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Effect of short-term hypothyroidism on reproduction in the bitch

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Abstract

Hypothyroidism in bitches has been reported to cause a variable interestrus interval, infertility, abortion, and stillbirth. The objective of this study was to evaluate the effect of experimentally induced hypothyroidism in bitches on fertility, pregnancy, parturition, and neonatal health. Eighteen healthy multiparous bitches were used; hypothyroidism was induced (by radioiodine administration) in nine bitches and the remaining nine served as untreated controls. After breeding, bitches were evaluated for pregnancy, fetal resorption, gestation length, litter size, duration and strength of uterine contractions (during parturition), interval between delivery of pups, viability of pups at birth, periparturient survival, and weight of pups at birth through 4 weeks of age. Bitches were bred a median of 19 weeks after induction of hypothyroidism. All bitches became pregnant and delivered term litters. There was no difference in the interestrus interval, litter size, or gestation length between hypothyroid and control bitches. Duration of uterine contractions was longer, but contraction strength was weaker in hypothyroid than control bitches; however, the interval between delivery of pups was not affected. Periparturient puppy mortality was significantly higher in litters from hypothyroid bitches. Viability scores and weight at birth were significantly lower in pups from hypothyroid bitches than controls. There was no difference between groups in pup weight gain during the first 4 weeks, in the interval from birth to the eyes opened, or to the onset of walking. Although hypothyroidism of relatively short duration did not affect fertility, it prolonged parturition and reduced pup survival in the periparturient period.

Keywords: Hypothyroidism; Bitch; Infertility; Parturition; Neonatal period

1. Introduction

Hypothyroidism is reported to be a common cause of reproductive failure in dogs. Unfortunately, this manifestation of hypothyroidism is not well documented [1,2]. Infertility, prolonged interestrus interval, abortion, and stillbirth were reported in a colony of Borzoi bitches with lymphocytic thyroiditis and hypothyroidism [3]. In addition, abnormal estrus was reported in 5 of 53 hypothyroid bitches, although the number of intact versus spayed bitches was not mentioned [4]. A brief report suggested that hypothyroidism was a common cause of infertility in bitches, causing anestrus and a shortened interestrus interval [5]. Contrary to this evidence, a study comparing thyroid function in 36 greyhound brood bitches with poor reproductive performance versus 60 with good performance failed to show evidence that hypothyroidism was related to infertility [6]. These conflicting results leave the question of the relationship

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between hypothyroidism and infertility in the bitch unanswered.

Infertility and menstrual irregularities are common in women with untreated hypothyroidism. Oligomenorrhea, amenorrhea, or excessive menstrual bleeding occur in 24–68% of hypothyroid women [7,8]. Failure to ovulate is also common in hypothyroidism [9]. When pregnancy is established in hypothyroid women, they frequently suffer several problems, including placental abruption, abortion, premature birth, and fetal distress during labor [10]; abortion occurred in 60–71% of hypothyroid women when levothyroxine dosage was inadequate, compared with no abortion in women with adequate treatment of hypothyroidism [11]. Overall, between 20 and 40% of hypothyroid women experience complications with pregnancy [12]. Hypothyroidism affects other species as well, causing infertility, abnormal estrous cycles, and a decrease in viable offspring [13–15].

Based on findings in other species and poorly documented reports in dogs, we hypothesized that experimentally induced hypothyroidism will result in prolonged or irregular interestrus interval and infertility. When pregnancy occurred, we anticipated increased fetal death, abortion, stillbirth, and a smaller number of pups per litter in hypothyroid bitches.

2. Materials and methods

2.1. Animals

Eighteen healthy bitches and two male mongrel dogs, aged 25–39 months and weighing 7.5–12 kg were studied. The animals were obtained from a commercial breeder. The bitches had been bred twice ($n = 13$) or three ($n = 5$) times and had normal litters on the two most recent breedings. The two male dogs had documented normal fertility, siring 11 and 17 normal litters, respectively. Semen quality was assessed by manual ejaculation and routine evaluation of volume, concentration, motility, and morphology. Both males had >90% motility and >70% morphologically normal sperm, based on phase-contrast light microscopy, as well as normal semen volume and sperm concentration. All bitches were be determined to be normal, based on lack of significant abnormalities on physical examination, complete blood count, serum chemistries, urinalysis, heartworm antigen test, and zinc sulfate fecal floatation. Serum concentrations of total thyroxin (T4), free T4 by equilibrium dialysis, and endogenous canine thyroid stimulating hormone (TSH) were within respective reference ranges. The study was approved by the Virginia Tech Animal Care and Use Committee.

2.2. Induction of hypothyroidism

Following 12–18 weeks of acclimation and data collection, hypothyroidism was induced in nine randomly selected bitches by intravenous administration of 1 mCi/kg of ^{131}Iodine (Cardinal Health, Charlottesville, VA, USA). Hypothyroidism was confirmed 9 weeks and again 38–45 weeks after ^{131}I by measurement of serum T4 concentrations before and 4 h after administration of 50 μg human recombinant TSH (Thyrogen®, Genzyme Corp., Framingham, MA, USA) below 7 nmol/L [16,17]. This model of experimental hypothyroidism has been used previously to successfully induce hypothyroidism [2,18]. The remaining nine untreated bitches acted as controls.

2.3. Reproductive studies

The interestrus interval was calculated from recorded observations after arrival, as well as those from the facility where the dogs were purchased. Bitches were monitored daily for signs of proestrus (vulvar swelling and bleeding). Day 1 of proestrus was defined as the first day of bloody vaginal discharge. Beginning 5 days after the onset of proestrus, vaginal cytology and blood samples were collected every second day through the day of ovulation; blood sampling was discontinued when the serum progesterone was ≥5 ng/mL. Immediately after blood collection, serum progesterone concentrations were determined with a commercial semi-quantiative enzyme-linked immunosorbent assay (TARGET®, Biometalics, Inc., Princeton, NJ, USA). In addition, to determine ovulation, serum progesterone concentration was measured using a radioimmunoassay (Diagnostic Products, Corp, Los Angeles, CA, USA); ovulation was defined as the first day the serum progesterone concentration was ≥5 ng/mL.

Bitches were bred by one of two studs every other day for at least two breedings in the periovulatory period, with at least one breeding occurring after ovulation. Receptiveness to the stud was subjectively assessed to address any decrease in libido induced by hypothyroidism. Libido was graded as good if the bitch actively solicited attention from the stud, fair if receptive but only mildly solicitous of the male, and poor if the bitch did not initiate interaction with the male but was receptive to mating.

Ultrasonographic examinations were performed weekly, beginning 21 days after ovulation, to confirm

Table 1
Viablility score for puppies (assessed immediately after birth); total score equals the sum of all five end points

End point	Score		
	0	1	2
Activity, muscle tone	Flaccid, weak	Some tone to extremities	Active movement, flexion
Pulse (beats/min)	Absent or <110	<220	>220
Irritability reflex	Absent	Some movement	Screams, agitated
Appearance	Pale, cyanotic	Moderate cyanosis	Pink
Respiration	Absent	Weak, arrhythmic	>15/min, regular

Adapted from personal communications with Dr. Antonio Prats Esteve, Barcelona, Spain.

pregnancy, fetal number and viability, and to identify fetal resorption. Gestation length was defined as the number of days from ovulation to the birth of the first pup of the litter.

Strength of uterine contraction was graded subjectively according to the following scale: strong = 5; good = 4; moderate = 3; fair = 2; poor = 1; absent = 0. Immediately after birth, postpartum pup viability was assessed using a subjective scoring system (Table 1) and the number of live and stillborn pups were recorded. For purposes of determining periparturient mortality, the periparturient period was defined as the onset of parturition to 48 h after birth of the last puppy in the litter. Body weight was measured immediately after whelping, daily for 2 weeks, then weekly until 4 weeks of age.

2.4. Statistical analysis

A Student's *t*-test was used to detect differences between control and hypothyroid bitches with regard to interestrus interval, litter size, duration of contractions for each pup, interval between delivery of pups, age that pups opened their eyes and began to walk, index of pup viability, and survival of pups in the periparturient period. A repeated measures ANOVA was performed for body weight of pups at birth and 7, 14, 20, and 28 days of age. Effects of each treatment were further evaluated using the Tukey test to compare results between time periods. Data is expressed as the mean ± S.D. (unless otherwise noted). The level of significance was set at $P < 0.05$. Statistical analyses were performed using a commercial statistical software package (SAS Enterprise, SAS Institute Inc., Cary, NC, USA).

3. Results

There was no significant difference between control and hypothyroid bitches for age, body weight, or number of litters prior to study. Bitches were bred a median of 19 weeks (range 16–32 weeks) after ^{131}I administration. Five bitches in the control and four bitches in the hypothyroid group were bred to Sire 1; the remainder were bred to Sire 2.

There was no difference in the interestrus interval in control versus hypothyroid bitches (36.9 ± 16.8 weeks versus 31.1 ± 9.3 weeks). Subjectively, there was no difference between the two groups in the character of proestrual bleeding. Libido was considered good in all nine control and seven hypothyroid bitches, fair in one hypothyroid bitch, and poor in one hypothyroid bitch.

All bitches became pregnant and carried their litters to term. Fetal resorption was noted in a single fetus in two bitches in the control group and in none in the hypothyroid group. Gestation length did not differ significantly in control versus hypothyroid bitches (62.9 ± 1.3 days versus 64 ± 2.8 days, respectively). Ninety-five pups were born during the study, 49 to controls and 46 to hypothyroid bitches (no significant difference between groups in litter size). Subjective uterine contraction scores, duration of contractions during parturition, and interval between delivery of pups was recorded in 36 pups from control bitches and 26 pups from hypothyroid bitches. Contraction strength scores were higher ($P < 0.01$) in the control group (4.0 ± 0.7) than the hypothyroid group (3.3 ± 1.1). Duration of contractions was shorter ($P < 0.05$) in the control versus hypothyroid bitches (11 ± 12.1 min versus 21 ± 21.9 min), but there was no difference in the interval between delivery of pups (42 ± 24.6 min versus 47 ± 30.7 min).

In the hypothyroid group, one pup from each of three litters, two from a litter of five, and all three pups in a litter of three died. The hypothyroid bitch that suffered 100% puppy mortality had a gestation length of 68 days. The pups were stillborn; they had severe autolysis, consistent with intrauterine death. Two pups from another hypothyroid bitch died on the second day with cerebral hemorrhage as the most noteworthy finding on

necropsy. One other pup in the hypothyroid group required manual assistance for delivery after prolonged parturition and was stillborn; brainstem hemorrhage was found on necropsy (thought to be secondary to trauma during parturition). The other two pups from a single dam died the day after birth and no cause was found on necropsy. No pups in the control group died spontaneously, but two from different dams were euthanized due to a cleft palate. Periparturient mortality was higher in litters from hypothyroid bitches than control bitches ($P = 0.014$).

Postpartum pup viability scores were recorded immediately after birth in 78 pups (40 in the control group and 38 in the hypothyroid group). The score was higher ($P < 0.01$) in the control (8.8 ± 1.4) compared with the hypothyroid bitches including (7.0 ± 3.3) or excluding (7.6 ± 2.7) the three pups that were stillborn from a single bitch.

Pups born to the control bitches weighed more than those born to hypothyroid bitches (0.67 ± 0.10 kg versus 0.48 ± 0.16 kg; $P < 0.0001$). Body weight of the pups remained higher in the control versus hypothyroid group at 7, 14, 20, and 28 days postpartum (all $P < 0.0005$). However, the amount of weight gained weekly was not significantly different between the groups through day 28. There was no significant difference between groups for age that eyes opened or they first began to walk. There were no obvious developmental physical or behavioral problems in any of the pups during the interval from 7 to 9 weeks after birth.

4. Discussion

Results failed to support hypothyroidism as a substantial cause of infertility in the bitch. Anestrus, prolonged interestrus interval and infertility were not documented as has previously been suggested to occur in hypothyroid bitches [3,5,19]. Evidence to support reproductive dysfunction in canine hypothyroidism is very limited; most retrospective studies of hypothyroidism have failed to note infertility or other reproductive disorders in bitches [20–23]. A single case of abortion and one of irregular interestrus interval were reported in two studies of 66 and 50 dogs, respectively [21,23]. Abnormal estrus was reported in 5 of 53 bitches in one study, but hypothyroidism was not well documented [4]. In greyhound bitches, mean T4 was higher in dogs with poor reproductive performance than those with adequate performance, suggesting that infertility was not due to hypothyroidism [6].

Clinical hypothyroidism commonly causes infertility due to ovulatory failure in women [9,10] In addition, menstrual abnormalities (including oligomenorrhea, amenorrhea, and menorrhagia) are common in hypothyroid women [7]. Similar effects of hypothyroidism were not detected in bitches in the present study. The interestrus interval, conception rates, and litter size in hypothyroid bitches were not different from controls. Libido was considered fair or poor in 2 of 9 hypothyroid bitches; this has been previously reported to occur with this disease [24].

Although all hypothyroid bitches carried litters to term, they had weaker contractions and longer duration of contractions before delivery of pups. In addition, periparturient mortality was greater in pups from hypothyroid dams. Abortion, preterm birth and resultant low birth weight are increased in hypothyroid women [11,12,25,26], but were absent in the present study. Perhaps the weaker and more prolonged uterine contractions during delivery were a manifestation of the muscle weakness and decreased metabolic rate that occurs in hypothyroid dogs [22].

It is unclear why bitches in this study did not develop the common reproductive abnormalities associated with hypothyroidism in women [9,10,25]. Perhaps the duration of hypothyroidism in the present study was too short to have the reproductive abnormalities of hypothyroidism be manifest. It is likely that thyroid failure develops more gradually in spontaneous hypothyroidism in dogs, compared with the abrupt severe hypothyroid state induced experimentally in this study. Therefore, a more prolonged hypothyroid state may occur in natural disease as it progresses from subclinical to more severe, overt disease.

Although the authors assert that their experimental model was appropriate for study of the effects of hypothyroidism on reproduction, it did not address the immune-mediated nature of the disease that occurs in approximately 50% of dogs with spontaneous hypothyroidism [27]. Premature ovarian failure, presumably due to ovarian autoimmunity, has been reported in women to be associated with other autoimmune endocrinopathies, including lymphocytic thyroiditis [28]. The authors are not aware that autoimmune ovarian failure has been documented to occur in bitches. Although a single bitch with hypothyroidism was noted to have a plasmacytic oophoritis [29], ovaries from beagles with lymphocytic thyroiditis and hypothyroidism were reported to be free of histopathologic lesions [30].

Maternal hypothyroidism impacts the health of offspring due to the contribution of maternal T4 to the fetus. Maternal thyroid hormones are the source of

fetal thyroid hormones until the middle of gestation [31]. Low birth weight occurred in rats born to hypothyroid dams [13], consistent with the pups in the present study. In addition, the lower postpartum pup viability scores found in pups born to hypothyroid dams may indicate delayed development. Conversely, the longer active parturition that occurred in hypothyroid bitches may have resulted in more fetal distress and contributed to the lower viability scores. The higher periparturient mortality found in the hypothyroid group was an indication that hypothyroidism has important effects on health of pups during and for a short interval after parturition. Because the rate of growth through 4 weeks of age and subjective assessment of the health of the pups through 7–9 weeks of age was similar between groups, we concluded that maternal hypothyroidism did not have substantial effects after the periparturient period.

In conclusion, hypothyroidism of relatively short duration did not affect interestrus interval, conception, litter size, or gestation length in bitches. However, parturition was prolonged, pups were smaller and more distressed at birth. In addition, hypothyroid bitches were predisposed to stillbirth and their pups were more likely to suffer perinatal mortality.

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