) who found significant (P<0.001) positive correlation among dark red valva color and receptivity percent, productive and reproductive performance in NZW rabbit does.

A 2nd method to detect proper time of natural mating is the observation of the physiological status of the doe, especially blood serum E2 and calcium

concentration. Our results showed significant ($P<0.001$) positive correlations between E2, calcium and doe receptivity, productive and reproductive performance (Table 4). The highest sexual receptivity, conception rate, litter size at birth were obtained in females with higher concentration of blood serum E2 and calcium concentration. These results are in agreement with Beyer *et al.* (1970) and Elsaesser (1980) who reported that estrogen is necessary for receptivity in the doe rabbits. Also, Kishk *et al.* (2006) found significant ($P<0.001$) positive correlations between serum concentration of E2 and doe receptivity, productive and reproductive performance in NZW rabbit does.

The highest blood serum concentration of E2 may be due to mature follicles which exist on the ovarian surface and could be expected to increase production of estrogens as described by Daniel *et al.* (1988). Also, McNitt (1992); Ferguson *et al.* (1997) and Hoy (2000) reported that there was a diurnal peak of does receptivity, this ability of doe is controlled mainly with E2 level in blood. On the other hand, Parvizi (2000); Boiti (2004) showed that in spontaneous ovulator species, many of the promoter neurons are estrogen-sensitive and are therefore critical for mediating the effects of blood estrogen and progesterone on the gonadotropin releasing hormone (GnRH) neural network within the cyclic LH surge-generator subsystem. Gonadotropin stimulation affects steroidogenesis, receptor development and mitosis in target cells, as well as meiotic maturation of oocytes (Amsterdam *et al.*, 1981; Knecht and Catt, 1982; Yoshimura *et al.*, 1994).

In addition, results of this experiment showed significant ($P<0.001$) positive correlation between E2 concentration and calcium level in blood serum. Brunette and Leclerc (2001) found that estrogen by itself regulates electrolyte reabsorption by the distal tubule luminal membrane. Nordin *et al.* (1991&1994); Adami *et al.* (1992); McKane *et al.* (1995); Heshemati *et al.* (1998) suggested that estrogen enhances calcium ion reabsorption by stimulation of parathyroid hormone (PTH) secretion and activation of vitamin D which plays an important role in increasing calicum intestinal absorption, more efficient renal calcium reabsorption and inhibition of bone resorption by PTH (Russel *et al.*, 1986; Silver *et al.*, 1986; Cheema *et al.*, 1989; McKane *et al.*, 1995; Gill and Christakos, 1995 and Karen and Rosenthal, 2006). These results may be explained on the bases of high E2 level and its direct effect on body metabolism. Also, high activity during this time could be responsible for higher body metabolism which is mainly subjected to body hormonal control especially thyroxin and adrenal cortical hormones, which prepare the females to high reproductive performance. These results are confirmed by results obtained by Shan *et al.* (1994); Grohé *et al.* (1996); Barry *et al.* (1997) who reported that, estrogen hormone can control the expression of many cellular regulators such as vascular smooth muscle and cardiac myocytes in vitro by regulation of cellular calcium. Also, Ahmad *et al.* (1990) found that estrogen hormone increases the blood serum level of calcium in rabbit and added that oral administration of calcium gluconate in a dose of 100 mg/kg body weight to adult non-pregnant female rabbits produced a significant increase of E2 and FSH hormones.

It is worthy to note that calcium plays an important role in stimulus-secretion coupling with most exocrine and endocrine glands (Douglas, 1968). Calcium movement into the cell and release from intracellular sites is vital for the coupling of receptor-stimulated cell surface events to cellular responses (Zaloga and Chernow, 1989). Also, Tsang *et al.* (1988) stated that calcium plays an important role in conversion of estradiol-17β -3-sulfate to estradiol-17α -3-sulfate. Moreover, Ahmad *et al.* (1990) reported that administration of oral calcium to female rabbits led to a higher percentage of conception compared with controlled female rabbits. This may be explained by the fact that calcium maintained a higher serum level of FSH leading to increased rate of ovulation rate.

In this respect, significant differences were found in physiological body reactions such as respiration rate and pulse rate. The higher rates of respiration and pulse rate could be attributed to the higher level of estrogen. Some of the body hormones can affect directly heart rate, respiration rate, blood pressure and blood stroke volume. These hormonal effects are mainly depending on hormone type, hormone receptor and hormone dose (Perrier *et al.*, 2000). Estrogen controls the expression of many cellular regulators such as vascular smooth muscle and cardiac myocytes in vitro by regulation of calcium cellular (Shan *et al.*, 1994; Grohé *et al.*, 1996; Barry *et al.*, 1997).

The last method to detect proper time of natural mating is behavioral status of rabbit does. Our results showed significant ($P<0.001$) positive correlation between relaxation behavior (sitting %) and most studied traits (Table 4). The highest sexual receptivity, conception rate and litter size at birth were obtained in females which spent more time sitting down %. Our results also suggest that the optimal time of natural mating characterized by higher relaxation behavior (decreasing in walking, standing, head moving and increasing sitting) which makes the female accept the male. These results indicate that estrogen hormone could affect behavioral activities of rabbit does. It causes more relaxation behavior of doe before natural mating. Kishk *et al.* (2008) reported that injection of female rabbits with GnRH caused significant ($P<0.001$) increase in relaxation behavior and increased productive and reproductive performance of NZW rabbit does.

In conclusion, the results of this experiment indicated that, there are positive correlations among most studied traits such as (vaginal vulva coloration, level of E2, receptivity percent & conception rate and litter size at birth). These positive correlations between vulva coloration and increased E2 concentration in blood serum was followed-up by an increasing in heart rate and blood flow into the external genitalia (vulva) which causes coloration changes of vulva. Moreover, increasing of vulva coloration reflects the high receptivity and conception rate in the female rabbits. This is an important indicator for the optimal or proper time of mating in the female rabbits.

Therefore, it could be concluded that, E2 levels,

vulva coloration, sexual behavior, blood metabolites could be used as a tool to determine proper time of natural mating. From the practical point of view, the optimal or proper time for natural mating in order to achieve maximum productivety of NZW rabbit does when vulva color was rose or turgid dark red.

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دراسة العلاقة بين لون وحالة الشفرين وبعض الصفات الفسيولوجية، السلوكية، التناسلية و الإنتاجية فى إناث أرانب النيوزيلاندى الأبيض

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أجريت هذه التجربة على مائة وأربعة عشر أنثى من أرانب النيوزيلاندى الأبيض بهدف دراسة العلاقة بين لون وحالة الشفرين عند التلقيح الطبيعى وبعض الصفات الفسيولوجية، السلوكية، التناسلية والإنتاجية. وذلك لتحديد أنسب ميعاد للتلقيح الطبيعى للحصول على أعلى كفاءة تناسلية وإنتاجية لإناث الأرانب. تم تقسيم الحيوانات الى أربعة مجاميع وفقاً الى لون وحالة الشفرين: باهت، أحمرباهت، أحمروردى وأحمر داكن متضخم. وتم تقدير تركيز مستوى كلٌ من هرمون الإستروجين، الكالسيوم، الفسفور الغير عضوى والحديد فى مصل الدم لكل الإناث. كما تم تسجيل بعض الصفات السلوكية للإناث قبل التلقيح الطبيعى. بالإضافة الى حساب معدل قبول الإناث للتلقيح، معدل الحمل، ووزن وحجم البطن عند الميلاد. وكانت أهم النتائج المتحصل عليها ما يلى:

أظهرت النتائج وجود فروق معنوية ($P<0.05$) بين المجاميع الأربعة فى معظم الصفات المدروسة. حيث تحسنت معظم الصفات معنوياً بزيادة درجة لون الشفرين. حيث زادت تفاعلات الجسم الفسيولوجية معنوية ($P<0.05$) متمثلة فى معدلى التنفس والنبض لمجموعتى الإناث ذات لون الشفرين الوردى والأحمر الداكن المتضخم مقارنتة بالمجموعتين الأخيرتين. كما لوحظ أن مجموعة الإناث ذات لون الشفرين الأحمر الداكن المتضخم كانت الأعلى معنوياً فى كلٌ من مستوى تركيز هرمون الاستروجين، الكالسيوم والفسفور الغير عضوى عن باقى المجاميع التجريبية ($P<0.05$). كما شوهدت فروق معنوية بين المجاميع الأربعة فى صفات النشاط السلوكى، حيث تميزت إناث المجموعة الرابعة ذات لون الشفرين الأحمر الداكن المتضخم الأقل معنوياً ($P<0.05$) فى كلٌ من نسب البقاء قائمة، حركة الرأس وحك الجسم بالفم كما كانت الأعلى معنوياً ($P<0.05$) فى نسبة البقاء راقدة مقارنة بباقى المجاميع التجريبية. كما أوضحت النتائج فروق معنوية لكل من الصفات التناسلية والانتاجية، حيث سجلت ايضاً المجموعة الرابعة ارتفاعاً معنوياً($P<0.05$) فى كلٌ من معدلى قبول الذكر، والخصوبة وكذلك حجم البطن عند الميلاد مقارنة بباقى المجاميع الأخرى. كما أوضحت النتائج وجود إرتباطاً معنوياً ($P<0.01$) موجباً وعالياً بين معظم الصفات المدروسة خاصاً بين لون الشفرين ومستوى تركيز هرمون الإستروجين ومعظم الصفات الإنتاجية والتناسلية المدروسة.

وخلاصة هذه الدراسة توضح أن أنسب وقت للتلقيح الطبيعى للحصول على أعلى إنتاجية فى إناث أرانب النيوزيلاندى الأبيض عندما يكون لون وحالة الشفرين أحمر وردى أو أحمر دكن متضخم.