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Development of the independent function of fetal thyroid glands in the dog in connection with iodothyronine concentrations in pregnant bitches, fetal fluids, and fetal serum

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ABSTRACT

Thyroxine (T_4) and triiodothyronine (T_3) concentrations in pregnant and nonpregnant bitches were measured. The allantoic and amniotic fluid samples were collected separately in the third week of pregnancy, and fetal blood samples were collected in the fourth week of pregnancy. There was no difference between T_4 results in the pregnant and nonpregnant animals, but the measured serum concentrations exceeded the healthy range for normal adults. Serum T_4 concentrations were lower in the fetus than those in adults ($P < 0.01$). Fetal T_4 concentrations continuously increased and reached 13.38 ± 6.19 nmol/L before birth. The fetal serum T_4 concentrations were lower than the T_4 concentrations in allantoic and amniotic fluid until the seventh week, and the fetal serum T_3 concentrations were lower than those in fetal fluids throughout the pregnancy ($P < 0.01$). Maximum T_3 concentrations in allantoic and amniotic fluid exceeded the concentrations in the fetal and maternal serum. It is conceivable that the considerable differences between maternal and fetal serum T_4 concentrations in healthy animals are explained by the T_4 impermeability of the placenta. Extremely high maternal T_4 (193.5 nmol/L) in 1 bitch was associated with T_4 concentrations under the detection limit in the fetal fluids and serum suggesting an inhibitory effect. The T_4 concentrations in all the fetal fluids and serum were under the detectable concentration that can be defined by 3.0 nmol/L in that bitch. We have demonstrated that fetal thyroid glands start functioning independently at the same time as thyroid cell formation in the dog, but the overproduction of maternal T_4 may have a suppressive effect on fetal iodothyronine production.

1. Introduction

Pregnancy involves endocrine and metabolic changes, which lead to the formation of a physiologic boundary between mother and fetus. The endocrine and metabolic changes occurring during pregnancy are directly attributable to hormonal signals of maternal and fetal origin. The fetus develops in an environment where respiration, alimentation, and excretory functions are provided by the mother through the placenta, and as a result of fetal development, newborns are capable of independent life. The placenta of domestic carnivores is endotheliochorial and provides a close connection between the maternal and fetal circulations. This relationship allows the placenta to efficiently transfer some substances to the fetus, although simultaneously serves as a barrier between the fetus and the mother

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[1]. Fisher et al (1977) demonstrated that the hypothalamus-pituitary-thyroid axis of human fetuses functions independently of the maternal endocrine system and that the human placenta is impenetrable for maternal thyroxine (T_4) [2,3]. Deiodinase activity of the placenta is documented in human subjects, experimental rodents, pigs, and dogs [4–7].

The aim of our study was to investigate fetal development of thyroid function in dogs by measuring T_4 and triiodothyronine (T_3) concentrations in different fetal fluids and comparing them to the maternal concentrations.

2. Materials and methods

Animal care and experimental procedures described in this study were performed in accordance with the Guidelines for Animal Experiments of Szent István University with the approval of the Institutional Animal Care and Use Committee (PEI/001/652-2/2015). Serum samples from 83 pregnant mismated bitches, that were being ovariohysterectomized at the request of their owners, were collected between 8 and 9 AM, before induction of anesthesia. The patients were healthy and free from any concomitant metabolic disease based on preanesthetic examination. Weeks of pregnancy were calculated from the date of unwanted mating, which was observed by the owners. The number of fetuses varied between 3 and 9 in the pregnant animals. Altogether, 513 fetuses were examined, representing 4 to 11 fetuses per pregnant animals. A mean of 9.5 (range of 7–12) different mothers were sampled at each week of pregnancy as described below. The allantoic and amniotic fluid samples were collected separately from the uterus after ovariohysterectomy. Fetal fluid samples were collected from the third week of pregnancy and, due to technical reasons, fetal blood samples were collected from the fourth week of pregnancy. Blood samples of fetuses were collected by cardiac puncture. Control blood samples were collected from 47 mismated but nonpregnant bitches at the time of elective neutering. These dogs were confirmed as being in metestrus by vaginal cytology and a serum progesterone (P_4) concentration greater than 2 ng/mL [8,9]. The number of weeks of pregnancy or metestrus in both groups was calculated from the date of mismating and recorded. Part weeks were included in the subsequent week so, for example, 10 d after misalliance was classified as week 2. To separate serum, blood was centrifuged (2000 × *g*, 5 min, room temperature). The P_4 concentrations of adult serum samples were determined within 2 h of collection, but the adult thyroid hormone and fetal serum hormone concentrations were measured at a later time. The separated sera and fetal fluids were frozen and stored at −20°C until T_4 and T_3 measurement. Serum T_4 and T_3 concentrations were measured with enzyme immunoassays, validated for dogs, according to the instructions of the manufacturer (unpublished data) (DRG Total T_4 and DRG Total T_3 Diagnostic Systems Laboratories, Inc Webster, USA). The sensitivity of the T_4 ELISA was 3 nmol/L, the interassay coefficient of variation (CV) was 2.80%, and the intra-assay CV was 2.50%. The sensitivity of T_3 was 0.2 ng/mL, the interassay CV was 1.4%, and the intra-assay CV was 3.2%. The normal T_4 concentration in healthy adult dogs is 20 to 45 nmol/L, and the normal T_3 concentration is 0.6 to 2.5 ng/mL in these assays.

Data were analyzed by SigmaPlot 12.0 software (Systat Software, San Jose, CA, USA). The level of significance was $P < 0.01$ in repeated-measurement 1 way ANOVA. The correlation of changes in maternal and fetal iodothyronine concentrations was tested by Pearson's product-moment correlation coefficient.

3. Results

Mean T_4 concentrations of pregnant females and nonpregnant females exceeded the normal healthy range in every week of the luteal phase. The adult serum T_4 concentrations decreased into the normal range only at the ninth week of pregnancy or metestrus. The means ± SD varied in pregnant animals between 43.33 ± 10.92 and 52.39 ± 15.55 nmol/L in the different weeks of pregnancy. Means ± SD of nonpregnant animals in the luteal phase fluctuated between 48.34 ± 5.44 and 55.44 ± 11.53 nmol/L. There were no differences among the results of pregnant and nonpregnant animals (Fig. 1). The mean serum T_4 concentrations were lower in fetuses (1.17 ± 1.16 nmol/L) than those in adults ($P < 0.01$; Fig. 1). A slow increasing tendency was observable in T_4 concentrations from the fourth week of pregnancy, but a remarkable increase was measured in 8- and 9-wk-old fetuses (mean ± SD, 9.19 ± 3.25 nmol/L and 13.38 ± 6.19 nmol/L; Fig. 2). Although the fetal serum T_4 concentrations at fourth and fifth week differed from results of any other weeks and the results of sixth and seventh week were different from concentrations of ninth week, at the level of significance, but the allantoic and amniotic T_4 concentrations were not different from week to week (Fig. 3). In contrast to T_4, an opposite

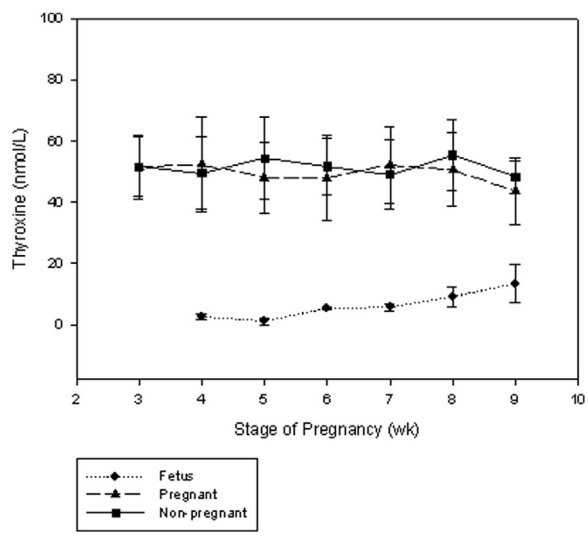

Fig. 1. The weekly changes in T_4 concentrations in adult bitches in the luteal phase exceeded the normal reference range (20 to 45 nmol/L), in each stage of the pregnancy. Fetal T_4 concentrations were much lower than that of the adults and were lower than the normal range but increased continuously until birth. Thyroxine concentrations of adults differed from fetal results at each week of pregnancy ($P < 0.01$).