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Thyroxin and progesterone concentrations in pregnant, nonpregnant bitches, and bitches during abortion

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ABSTRACT

Serum progesterone and thyroxin concentrations were measured weekly until 61 to 62 days after ovulation in 24 pregnant bitches and in the control group of nine nonpregnant bitches in the luteal phase. Fourteen of the 24 dogs had a normal pregnancy and parturition. Ten of the 24 dogs showed mucinous or colored vaginal discharge, decreased appetite, or lethargy. These initial signs of abortion or fetal resorption were noted during the fourth week of pregnancy, and the process occurred over the next 2 weeks. Progesterone and thyroxin concentrations were measured by quantitative ELISAs validated to dog serum. The serum progesterone concentrations of the group going through abortions differed significantly from the third week until the end of the eighth week. The mean serum thyroxin concentrations of healthy pregnant and nonpregnant groups significantly exceeded the reference range (20–45 nmol/L). The serum thyroxin concentrations in the abortion group were between 16.15 ± 3.17 and 40.78 ± 8.97 nmol/L. The values in this group were significantly different from the other two groups at the third week of the luteal phase. Clinical signs of abortion or fetus resorption manifested in midpregnancy. The clinical signs of abortion coincided in each case with a low serum progesterone concentration (<10 ng/mL). This phenomenon indicated, in contrast with other studies, that the decrease of serum progesterone below 10 ng/mL at the fourth week of pregnancy may signal impending abortion. In the second half of pregnancy, the thyroid gland was not able to respond adequately to the elevated requirement in thyroid hormone, although in other periods of the ovarian cycle, there were no clinical signs of hypothyroidism.

1. Introduction

Numerous hormonal changes and metabolic demands occur during pregnancy, resulting in complex changes in maternal thyroid function. Despite these changes in thyroid function, normal pregnancy is recognized as a euthyroid state. The prevalence of hypothyroidism in women of childbearing age is relatively high and is associated with menstrual abnormalities, anovulation, and hyperprolactinemia in some cases [1]. The decreased thyroid hormone concentration inhibits follicular development, and therefore, ovulation might fail. The incidence of hypothyroidism during pregnancy in human subjects ranges between 0.3% and 0.7% [2] and carries certain risks, such as increased incidence of miscarriage, placental abruption, and poor perinatal outcome with low birth weight [2–4]. Pregnancy is a state of dynamic changes in metabolism, with accumulation of lipids and nutrients during about the first half. During late pregnancy and lactation, these accumulated reserves are used for fetal growth and subsequently for milk production. Thyroid hormones markedly influence these processes. Thyroid hormones also have documented actions on the secretion of hormones involved

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in reproduction and the maintenance of pregnancy. The action of thyroid hormones is explained by the presence of thyroid-stimulating hormone receptors and thyroxin in ovarian tissue in human subjects [5]. Thus, some of the harmful effects of hypothyroidism on pregnancy may be due to changes in the hormonal balance rather than direct effects of hypothyroidism on metabolism. Overt abnormalities in thyroid function are common endocrine disorders affecting more than 19.2% of pregnant women in certain geographic areas of Hungary [6]. Pregnancy increases maternal requirements for thyroxin, which necessitates a 25% to 50% increase in maternal thyroxin production in euthyroid women. The amount of excess thyroxin increases by the end of the first trimester because of the increase in serum concentration of thyroid-binding globulin. In humans, the placenta produces large amounts of hCG, which has some thyrotropin-like bioactivity [7,8]. Human chorionic gonadotropin concentrations increase rapidly after implantation, ensuring sufficient progesterone (P4) concentrations to maintain the pregnancy until placental production is adequate.

In dogs, P_4 continuously increases after ovulation, reaching a peak around Days 20 to 30 of pregnancy; then, it slowly decreases until before whelping when it rapidly breaks down. The CL is the primary source of P_4 during pregnancy in dogs, as the placenta does not secrete P_4 [9]. Our knowledge is narrow concerning roles of placenta-derived hormones in dogs. The well-known, important luteotroph factor is prolactin, which helps to maintain pregnancy in dogs along with a decrease in P_4. Regardless of slow decrease, P_4 concentration is high until the last days of pregnancy. Although few case reports deal with decreased CL function, there has to be an explanation for it. The aim of our study was to examine the possibility of a correlation between ovarian and thyroid function.

2. Materials and methods

2.1. Animals and samples

Twenty-four pregnant, privately owned bitches from different breeds were examined. Blood samples were collected weekly, and results were compared with results of nine nonpregnant bitches in the luteal phase as controls. The age, body weight, and breed of the animals are detailed in Table 1. Concerning the serum thyroxin (T_4) concentrations, it was a blinded study as selection criteria for inclusion in the study were the pregnancy and nonpregnant luteal phase. Only the P_4 concentrations were known in the time of follow-up, and the three study groups were defined as healthy pregnant group (P), healthy nonpregnant control group (NP), and abortion group (A). Blood samples were collected weekly until 61 to 62 days after ovulation (Day 0). Fourteen dogs had a normal pregnancy and parturition. Ten dogs of the 24 dogs did show mucinous vaginal discharge, decreased appetite, or lethargy. In spite of suspected diminished CL function in those 10 dogs, the owners refused treatment with natural P_4 to prevent the impending abortion. The first sign of abortion or fetal resorption was noted at the fourth week of pregnancy, and the process was continued over the following 2 weeks. Samples from nonpregnant, control bitches were collected from the time of ovulation (Day 0), which was determined by vaginal cytology and measurement of serum P_4 concentration. The reproductive history of the dogs in the three groups is summarized in Table 2.

Table 1
Breed, average body weight, and average age of dogs in the three examined groups.

Group (N)	Age (y), mean (range)	Body weight (kg), mean (range)	Breed (n)
Pregnant (14)	3.95 (2–6.25)	32.11 (15.3–62.4)	Golden Retriever (4) Hungarian Vizsla (2) Irish Setter (2) Samoyed (2) Collie (1) Kerry Blue Terrier (1) English Bulldog (1) Mastino (1)
Abortus (10)	4.35 (2.25–6.00)	32.00 (15.9–56.9)	Golden Retriever (3) English Bulldog (2) Border Collie (1) Malamute (1) Irish Setter (1) Bohemier (1) Samoyed (1)
Nonpregnant (9)	4.39 (2.75–6)	32.57 (11.2–56.2)	English Bulldog (2) Golden Retriever (1) German Shepherd (1) Pointer (1) Samoyed (1) French Bulldog (1) Bordeaux Dog (1) German Shepherd (1)

The animals were privately owned bitches, kept for breeding purposes. There were no significant differences in age or body weight of animals between the three groups, and the breeds are common, popular breeds in Hungary.

Animal care and experimental procedures described in this study were done 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). Blood samples were collected weekly from the cephalic vein in vacuum tubes without anticoagulants (Vacuette; Greiner Labortechnik), between 8 AM and 9 AM. To separate serum, blood was centrifuged (2000 × *g*, 5 minutes, room temperature). The separated serum was stored at 4 °C and assayed by P_4 ELISA within 4 hours [10]. Samples were frozen and kept at −18 °C until the T_4 assay. The pregnancy was diagnosed and monitored by ultrasonography (ALOKA SSD-3500).

2.2. ELISA

Serum P_4 concentration was measured by Quanticheck ELISA validated for dog serum (Veterinorg Ltd., Budapest, Hungary); the interassay coefficient of variation (CV) was 5.25%, and the intra-assay CV was 2.50% [10].

Serum T_4 concentration was determined by an ELISA test validated for dog serum (DRG Total T_4; Diagnostic Systems Laboratories, Inc., Webster, USA); the interassay CV was 2.80%, and the intra-assay CV was 2.50%.