RELATIONSHIP BETWEEN LIPTEN HORMONE CONCENTRATION AND RABBIT REPRODUCTIVITY

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Abstract

The present work was aimed to study the relationship between Lipten hormone concentration and rabbit reproductively in two rabbit breeds (Baladi Black and New-Zealand White). Lipten hormone concentrations were determined in blood, milk and semen of rabbits. Sixty mature rabbits at twelve months of age were used. Results obtained showed that, Leptin hormone concentration in blood serum of ordinary fertile doe and buck rabbits were significantly (P≤0.5 or 0.1) higher than those recorded in low fertile ones, in both BB and NZW rabbits. BB rabbits recorded leptin hormone levels insignificantly higher than those obtained by NZW rabbits. Ordinary fertile BB and NZW lactating rabbit does produced milk with Leptin hormone concentration significantly (P≤ 0.1) more than recorded by low fertile rabbits. Leptin hormone concentrations were arranged in descending order insignificantly as obtained during, the 3rd; 2nd; 4th; 1st then the 5th week of lactating period, respectively. Semen ejaculated by ordinary fertile BB and NZW rabbit bucks included Leptin hormone levels more significantly (P≤0.1) than those of low fertile bucks. BB rabbit bucks and second ejaculates characterized by Leptin hormone levels insignificantly higher than those obtained by NZW rabbit bucks and first ejaculates. It could be concluded that, there was strongly correlation between Leptin hormone concentration in blood, semen, milk and rabbit reproductivity. BB rabbits may be capability more than NZW rabbits.

Key words: rabbit; Leptin; fertility; blood; reproduction

INTRODUCTION

Developing countries are often suffered from protein deficiency. This is due to the small number of existing farm animals as compared to rapid growth of human population, and the low productivity and reproductivity of these animals [4, 14]. Increasing in animal protein production may come from short-life cycle animals like rabbits [8]. Rabbits had gradually increased attention in the last few months for meat production [13, 16, 17, 22].

Zhang et al. (1994) demonstrated that, lipten has additional physiological activities, including activation of the sympathetic nervous system, regulation of reproductive function, and activation of the immune system. Houseknecht et al. (1997) reported that, lipten levels in whole and skim milk were correlated with maternal plasma lipten concentrations. From early to mid pregnancy, circulating lipten levels increased and remained elevated until late pregnancy.

Are few researches in Egypt to evaluate the relationship between Leptin hormone level and reproductive performance in rabbits.

The present work was planned to evaluate the relationship between Lipten hormone levels and rabbits' reproductivity.

MATERIAL AND METHOD

The present study was conducted in the Faculty of Agriculture, Ain Shams University, Egypt. The study lasted three months, from January till March, 2012.

Two rabbit breeds (Baladi Black "BB" as a native breed and New-Zealand White "NZW" as an exotic breed) were used in the present study. Lipten hormone concentrations were determined in blood, milk and semen of ordinary and low fertile rabbits.

Rabbits were fed *ad libitum* a commercial diet according to NRC (1994).

The experimental work included two experiments as follows:

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1st experiment was designed to separate low-fertile does from ordinary-fertile ones of BB and NZW rabbits. The separation depended on the records in Rabbitry represented in rates of each of abortion, conception and kindling and values of each of litter size and weight at birth and at weaning, in addition to bunny weight at birth and at weaning. The pattern used to determine the fertility traits of rabbit does according to Rabbitry records are shown in Table 1. BB and NZW rabbit bucks characterized by low-fertilizing ability were also separated from those with ordinary-fertilizing ability using libido and physical semen characteristics, in addition to mating activity, scrotal circumference and testicular index. The pattern used to evaluate fertilizing ability of rabbit bucks as recorded by physical semen characteristics are shown in Table 2.

Table 1 The pattern used to determine the fertility traits of rabbit does according to Rabbitry records

Items	Fertility traits	
	Low-fertile	Ordinary-fertile
Abortion rate (%)	≥ 03.00	< 03.00
Conception rate (%)	≤ 60.00	> 60.00
Kindling rate (%)	≤ 57.00	> 57.00
Litter size at birth (No.)	≤ 04.75	> 04.75
Litter weight at birth (g)	≤ 200.0	> 200.0
Bunny weight at birth (g)	≤ 40.00	> 40.00
Litter size at weaning (No.)	≤ 04.00	> 04.00
Litter weight at weaning (g)	≤ 2250	> 2250
Bunny weight at weaning (g)	≤ 0550	> 0550

Table 2 The pattern used to evaluate fertilizing ability of rabbit bucks

Items	Fertilizing ability	
	Low-fertile	Ordinary-fertile
Libido (Sec.)	≥ 40.00	< 40.00
Mass sperm motility (Score)	≤ 02.50	> 02.50
Advanced sperm motility (%)	≤ 50.00	> 50.00
Dead spermatozoa (%)	≥ 35.00	< 35.00
Sperm abnormalities (%)	≥ 30.00	< 30.00
Acrosomal damages (%)	≥ 25.00	< 25.00
Semen ejaculate volume (ml)	≤ 00.40	> 00.40
Sperm-cell concentration (N X 106/ml)	≤ 450.0	> 450.0
Total-sperm output (N X 106/ejaculate)	≤ 180.0	> 180.0
Mating activity (no. of mating/20 minutes)	≤ 02.50	> 02.50
Scrotal circumference (Cm)	≤ 07.00	> 07.00
Testicular index (Cm^3)	≤ 06.50	> 06.50

Sixty sexually mature rabbits (30 each of low-fertile and ordinary-fertile) of 12 months age (10 does& 5 bucks of each breed of BB and NZW) were used in this study.

2nd experiment was planned to measure Lipten hormone level in blood serum of doe and buck of BB and NZW rabbits. Lipten hormone levels in milk, during lactating period of rabbit does were recorded weekly up to the 5th week, as well as, Lipten hormone levels were estimated in 1st and 2nd semen ejaculated by rabbit bucks. Lipten hormone levels were measured in ordinary and low-fertile of two rabbit breeds used in the study. Buck and doe rabbits in this experiment did not expose to any treatment. Five bucks and ten does within both ordinary and low-fertile of each rabbit breed used in the study were conducted to determine Lipten hormone levels in blood, semen or milk.

Measurement of Leptin hormone levels: Leptin hormone levels in blood and milk serum and in seminal plasma was done using DRG® Leptin (Sandwich) ELISA (EIA-2395). The DRG® Leptin ELISA commercial kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle according to Considine and Sinha (1996) and Guillaume and Bjorntorp (1996).

Measuring and assaying of Leptin hormone concentration was done as follows:

- 15 µl of each standard were dispensed, controls and samples with new disposable tips into appropriate wells.
- 100 µl assay buffer into each well were dispensed and completely mixed thoroughly for 10 seconds.
- For 120 minutes at room temperature (without covering the plate) incubated, and then the contents of the wells were briskly shacked out.

Procedure of Kit Company recommended that, the sensitivity and precision of this assay is markedly affected by the correct performance and number of times of the washing procedure so we washed one more time to be sure of its performance than written in the kit's brochure (3 times).
- The wells were rinsed 4 times with diluted wash solution (300 µl per well) and strike sharply on absorbent paper to remove residual droplets.
- 100 µl of antiserum were added to each well then incubated for 30 minutes at room temperature and briskly shacked out the contents of the wells.
- The wells rinsed 4 times with diluted wash solution (300 µl per well) and strike the sharply on absorbent paper to remove residual droplets.
- 100 µl enzymes complex were dispensed into each well then incubated for 30 minutes at room temperature.
- The contents were briskly shacked out of the wells and rinsed 4 times with diluted wash solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
- 100 µl of substrate solution were added to each well then incubated for 15 minutes at room temperature.
- Finally the enzymatic reaction was stopped restrictedly by adding 50 µl of stop solution to each well.
- The reading of sample was done at OD 450 ± 10 nm with a micro titer plate reader ELISA within 10 minutes after adding the stop solution.

The results were calculated as an average absorbance values for each set of standard, controls and samples. A standard curve was constructed by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical axis (Y), and concentration on the horizontal axis (X). The mean absorbance value for each sample was used to obtain the corresponding concentration from the standard curve.

Blood collection: Blood samples were collected from the marginal ear vein after shaving and cleaning with alcohol. A spring lancet made of steel sheets puncture was used to get an adequate but not profuse flow of blood. Blood samples were collected at the morning between 8.00 to 10.00 a.m. as stated by Thompson and Proctor (1984).

Blood samples were collected into dry clean centrifuge tubes. Blood serum was separated by centrifugation at 3000 r.p.m. for 20 minutes and kept in a deep freezer at -20°C until biochemical analysis.

Semen collection: Semen was collected from rabbit bucks by means of an artificial vagina between 08.00 to 10.00 a.m., some rabbit does were used for that particular purpose. The temperature of inner lining rubber sleeve of A.V. was adjusted to 41- 43°C. Lubrication of the inner sleeve was performed using medical white vaseline with sterile rode. The pressure in the lumen of A.V. was adjusted to suit individual rabbits. Most of the ejaculate passes into the collecting tube, but the entire yield can be obtained by inverting the A.V. and opening the clap to allow the water to run out by reducing the pressure in the inner sleeve. Each ejaculate was kept separately in a water bath at 37°C for examination. All the equipment's of the A.V. were washed thoroughly and sterilized before every collection of semen to avoid contamination.

Semen evaluation: Libido and physical semen characteristics represented in values of each of semen-ejaculate volume; mass and advanced sperm motility; percentages of sperm abnormalities; dead spermatozoa and acrosomal damages and sperm-cell concentration per ml and per ejaculate were evaluated. Physical semen characteristics were evaluated according to according to Campbell et al. (1956); Hackett and Macpherson (1965); Watson (1975); Salisbury et al. (1978) and Seleem et al. (2009).

Mating activity of rabbit buck was done as studied by Badawi et al. (2010). Scrotal circumference was measured by measuring the circumference of scrotum of each testis of

each BB and NZW rabbit bucks as the method described by Mickelsen et al. (1982). Testicular index (length x width x depth) was calculated in cubic centimeters as recorded by El-Kholy et al. (2012).

Statistical analysis: Data were statistically analyzed using Least Squares Analysis of Variance according to Snedecor and Cochran (1967). Percentage values were transformed to arcsin values before being statistically analyzed. Duncan's Multiple Range Test [5] was used to compare the differences between significant means. Conception and kindling rates were analyzed using Contingency tables according to Everitt (1977).

RESULTS AND DISCUSSIONS

Leptin Hormone levels in blood: Data presented in Tables (3 and 4) showed that, Leptin hormone concentration in blood serum of ordinary fertile doe and buck rabbits were significantly (P≤0.5 or 0.1) higher than those recorded in low fertile ones. This trend was recorded in both two studied breeds (BB and NZW) rabbits. It is interested to note that, in each status studied, ordinary and low fertile, and doe and buck rabbits, BB rabbits recorded Leptin hormone levels insignificantly higher than those obtained by NZW rabbits.

Leptin Hormone levels in milk: Table 5 clearly showed that, ordinary fertile BB and NZW lactating rabbit does produced milk with Leptin hormone concentrations significantly (P≤0.5 or 0.1) higher than those recorded by low fertile rabbits, during nursing period from kindling up to 5 weeks. BB rabbit does gave values of leptin hormone concentrations insignificantly superior than those recorded by NZW ones. Leptin hormone concentrations were insignificantly arranged in descending order as recorded during, the 3rd; 2nd; 4th; 1st then the 5th week of lactating period, respectively.

Leptin Hormone levels in semen: Data presented in Table 6 showed that, semen ejaculated by ordinary fertile BB and NZW rabbit bucks included Leptin hormone concentrations significantly (P≤0.5 or 0.1) higher than those of low fertile bucks. BB rabbit bucks and second ejaculates characterized by Leptin hormone levels insignificantly higher than those obtained by NZW rabbit bucks and first ejaculates, respectively.

Zhang et al. (1994) identified the mutated protein, lipten, in mice, which is assumed to be the circulating satiety signal. Since the identification of this protein, many studies demonstrated that, lipten has additional physiological activities, including activation of the sympathetic nervous system, regulation of reproductive function, and activation of the immune system (Zhang et al., 1994). In human; Houseknecht et al. (1997) reported that, lipten concentrations in whole and skim milk were correlated with maternal plasma lipten concentrations. From early to mid pregnancy, circulating lipten levels increased and remained elevated until late pregnancy in sheep. In mares, Romagnoli et al. (2007) found that, the highest cholesterol lipten level recorded during the week of parturition compared with two weeks before parturition. On the other hand, in sows, Huszenicza et al. (2002) and Smith and Grove (2002) reviewed that negative energy balance during lactation is reflected by decreases in serum lipten and thyroid hormone levels. At this time, it is unclear and poorly documented, what mechanisms may be responsible for the suppression of thyroid hormones and whether there is a link to the suppression of lipten.

Table 3 Leptin hormone levels in blood (ng/dl) of low and high fertile rabbit does (Means ± SE)

Breeds	Fertility traits		Means ± SE
	Low fertile	High fertile	
BB	2.47±0.42	3.84±0.51	3.16±0.44
NZW	2.36±0.37	3.61±0.54	2.99±0.49
Means ± SE	2.42±0.63 b	3.73±0.53 a	3.07±0.57

Means bearing different letter superscripts within the same row are significantly (P ≤ 0.05 or 0.01)

Table 4 Leptin hormone levels in blood (ng/dl) of low and high fertile rabbit bucks (Means ± SE)

Breeds	Fertility traits		Means ± SE
	Low fertile	High fertile	
BB	2.34±0.37	3.62±0.52	2.98±0.41
NZW	2.24±0.33	3.43±0.54	2.84±0.40
Means ± SE	2.29±0.34 b	3.53±0.50 a	2.91±0.46

Means bearing different letter superscripts within the same row are significantly (P ≤ 0.05 or 0.01)

Table 5 Leptin hormone concentration in milk (ng/dl) of low and high fertile rabbit does (Means ± SE)

Milk period	Breeds	Fertility traits		Means ± SE
		Low fertile	High fertile	
1st week	BB	1.93±0.27	3.36±0.59	2.65±0.36
	NZW	1.84±0.19	3.28±0.62	2.56±0.21
Means ± SE		1.89±0.26 b	3.32±0.54 a	2.61±0.33
2nd week	BB	2.51±0.32	3.69±0.70	3.10±0.42
	NZW	2.32±0.34	3.64±0.63	2.98±0.52
Means ± SE		2.42±0.33 b	3.67±0.68 a	3.04±0.51
3rd week	BB	2.64±0.44	3.99±0.58	3.32±0.54
	NZW	2.41±0.51	3.92±0.48	3.17±0.47
Means ± SE		2.53±0.46 b	3.96±0.56 a	3.24±0.50
4th week	BB	2.11±0.38	3.47±0.47	2.79±0.40
	NZW	2.01±0.30	3.18±0.40	2.60±0.34
Means ± SE		2.06±0.31 b	3.33±0.46 a	2.70±0.33
5th week	BB	1.86±0.25	3.14±0.59	2.50±0.29
	NZW	1.79±0.18	2.95±0.46	2.37±0.22
Means ± SE		1.83±0.22 b	3.05±0.51 a	2.44±0.33
Overall means ± SE		2.15±0.24 b	3.47±0.46 a	2.81±0.27

Means bearing different letter superscripts within the same row are significantly (P ≤ 0.05 or 0.01)

Table 6 Leptin hormone concentration in semen (ng/dl) low and high fertile rabbit bucks (Means ± SE)

Semen ejaculated	Breeds	Fertility traits		Means ± SE
		Low fertile	High fertile	
1st ejaculate	BB	2.07±0.28	3.21±0.42	2.64±o.33
	NZW	2.02±0.31	3.09±0.33	2.56±0.30
Means ± SE		2.05±0.28 b	3.15±0.36 a	2.60±0.31
2ndejaculate	BB	2.22±0.19	3.48±0.44	2.85±0.26
	NZW	2.16±0.24	3.26±0.29	2.71±0.22
Means ± SE		2.19±0.20 b	3.37±0.33 a	2.78±0.24
Overall means ± SE		2.12±0.17 b	3.26±0.33 a	2.69±0.21

Means bearing different letter superscripts within the same row are significantly (P ≤ 0.05 or 0.01)

New Zealand White rabbits as well known in Egypt as a meat purpose breed that intensively spread allover the country Ayyat and Marai (1998) have indicated that, Baladi Black rabbits as a local meat type breed are widely and more adapted with Egyptian environmental conditions (Khalil, 1999 and Youssef, 2004).

Khalil et al. (1988) and Hilmy (1991) scored that, local Baladi Black rabbits which assumed to be adapted to the Egyptian conditions have different genetic basis among foreign breeds. On the other hand, El-Desoki (1991) found NZW rabbit breed characterized by productive and reproductive superior than Baladi Black ones. Direct genetic effects of exotic breeds compared to Baladi ones are favorable in letters at birth and during the first 21 days of sucking but not at weaning (Afifi and Khalil, 1991). New Zealand white bucks generally produce litters with larger size and heavier weight along with heavier mean bunny weight at birth and at 21 days of age than do the Baladi bucks (Youssef, 1992).

CONCLUSIONS

The study concluded that, there was strongly correlation between Leptin hormone concentration in each of (blood, semen and milk) and rabbit reproductivity. BB rabbits recorded Leptin hormone levels insignificantly higher than those of NZW rabbits, so BB rabbits may be characterized by reproductive capability more than NZW rabbits, under the environmental conditions of conducting that experiment.

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