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10*20+10SOME RABBITS' PHYSI543-OLOGICAL, BIOCHEMICAL AND HORMONAL PARAMETERS IN RESPONSE TO DIFFERENT PHYSIOLOGICAL STATUS

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ABSTRACT:A total of 24 primiparous rabbit does of California (CAL) breed in three different reproductive statuses were used to assess the profile of some physiological, biochemical and hormonal parameters. The 3 reproductive statuses were before mating, during pregnancy and after parturition. Feed and water were provided *ad libitum*. Does body weights were weighed weekly. Rectal temperatures (RT), respiratory rate (RR) and pulse rate (PR) of does were recorded every other day. Serum total protein, albumin, cholesterol, glucose, urea and creatinine concentrations were determined twice a week. Growth hormone (GH), Estrogen (E_2), Progesterone (P_4), and Triiodotheronine (T_3) were determined in rabbit does within each of the 3 physiological statues.

Body weight increased in pregnant rabbits than those before pregnancy and after parturition. No significant variations were found among biochemical parameters during these 3 physiological conditions except for albumin value that was lower after parturition in comparison to the other 2 status. No significant differences were observed in hormone values among rabbit groups except for progesterone that was higher during pregnancy in comparison to the other 2 status.

It could be concluded that rabbit does adjust their internal homeostasis during pregnancy and suckling. In addition, there is a positive correlation between some of the studied parameters indicating healthy status of the experimental does.

Keywords: Rabbit, Reproduction, Serum constituents, Hormones.

INTRODUCTION

Published data on rabbit does indicated changes in hematological, biochemical and hormonal parameters change during pregnancy with a sometimes dramatic return to baseline following parturition. Hormonal and metabolite analyses represent a good tool for understanding the physiological mechanisms required to meet higher reproductive performance (Cardinali *et al*., 2009).

Therefore, blood examination gives the opportunity to investigate the presence of several metabolites and other constituents and helps detect conditions of stress, which can be nutritional, environmental or physical (Aderemi, 2004). Hoy and Verga (2008) stated that physiological parameters such as hormones, heart rate and immune reactions, when considered in relation with behavioral parameters can be used as a welfare indicator. Although there are a lot of studies on blood parameters of various domestic animals, few data are available about biochemical and hormonal values of rabbits during different physiological conditions. Therefore, the present study was aimed to determine the changes in physiological, biochemical, and hormonal parameters occurred in rabbit does before and during pregnancy and after delivery (during suckling). In addition, to investigate the correlation that might exist among the determined parameters.

MATERIALS AND METHODS

Animals and husbandry

The experiment was carried out at the rabbitry of the Experimental Farm belonging to Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. A total of 24 primiparous rabbit does of California (Cal) breed in three different reproductive statuses were used. The experiment aimed to observe the profile of some physiological, biochemical and hormonal parameters during fifteen days within each of the three reproductive conditions. The reproductive statuses were as follows: two weeks before mating, during pregnancy (from day 15 to 28 gestation) and after parturition (first two weeks of suckling). The animals were apparently healthy and free of any external parasites or skin diseases. All experiments on animals were performed in accordance with University animal care and use committee guidelines. Age of rabbit does ranged between 18 and 24 months and their weight averaged 3.24±0.36 kg. 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. The day of mating was considered day 0 of pregnancy. Manual abdominal palpation of the uterus was performed 12 days post mating to determine pregnancy. Ambient temperature and relative humidity inside rabbitry were recorded daily during the experiment periods by using thermometer and hygrometer. The ambient temperature and relative humidity were 23±0.5 ^{o}C and 45±2 % RH, respectively.

Data collection:

Body weight and physiological body reaction

Does were weighed weekly. Thermo-cardio-respiratory responses including, rectal temperatures (RT), respiratory rate (RR) and pulse rate (PR) of does were recorded every other day in the morning between 8:00-10:00 am for each animal. RT (^{o}C) was measured for one minute in animals by using clinical thermometer

which inserted into the rectum for about 2cm from anus orifice after liniment with pure vaseline. RR was measured by counting the movements of the chest fleece for one minute. PR was measured by counting pulses in the femoral artery with aid of a finger for a minute (Khalil *et al*., 2014).

Blood sampling and analysis

Blood samples were collected (twice a week) during experiment in the morning between 8:00-10:00 am. Blood samples were obtained from the marginal ear vein of each doe by using a 3 ml syringe. Serum was obtained by blood samples centrifugation at 3000 rpm for 20 min. and stored at -20^{o}C until analysis. Serum total protein (TP), albumin (Al), globulin (Glo), glucose (Glu), cholesterol (Ch),urea (Ur) and creatinine (Cre) concentrations were measured spectrophotometrically (T 80 UV/VIS Spectrometer, PG Instrument Ltd) in serum using SPECTRUM commercial kits (MDSS GmbH, Hannover, Germany). Also, serum growth hormone (GH), estradiol-17β (E_2), progesterone (P_4) and triiodotheronine (T_3) hormones were analyzed by ELIZA kits manufactured by DiaMetra, Spello-Perugia, Italy.

Statistical analysis

Data were statistically analyzed using the General Linear Model (GLM) procedure of SAS (SAS., 2004). Differences among means were detected using Duncan's new multiple test (Duncan 1955). Correlation coefficients among traits were estimated. The statistical model used for data analysis was:

$Y_{ij} = \mu + s_i + e_{ij}$

Where:

Y_{ij} = the observation on the j^{th} individual from the i^{th} does status.

M = the overall mean.

s_i = the fixed effect of the i^{th} does status.

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

RESULTS

Tables 1, 2 and 3 show the physiological, biochemical and hormonal parameters levels in California rabbit does during different physiological conditions, respectively. Rabbit does body weights increased significantly (P<0.001) during pregnancy more than those before pregnancy and after parturition due to weight of pregnancy (Table 1).Physiological body reactions: RT, RR, and PR levels showed changes in the three physiological statuses due to pregnancy (Table 1). General elevation of these parameters was observed in rabbits during pregnancy than before pregnancy and returned to normal levels after parturition. However, this increase was only significant (P<0.001) in respiration rate.

Table 2 shows values of serum biochemical parameters: TP, Al, Glo, Glu, Ch, Ur and Cre in rabbit does serum before pregnancy, during pregnancy and after parturition. No significant variations were found among biochemical parameters during these three physiological conditions except for albumin value. Albumin level showed significant (P<0.001) decrease during pregnancy than those before pregnancy and after parturition. It is important to note that glucose level showed non-significant higher values during pregnancy and after parturition (189.33 and 186.95 mg/ml) than before pregnancy (163.93 mg/ml), respectively.

Table 3 shows levels of some hormones (GH, Estrogen, Progesterone, T_3) in rabbit does during different physiological conditions. No significant differences were observed in hormone values among rabbit groups except for progesterone. Progesterone level showed significant (P<0.001) higher value (8.29 ng/ml) during

pregnancy than those before pregnancy and after parturition (1.74 and 2.23 ng/m, respectively). Estrogen and T_3 hormones showed non-significant decreasing trend (P=0.93 and 0.45 respectively) in pregnant and lactating rabbits than before pregnancy. However this trend was reversed with also the absence of significance (p=0.464) for GH level recording the highest value for lactating does followed by the pregnant ones.

Table 4 showed correlation coefficients among some studied traits.There was a significant (P<0.05) positive correlation between body weight of rabbit does and each of RR, PR, Glo and progesterone levels. In addition, significant (P<0.05) positive correlation was found between pulse rate and TP, Al and Ch. Also, between RR and Ur and progesterone concentrations. In contrast, significant (P<0.05)negative correlation was found between body weight and Cre level; between PR and Ur level; and between Al, Ur and Cre concentrations

Awojobi *et al*. (2004) reported that nutrient intake was higher in lactating rabbits than in pregnant and non-pregnant rabbits. The present study showed no significant differences were found between doe weights before pregnancy and after parturition. However, rabbit body weight increased significantly during pregnancy due to pregnancy weight as a result of the increase in feed consumption. On the other hand, Szendr˝o and Maertens (2001) reported that nutrient supply to embryos and fetuses is exclusively dependent upon the mother. Consequently, as the body size of the does decreases, the litter weight at kindling decreases (Holdas and Szendro, 2001). Furthermore, reduction of total caloric intake in certain cases i.e., during pregnancy, eating disorders or other related indicators resulted in low pregnancy weights of does in association of a significant proportion of intrauterine growth retardation (Kramer, 1993).

Changes in animal body homeostasis during various reproductive statuses (pregnancy or suckling) have been determined by measuring physiological body reaction parameters (RR, PR, RT) of rabbit does. In addition, serum biochemical and hormonal parameters are becoming increasingly important diagnostic tools in veterinary medicine and production traits. Determination of the abovementioned parameters in animals gives clues and interpretation for the effect of breed, gender, age, reproductive status and seasonal variations (Gill and Wanska, 1978; Mira and Mathias, 1993; Ozegbe, 2001and Wells *et al*., 1999). Physiological body reaction parameters determined in the present study showed only significant elevation in RR in pregnant than those observed in non-pregnant and suckling ones. This elevation in RR is suggested to be due to stress of pregnancy. Thus during pregnancy, multiple physiological and anatomical adjustments are activated to maintain normal maternal homeostasis. No significant alterations in RT and PR were found among animal groups. In non-pregnant individuals, many of these alterations would be considered pathological rather than physiological. Latner (1975) found in pregnant rabbits high progesterone levels which caused hyperventilation with a consequent greater rapidity of carbon dioxide clearance. This interprets the current elevation of RR in the pregnant group. In a previous report conducted on rabbit does during similar reproductive conditions, Awojob et al. (2004) stated that there were no significant differences in hematological indices for the three physiological states in rabbits. Many authors reported of heamodilution and alterations in renal plasma flow, glomerular filtration rate, plasma volume as well as gastro-intestinal absorption efficiency during pregnancy (Sims and Krantz, 1958

and MacDonald and Good, 1971b). These observed changes during pregnancy further resulted in alterations in hematological and plasma biochemical profiles (MacDonald and Good, 1971a,b; Rowlands *et al*., 1975; Felbinger, 1987 and Vihan and Rai, 1987). In the present study it was observed that there were no significant differences between all biochemical parameters except for albumin. Total protein was insignificantly lower, but albumin was significantly lower in lactating does than those not pregnant or pregnant ones.

Çetin *et al.*(2009) found that total protein, albumin, triglyceride, and cholesterol were significantly ($P<0.05$) lower in pregnant Angora rabbits than in other females, while the mean glucose level was significantly higher ($P<0.05$) in pregnant rabbits compared with non pregnant females. In cows, there were differences in total protein, albumin and globulin fractions between pregnant and non-pregnant cows (Zvorc *et al.,* 2000). However, values of biochemical parameters (Table 2) in the present study were in normal ranges and comparable for rabbits in the three physiological statuses and were in agreement with several studies in farm animals such as Zvorc *et al*. (2000) in cattle, Ozegbe (2001) in rabbits, Milinkovic-Tur *et al*. (2005) in mares and Waziri *et al.* (2010) in goats. The marked increase (not significant) in glucose and urea after parturition could be due to increase nutrient metabolism for milk synthesis and production. Awojobi *et* al. (2004) found that blood glucose and total proteins were significantly ($P < 0.05$) lower in lactating does compared to the pregnant and non-pregnant does. Furthermore, they found that globulin was however significantly ($P < 0.05$) higher in pregnant and lactating does than in the non-pregnant. These findings agreed with our study except for glucose level which was insignificantly high during pregnancy and lactation.

Rabbit does cannot completely satisfy the high nutritional requirements during lactation that is exceptionally high (Pascual *et al*., 1999). Several hormones work together in linking growth, metabolism, energy homeostasis and reproduction functions (Hornick *et al*., 2000). They stated that T_3 blood concentration is an important key to analyze the metabolic adaptation and with the glucose level are good indicators of the energy balance. Cardinali *et al*. (2009) found in rabbits that insulin levels increased during pregnancy as a consequence of the growing of the fetuses. They further added that glucose levels did not show any significant increase in pregnant group and this can explain the homeostatic mechanisms that controlling the glycaemia. In addition, Brecchia *et al.* (2005) stated that Insulin is also a key player in the control of intermediary metabolism and exerts an important role in ovarian function.

Results of the present study showed a slight increase in GH level during pregnancy and lactation than that before pregnancy. Ovarian steroids, GH and Prolactin stimulate mammogenesis but several other hormones play a permissive and supportive role in mammary growth.

In conclusion, this study showed variation in some parameters in rabbit does during three physiological statuses. These alterations attributed to adjustment of internal homeostasis of rabbits during pregnancy and suckling. The positive correlation found between some of the studied parameters indicated the suitable good husbandry and proper feeding requirements provided during experiment.

Table(1): Body weight and physiological body reactions with respect to different physiological status of rabbit doe

Trait	Doe Status			*P*-value
	Before pregnancy N = 32	During pregnancy N = 56	After parturition N = 32	
Body weight (kg/doe)	3.34±0.08[b]	3.74±0.06 [a]	3.47±0.07 [b]	0.001
Respiration rate (no/min)	99.31±1.61[b]	128.00±1.60[a]	97.33±2.47[b]	0.001
Pulse rate (no/ min)	106.75±2.55	107.29±2.55	102.40±2.24	0.398
Rectal temperature (ºC)	38.95±0.10	38.79±0.05	39.00±0.08	0.077
a,b Means in the sam row with no common superscript differ (*P<0.05*).				

Table(2): Serum biochemical constituents with respect to different physiological status of rabbit doe

Trait	Doe Status			*P*-value
	Before pregnancy N = 32	During pregnancy N = 56	After parturition N = 32	
Total protein g/dL	8.42±0.22	8.51±0.26	7.86±0.35	0.240
Albumin g/dL	4.94±0.07[a]	4.85±0.08 [a]	4.37±0.08 [b]	0.001
Globulin g/dL	3.48±0.23	3.66±0.26	3.52±0.38	0.888
Glucose mg/dL	163.89±5.39	189.33±8.93	186.95±12.48	0.147
Cholesterol mg/dL	105.93±6.66	105.46±7.04	102.04±7.88	0.935
Urea mg/dL	64.22±7.11	62.78±11.44	111.95±35.89	0.178
Creatininemg/dL	1.19±0.10	1.22±0.09	1.26±0.12	0.909
[a,b] Means in the sam row with no common superscript differ (*P<0.05*).				

Table 3: Hormonal profile with respect to different physiological status of rabbit doe

Trait	**Doe Status**			***P*-value**
	Before pregnancy N = 32	**During pregnancy N = 56**	**After parturition N = 32**	
GH (ng/ml)	0.36±0.06	0.42±0.09	0.54±0.09	0.464
E_2 (pg/ml)	18.49±1.66	17.66±1.46	17.75±1.31	0.925
P_4 (ng/ml)	1.74±0.81^b	8.29±0.54^a	2.23±0.47^b	0.001
T_3 (ng/ml)	0.80±0.06	0.77±0.07	0.66±0.02	0.448
^{a,b} Means in the sam row with no common superscript differ (*P<0.05*).				

Table 4: Correlation coefficients among some studied traits

R	**PR**	**RR**	**RT**	**TP**	**Al**	**Glo**	**Glu**	**Ch**	**Ur**	**Cre**	**E2**	**P4**
BW	.617*	.309	-.313	.072	.354	.694**	.975**	.082	-.268	-.547*	.116	.782**
PR	1.000	.152	.006	.747**	.804**	.444	-.204	.836**	-.557*	-.198	.063	-.233
RR		1.000	-.107	.448	.292	.028	.397	-.039	.531*	-.277	.171	.521*
TP				1.000	.727**	.241	.081	.503	-.802**	-.242	-.150	.173
Al					1.000	.648**	.260	.867**	-.767**	-.578*	.232	.219
Glo						1.000	.560*	.613*	-.218	-.393	.204	.291
Glu							1.000	-.089	-.318	-.547*	.060	.869**
Ch								1.000	-.400	-.308	.271	-.210
Ur									1.000	.620*	-.056	-.514
Cre										1.000	-.487	-.539*
E2											1.000	-.056

R: Correlation coefficient, BW= Body weight of doe, PR=Pulse rate, RR: Respiration rate,TP= Total protein, Al= Albumen, Glo= Globulin, Glu=Glucose, Ch= Cholesterol, Ur= Urea, Cre=Creatinine, E2= Estradiol-17β, P4= Progesterone, *p≤0.05, **p≤0.01

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الملخص العربي

التغيرات البيوكيميائية و الهرمونية في الأرانب كإستجابة لحالات فسيولوجية مختلفة

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هدفت التجربة إلي تتبع ووضع تصور لتغيرات بعض القياسات الفسيولوجية، البيوكيميائية و الهرمونية في إناث الأرانب خلال ثلاث حالات فسيولوجية مختلفة.

تم استخدام ٢٤ ام ولودة من نوع كاليفورنيا للمقارنة بين ثلاث مراحل فسيولوجية مختلفة وهي قبل التلقيح، اثناء الحمل و بعد الولادة. تم إتاحة الغذاء والماء للأرانب بصورة حرة اثناء التجربة وتم وزن الأمهات اسبوعيا، كذلك تم تسجيل الاستجابات الفسيولوجية للأمهات متمثلة في معدل التنفس، معدل النبض و درجة حرارة المستقيم يوم بعد يوم. تم تقدير البروتين الكلي، الألبومين، الكوليسترول، الجلوكوز، اليوريا و الكرياتنين في سيرم الدم مرتين أسبوعيا و كذلك تم تقدير مستويات هرمون النمو، الأستروجين، البروجستيرون و الثيروكسين في إناث الأرانب خلال المراحل الفسيولوجية المختلفة. كما تم تقدير معامل الأرتباط بين بعض الصفات تحت الدراسة.

لوحظ وجود زيادة معنوية في وزن جسم الأمهات اثناء الحمل عنه قبل الحمل و بعد الولادة. لم يكن هناك اختلافات معنوية بين المقاييس البيوكيميائية المدروسة خلال المراحل التناسلية المختلفة فيما عدا قيم الألبومين. كذلك تركيزات الهرمونات محل الدراسة لم تختلف معنويا فيما عدا مستويات البروجستيرون.

الاستنتاج:

أوضحت الدراسة وجود بعض التغيرات في بعض القياسات المقدرة في إناث الأرانب خلال المراحل الفسيولوجية المختلفة.أعزت هذه الاختلافات إلي حدوث تعديلات فى التوازن الداخلي للأرانب اثناء الحمل والولادة. وجود ارتباط موجب بين الصفات المدروسة وبعضها أكد علي حيوية الأرانب الجيدة للأمهات اثناء التجربة.

كلمات مفتاحية: أرانب ، تناسل ، مكونات السيرم ، هرمونات