Theriogenology 97 (2017) 154–158

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Serum concentration dynamic of energy homeostasis hormones, leptin, insulin, thyroid hormones, and cortisol throughout canine pregnancy and lactation

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ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form 20 April 2017
Accepted 24 April 2017
Available online 28 April 2017

Keywords:
Bitch
Pregnancy
Lactation
Leptin
Thyroid hormones

ABSTRACT

Pregnancy and lactation represent critical periods of canine reproductive life and different hormones are required to maintain homeostasis and the correct energy requirements. The aim of this study was to evaluate leptin, insulin, triiodotyronine (T3), thyroxin (T4) and cortisol serum concentrations to better describe the canine maternal energy metabolism. Twelve Bloodhound bitches that had at least one litter and were considered to be healthy submitted blood samples every 15 days starting from day 0 (ovulation) throughout pregnancy up to the end of lactation (day 120). We found that leptin concentrations increased from day 0 to reach maximum value at day 45 ($P < 0.001$), decreased at day 60 ($P < 0.05$) and rose again at day 105 and day 120 ($P < 0.05$ and $P < 0.01$ respectively). Insulin concentrations significantly increased during pregnancy peaking at day 60 ($P < 0.05$). After parturition dropped and reached progressively lower values similar to those at day 0 at the end of lactation. During pregnancy Body Weight was in low correlation with leptin ($r = 0.32$, $P < 0.01$) and there was no correlation with insulin ($r = 0.13$, $P > 0.1$); moreover, no correlation was found during lactation. T3 concentrations showed a U-shaped trend with a significant reduction at day 75 ($P < 0.05$). T4 concentrations fluctuated without any significant change (ranging from 27.0 ± 1.6 nmol/l to 32.1 ± 1.9 nmol/l). Cortisol concentrations ranged within the reference values (minimal value at day 30 (49.8 ± 6.3 nm/L) and maximal value at day 45 (72.5 ± 16.1 nm/L)). Our study states that canine pregnancy and lactation evoke many hormonal changes necessary to mother and fetuses. In particular, this is the first report regarding the pattern of leptin during whole pregnancy and lactation. The present work lays the groundwork for further studies on endocrine homeostatic mechanisms using the dog as a model for human medicine.

1. Introduction

During pregnancy in mammals, a positive energy balance is required to satisfy the fetal development and lactation [1]. During this period, the hormones involved in the overall regulation of energy metabolism (particularly leptin, thyroid hormones, insulin, and cortisol) undergo significant homeostatic adaptations [2–6].

Leptin, a protein encoded by the obese gene (OB) and produced mainly by adipocytes, exerts multiple pleiotropic actions in the control of reproduction, food intake, energy expenditure, and metabolism [7,8]. During pregnancy, leptin enhances the mobilization of maternal fat [9], supports the development of fetal neuronal-hypothalamic networks involved in the energy homeostasis [10], and acts as a growth factor for the embryos [11]. The peripheral blood profile of leptin during pregnancy and lactation has been described in different mammal species [6,12–15] but not yet in dogs.

Pregnancy is known to affect the glucose regulatory mechanisms. In human, the phenomenon of pregnancy-associated insulin resistance is characterized by intracellular energy deficiency and hyperglycemia [16]. The same condition was reported in bitches

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http://dx.doi.org/10.1016/j.theriogenology.2017.04.040
0093-691X/© 2017 Published by Elsevier Inc.

[17] which consequently have been proposed as model to study the human pregnancy-associated metabolic changes [18].

Like leptin and insulin, thyroid hormones are well-known regulators of metabolism and energy balance including during pregnancy [19]. In humans, thyroid activity builds up due to an increased production of thyroid binding globulin, to a secretion of thyrotrophic factor by the placenta, to an enhanced responsiveness of the pituitary thyrotropins to the hypothalamic releasing hormone TRH, and to changes in the maternal thyroid hormone metabolism [20–22]. In pregnant bitches, thyroxin (T4) blood concentrations significantly exceed the reference range found in non-pregnant animals [23]; however no information is available concerning the changes occurring during lactation.

In many species, cortisol is essential for the development and maturation of fetal organs and participate in the induction of parturition [24,25]. In dogs, the changes of cortisol concentration during pregnancy have been described by Concannon et al. [2] but these studies only focused on the peri-partum period. To our knowledge, no information is available on cortisol concentrations during the whole pregnancy and lactation.

As only incomplete reports exist concerning the hormonal changes involved in the dog energy homeostasis and no data specifically exist concerning the leptin profile during pregnancy and lactation, the aim of this work was to evaluate at the same time in a longitudinal descriptive study leptin, insulin, triiodothyronine (T3), thyroxine (T4), and cortisol serum concentrations throughout canine pregnancy and lactation.

2. Materials and methods

2.1. Animals

The study included 12 Bloodhound bitches ranging from 2 to 8 years (median = 4.5 years) and weighing 43.3 ± 4.7 kg (mean ± SD). The privately owned dogs were followed by the Obstetric and Gynecology Service of the Veterinary Teaching Hospital of the University of Perugia. The study was performed during the period April to September 2015. All experimental procedures were carried out in accordance with European Directives approved by the Institutional Animal Care and Use Committee of Perugia University and included the owner informed consent.

Based on clinical examinations and reproductive history, the dogs were considered to be healthy with normal inter-estrus intervals for previous cycles. All dogs had had at least one litter. All bitches were evaluated from the beginning of proestrus, throughout pregnancy and lactation until weaning. From the first appearance of vulvar serosanguineous discharges, indicating the onset of proestrus, vaginal smears were taken daily from each bitch to identify the end of proestrus and start evaluating progesterone. All subjects were mated naturally twice at two day interval starting 24–48 h post ovulation when serum progesterone concentration was over 10 ng/ml [26]. Pregnancy was diagnosed 22–25 days post ovulation by ultrasonography using a MyLab 30 Gold system (Esaote; Genoa, Italy) equipped with a 5.5–7.5 MHz-microconvex probe for B-Mode. Sonographies were then repeated every 15 days to monitor fetal health.

Dogs were fed twice daily (at 8.00 a.m. and 8.00 p.m.) with a commercial diet according to the following average energy demand: 132 kcal/kg BW ^{0,75} during the first four week of pregnancy and then 132 kcal/kg BW^{0,75} + 26 kcal/kg BW up to parturition. During lactation and up to weaning food was provided ad libitum. These amounts were sufficient to meet the metabolic energy needs based on F.E.D.I.A.F. recommendations (Nutritional guidelines for complete and complementary pet food for cats and dogs, August 2011). Food intake was assessed daily by weighing food before and after feedings. Water was provided ad libitum. From the beginning to the end of the study, the body weights of all dogs were recorded every 15 days at 7.00 a.m. before food administration.

2.2. Blood sampling

Blood samples were collected from the cephalic vein every 15 days starting from ovulation (day 0) throughout pregnancy and up to the end of lactation (day 120). Blood samples were drawn into plastic tubes without anticoagulant, centrifuged at 3000*g* for 15 min, and sera stored at −20 °C until assayed.

Moreover, in order to detect day 0, blood samples (2 ml) were collected daily from late cytological proestrus until progesterone was over 10 ng/ml [26].

2.3. Measurements of hormones

Serum progesterone concentrations were evaluated daily from the end of proestrus up to ovulation by ELFA kits (MiniVidas bioMérieux, Florence, Italy) to predict the optimal breeding time for each bitch.

Serum leptin concentrations were measured by double antibody RIA using the multi-species leptin kit (Linco Research Inc., St. Charles, MO, USA). The limit of sensitivity was 1.0 ng/ml and intra- and inter-assay coefficients of variation were 3.4 and 8.7%, respectively.

Serum insulin concentrations were determined by the double antibody technique using an insulin IRMA kit (Immunotech, Prague, Czech Republic). The limit of sensitivity was 1.35 μU/ml and intra- and inter-assay coefficients of variation were 4.0 and 4.8%, respectively.

Total T3 and T4 were assayed by RIA (Immunotech, Prague, Czech Republic). The sensitivity of the assay was 0.26 nmol/l and 10.63 nmol/l for T3 and T4 kits, respectively. The intra- and inter-assay coefficients of variation were 6.3% and 7.7%, respectively, for T3 and 3.29% and 7.53%, respectively, for T4.

Cortisol concentrations were evaluated by RIA, using the cortisol RIA kit (Immunotech, Prague, Czech Republic). The limit of sensitivity was 5 nM and intra- and inter-assay coefficients of variation were 5.8% and 9.2%, respectively.

2.4. Statistical analysis

Data were analyzed using repeated measures analysis of variance (General Linear Model procedure) with 5 and 4 time points during pregnancy and lactation, respectively. We used Mauchly's test to assess sphericity and corrections of Greenhouse - Geisser or Huynh - Feldt when assumption of sphericity had been violated [27]. We planned simple contrasts and performed tests for linear (straight-line relationship) and quadratic (U-shaped relationship) trends of means over time by using the orthogonal polynomial comparisons method [27]. Diagnostic graphics were used to check assumptions. Results were expressed as estimated marginal means ± standard error (SE) while row data were presented in figures. The Pearson test (r) was used to evaluate the correlation between BW and hormones. Correlation was defined as high when absolute value of r > 0.5, medium when r ranged from 0.3 to 0.5, and low when r < 0.3 [27]. Statistical analyses were performed with SPSS Statistics version 20 (IBM, SPSS Inc., Chicago, IL, USA). P ≤ 0.05 was defined as significant and P < 0.1 as a trend.

3. Results

All dogs became pregnant and whelped healthy puppies naturally 61–65 days post ovulation (median = 63 days). Puppies were