Metabolic and endocrine characteristics of pregnancy toxemia in the ferret

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ABSTRACT: Metabolic and endocrine characteristics of pregnancy toxemia are well documented in small ruminants, but less known in other species. The objective of this study was to measure plasma levels of certain metabolites and metabolic hormones related to the energetic status in blood from sick and healthy, non-pregnant (control) ferrets. Blood was collected from moribund, hypothermic, late pregnant females suffering from pregnancy toxemia (n = 4) and from healthy female ferrets (n = 14) to measure glucose, ketone (βOH-butyrate, BHB), insulin, thyroxine (T\(_4\)) and 3,3',5-triiodothyronine (T\(_3\)) concentrations. In contrast to healthy animals, hypoglycemia, hyperketonemia, hypoinsulinemia and decreased T\(_4\) and T\(_3\) levels were detected in females with pregnancy toxemia and necropsy showed excessive hepatic lipidosis. In summary, it can be concluded that pregnancy toxemia caused by a negative energy balance in ferrets resembles the late-gestational hyperketonemia of twin-pregnant ewes, and moreover that similar endocrine changes may occur.

Keywords: gestational ketosis; insulin; thyroxine (T\(_4\)); 3,3',5-triiodo-thyronine (T\(_3\))
glucose deficiency with intermittent hypoglycemia and accumulation of ketone bodies. The shift of energy metabolism in a catabolic direction is characterized by a wide range of endocrine changes, such as insufficient pancreatic β-cell function with a coinciding increase in insulin resistance, impairments in growth hormone – insulin-like growth factor-1 (IGF-1) axis, and increased peripheral inactivation of thyroid hormones. As a result, low levels of insulin, IGF-1, leptin, thyroxine (T₄), and 3,3',5-triiodothyronine (T₃) are measured in the blood. These endocrine consequences are clearly demonstrated in peri-parturient and postpartum dairy cows (Pethes et al., 1985; Sartin et al., 1988; McGuire et al., 1991; Harmon, 1992; Bauman, 2000; Kadokawa et al., 2000; Kahl et al., 2000; Meikle et al., 2004; Balogh et al., 2008; Bossaert et al., 2008; Lucy, 2008), and during late gestation also in twin-pregnant, hyperketonemic ewes (Henze et al., 1998; Van Saun, 2000; Kulcsar et al., 2006).

Whilst the pathology and clinical biochemistry of pregnancy toxemia are known in ferrets, our understanding of the endocrine background is still incomplete. We were interested in whether the endocrine changes described in the pregnancy toxemia of ewes are present in the ferret. The objective of the current study was to measure the levels of glucose, ketone bodies (βOH-butyrate, BHB) and three hormones (insulin, T₄ and T₃) regulating the energy homeostasis in blood from sick females, and to compare these parameters to those of healthy, non-pregnant female ferrets (control).

MATERIAL AND METHODS

Animals, study design and sampling

The study was conducted on domesticated female ferrets (Mustela putorius furo) (n = 18; age: 9 to 35 months; body weight: 800 to 1 000 g) of mild to moderate body condition, from private veterinary practices in Budapest. Owners gave their permission for blood sampling and necropsy in all cases. Four animals showed the pathognostic signs of pregnancy toxemia (severe lethargy, dehydration, hypothermia, hair loss, uterus full of foetuses) on days 40 to 42 of their first (n = 2), second (n = 1) and fourth (n = 1) pregnancy. At the time of veterinary examination they were in the final comatose stage of this disease, and died spontaneously within minutes, before preparation for cesarean section.

Samples were taken just at dying, as the final event of the emergency situation. As females were in a comatose stage, it was not necessary to anesthetize them before blood sampling. These animals were necropsied immediately. During necropsy the urinary pH and urobilinogen, bilirubin and ketone contents were also estimated (Medi-Test Combi-9 strips, Macherey-Nagel, Duren, Germany).

Healthy female ferrets (n = 14) were used as control animals. These ferrets were hospitalized for ovariectomy, either 3 to 10 days after the beginning of heat (n = 5), or 9 to 21 days after, with hCG inducted ovulation (n = 6), or out of breeding season (in winter when females were in anoestrus; n = 3). The stage of ovarian function was confirmed by morphological examination of the removed ovaries. Blood samples were taken at the beginning of the operative manipulation, after a 6 to 8 h-long food deprivation, using an intramuscular combination of ketamine (2.5 mg/100 g body weight; SBH-Ketamine inj., Produlab Pharma BV, Raamsdonksveer, The Netherlands) and xylazine (0.2 mg/100 g body weight; Rompun inj., Bayer AG, Leverkusen, Germany) for immobilization and anesthesia.

Handling of blood samples. Laboratory and statistical procedures

Blood samples (2 ml) from each female were taken from the jugular vein into heparinized and fluoride-containing tubes. Samples were cooled and centrifuged within three hours. Plasma was harvested and stored at either +4°C (≤ 48 h) until BHB and glucose determinations or at –20°C until insulin, T₄ and T₃ measurements.

All metabolites and hormones were determined with commercial kits adapted for assaying bovine and ovine plasma samples in our lab (Meikle et al., 2004; Kulcsar et al., 2006; Balogh et al., 2008). Glucose was measured using the GOD-POD reaction (Glucose kit, Cat. #40841, Diagnosztikum Co. Ltd., Budapest, Hungary), BHB was measured using the βOH-butryate-dehydrogenase reaction (D-3-Hydroxybutyrate kit, Cat. #RB 1007, Randox Laboratories Ltd., Ardmore, UK), insulin was determined using the 125I-Insulin RIA CT kit (CIS Bio International Ltd, Gif-Sur-Yvette, France), T₄ was measured using the 125I-T₄ RIA-Spec MIS kit (Institute of Isotopes Co. Ltd., Budapest, Hungary), and T₃ was measured using the 125I-T₃ RIA MIS kit.
However, before assaying the current samples, all these endocrine analytical procedures were validated for ferret plasma: the binding pattern of two serially diluted sample pools was parallel to that of the standard curves, and the recovery rates of added known quantity of hormones were between 85 and 106%. The sensitivity and intra- and interassay CV of procedures were within the acceptable range (insulin: 7.75 µIU/ml, 5.5–8.4% and ≤ 8.8%; T4: 1.46 nmol/l, 6.6–8.5% and ≤ 7.7%; T3: 0.18 nmol/l, 6.2–8.8% and ≤ 6.7%, respectively).

For presentation of results the group means and their standard errors (SEM) were calculated. The results were subjected to Student’s t-test (pairwise comparison of group means), or a single trait analysis of variance (ANOVA; for comparison of three group means in healthy controls). If ANOVA revealed significant differences, the least significant differences were planned to be calculated at 5% (LSD$_{p<0.05}$) for further comparison.

### RESULTS

Necropsy confirmed the suspected diagnosis of pregnancy toxemia: mild icterus and severe form of hepatic lipidosis, furthermore ketonuria, low pH and increased urobilinogen and bilirubin contents in the urine were observed in all the four cases. Two of these were primiparous females carrying 9 and 10 kittens. The third ferret was in its second pregnancy and was carrying 14 kittens while the fourth female was in its fourth pregnancy with 13 kittens. The morphological examination of ovaries from healthy ferrets showed the expected physiological characteristics. Ovaries of females in heat ($n = 5$) contained 3 to 9 tertiary follicles, whilst those of ferrets treated with hCG had 2 to 10 well developed corpora lutea. Animals, spayed in anoestrus, did not have any follicle or corpus luteum-like tissue on their ovaries.

In all animals with pregnancy toxemia, glucose levels were lower and BHB concentrations were

![Figure 1](image_url)
higher than those found in healthy controls ($P < 0.034$ and $0.001$; Table 1 and Figure 1). Significant differences were also found in the endocrine parameters (Table 1 and Figure 2): the insulin, T$_4$ and T$_3$ contents were lower in the four sick female ferrets than in the 14 controls ($0.0001$, $0.024$ and $0.001$, respectively). There were no differences among the three sub-groups of controls (data not shown).

DISCUSSION

In this study, the endocrine characteristics of ketonemia in ferrets with pregnancy toxemia were studied. Plasma glucose, BHB, insulin, T$_4$ and T$_3$ levels in sick animals were compared to those in healthy ferrets. Using comparison with healthy controls, significant differences were found in all these parameters. However, due to the low number of animals, the results should be treated with caution. These tendencies were comparable to those of ruminant ketonemia in late pregnancy. Although, our results generally correlated with reference ranges found in the literature (Garibaldi et al., 1988; Fox, 1998), variability was seen, probably due to the several different technical procedures among laboratories.

Whereas hematological and clinical biochemical parameters (among others, hyperketonemia, hypoglycemia and ketonuria) in pregnancy toxemia are well described in the ferret (Fox et al., 1998; Batchelder et al., 1999; Dalrymple, 2004; Lewington, 2007), the endocrine response to relative or real fasting during late pregnancy in female ferrets carrying large litters (changes in the insulin, T$_4$ and T$_3$ blood concentration) had not been studied to date.

During pregnancy, fetuses have a large glucose demand that is satisfied by the mother. If the fetal demand and the mother supply become imbalanced due to fasting of the mother or the increased nutritional demands of the rapidly developing fetal placental unit, females suffer from negative energy balance and succumb to severe hypoglycemia (Batchelder et al., 1999; Dalrymple, 2004). In our sick animals, the blood glucose concentration was lower than the normal fasting blood glucose level although the differences between the sick and healthy animals only just reached the significance level. This was most likely due to the terminal stage of the sick animals. In this disease, initially, glucose concentration is low, but in the terminal stage, it may rise even above normal levels (Fox et al., 1998).
During the decompensation of the negative energy balance (fat mobilization), liver function may be overwhelmed and hepatic lipidosis may develop. The impaired function of the liver together with the enhanced mobilization of free fat acids from triglycerides causes ketogenesis. Consequently, the level of ketones increases in the blood. This phenomenon has been observed previously (Lewington, 2007), and was also confirmed in our study. In female ferrets with pregnancy toxemia, the plasma levels of this parameter (BHB) related to the energy metabolism was elevated (1.13 ± 0.07 mmol/l) compared to the control group (0.25 ± 0.02 mmol/l). Moreover, in the sick female ferrets, hepatic lipidosis was observed post-mortem.

The response to fasting (negative energy balance) incorporates hormonal signals which initiate energy preservation. Insulin, T₄ and T₃ are important hormones in the regulation of energy homeostasis. Our data in ferrets were pro-rata similar to the ewes with pregnancy toxemia (Kulcsar et al., 2006) in that hypoinsulinemia and decreases in the T₄ and T₃ concentrations were detected. In ferrets, hypoinsulinemia may be linked to the lower than normal secretory capacity of pancreatic β-cell function in the same way as in ruminants (Harmon, 1992; Henze et al., 1998; Van Saun, 2000). In pregnant ewes, the therapeutic effect of insulin treatment demonstrated that insulin plays a causative role in the pathogenesis of ovine ketosis (Henze et al., 1998). Based on this observation, the survival rate of the affected ferrets could probably be increased with the inclusion of insulin treatment in therapeutic protocol.

The peri-parturient changes in thyroid hormones, and further, their involvement in the metabolic adaptation to negative energy balance, and in the pathogenesis of ketosis have been intensely studied in ruminants (Pethes et al., 1985; Meikle et al., 2004; Huszenicza et al., 2006). In these studies, decreases in thyroid hormone levels have been described and explained by the increased degree of their inactivation in peripheral tissues, and/or alternatively by the decreased capacity of T₄ activation, diminishing the transformation of T₄ to T₃. Decreases in the T₄ and T₃ concentrations were also detected in our ferrets with pregnancy toxemia.

To summarize the results, it can be concluded that pregnancy toxemia caused by a negative energy balance in ferrets resembles the metabolic disease of ketonemia in late pregnant ruminants and that similar endocrine changes may occur. Since the endocrine and metabolic background of pathophysiological changes has not yet been fully elucidated in ferret pregnancy toxemia, further investigations are needed to confirm our suggestion that there exists a similarity to ruminant ketosis.

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