Comparison of estradiol and progesteron serum levels in ferrets suffering from hyperoestrogenism and ovarian neoplasia

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ABSTRACT: The aim of this study was to determine whether serum levels of 17beta-estradiol and progesterone are significant diagnostic tools for the confirmation of specific reproductive diseases associated with hyperestrogenism. Thirty-one adult ferrets (*Mustela putorrius furo*) were divided into five groups. The levels of serum 17beta-estradiol differed significantly when comparing females with ovarian tumors (817.80 ± 433.90 pmol/l) to intact (83.50 ± 32.53 pmol/l) and spayed, healthy females (73.17 ± 0.41 pmol/l) as well as to females with prolonged estrus (274.75 ± 192.40 pmol/l). Concentrations of serum progesterone differed significantly when comparing females with ovarian tumours (2.10 ± 1.85 ng/ml) to intact (0.40 ± 0.40 ng/ml) and spayed, healthy females (0.30 ± 0.12 ng/ml). Our study has made it clear that a determination of serum concentrations of 17-beta estradiol and progesterone is not sufficient for distinguishing between prolonged estrus and the presence of ovarian tumours. Therefore, it is advisable to employ other clinical procedures, such as ones allowing organ visualization. Increased concentrations of 17beta-estradiol in ferrets persisted for two weeks after hCG administration. This is particularly important in clinical practice, as negative effects of estrogens on bone marrow could persist for more than 14 days. Therefore, ferrets should be clinically monitored for a longer period of time.

Keywords: hyperestrogenism; neoplasia; hormonal disease; castration

Various types of endocrinopathies are among the most serious health complications frequently diagnosed in pet ferrets (Fox et al., 1987; Rosenthal et al., 1993; Lloyd, 1999; Prohaczik et al., 2009a,b). Prolonged estrus is one of the most common hormonal diseases in ferrets (Cooper, 1985; Kelleher, 2001; Pollock, 2003). Ferrets are seasonally polyestric animals with an induced ovulation (Kociba and Caputo, 1981; Brown, 1997). In the absence of male stimulation, a certain percentage of ferrets develop persisting follicles with a prolonged estrogen phase.

Pollock (2003) recorded ranges of physiological serum concentrations of estradiol from 122.0 to 210.0 pmol/l in intact, and 30.0 to 108.0 pmol/l in spayed, healthy females. Fox and Marini (1998) described the physiological range of plasma concentrations of estradiol and progesterone as 85.89 ± 73.7 pmol/l and 0.2 ± 0.3 nmol/l, respectively.

Partially supported by the Ministry of Education, Youth and Sports (Grant No. MSM 161700002).

Another possible cause of hyperestrogenism in ferrets could be an incomplete removal of ovarian tissues during castration (Brown, 1997). Patients with endocrine active tumors of the genital system represent another special group of cases (Li and Fox, 1996). Estrogen activity can be also detected in patients suffering from adrenal diseases (Rosenthal and Peterson, 1996; Rosenthal, 1997; Schoemaker et al., 2002).

For determining an optimal therapy, it is desirable to reliably and promptly distinguish between different forms of hyperestrogenism in ferrets (Ryland, 1982; Lawrence et al., 1993; Benson et al., 2000). Apart from the assessment of an anamnestic protocol and clinical examination, other possible techniques include ultrasound examination of abdominal organs and the determination of sex hormone concentrations in the blood (Neuwirth et al., 1993; Ackermann et al., 1994; Wagner and Dorn, 1994; O'Brien et al., 1996; Besso et al., 2000).

The aim of this study was to determine whether serum levels of 17beta-estradiol and progesterone are significant for the confirmation of specific reproductive diseases which are associated with hyperestrogenism.

MATERIAL AND METHODS

Animals

The ferrets included in this study were client-owned animals. A total of 31 female ferrets were divided into five groups. Group 1 comprised five patients with ovarian tumors (two ovarian leiomyomas, granulothecal ovarian tumor, ovarian cystadenocarcinoma, papillary ovarian adenocarcinoma). Group 2 consisted of eight females with clinical hyperestrogenism (prolonged estrus). Prolonged estrus was determined as a long term estrus (more than three weeks), which was clinically manifested in the form of an edematous vulva and alopecic changes. In six females from Group 3 showing symptoms of a prolonged estrus, ovulation was stimulated by the administration of hCG 7 days before determination of the 17beta-estradiol and progesterone concentrations. Group 4 included six intact healthy females. Group 5 was composed of six healthy spayed females. Hyperadrenocorticism was ruled out in all the cases, based on clinical (abdominal palpation) and ultrasonographic examination.

Blood analyses

Blood was obtained from the cranial vena cava (Jekl et al., 2005). The serum levels of 17beta-estradiol and progesterone were determined using chemiluminiscence (LEIA) detection with the Immulite Analyzer (DPC Biermann GmbH Germany). Possible haemolysis did not cause any interference. The limit ranges of 73.0–7342.0 pmol/l and 0.2–127.0 nmol/l were set for the assessment of 17beta-estradiol and progesterone, respectively. Analytical sensitivity for 17beta-estradiol was determined as 55.0 pmol/l, and 0.1 nmol/l for progesterone.

Haematology

Basic haematological tests were performed with an automatic counting system (ACT diff, Beckman Coulter); the white blood count was determined on the basis of assessment of Pappenheim-stained blood films.

Histology examination

Ferrets in Group 1 underwent laparotomy with neoplastic mass excision. Ovariohysterectomies were conducted in all animals from Group 5. Histology samples were obtained from all the parts of the genital system and from the neoplastic masses. Results were evaluated by two independent laboratories.

Statistical analysis

Results were processed with non-parametric tests. A Kruskal-Wallis test was employed in the whole patient set and a Steel-Dwass test was used for the pair-wise comparison of the groups (program KyPlot).

RESULTS

The average serum levels of 17beta-estradiol and progesterone in ferrets from the different groups are shown in Table 1.

The female ferrets diagnosed with ovarian tumors showed increased serum levels of progester-

Table 1. Concentration of 17beta-estradiol and progesteron in ferrets

Group No.	Characteristic	17beta-estradiol (pmol/l)		Progesterone (ng/ml)	
		min–max (range)	$\bar{x}$ ± SD	min–max (range)	$\bar{x}$ ± SD
1 (n = 5)	females with ovarian or uterine tumors	170–1 377.00	817.80 ± 433.9	0.67–5.00	2.10 ± 1.85
2 (n = 8)	females with clinical symptoms of hyperestrogenism	133–716.00	274.75 ± 192.4	0.20–7.50	1.10 ± 2.58
3 (n = 6)	females with clinical symptoms of hyperestrogenism treated with hCG	< 73–215.00	144.00 ± 48.33	0.70–20.00	15.3 ± 6.31
4 (n = 6)	intact healthy females	< 73–136.00	83.50 ± 32.53	0.20–0.30	0.40 ± 0.40
5 (n = 6)	spayed healthy females	< 73–74.00	73.17 ± 0.41	0.20–0.50	0.30 ± 0.12

one (2.10 ± 1.85 ng/ml), and very high levels of 17beta-estradiol (817.80 ± 433.90 pmol/l). For the individual tumors, the estradiol levels were ovarian leiomyoma (1 377.00 pmol/l), granulothecal ovarian tumor (946.00 pmol/l), papillary ovarian adenocarcinoma (756.00 pmol/l) and ovarian cystadenocarcinoma (840.00 pmol/l). Serum concentrations of 17beta-estradiol in females suffering from ovarian tumors reached the highest levels (817.80 ± 433.90 pmol/l). These values differed significantly ($P < 0.05$) from serum levels in the intact /spayed healthy females, but were not significantly different from estradiol levels in the ferrets with prolonged estrus. Significant differences ($P < 0.05$) were also detected between Group 2 (females with clinical hyperestrogenism) and Groups 4 and 5 (intact healthy females, spayed females).

There were significant differences ($P < 0.05$) after pair-wise comparisons of serum concentrations of progesterone between Group 1 and Groups 4 and 5; and between Group 3 (ferrets with clinical hyperestrogenism treated with hCG) and Groups 2, 4 and 5 ($P < 0.0$).

All haematological parameters of all the ferrets were within reference ranges (Brown, 1997).

DISCUSSION

In our study, the average serum concentration of 17beta-estradiol in intact healthy females was 83.50 pmol/l and 73.17 pmol/l in spayed healthy females. These results are similar to the ones described by Fox and Marini (1998).

Extremely high estrogen concentrations, and, in particular, its long-term effect on the bone marrow may result in aplastic, possibly fatal anaemia (Bernard et al., 1983; Sherill and Gorham, 1985; Pearson, 1999; Pollock, 2003). However, in our study, we did not record anaemia in ferrets with extremely high estradiol levels suffering from ovarian tumors.

We observed that females showing clinical symptoms of hyperestrogenism without the occurrence of tumors had average serum levels of 17beta-estradiol (274.75 ± 192.40 pmol/l), which were significantly higher than in the spayed and in the intact healthy females. The estradiol concentrations were significantly lower (to 144.00 ± 48.33 pmol/l) and at the same time the concentrations of progesterone were higher (15.30 ± 6.31 ng/mol) in ferrets with hyperestrogenism after hCG treatment than in ferrets with hyperestrogenism without treatment. This implies an influence of hCG on the ovulation and accession of the luteal phase. The difference in the concentrations of 17beta-estradiol between Group 2 and 3 was obvious, but not statistically significant. These results suggest that increased concentrations of 17beta-estradiol in ferrets could have a long-term effect after hCG administration. This is particularly important in clinical practice, as negative effects of estrogens on bone marrow could persist for more than 14 days. Therefore, ferrets should be clinically monitored for a longer period of time.

From the clinical point of view, it is important to distinguish between patients with functional ovaries/persisting follicles and those which develop ovarian tumors. Our study has made it clear that a determination of serum concentrations of 17-beta estradiol and progesterone is not sufficient to differentiate between the different diseases.

Therefore, it is advisable to use other clinical procedures, such as ultrasonography. However,

the presence of ovarian tumors should still be suspected at serum estradiol levels of more than 500 pmol/l.

Acknowledgments

The authors are grateful to Ms. Paulikova and Ms. Karesova (University of Veterinary and Pharmaceutical Sciences, Brno) for their skillful technical assistance. The authors would like to thank the Departments of Pathology at the Universities of Brno and Utrecht for the histological examinations.

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Received: 2009–03–09
Accepted after corrections: 2009–11–11

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