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SHORT COMMUNICATION

Free Estriol Periconceptional Serum Concentrations in American Mink

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A B S T R A C T

An attempt was made to determine the concentration of unbound estriol (E3) in the blood of female mink in the periconceptional period in terms of the possible use of this hormone in the diagnosis of pregnancy. The studies involved 54 Black female mink. Blood was collected before mating, on 27 February (non-pregnant females), and after mating, on 5 April. The E3 serum concentrations before mating were statistically lower ($P \leq 0.01$) compared to those measured after mating. No significant differences in E3 concentration between females of different gestational status were found. Striking is the high number of females that had been marked as mated, yet never gave birth (approx. 31%). Free estriol serum concentrations over the first 10 days of putative gestation is not a good tool in mink pregnancy detection.

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INTRODUCTION

Estrogens, the most important female hormones, include estriol, estron, and estradiol. Estriol, the weakest of estrogens, is an important biochemical marker used in the diagnosis of fetal health and wellbeing in human pregnancy (Kaur *et al.*, 2013; Olsen *et al.*, 2014). In women, there is a direct correlation between estriol concentrations and gestational age at the time of delivery (Olsen *et al.*, 2014). Hydroxylation of DHEA, a subsequent stage in estriol synthesis, takes place nearly entirely in the fetal liver and results in the synthesis of 16-alpha-hydroxy-dehydroepiandrosterone. This is transferred to the placenta to be hydrolyzed under placental sulfatase into free alpha-hydroxy-dehydro-epiandrosterone, which – still in the placenta – undergoes final aromatization into estriol (E3) (Yaron *et al.*, 1999).

The available literature lacks information on periconceptional estriol in animals in terms of its diagnostic application. Hence, this attempt was made to clarify whether pregnancy in mink can be detected by periconceptional levels of estriol. A positive answer to this question should also demonstrate whether estriol serum levels in a pregnant female could allow estimations of the prospective litter size. This assumption is justified, since fetuses participate in estriol production; hence, their number might hypothetically have an effect on the dam's serum concentrations of this hormone.

Considering the knowledge resulting from studies on women, an attempt was made to determine the concentration of unbound estriol in the blood of female mink before and during the periconceptional period in terms of the possible use of this hormone in the diagnosis and monitoring of pregnancy.

MATERIALS AND METHODS

Animals and blood sampling: The studies involved 54 Black female mink bred on a fur farm in north-western Poland. All the animals remained under the same environmental conditions and were offered the same feeding throughout the period of the experiment. The diet was based on a standard feed containing in the wet weight approx. 30% dry matter, 3% ash, 14% protein, 7% fat, and 8% carbohydrates, as well as about 5 MJ metabolizable energy.

Blood was collected twice. On 27 February blood was drawn from 40 females which were considered non-pregnant (control), and on 5 April blood was sampled from 37 mated females, the same as those sampled on the previous date plus 17 other females considered unmated. All the studied females were mated starting from 5 March. Two of the 37 mated females were culled from the herd during the study period. The gestational status of the females sampled for blood was on the second date unknown, and was retrospectively determined after whelping; the females that finally delivered offspring

were marked as pregnant, whereas those that failed to give birth were considered non-pregnant. The date of implantation was calculated by subtraction of 31days from the date of whelp. The procedure of blood sampling have been described in our previous report (Lasota *et al.*, 2014). The collected blood samples were used to obtain serum, which was next frozen until further analyses.

Estriol measurement: For free estriol quantitative measurements, we used the Free Estriol ELISA (DiaSource, Belgium) kit. The sensitivity of the assay is 0.26 nmol/l in the range of 0-138,66 nmol/l. Two readings were done for each sample using the ELx800 microplate reader (Bio-Tek).

Statistical analysis: Statistical analysis was performed using STATISTICA v.10 (StatSoft, Poland). The data were analyzed using cross-sectional descriptive statistics, the Levene test of homogeneity of variance, and the Shapiro-Wilk normality of distribution test. In consequence, we applied nonparametric statistics: Spearman's rank correlation, Kruskal-Wallis ANOVA, and median test in order to compare the assays and correlations.

RESULTS AND DISCUSSION

Estriol concentration before the mating season, on 27 February, when the females were certainly non-pregnant, was 1.25±0.69 nmol/l and was significantly lower ($P \leq 0.01$) than on 5 March, when it was 1.59±0.66 nmol/l. Table 1 presents serum E3 concentrations in females differing by reproductive status. Striking is the high number of the females that had been marked as mated, yet never gave birth (approx. 31%), a wrong qualification to the group of unmated females occurred only in a single case. No significant differences in E3 concentration between females of different gestational status were found. The gestation period did not significantly differentiate serum E3 levels on 5 April in the studied mink, which probably resulted from a small group of samples on each day of pregnancy (Fig. 1).

There is very little information in the subject literature on E3 concentration in animal blood, and virtually no information on any studies of this hormone in mink in the periconceptional period. In this study we simply observed a significant increase in serum E3 levels between samples collected on 27 February and 5 April. On the first date the females were with absolute certainty non-pregnant, whereas the gestational status of the females on the latter date was diverse. However, the gestational status of the females was unrelated with the E3 concentrations. The observed E3 concentrations with a strong probability exclude a contribution of placenta/fetal estriol in the total level of this hormone in mink females. This presumably results from the fact that the placenta does not achieve its full functional capacity within the 1st week following implantation. Studies by Pfarrer *et al.*, (1999) on mink show that placenta in early pregnancy is not morphologically fully developed. The literature lacks information on when the placenta becomes fully functional in mink.

Table 1: Reproductive status and E3 serum concentration in mink

Status	Number (%) of animals	Mean±SD (median) [nmol/l]
Mated	37* (100)	1.42±0.62 (1.21)
Whelping	24 (68.6)	1.32±0.55 (0.32)
Non-whelping	11 (31.4)	1.49±0.59 (0.40)
Unmated	17 (100)	1.70±0.76 (0.44)
Whelping	1 (6)	1.66±0.00 (1.66)
Non-whelping	16 (94)	1.70±0.76 (1.52)
Total		
Whelping	25**	1.32±0.55 (1.11)
non-whelping	27	1.63±0.69 (1.42)

*2 females culled from herd; **including whelping female previously marked as non-mated

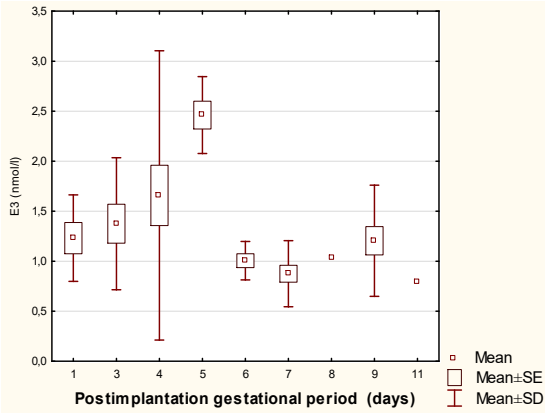

Fig. 1: Mean concentration of E3 (nmol/l) on 5 April in relation to post-implantation gestational period

In the present study, the earliest implantation took place on 23 March, hence the longest gestation period on the day of blood sampling (5 April) was 11 days and most females (76%) was in the first week of gestation. Consequently, this can be assumed to be the first trimester of pregnancy in mink. In women, estriol is first detected at 9 weeks of gestation and peaks at 31-35 weeks (Yaron *et al.*, 1999). Therefore, by analogy to human estriol pattern of changes, one cannot expect a significant increase in mink fetal estriol during this period. Nevertheless, studies on human pregnancy also show low estriol levels in the 2nd trimester, similar to those in mink in the first week after implantation. Boulis *et al.*, (2014) recorded E3 in the concentration of 1.05 nmol/l. Tang *et al.*, (2013) observed in the 14th week the concentration of unconjugated E3 of 3.45 nmol/l). The difference in the concentration patterns of E3 between pregnant and non-pregnant female mink and women may be a result of different types of placenta. Carnivores, which include mink, have a zonary endotheliochorial placenta (Wooding and Burton, 2008). According to Hoffmann and Schuler (2002), estrone-3-sulfate is the major placental estrogen in cows. This species has a different type of placenta, thus the finding of these authors allow us to propose that the varied relationship between particular estrogens may be a result of the difference in the construction of the placenta.

The large discrepancy between the estimated number of females that had been fertilized (mated) and the number of females that later failed to deliver offspring (about 31% females) may have resulted from either pre- or post-implantation embryonic mortality. McRae (1992) believes that the mortality of embryos after implantation may be 40%.

Conclusions: E3 concentration increased significantly between the preconceptional and periconceptional periods, though the increase probably results from an elevated production level of this hormone by the females. No significant increase in E3 has been observed over the first 10 days of pregnancy. There were no significant differences in serum E3 concentrations between mated and unmated mink in the periconceptional period. Consequently, free estriol serum concentrations over the first 10 days of putative gestation is not suitable for a mink farmer as a tool of pregnancy testing. Further studies are recommended in order to find out why a high number of females that are considered as mated do not actually give birth.

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