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Article

Correlations between hypothyroidism and ovarian cysts in bitches

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Abstract: This study involved 48 canine individuals with ovarian cystic formations larger than 4–5.5 mm in diameter, as identified by ultrasound, who were in the diestrus or anestrus phase of the sexual cycle. Both T4 and fT4 levels were measured in all 48 cases. The imaging diagnosis of ovarian cystic formations was performed using the stationary ultrasound equipment Esaote MyLab X5, and with the owners' consent, samples were collected and the obtained data processed. Peripheral venous blood samples were collected in coagulation activator-lined tubes. Hormone assays were performed using the Biomerieux MiniVidas hormone analyzer. The results are automatically calculated by the machine using stored calibration curves and are expressed in µg/dL for T4 and in ng/dL for fT4. The objective of this study is to determine the prevalence and extent of hypothyroidism in polycystic ovarian syndrome in bitches by measuring the levels of both T4 and fT4 in 48 patients with ovarian cysts. Considering clinical symptoms, borderline values were classified as indicative of hypothyroidism. 27% (n = 13) were diagnosed with hypothyroidism, while 73% (n = 35) had euthyroidism. Statistically significant differences were found in the results of both T4 and fT4 between the euthyroid and hypothyroid groups. This study found a high prevalence of hypothyroidism in female dogs with ovarian cysts. Given the impact of hypothyroidism on ovulation in women and its potential effects in dogs, thyroid function testing is recommended for female dogs with infertility.

Keywords: cysts, hypothyroidism, infertility, bitch

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1. Introduction

Polycystic ovarian syndrome (PCOS) is the leading global cause of infertility in women, associated with a high level of comorbidities. This syndrome is linked to excessive ovarian and/or adrenal androgen hormone secretion, anovulation, and often insulin resistance and other associated metabolic disorders [1]. PCOS is thus a heterogeneous condition involving changes in the reproductive and cardiovascular systems, as well as metabolic and oncological implications, with serious health consequences [2].

Thyroid dysfunction in women can impact fertility through various mechanisms, resulting in anovulatory cycles, luteal phase defects, elevated prolactin levels (PRL), and disruptions in sex hormone balance. As a result, proper thyroid function is essential for fertility, pregnancy, and the maintenance of a healthy pregnancy, even in the early stages [3].

Thyroid hormones influence the function of nearly every organ in the body; therefore, canine hypothyroidism can present a wide range of clinical signs. One factor in determining the effects of the disease is the challenge of confirming a diagnosis of hypothyroidism in dogs. Establishing the diagnosis is hindered both by the lack of specificity of thyroxine (T4) analysis and the insensitivity of thyrotropin testing. Given that purebred

dogs are intentionally bred, the impact of hypothyroidism on reproduction holds significant clinical importance. While a wide range of abnormalities occur in women with this disorder, there is limited

knowledge about the effects of thyroid hormone deficiency on reproductive performance in female dogs [4].

The metabolically active fraction of thyroxine (T4), known as free T4 (fT4), is widely acknowledged to more closely reflect the thyroid status compared to T4. Determining fT4 through equilibrium dialysis has been proposed as an alternative, non-invasive method, and is considered by some authors to be more sensitive and specific than measuring T4. This is because fT4 represents the amount of thyroid hormone available for use by the body's cells, whereas the total T4 level can be influenced by binding proteins [5]. In the same reference the authors state that the specificity of the low fT4 is between 93–94%.

In small animal breeding programs, infertility is one of the most critical issues that poses a challenge to successful management. Fertility involves achieving conception, followed by a pregnancy through implantation and successfully carrying the pregnancy to full term.

This study aims to establish the prevalence and degree of involvement of hypothyroidism in polycystic ovarian syndrome in the canine species, by testing the levels of both T4 and fT4 in 48 patients with infertility.

2. Materials and Methods

In this study, 48 individuals belonging to the female canine species were included, in which ovarian cystic formations larger than 4–5.5 mm in diameter were identified by ultrasound, and they were in the diestrus or anestrus phase of the sexual cycle. In all 48 cases both T4 and fT4 levels were measured.

The diagnosis of females with ovarian cysts, as well as the collection of samples for thyroid hormone measurements, were conducted at the Faculty of Veterinary Medicine in Cluj-Napoca within the Reproduction, Obstetrics, and Reproductive Pathology Department (small animal clinic), as well as at the specialized private veterinary clinic Quantas Repro Vet in Cluj-Napoca.

The imaging diagnosis of ovarian cystic formations was conducted using the stationary ultrasound equipment Esaote MyLab X5, with a microconvex probe, followed by measuring the cystic structures using the ultrasound machine's software. After diagnosing cases of ovarian cysts, with the owners' consent to collect samples and process the obtained data, peripheral venous blood samples were collected in coagulation activator-lined tubes.

The collected samples were centrifuged at 5000 rotations per minute to obtain serum. The liquid fraction was transferred into 1 ml Eppendorf tubes and stored at a freezing temperature of –18 degrees Celsius to conduct the thyroid profile. In most cases, the thyroid profile was conducted for diagnostic purposes, so the owners agreed to have T4 and fT4 levels measured. In this scenario, the processing of the samples was done directly after collection. Processing samples stored in the freezer involves first allowing them to thaw at room temperature for 15 minutes. After thawing, the samples need to be homogenized before testing. Hormone assays were performed using the Biomerieux MiniVidas hormone analyzer. This is an automated analyzer that utilizes immunoassay techniques for testing infectious markers (viral, bacterial), tumor markers, hormones, markers for cardiovascular conditions and hemostasis pathology, drug assays, allergology, etc. Special kits from the supplier are required for each assay.

The results are automatically calculated by the machine using stored calibration curves and are expressed in nmol/L. Samples with concentrations greater than 320 nmol/L need to be retested after dilution with 1/2 in T4-free serum (1 volume of sample and 1 volume of T4-free serum) and retested in the VIDAS T4 test. The results for fT4 are automatically calculated by the machine using stored calibration curves and are expressed in pmol/L.

The obtained fT4 and T4 data were examined for normality using the Shapiro-Wilk test. For the normal distribution results comparison, the independent samples t-test was applied. In case the normality was rejected for the examined data, the Mann-Whitney test was applied. Values of $p < 0.05$ were considered as statistically significant. The statistical analysis was performed using MedCalc® Statistical Software version 22.032 (MedCalc Software Ltd, Ostend, Belgium).

3. Results

In the following table the levels of both T4 and fT4 in the studied cases are presented (Table 1). The prevalence of hypothyroidism among female dogs diagnosed with ovarian cysts and who underwent thyroid hormone testing (n = 48) was 12% (n = 6). In 15% of cases (n = 7), thyroid hormone levels were at the lower limit. Such cases close to the lower limit were included in the borderline group and were considered

along with those with hypothyroidism, as the clinical history revealed signs of thyroid insufficiency. From a clinical perspective, considering that these levels should be interpreted in conjunction with the clinical symptoms, we classified the borderline values as indicative of hypothyroidism. Therefore, 27% (n = 13) of cases were diagnosed with hypothyroidism and a percentage of 73% (n = 35) of cases were diagnosed with euthyroidism.

Table 1. The levels of the T4 and fT4 in the studied cases

NR.CRT	Breed	Age	T4 µg/dl	FT4 ng/ml	T4 REF. µg/dl	FT4 REF. ng/ml
1	Central Asian Shepherd	3	1.56	1.11	1.50–4	0.80–3
2	Romanian Bocovina Shepherd	4	2.4	1.35	1.50–4	0.80–3
3	Labrador Retriver	11	1.57	1.05	1.50–4	0.80–3
4	Bernese Mountain Dog	6	1.37	0.86	1.50–4	0.80–3
5	American Bully	2	3.34	3.28	1.50–4	0.80–3
6	Tibetan Mastiff	3	1.31	0.91	1.50–4	0.80–3
7	French Bulldog	7	2.63	2.21	1.50–4	0.80–3
8	Central Asian Shepherd	8	1.99	2.79	1.50–4	0.80–3
9	Malinois Shepherd	7	2.05	2.11	1.50–4	0.80–3
10	Siberian Husky	2	2.77	1.89	1.50–4	0.80–3
11	Central Asian Shepherd	8	4.03	2.8	1.50–4	0.80–3
12	Bernese Mountain Dog	8	1.32	0.74	1.50–4	0.80–3
13	Cane Corso Italiano	4	1.79	1.83	1.50–4	0.80–3
14	Belgian Shepherd	4	3.56	2.81	1.50–4	0.80–3
15	Belgian Shpeherd	4	2.67	1.85	1.50–4	0.80–3
16	American Bully	4	0.93	0.7	1.50–4	0.80–3
17	Pero de Pressa Canario	9	1.78	1.35	1.50–4	0.80–3
18	Akita Inu	10	1.26	1.10	1.50–4	0.80–3
19	Middle Mixed breed	12	0.74	0.75	1.50–4	0.80–3
20	Malinois Shepherd	5	2.48	2.31	1.50–4	0.80–3
21	Pug	4	2.59	1.79	1.50–4	0.80–3
22	German Shepherd	9	2.52	1.74	1.50–4	0.80–3
23	German Shepherd	3	1.55	0.77	1.50–4	0.80–3
24	Bull Terrier	9	2.39	1.85	1.50–4	0.80–3
25	Middle Mixed Breed	12	1.23	1.02	1.50–4	0.80–3
26	Malinois Shepherd	7	1.32	1.85	1.50–4	0.80–3
27	American Bully	2	1.97	1.6	1.50–4	0.80–3
28	Bichon Maltese	8	2.23	1.72	1.50–4	0.80–3
29	American Bully	1	4.46	1.67	1.50–4	0.80–3
30	American Bully	3	2.25	1.23	1.50–4	0.80–3
31	English Bulldog	1	2.39	1.64	1.50–4	0.80–3
32	Caucasian Shepherd	7	1.7	1.3	1.50–4	0.80–3
33	Bernese Mountain Dog	4	1.48	0.87	1.50–4	0.80–3
34	Wired Dachshund	7	0.57	0.37	1.50–4	0.80–3
35	American Bully	1	2.25	1.91	1.50–4	0.80–3
36	American Bully	1	1.31	0.87	1.50–4	0.80–3

NR.CRT	Breed	Age	T4 µg/dl	FT4 ng/ml	T4 REF. µg/dl	FT4 REF. ng/ml
37	Beagle	11	2.28	1.34	1.50–4	0.80–3
38	Yorkshire Terrier	6	2.74	1.4	1.50–4	0.80–3
39	American Bully	1	1.12	1.07	1.50–4	0.80–3
40	Central Asian Shepherd	5	0.87	0.7	1.50–4	0.80–3
41	Middle Mixed Breed	16	2.31	1.14	1.50–4	0.80–3
42	Mini Bullterrier	1	3.2	1.57	1.50–4	0.80–3
43	American Bully	2	3.49	2.23	1.50–4	0.80–3
44	German Shepherd	4	2.69	1.41	1.50–4	0.80–3
45	Alaskan Malamut	6	1.3	1.16	1.50–4	0.80–3
46	American Bully	4	2.03	1.25	1.50–4	0.80–3
47	Wired Dachshund	4	1.18	0.91	1.50–4	0.80–3
48	French Bulldog	1	2.69	1.87	1.50–4	0.80–3

The T4 values for canine females diagnosed with ovarian cysts were compared in the euthyrotic (n = 35) and hypothyrotic (n = 13) groups. Since the normality of the T4 values in both groups was accepted, the independent sample t-test was applied. The results indicated a statistically significant difference ($p = 0.001$) in T4 values between the euthyrotic group and the hypothyrotic group.

The fT4 values for canine females diagnosed with ovarian cysts were compared in the euthyrotic (n = 35) and hypothyrotic (n = 13) groups. Since the normality of the data was rejected for the fT4 in the hypothyrotic group, the Mann-Whitney test was applied. The results indicated a statistically significant difference ($p = 0.0001$) in fT4 values between the euthyrotic group and the hypothyrotic group.

Six cases of hypothyroidism have been identified in females with ovarian cysts, and 7 cases with thyroid parameter values at the lower limit, borderline, classified as hypothyroidism. The cases of hypothyroidism and diagnosed cystic formations (n = 13) were from the categories of giant breeds (n = 5), large breeds (n = 2), medium breeds (n = 4), and small breeds (n = 2). Thus, we observed a prevalence of 38.5% in giant breed individuals, 30.8% in medium-sized females, and 15.4% in patients of large and small sizes.

4. Discussion

Fertility issues are typically categorized into one of four groups: abnormal estrous cycles, normal estrous cycles, unsuccessful breeding, or failure to carry a litter to full term. This classification system helps in creating a list of potential causes and conducting a systematic evaluation of all possibilities [6].

Thyroid hormones influence the function of nearly every organ in the body; therefore, canine hypothyroidism can present a wide range of clinical signs.

In women, hypothyroidism is linked to a wide range of reproductive disorders, from abnormal sexual development to menstrual irregularities and infertility. The importance of this disorder on the menstrual cycle, in women, has been studied and know since 1950s [7]. Hypothyroidism disrupts the normal physiological secretion of GnRH, which is essential for normal follicular development and ovulation. A delay in LH response can result in insufficient progesterone secretion by the corpus luteum [7]. Both gonadotropins and thyroxine seem to be essential for achieving optimal fertilization rates and blastocyst development [7]. On a cellular level, thyroid hormones work together with FSH to directly stimulate granulosa cell functions, including morphological differentiation. Thyroid hormones assist in FSH-induced LH/HCG receptor activation and progesterone secretion. Therefore, insufficient availability of thyroid hormones at the ovarian level may contribute to gonadal dysfunction [8]. In a study, the author observed primary and secondary infertility in 6.2% of 16 overtly hypothyroid women. This prevalence was similar, 4.8% in the euthyroid group with goiter and 2.4% in normal control women (unknown thyroid function) [9].

The clinical signs of canine hypothyroidism include low metabolic rate, dermatological conditions (dermatological abnormalities are reported in 60–80% of hypothyroid dogs), reproductive abnormalities (prolonged interestrus, silent estrus, lack of cyclicality, spontaneous abortion, small size puppies or low postpartum weight compared to the breed characteristics, uterine inertia, and weak or stillborn puppies);

however, evidence for this association is limited. If hypothyroidism is a cause of female reproductive dysfunction, it seems to frequently pass undiagnosed in veterinary practice. For example, a single case of hyperprolactinemia in a dog with primary hypothyroidism has been reported in the veterinary literature [10].

A study assessed the influence of short-term induced hypothyroidism on fertility, gestation, parturition, and neonatal health in female dogs. There was no difference in the estrous cycle interval, litter size, or gestation length between the hypothyroid group and the control dogs. The duration of uterine contractions was longer, but the strength of contractions was weaker in the hypothyroid group compared to the control dogs; however, the interval between puppies was not affected. Postpartum puppy mortality was significantly higher among females with hypothyroidism [4]. It is not clear why the female dogs in this study did not develop the common reproductive abnormalities associated with hypothyroidism in women. One possible reason could be the relatively short duration of the present study and, consequently, of the hypothyroidism, for reproductive abnormalities associated with hypothyroidism to manifest. It is possible that thyroid insufficiency gradually develops in the case of spontaneous hypothyroidism, compared to the sudden induction of severe hypothyroid status for experimental purposes discussed in this study. Therefore, prolonged hypothyroidism may occur in natural disease as it progresses from subclinical to more severe manifestations, becoming evident over time [4].

Infertility and insulin resistance are the result of glucose metabolism abnormalities in women with Polycystic Ovary Syndrome (PCOS). PCOS is the most common cause of anovulatory infertility, while hyperinsulinemia in women leads to increased androgen production and the presence of insulin resistance (IR). Patients with IR and PCOS may have elevated plasma levels of homocysteine, which can influence both short-term reproductive function and long-term cardiovascular complications associated with insulin-resistant PCOS [11]. Patients with hypothyroidism frequently experience insulin resistance, which results in increased serum gonadotropin (LH) levels. This stimulates the ovaries to produce excessive androgens, thus triggering or worsening PCOS [12]. A study discovered that 22.5% of PCOS patients also had subclinical hypothyroidism (SCH), in contrast to only 8.3% of the normal control group [13] compared to another study that the prevalence of subclinical hypothyroidism (SCH) in PCOS patients was 43.6% [12]. Xing et. Al [14] reported an increased prevalence of SCH in PCOS women at 26.97%. In our investigation, among all the cases diagnosed with ovarian cysts and infertility, 27% had T4 and fT4 values indicative of hypothyroidism, which aligns with the previous mentioned studies. Statistically, there was a significant difference in results for both T4 and fT4 between the euthyroid and hypothyroid groups. On the canine species we did not find studies that indicate a correlation between these 2 disorders. The limitations of our study are comparable to those of Fatima et al.'s study [12], such as the absence of a temporal understanding between the two disorders, clinical outcomes following hypothyroidism treatment, and the correlation with other disorders like diabetes or obesity.

5. Conclusions

Given the recognized significance and impact of hypothyroidism on the physiological process of ovulation in women, and its lesser-studied effects in canine species, there should be increased consideration of this disorder in the pathological evaluation of infertility in female dogs. This study revealed a notable prevalence of hypothyroidism in female dogs with ovarian cysts. Specifically, one-third of the canine females in the research group with ovarian cysts were also diagnosed with hypothyroidism, indicating a significant correlation between the two conditions. As ovarian cystic pathology can lead to infertility, this study suggests testing thyroid functions in female dogs with a clinical history of infertility. Another noteworthy finding in this study is that middle and giant breeds are more prone to hypothyroidism. Therefore, there is a high likelihood that hypothyroidism could be the underlying cause of infertility in these breeds. Cases exhibiting clinical signs such as infertility, skin lesions, obesity tendency, high blood pressure, etc., along with borderline values of the T4 and fT4 indicators, should be regarded as hypothyroidism cases.

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