Metabolic and endocrine characteristics of pregnancy toxemia in the ferret

A. Prohaczik, M. Kulcsar, Gy. Huszenicza

Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary

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)

Pregnancy toxemia (syn. gestational ketosis) caused by negative energy balance in late gestation is commonly observed in ewes and does (Henze et al., 1998; Rook, 2000; Van Saun, 2000; Kulcsar et al, 2006), occasionally in beef cows (Rook, 2000), and also in monogastric species (rabbits, guinea pigs, dogs and in ferrets; Bell, 1997; Pare and Murphy, 1997; O'Rourke, 1997; Batchelder et al., 1999; Dalrymple, 2004; Lewington, 2007). The background of the disease is the result of the fetal carbohydrate- or energy-demand exceeding maternal supply during the last trimester of pregnancy. In the ferret, pregnancy toxemia usually occurs between days 32 and 42 of gestation, especially just before the whelping date. It is more common in primiparous female ferrets carrying > 10 kits (Batchelder et al., 1999; Lewington, 2007) or among females carrying average litters and fed adequate diets, but where an accidental fast occurs during this period (Bell, 1997). Even one overnight fast can induce toxemia in females with a large litter. In the affected animals, clinical signs include a sudden onset of lethargy, hypothermia, dehydration, sternal recumbency with decreased awareness, open glazed eyes, black tarry stools and a doughy feeling to the skin (Wallach and Boever, 1983; Batchelder et al., 1999; Lewington, 2007). At the veterinary examination, females are usually in a critical condition; coma and death often ensue (Batchelder et al., 1999; Lewington, 2007). Laboratory testing often reveals anemia, azotemia, bilirubinemia, hypoglycemia, hypoproteinemia, high levels of hepatocellular serum enzymes, hyperketonemia and ketonuria (Batchelder et al., 1999; Lewington, 2007). Serum glucose is initially low (usually below 2.77 mmol/l), but at a terminal stage it may rise above normal to 8.32 mmol/l or more. Urea nitrogen is often greater than 35.7 mmol/l. Despite clinical dehydration, the haematocrit is usually below 30% (reference values for a pregnant ferrets are generally over 40%; Fox, 1998). At necropsy, the uterus is full of a large number of foetuses. The liver is enlarged, yellowish, and mild icterus is encountered. Histopathology reveals excessive hepatocellular lipid accumulation (Batchelder et al., 1999; Lewington, 2007).

Negative energy balance increases lipid mobilization, which results in hepatic lipidosis with subsequent impairment of hepatocellular function,

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 (T_4), and 3,3',5-triiodothyronine (T_3) 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, T_4 and T_3) 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, Leverkusesen, 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 immediately, and stored at either +4°C (≤ 48 h) until BHB and glucose determinations or at –20°C until insulin, T_4 and T_3 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-butyrate-dehydrogenase reaction (D-3-Hydroxybutyrate kit, Cat. #RB 1007, Randox Laboratories Ltd., Ardmore, UK), insulin was determined using the ^{125}I-Insulin RIA CT kit (CIS Bio International Ltd, Gif-Sur-Yvette, France), T_4 was measured using the ^{125}I-T4 RIA-Spec MIS kit (Institute of Isotopes Co. Ltd., Budapest, Hungary), and T_3 was measured using the ^{125}I-T3 RIA MIS kit

Table 1. Metabolic and endocrine characteristics of female ferrets with pregnancy toxemia and healthy female ferrets

	Female ferrets with pregnancy toxemia (n = 4)		Healthy female ferrets (n = 14)	
	min.	max.	min.	max.
Glucose (mmol/l)	3.07	5.03	5.07	6.12
BHB (mmol/l)	0.96	1.31	0.16	0.33
Insulin (μIU/ml)	1.29	2.55	9.41	19.17
T_4 (nmol/l)	12.29	26.58	23.44	37.19
T_3 (nmol/l)	0.69	0.93	0.98	1.36

(Institute of Isotopes Co. Ltd., Budapest, Hungary). 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%; T_4: 1.46 nmol/l, 6.6–8.5% and ≤ 7.7%; T_3: 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

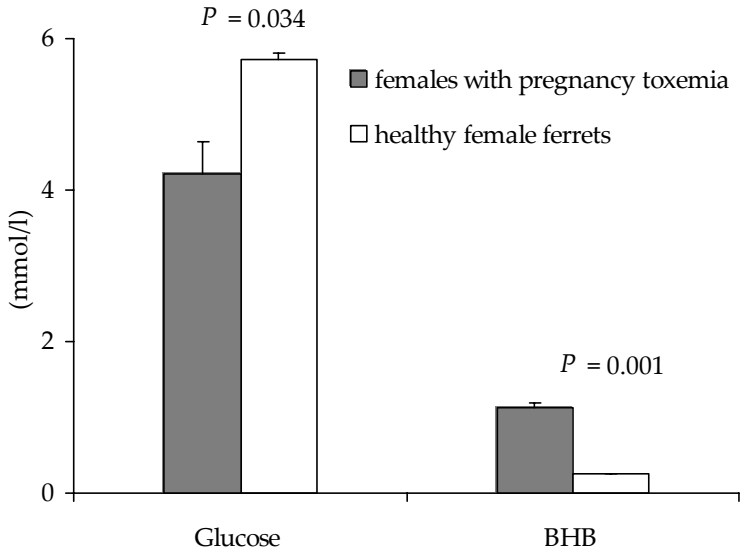

Figure 1. The plasma glucose and ketone (βOH-butyrate, BHB) levels in late pregnant ferrets suffering from pregnancy toxemia (n = 4) and from healthy non-pregnant (control) female ferrets (n = 14) (mean ± SEM)

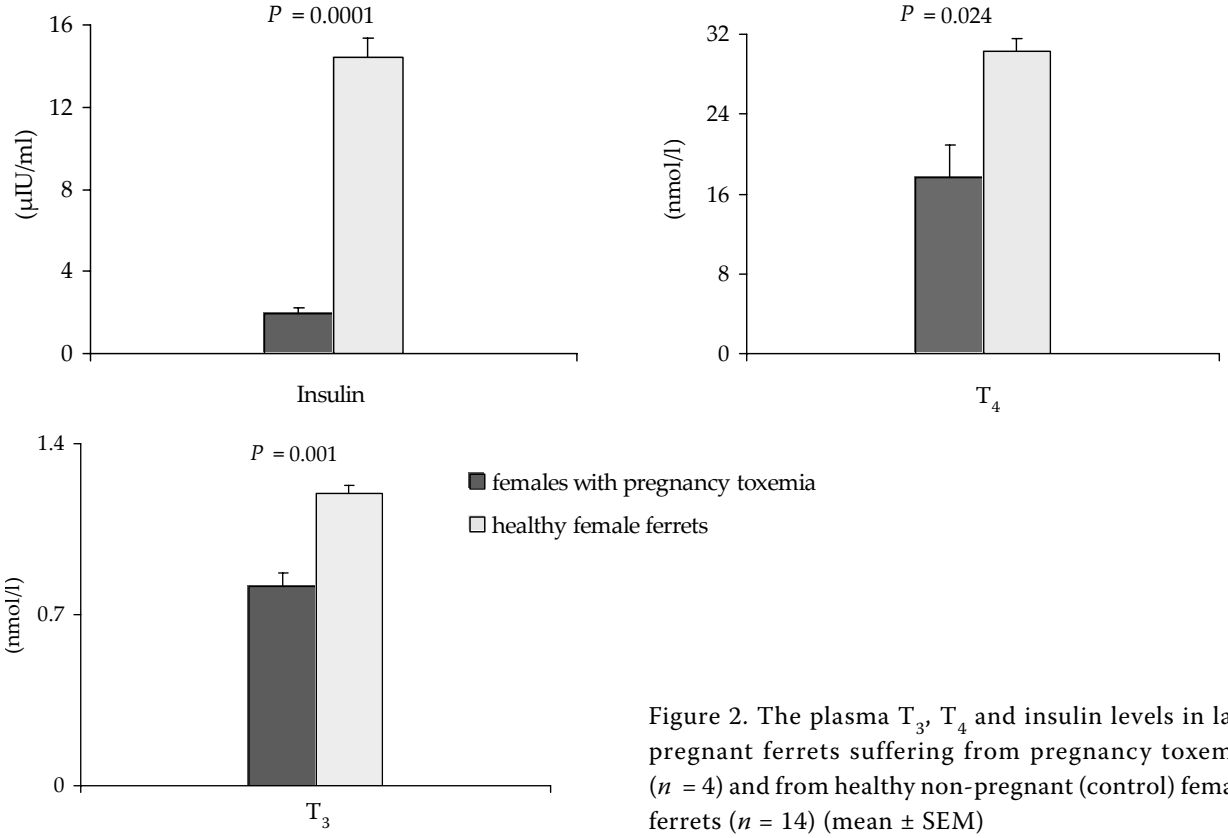

Figure 2. The plasma T_3, T_4 and insulin levels in late pregnant ferrets suffering from pregnancy toxemia (n = 4) and from healthy non-pregnant (control) female ferrets (n = 14) (mean ± SEM)

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, T_4 and T_3 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 T_4 and T_3 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 T_4 activation, diminishing the transformation of T_4 to T_3. Decreases in the T_4 and T_3 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.

REFERENCES

Balogh O., Szepes O., Kovacs K., Kulcsar M., Reiczigel J., Alcazar J. A., Keresztes M., Febel H., Bartyik J., Fekete S., Fesus L., Huszenicza Gy. (2008): Interrelationships of growth hormone *Alu*I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. Veterinarni Medicina, 53, 604–616.

Batchelder M.A., Bell J.A., Erdman S.E., Marini R.P., Murphy J.C., Fox J.G. (1999): Pregnancy toxemia in the European ferret (*Mustela putorius furo*). Laboratory Animal Science, 49, 372–379.

Bauman D.E. (2000): Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revised. In: Cronje P.J. (ed.): Ruminant Physiology: Digestion, Metabolism and Growth and Reproduction. CAB Publishing, New York. 311–327.

Bell J.A. (1997): Periparturient and neonatal diseases. In: Hillyer E.V., Quesenberry K.E. (eds.): Ferrets, Rabbits, and Rodents. WB Saunders, Philadelphia. 53–62.

Bossaert P., Leroy J.L.M.R., de Vliegher S., Opsomer G. (2008): Interrelation between glucose-induced insulin response, metabolic indicators, and the time of first ovulation in high-yielding dairy cows. Journal of Dairy Science, 91, 3363–3371.

Dalrymple E.F. (2004): Pregnancy toxemia in a ferret. The Canadian Veterinary Journal, 45, 150–152.

Fox J.G. (1998): Normal clinical and biological parameters. In: Fox J.G. (ed.): Biology and Diseases of the Ferret. 2nd ed. Williams and Wilkins, Philadelphia. 183–210.

Fox J.G., Pearson R.C., Bell J.A. (1998): Disease of the genitourinary system. In: Fox J.G. (ed.): Biology and Diseases of the Ferret. 2nd ed. Williams and Wilkins, Philadelphia. 247–272.

Garibaldi B.A., Pecquet Goad M.E., Fox J.G., Murray R. (1988): Serum thyroxine and triiodothyronine radioimmunoassay values in the normal ferret. Laboratory Animal Science, 38, 455–458.

Harmon D.L. (1992): Impact of nutrition on pancreatic exocrine and endocrine secretion in ruminants: a review. Journal of Animal Science, 70, 1290–1301.

Henze P., Bickhardt K., Fuhrmann H., Sallmann H.P., (1998): Spontaneous pregnancy toxaemia (ketosis) in

sheep and the role of insulin. Zentralblatt fur Veterinarmedizin, 45, 255–266.

Huszenicza Gy., Kulcsar M., Korodi P., Bartyik J., Rudas P, Ribiczei-Szabo P., Nikolie J.A., Samanc H., Ivanov I., Gvozdie D. (2006): Adrenocortical and thyroid function, hormone and metabolite profiles and the onset of ovarian cyclicity in dairy cows suffering from various forms of ketosis. Acta Veterinaria (Beograde), 56, 25–36.

Kadokawa H., Blache D., Yamada Y., Martin G.B. (2000): Relationships between changes in plasma concentrations of leptin before and after parturition and the timing of first post-partum ovulation in high-producing Holstein dairy cows. Reproduction, Fertility and Development, 12, 405–411.

Kahl S., Elsasser T.H., Blum J.W. (2000): Effect of endotoxin challenge on hepatic 5'-deiodinase activity in cattle. Domestic Animal Endocrinology, 18, 133–143.

Kulcsar M., Danko G., Delavaud C., Mircu C., Nikolic A.J., Gaspardy A., Cernescu H., Chilliard Y., Cseh S., Rudas P., Huszenicza Gy. (2006): Endocrine characteristics of late pregnant hyperketonaemic ewes and their reproductive performance following the induction of ovarian cyclicity out of the breeding season. Acta Veterinaria Hungarica, 54, 235–249.

Lewington J.H. (2007): Pregnancy toxaemia. In: Lewington J.H. (ed.): Ferret Husbandry, Medicine and Surgery. 2nd ed. Saunders Elsevier, Edinburgh. 94–95.

Lucy C.M. (2008): Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: Implications for postpartum nutrition and reproduction. Reproduction in Domestic Animals, 43 (Suppl. 2), 31–39.

McGuire M.A., Beede D.K., Collier R.J., Buonomo F.C., DeLorenzo M.A., Wilcox C.J. (1991): Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating Holstein cows. Journal of Animal Science, 69, 2050–2056.

Meikle A., Kulcsar M., Chilliard Y., Febel H., Delavaud C., Cavestany D., Chilibroste P. (2004): Effects of parity and body condition score at calving on endocrine and reproductive parameters of the dairy cow under grazing conditions. Reproduction, 127, 727–737.

O'Rourke D.P. (1997): Disease problems of Guinea pigs. In: Hillyer E.V., Quesenberry K.E. (eds.): Ferrets, Rabbits, and Rodents. WB Saunders, Philadelphia. 245–254.

Pare J.A., Murphy J.P. (1997): Disorders of the reproductive and Urinary Systems. In: Hillyer E.V., Quesenberry K.E. (eds.): Ferrets, Rabbits, and Rodents. WB Saunders, Philadelphia. 183–193.

Pethes Gy., Bokori J., Rudas P., Frenyo V.L., Fekete S. (1985): Thyroxin, triiodothyronine, reverse-triiodothyronine, and other physiological characteristics of periparturient cows fed restricted energy. Journal of Dairy Science, 68, 1148–1154.

Rook J.S. (2000): Pregnancy toxemia of ewes, does, and beef cows. The Veterinary Clinics of North America. Food Animal Practice, 16, 293–317.

Sartin J.L., Kemppainnen R.J., Cummins K.A., Williams J.C. (1988): Plasma concentrations of metabolic hormones in high and low producing dairy cows. Journal of Dairy Science, 71, 650–657.

Van Saun R.J. (2000): Pregnancy toxaemia in a flock of sheep. Journal of the American Veterinary Medical Association, 217, 1536–1539.

Wallach I.D., Boever W.J. (1983): Diseases of Exotic Animals Medical and Surgical Management. WB Saunders, Philadelphia. 93 pp.

Received: 2009–02–05
Accepted after corrections: 2009–02–15

Corresponding Author:

Gyula Huszenicza, Szent Istvan University, Faculty of Veterinary Science, Istvan u. 2., H-1078 Budapest, Hungary
Tel. +36 1 478 4202, E-mail: huszenicza.gyula@aotk.szie.hu