STUDY OF BODY CONDITION, ENERGY MOBILIZATION AND LEPTIN PROFILE IN REPRODUCTIVE FEMALES

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ABSTRACT

This study examined the effect of reproductive and lactating status, and gestation order on body condition, non-esterified fatty acids (NEFA) and leptin concentration in rabbit females. A total of 26 rabbit females from a synthetic line were used in the experiment. Body weight, perirenal fat thickness, NEFA and leptin concentration were measured at mating and at 12 days of gestation. The model used included the effects of reproductive status (mating or 12 days of gestation), lactation status at mating (lactating or not lactating), gestation order (second and third), and female effect. All statistical analysis were performed by Bayesian methodology. Body weight and perirenal fat thickness were -5 % and -12 % lower at mating than at 12 days of gestation (P=1.00), but NEFA were similar at both status (P=0.81). Leptin concentration was +18 % higher at mating than at 12 days of gestation. Lactating females showed higher body weight (+3 %; P=0.92) and perirenal fat thickness (+8 %; P=0.96) than non-lactating females. NEFA were -50 % lower in lactating females than non-lactating females and leptin concentration was similar (P=0.70). All traits were lower in the second gestation than in the third gestation. In conclusion, reproductive status, lactation-gestation overlap and gestation order affect body condition, energy mobilization and leptin concentration.

Key words: Gestation, Lactation, Leptin, Mating, NEFA, Perirenal Fat Thickness.

INTRODUCTION

Several metabolites, as NEFA, and hormones, as leptin, have been proved to play a role in the relationship between energy balance and reproductive efficiency (Castellini et al., 2010). Body weight and perirenal fat thickness are good predictors of body condition and non-esterified fatty acids (NEFA) as well as leptin concentrations provide an accurate measurement of energy mobilization (Jorritsma et al., 2003; Calle et al., 2017). NEFA act at the ovarian level by modifying endocrine, paracrine, and autocrine regulation, which permit follicle growth, ovulation, and development of the corpus luteum in rabbits (Fortun-Lamothe, 2006). Leptin is a cytokine involving the control of satiety and energy metabolism. Specifically, leptin acts as the critical link between adipose tissue and the reproductive system (Moschos et al., 2002) and it is implicated in steroidogenesis (Brannian et al., 1999), ovulation (Ryan et al., 2002; Sakr et al., 2010), pregnancy (Menchetti et al., 2015) and lactation (Mukherjea et al., 1999).

To know body condition, energy mobilization and leptin profile in different reproductive conditions would improve management strategies of females. The objective of the present work was to study the effect of reproductive and lactating status, and gestation order on body condition, NEFA and leptin concentration in rabbit females.

MATERIALS AND METHODS

All experimental procedures involving animals were approved by the University Miguel Hernández of Elche Research Ethics Committee.

Animals and experimental design

A total of 26 rabbit females from a synthetic line were used in the experiment (García et al., 2010). The females were held in the experimental farm at the University Miguel Hernández de Elche (Spain). All animals were reared in individual cages and were fed a commercial diet. The photoperiod used was 16 h light: 8 h dark. Females were mated first at 18 weeks of age and thereafter at 10 days after each parturition. The weaning at 28 days was standard procedure. Sexual receptivity was checked on the day of mating by evaluation vulva colour; does were scored either receptive when their vulva was red and turgid, or non-receptive in all other cases. Only females classified as receptive were mated. Does were stimulated to ovulate with an intramuscular injection of 1 μg busereline acetate (Suprefact, Hoechst Marion Roussel S.A., Madrid, Spain) when the mating was performed.

Females were controlled to second and third effective mating and at 12 days of second and third gestation. Blood samples were collected from the margin ear vein into tubes containing EDTA. Immediately, plasma was obtained after centrifugation at 3000 g for 15 min at 4 ºC and stored at -20 ºC until the hormones and metabolites assays were performed. Females were weighted each time that blood samples were collected.

Body condition

Perirenal fat thickness was measured by ultrasound imaging as described by Pascual et al. (2004), using Justvision 200 SSA-320A Toshiba ultrasound equipment.

Metabolite assays

The non-esterified fatty acids (NEFA) concentrations were analyzed using two reactions, enzymatic based colorimetric assay from WAKO (NEFA-C, Wako Chemicals GmbH, Neuss, Germany) based on the ability of NEFA to acylate coenzyme A in presence of CoA synthetase.

Hormone assays

Leptin concentrations were measured by double antibody RIA using multi-species leptin kit (Linco Research Inc., St. Charles, USA). The limit of detection was 1-50 ng/mL Human Equivalent (HE).

Statistical Analysis

The traits analyzed were body weight (g), perirenal fat thickness (mm), NEFA (mmol/L) and leptin (ng/mL HE). The model used included the effects of reproductive status (mating or 12 days of gestation), lactation status (lactating or not lactating), gestation order (second or third), and female effect. All analyses were performed using Bayesian methodology. Bounded uniform priors were used for all effects. Residuals were a priori normally distributed with mean **0** and variance $\mathbf{I}\sigma^2_e$, where **I** is a unity matrix and σ^2_e is the variance of the error. The prior for the variance was also bounded uniform with exception of the female effect, which was considered normally distributed with mean **0** and variance $\mathbf{I}\sigma^2_f$, where σ^2_f is the variance of the female. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for all procedures. We used a chain of 60,000 samples, with a burn-in period of 10,000. Only one out of every 10 samples was saved for inferences.

RESULTS AND DISCUSSION

Table 1 shows the features of the marginal posterior distribution of the differences between mating and 12 days of gestation. Body weight and perirenal fat thickness were -5 % and -12 % lower at mating than at 12 days of gestation (P=1.00), but NEFA was similar at both status (P=0.84). Similar

results were found for body weight and perirenal fat thickness by Savietto et al. (2016) in primiparous females and for NEFA by Menchetti et al. (2015).

Table 1: Features of the marginal posterior distribution of the differences between mating and 12 days of gestation for body weight, perirenal fat thickness, NEFA and leptin.

	M	G	M-G	$HPD_{95\%}$	P
Body weight (g)	4,142	4,332	-190	-271; -109	1.00
Perirenal Fat Thickness (mm)	5.8	6.6	-0.7	-1.1; -0.4	1.00
NEFA (mmol/L)	0.31	0.27	0.04	-0.03; 0.11	0.84
Leptin (ng/mL HE)	4.5	4.0	0.5	-0.1; 1.1	0.94

M = median of Mating; G = median of 12 days of Gestation; M-G = median of the difference between mating and 12 days of gestation; $HPD_{95\%}$ = highest posterior density region at 95 %; P = probability of the difference being > 0 when M-G > 0, and probability of the difference being < 0 when M-G < 0.

Females had higher leptin concentration at mating than at 12 days of gestation. This result could be expected due to leptin is implicated in steroidogenesis and ovulation (Brannian et al., 1999; Ryan et al., 2002).

Table 2: Features of the marginal posterior distribution of the differences between lactating and non-lactating females for body weight, perirenal fat thickness, NEFA and leptin.

	L	NL	L-NL	$HPD_{95\%}$	P
Body weight (g)	4,306	4,168	138	-42; 344	0.92
Perirenal Fat Thickness (mm)	6.4	5.9	0.5	-0.1; 1.1	0.96
NEFA (mmol/L)	0.23	0.35	-0.12	-0.20; -0.04	1.00
Leptin (ng/mL HE)	4.1	4.3	-0.2	-1.0; 0.6	0.70

L = median of Lactating females; NL = median of Non-Lactating females; L-NL = median of the difference between Lactating and Non-Lactating females; $HPD_{95\%}$ = highest posterior density region at 95 %; P = probability of the difference being > 0 when L-NL > 0, and probability of the difference being < 0 when L-NL < 0.

Lactating females showed higher body weight (+3 %; P=0.92) and perirenal fat thickness (+8 %; P=0.96) than non-lactating females (Table 2). NEFA were -52 % lower in lactating females than non-lactating females (P=1.00) and leptin concentration was similar (P=0.70). Savietto et al. (2016) found higher perirenal fat thickness in lactating females than non-lactating females at 18 days of gestation but similar body weight. These results showed that rabbit female is adapted to reproductive management with lactation-gestation overlap since lactating females had a higher body condition that non-lactating females.

Table 3: Features of the marginal posterior distribution of the differences between second and third gestation order for body weight, perirenal fat thickness, NEFA and leptin.

	2^{nd}	3^{th}	2^{nd} - 3^{th}	$HPD_{95\%}$	P
Body weight (g)	4,172	4,302	-130	-261; 1.9	0.98
Perirenal Fat Thickness (mm)	5.5	6.8	-1.3	-1.8; -0.8	1.00
NEFA (mmol/L)	0.26	0.32	-0.06	-0.14; 0.01	0.94
Leptin (ng/mL HE)	3.8	4.7	-0.9	-1.65; -0.20	0.99

2^{nd} = median of second gestation; 3^{th} = median of third gestation; 2^{nd} - 3^{th} = median of the difference between second and third gestation; $HPD_{95\%}$ = highest posterior density region at 95 %; P = probability of the difference being > 0 when 2^{nd} - 3^{th} > 0, and probability of the difference being < 0 when 2^{nd} - 3^{th} < 0.

All traits were lower in the second gestation than in the third gestation (Table 3). Body weight of females at third gestation was similar than adult weight of maternal lines (Pascual et al., 2015) thus it could be expected that females were still growing at second gestation.

CONCLUSIONS

Reproductive status, lactation-gestation overlap and gestation order cycle affect body condition, energy mobilization and leptin concentration.

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