Correlation between sperm parameters and circulating thyroid hormones and testosterone concentrations in Labrador Retriever dog

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ABSTRACT
Thyroid hormones physiologically regulate the semen quality, by altering testosterone concentrations, and different seminal parameters, as well as sperm motility, viability and semen volume. Serum thyroxine (T4), free thyroxine (fT4), thyroid-stimulating hormone (TSH) and testosterone (T) concentrations were measured in 26 adult Labrador Retriever dogs (n. 20 normospermic and n. 6 azoospermic groups), aged 3 ± 0.5 years old, to determine their specific references, by taking into account the related conventional and kinematic sperm parameters and clinical ultrasound evaluations. The mean values of T4, fT4, TSH and T concentrations, as soon as those of sperm parameters and clinical evaluations of normospermic group were in line with dog’s physiological range of literature data. Normospermic group showed significant correlations between T4 and T (r = 0.681; p < .01), sperm progressive motility (%) (r = 0.623; p < .01), and sperm non-progressive motility (%) (r = 0.625; p < .02). The azoospermic group showed higher T4, fT4, TSH and lower T concentrations, compared to normospermic group, and a positive correlation between T4 and T (r = 0.8548; p < .046). The use of breed-specific hormonal ranges and sperm parameters will improve the knowledge of their interaction in Labrador Retriever dogs, adding a new segment of scientific literature.

HIGHLIGHTS
- Thyroxine (T4), free thyroxine (fT4), thyroid stimulating hormone (TSH) and testosterone (T) concentrations were measured in male dogs.
- Conventional and kinematic sperm parameters were also evaluated.
- Dogs were divided in 20 normospermic and n. 6 azoospermic Labrador Retrievers.
- Azoospermic group showed higher values of T4, fT4, TSH and lower values of T compared to normospermic group.
- Use of breed-specific hormonal ranges and sperm parameters will improve the knowledge of scientific literature.

Introduction
Thyroid hormones are vital for the physiological reproductive function of humans and animals. In dogs, changes in circulating thyroid hormone and thyrotropin (TSH) concentrations with age, sex, body size, and breed have been noted (Shiel et al. 2007, 2010; Piechotta et al. 2010; Hegstad-Davies et al. 2015). Hence, breed-specific laboratory reference intervals, for assessing the thyroid function of dogs in both physiological and pathological conditions, could be considered (Daminet et al. 2003; Segalini et al. 2009; Köhler et al. 2012).

Thyroid disorders have clinical effects on spermatogenesis regardless the breed and the purpose of its selection (Sengupta and Dutta 2018). Hence, an excess or deficit of thyroid hormones alters both the physiological testicular functions and interrupts the crosstalk between hypothalamic–pituitary–thyroid (HPT) and hypothalamic–pituitary–gonadal (HPG) axes, with decreased testosterone concentrations and altered semen quality in human and animal species (Sengupta and Dutta 2018).

It was reported that disorders of the thyroid gland may sometime affect the fertility in female and male dog (Panciera et al. 2007; Segalini et al. 2009; Sontas et al. 2014). In male dogs, hypothyroidism can induce a decreased libido, ejaculate volume and also semen quality (Johnson et al. 1999). Moreover, a study conducted on five breeds predisposed to hypothyroidism,
showed no reproductive alterations in subjects with lower levels of thyroxine, even though the hormone levels were lower in 70% of males od Dogue de Bordeaux compared to Great Dane and Leonberger. In addition, no significant differences in plasma thyroxine concentrations between fertile and hypofertile subjects were observed (Segalini et al. 2009). What is more, no effects of gonadectomy on the pituitary–thyroid axis in male dogs were observed (Gunzel-Apel et al. 2009). However, the role of thyroid hormones on male reproductive function and on sperm parameters is still unclear and the scientific data are conflicting.

The presence of thyroid hormone receptors (TRalpha1) in rat, expressed in proliferating Sertoli cell nuclei, in germ cells from intermediate spermatogonia to mid-cycle pachytene spermatocytes and in a subset of interstitial cells (Buzzard et al. 2000) and in human Sertoli cells (Jannini et al. 2000) suggests a possible interaction between thyroid hormones and fertility. Hence, hyperthyroidism or thyrotoxicosis also damages spermatogenesis, causing an arrest of maturation, sperm abnormalities, spermatogenesis’ interruption, reduction of sperm vitality, impairs mitochondrial activity, with an alteration of the antioxidant systems in rats, with lipid peroxidation (Choudhury et al. 2003; Sahoo et al. 2008; Romano et al. 2017), as well as asthenozoospermia in humans (Krassas et al. 2010). Some evidence suggested a negative effect of T4 on male fertility, inducing a damage on the human sperm motility through the activation of its thyroid receptor-dependent mechanism (Xian et al. 2017), decreasing the number of spermatocytes/spermatids in the seminiferous tubular lumen, and inducing alterations in the testis and seminal vesicles of animal models (Jacob et al. 2005). On the other hand, fT4 seems to have a potential protective effect, being inversely correlated with sperm DNA damage in men (Meeker et al. 2008).

In human, the in vitro effects of levothyroxin on conventional and bio-functional sperm parameters on male fertility were recently assessed, clarifying the involvement of thyroid function on fertility (Condorelli et al. 2019).

The hypothesis was the existence of a crosstalk between thyroid profile, testosterone and semen parameters in dogs. The purpose of the present study was to establish the circulating T4, fT4, TSH and testosterone ranges in Labrador Retriever dogs, and to verify and justify the existence of positive or negative correlations among hormonal pattern, semen parameters and clinical evaluations, by taking into account their involvement on reproductive performance.

Material and methods

The research complied with guidelines of Good Clinical Practices (EMEA, 2000) and of the Legislative and ethical aspects on use of canine artificial insemination (Quartuccio et al. 2020). This study was performed according to the ethical principles that have their origins in the Italian Veterinarians’ Ethical Code (Passantino 2007), and the Italian and European regulations on animal welfare (D.L. 26/2014; Directive 2010/63/EU 2020). The experimental design was approved by the Ethic committee of Department of Veterinary Sciences University of Messina, Italy (Ethics Reference No: 045/2020).

Animals and diets

The study was carried out on 26 adult dogs (n. 20 normospermic and n. 6 azoospermic groups), aged 3 ± 0.5 years old, belonging to the Labrador Retriever breed, enrolled in the Register of Italian Origin (ROI). Dogs were individually housed in different family home and the commercial dry food for large breed (Maxi adult, Royal Canin) was administered two times a day. Water was ad libitum.

The recruitment, enrolment, samples collection and analysis carried out along September 2019-February 2020 at the Veterinary Teaching Hospital of Messina University, Italy. Animal inclusion criteria were based on animals’ history, physical examination, as well as reproductive ultrasound of prostate gland and testicles, to exclude pathological conditions. Biochemical and haematological tests were previously performed in all subjects to exclude systemic alterations.

All dogs have no history of receiving medication during the previous 3 months or nutritional supplements, and management factors such as diet, veterinary care and husbandry are similar for all dogs to minimise the impact of heterogeneous factors.

The determination of body weight (BW), weight between 30.75 and 41.00 kg, was measured on fasted animals, in the morning at 9:00 am, by using a digital scale. The Body Condition Score (BCS), paired to 5.23 (± 0.90), was evaluated by a visual assessment and palpation adopting a 9 point scale system. Four classes of BCS were considered: BCS 1 to 3 = lean dog; BCS 4 and 5 = ideal dog; BCS 6 and 7 = overweight dog; and BCS ≥8 = obese dog (Ricci et al. 2007).

Blood samples and analyses

Owner consent was obtained before to blood samples and semen collections. Each dog was submitted to the
blood samples once a week for three consecutive weeks, for a total of 78 individual samplings. Blood samples were collected from the cephalic vein both in EDTA sterile tubes for haematological analysis and in glass sterile tubes for biochemical and hormonal analyses. For these purposes, serum was centrifugated within 60 min of collection and refrigerated at 4°C for biochemical and hormonal analyses, performed within 24 hours after collection, at the Veterinary Diagnostic Centre BIOGENE (Catania, Italy). Chemistry profile (Bt 3500 Chemistry Analyser, Biotechnic Instruments, Italy), included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), bilirubin (BIL), urea (URE), total protein (TP), albumin (ALB), total cholesterol (CHO), creatinine (CREA), electrophoretic protidogram (Sebia capillary electrophoresis, France). A complete blood cell count was also recorded (Xt 1800 Automated Haematology Analyser utilises the power of fluorescent flow cytometry and hydrodynamic focussing technologies, Sismex, Dubai).

Serum T4 concentrations were assayed using a homologous solid-phase, chemiluminescent enzyme immunoassay (Immulite® 2000 Canine Total Thyroxine, Siemens Medical Solutions-Diagnostics-USA), according to the manufacturer’s instructions. The intra-assay coefficient of variation (CV) and inter-assay CV were 10.8% and 4.4%, respectively, 13.8% and 6.8% at Thyroxine concentrations of 0.65 and 3.84 ng/dL, respectively. The lowest detectable amount of Thyroxine was 0.12 µg/dL.

Serum fT4 concentrations were assayed using of equilibrium dialysis, Nichols Institute Diagnostic, San Clemente, CA, according to the manufacturer’s instructions. The intra-assay CV was 8.3% at fT4 concentrations of 2.0 ng/dL determined in canine serum. The inter-assay Cvs were 8.7% and 6.9% at fT4 concentrations of 1.8 and 6.1 ng/dL, respectively, for canine serum. As determined by the manufacturer, the lowest detectable amount of fT4 was 0.15 ng/dL.

Serum TSH concentrations were assayed using a homogeneous solid-phase, two-site chemiluminescent immunometric assay (Immulite® 2000 Canine TSH, Siemens Medical Solutions-Diagnostics-USA), according to the manufacturer’s instructions. Intra-assay CVs were 5%, 4% and 3.8% at TSH concentrations of 0.2, 0.5 and 2.6 ng/mL, respectively. The inter-assay CVs were 6.3% and 8.2% at TSH concentrations of 0.16 and 2.8 ng/mL, respectively. The sensitivity of the assay was 0.03 ng/mL.

Serum testosterone concentrations were assayed using an immunoassay system based on the Enzyme Linked Fluorescent Assay (ELFA) principles by Mini VIDAS system (BioMerieux S.A., Lyon, France). The VIDAS Testosterone test is an enzyme-linked fluorescent assay. To establish the intra-assay variation of the VIDAS method, 2 canine samples with a high (6.39 ng/mL) and low (1.2 ng/mL) testosterone concentrations were analysed 10 times on the same day. The CV was calculated as S.D./mean × 100.

Andrological examinations and sperm samples

Before the assessment of semen quality, all subjects underwent the routine training for the manual seminal collection, using a teaser oestrus female as a mount. Semen was collected by manual manipulation as described by Linde-Forsberg (1991) in the presence of a teaser bitch in heat.

The animals were in good general conditions with normal sexual libido, without any disorders or abnormalities of the genital tract. Ultrasonic examinations of the testes and prostate were undertaken once on each dog, the equipment used was Mindray M9 ultrasound machine (Mindray, Italy), with a linear (testes) and microconvex (prostate) 6.6 to 13.5 MHz transducers. The testes were scanned in the sagittal (length), transverse (width) and dorsal (height) planes using the electronic callipers of the machine and the testicular volume was calculated using the formula for an ellipse (Paltiel et al. 2002). Total testicle volume (TTV) was calculated by adding up the volume of each testicle. Prostate volume (PV) was calculated using the formula reported by Kamolpatana et al. (2000).

The preliminary collection of semen, on three consecutive days, seemed sufficient for both training and to minimise the extragonadal sperm reserves in all dogs, in order to avoid a reduction in motility due to sperm aging and increased debris (Johnston et al. 2001). The three ejaculate fractions were collected separately into pre-warmed (36–38°C) sterile graduated conical tubes. Each normospermic dog was submitted to the semen collection once a week for three consecutive weeks, for a total of 60 individual semen collection; azoospermic dogs were submitted to the semen collection only once time, for a total of 6 individual semen collection. Specifically, the azoospermic dogs were affected by epididymitis caused by Escherichia coli, and submitted to therapy six months before the start of the present study. The semen sample was immediately examined at the laboratory of the Veterinary Teaching Hospital of the Department of Veterinary Sciences, Messina University for macro (volume, colour, smell and pH) and microscopic (motility,
concentration, morphology and vitality) evaluations, including kinematic parameters (velocity curved line: VCL, velocity straight line: VSL, velocity average pathway: VAP, percent linearity: LIN %, percent straightness: STR %, percent oscillation: WOB %, trajectory %, hyperactivity %).

The microscopic examination was performed by placing a 2 μL aliquot of seminal material of the 2nd fraction, using a micropipette (Eppendorf Reference variable 54fc digital camera (resolution 782 pixels; 54 frames per second) and computerised automatic semen analysis system SCA (Sperm Class Analyser, Microptic Automatic Diagnostic System) were used.

**Statistical analysis**

Differences of biochemical and hormonal parameters between groups (Normospermic vs Azoospermic dogs) were valued by ANOVA analysis using the GLM procedure of SAS/STAT® software (SAS Institute 2017). Pearson’s correlation coefficients (r) were used to measure the relationships, in normospermic dogs, between all measured parameters and performed by SAS/STAT® software (SAS Institute 2017) and differences were considered significant if p < .05. Results were reported as means ± standard deviation of the mean.

**Results**

Circulating T4, fT4, TSH and testosterone concentrations (mean ± SD) of normospermic and azoospermic groups are shown in Table 1.

The comparison between the two groups showed the highest T4 (p < .01), fT4 (p < .01), TSH (p < .01), and the lowest T (p < .05) concentrations in azoospermic group.

Azoospermic group showed a significant positive correlation between T4 and T (r = 0.8548; p < .046). The conventional sperm parameters (mean ± SD) of normospermic group are shown in Table 2. The semen volume produced by each dog ranged from 4 mL to 16 mL, and the sperm concentration from 90.44 to 171.89 10^6/mL; progressive sperm motility showed a wide value ranged from 59.07 to 82.77 (%), as soon as the non-progressive sperm motility ranged from 17.23 to 39.52 (%); also immobility (%) showed variable values ranged from 0.1 to 2.58 (%).

The kinematic sperm parameters, including the three different velocities (VCL, VLS VAP) and related percentages of trajectory and activity (LIN, STR, WOB) of normospermic dogs are shown as mean ± SD in Table 2.

TTV of normospermic dogs ranged from 9.51 to 13.42 cm³ and PV ranged from 9.20 to 24.70 cm³, respectively (Table 3). Related to the azoospermic dogs, TTV ranged from 8.76 to 12.74 cm³ and PV ranged from 9.10 to 20.86 cm³, respectively (Table 3). No significant differences were observed between

**Table 2.** Conventional and kinematic sperm parameters of normospermic Labrador Retriever dogs.

| Parameters                      | Mean ± SD | Min | Max |
|---------------------------------|-----------|-----|-----|
| Sperm volume mL                 | 8.61 ± 3.37 | 4  | 16  |
| Sperm concentration 10^6/mL     | 144.74 ± 56.08 | 90.44 | 171.89 |
| Progressive sperm motility %    | 68.80 ± 13.88 | 59.07 | 82.77 |
| Non Progressive sperm motility %| 30.08 ± 13.48 | 17.23 | 39.52 |
| Immobility %                    | 1.12 ± 0.12 | 0.10 | 2.58 |
| VCL μm/s                        | 113.66 ± 9.60 | 99.81 | 133.29 |
| VSL μm/s                        | 50.06 ± 6.61 | 40.70 | 61.21 |
| VAP μm/s                        | 75.44 ± 6.13 | 67.10 | 89.09 |
| LIN %                           | 44.72 ± 7.40 | 34.71 | 57.58 |
| STR %                           | 66.55 ± 9.06 | 57.66 | 88.23 |
| WOB %                           | 66.53 ± 4.43 | 60.20 | 73.50 |
| Circular trajectory %           | 64.87 ± 11.47 | 41.87 | 79.31 |
| Hyperactivity %                 | 33.59 ± 11.40 | 19.13 | 46.95 |

Min: Minimum; Max: Maximum.

**Table 3.** Clinical ultrasound evaluations in normospermic and azoospermic Labrador Retriever dogs.

| Parameters | Normospermic (20) | Azoospermic (6) | Dogs |
|------------|-------------------|-----------------|------|
| TTV cm³    | 11.35 ± 1.36      | 9.51 ± 1.42     | 10.59 ± 1.07 |
| PV cm³     | 15.95 ± 4.28      | 9.20 ± 2.86     | 13.81 ± 3.57 |

Min: Minimum; Max: Maximum.

Normospermic vs. Azoospermic dogs: no significant TTV: Total Testicular volume was calculated with electronic callipers of the ultrasound machine by formula of Paltiel et al. 2002. PV: Prostatic Volume was obtained by electronic callipers of the ultrasound machine by formula of Kamolpatana et al. 2000.

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**Table 1.** Hormonal parameters in normospermic and azoospermic Labrador Retriever dogs.

| Animals' number | Normospermic (20) | Azoospermic (6) | Dogs |
|-----------------|-------------------|-----------------|------|
| T₄ µg/dL        | 2.41 ± 0.55³      | 1.20 – 3.00     | 7.91 ± 0.17³ | 7.88 – 8.10 |
| fT₄ ng/mL       | 1.18 ± 0.39³      | 1.05 – 1.79     | 2.43 ± 0.41³ | 2.10 – 2.86 |
| TSH ng/mL       | 0.16 ± 0.08³      | 0.09 – 0.41     | 0.48 ± 0.10³ | 0.33 – 0.54 |
| Testosterone ng/mL | 3.47 ± 1.13³  | 2.67 – 5.57     | 2.25 ± 0.05³ | 2.19 – 2.60 |

³ Significant different for p < .05.

T₂ and TSH analyses were performed by chemiluminescent enzyme immunoassay.

fT₄ analysis was determined by equilibrium dialysis.

Testosterone test was assayed by enzyme-linked fluorescent assay.

Min: Minimum; Max: Maximum.
these clinical ultrasound evaluations in the two groups. Significant correlations among hormonal concentrations, conventional and kinematic semen parameters and reproductive ultrasound evaluations are observed. Specifically, T4 showed significant negative correlations with Testosterone (r = −0.681; p < .05) and with progressive sperm motility (r = −0.623; p < .05), and a significant positive correlation with non-progressive sperm motility (r = 0.625; p < .05).

Related to the testicular volume, it showed significant positive correlations with circular trajectory (r = 0.888; P < .01), sperm concentration (r = 0.597; p < .05), VCL (r = 0.784; p < .01) and negative with Immobility (r = −0.737; p < .05), VSL (r = −0.596; p < .05), LIN (−0.887; p < .01), STR (r = −0.946; p < .01) and WOB (r = −0.605; p < .05).

Discussion

This study represents the particular comprehensive research of thyroid and testosterone patterns and sperm parameters in a homogenous and representative group of breeding fertile Labrador Retrievers, represented by normospermic dogs. The obtained data were consistent with the initially hypothesis and objectivities of the present study. The haematological and biochemical parameters obtained in Labrador Retrievers are in line with physiological ranges recorded in dogs (Lawler et al. 2007). The comparison of hormonal data of normospermic group revealed that T₄, fT₄, and TSH are in agreement with literature data for physiological dogs’ ranges, and particularly for T₄: 1.53 – 2.25 μg/dL (Enzyme immunoassay by Bröm el et al. 2005; Chemiluminescent-immunometric-assay by Urhausen et al. 2009); T₃: 1.7 – 4.0 μg/dL (Radio-immuno-assay by Daninet et al. 2003; Chemiluminescence-immune-assay by Köhler et al. 2012; Enzyme-immuno-assay by Zeugswetter et al. 2013); fT₄: 0.98 – 1.57 ng/dL (Modified Equilibrium Dialysis technique by Bröm el et al. 2005; Chemiluminescent enzyme immune-assay by Hegstad-Davies et al. 2015); TSH: 0.06 – 0.26 ng/mL (Immuno-radio-metric-assay by Bröm el et al. 2005); TSH: 0.32 ± 0.05 ng/mL (Chemiluminescent enzyme immuno metric assay by Bhatti et al. 2006); TSH: 0.01–0.65 ng/mL (Chemiluminescent-immunometric assay) by Urhausen et al. 2009) and with recent data observed specifically in Labrador Retrievers of the Guide Dogs (T₄: 2.41 – 2.73 μg/dL; T₃: 32.06 ± 1.19 nmol/L – 33.91 ± 1.16 nmol/L) (Fluorescence enzyme immune assay by Chiofalo et al. 2019). On the other hand, in azoospermic dogs, these same hormones were higher compared to previous physiological literature data. Testosterone concentrations of normospermic and azoospermic groups were in agreement with those physiological wide ranges reported in literature for the dog (T:1-10 ng/mL) (Urhausen et al. 2009; Kaneko et al. 2010), but consistently lower in azoospermic dogs. Nevertheless, slight variations might be ascribed to differences among laboratories using different methods and instruments and are subjected of speculation; what is more, some differences may also be explained by different age, sex, size (height, weight, body mass index) and breed (Urhausen et al. 2009; Hegstad-Davies et al. 2015) and varying dietary contents (Larsson et al. 2015; Ober et al. 2016; Vitger et al. 2017; Schauf et al. 2018).

The evaluations of TTV and PV with ultrasound assistance are a simple tool for assessing the reproductive potential of male dog and they are recommended always to exclude the presence of any disorder which could be not clinically detectable.

The conventional and kinematic sperm parameters and reproductive ultrasound evaluations were in line with dogs’ physiological range of literature data (Niżański et al. 2011; Domoslawska et al. 2017), and with ranges specifically reported for male Labrador Retriever (Schäfer-Somi 2015; Hesser et al. 2017; de la Fuente-Lara et al. 2019).

Many aspects of the possible role of thyroid hormones are yet to be clarified in the dog, by taking also into account their involvement on the reproductive performance. To date, all in vitro researches were performed on animal models, and recently in vitro effects of thyroid hormones on sperm mitochondria, viability and DNA integrity were studied in humans (Condorelli et al. 2019).

We hypothesised a relationship between thyroid hormones and sperm parameters as previously described in rat (Jiang et al. 2000) and human (Meeker et al. 2008).

Considering that all dogs were healthy according to the clinical evaluation and did not show alterations in haematological and biochemical parameters, the significant differences observed between the two groups, with the highest T₄, fT₄ and TSH in azoospermic dogs, induced to considered them slightly hyperthyroid.

Obtained data showed that T₄ had significant negative correlation with both T (r = −0.681) and sperm progressive motility (%) (r = −0.623) and a positive correlation with sperm non-progressive motility (%) (r = 0.625). This apparent and controversial surprising result related to the interaction between T₄ and sperm progressive motility is
comparable with recent results obtained in human in vitro by Condorelli and colleagues, (2019) that observed that the levothyroxine (LT4) was able to increase sperm motility, as well as to improve the membrane mitochondrial potential (MMP) of spermatozoa, at a concentration of 0.9 pmol L\(^{-1}\), but at higher concentrations than 0.9 pmol L\(^{-1}\), sperm progressive motility significantly decreased. High T\(_4\) concentrations, which simulate an in vitro hyperthyroidism condition, damage spermatozoa, with decreased sperm motility, due to an excessive consumption of substrates. Hence, the previous Authors confirmed the relationship between these two sperm parameters, hypothesised that LT4 improving MMP also stimulated sperm motility until substrates are exhausted with a subsequent decline in motility itself. On this scientific evidence, it is possible to presume that the concentrations of thyroid hormones can induce a supposed positive or negative feedback on the sperm progressive motility also in dog, according to their concentrations and related metabolically active effects. The higher concentrations of thyroid hormones in azoospermic sperm, suggest a possible regulatory role of these hormones on sperm motility, according to the available studies that suggested that normal human prostate thyroid activity seems vital for maintaining semen quality via genomic or non-genomic mechanisms, either locally acting on Sertoli cells, Leydig cells or germ cells or by affecting crosstalk between the HPT axis and hypothalamic-pituitary-testicular axis (Sengupta and Dutta 2018). This result was corroborated by the highest T\(_4\), FT\(_4\) and TSH concentrations in slightly hyperthyroid and azoospermic dogs.

Related to the negative correlation between T\(_4\) and testosterone, it is well known that thyroid hormones basically regulate semen quality by altering serum testosterone level in men and boys (Meikle, 2004), promoting spermatogenesis, maintaining the sperm count (Wagner et al. 2009) and regulating also semen parameters, like sperm motility and sperm morphology (Kumar et al. 2014). What is more, hyperthyroidism is a condition of increased T\(_4\) concentration which were associated with an increase in sex hormone binding globulin (SHBG) in circulation and decrease in the metabolic clearance rate of testosterone (Abalovich et al. 1999; Schulte et al. 2000), as observed in hyperthyroid dogs of azoospermic group.

On this basis, it is possible to presume that physiological T\(_4\) concentrations could be associated with normal SHBG values and testosterone’s metabolic process; hence, the negative correlations between these two hormones could be due to the percentage of binding testosterone that results in low bioavailable free percentage, according to the changes of SHBG. Unfortunately, testosterone concentrations in the present study were assayed as total circulating testosterone and no data are available in literature for the dog.

In adult testicular cells of rat both desiodase 1 (D1) and D2 were described. Their relative activity indicate that D2 represents the predominant activating enzyme in this organ. It is noteworthy that the highest D2 expression might play a major role in the intracellular conversion of T\(_4\) to T\(_3\) (Buzzard et al. 2000). Based on this evidence it is possible to explain the negative and positive correlations observed among T\(_4\) and progressive and non-progressive percentages of spermatozoa.

Semen parameters showed minimal inter-individual variations, thus confirming the homogeneity of the group in terms of breed, age, well-monitored management and diet.

Overall, thyroid function leads to multiple effects of semen quality that include conventional and kinematic sperm parameters. Particularly, concerning the significant, multiple and frequently negative correlations among ejaculate concentrations circular trajectory and some sperm kinematic parameters, it is difficult to interpret them. The mechanism whereby thyroid hormones induce their affects on the semen quality is poorly understood in humans and unknown in dogs. It may result from a direct effect on sperm cell, as well as from an effect on non-germ cells, as recorded in man (La Vignera and Vita 2018).

In murine models and humans’ thyroid hormones evidently modulate the hypothalamic–pituitary–gonadal (HPG) axis through the crosstalk between HPG and hypothalamic–pituitary–thyroid (HPT) axis. Most of the studies cited in the present article were carried out in mice/rats and subsequently in humans. Therefore, thyroid function tests should be part of the diagnostic clinical workup in dogs undergo purposeful breeding, by taking into account the pivotal role of thyroid hormones on reproductive activity.

Conclusions

In this study, we consolidate the small amount of previously reported physiological data with an additional result that reflects a crosstalk between hormonal pattern and reproductive outcome. These data contribute to the overall information base on the physiological ranges of thyroid and androgen concentrations, and also contribute longitudinally to collect additional information on the sperm quality of breeding
Labrador Retriever dogs. New and future approaches to significant semen improvement in dogs could be done, describing the related seminal measurements of thyroid hormones and androgen, as previously described in men, to better understand their crosstalk and correlation with systemic concentrations.

Studies on a larger number of dogs and on specimens with different histories of infertility are still in process and will be subject for further publications.

**Author contributions**

Conceptualisation, M.Q., E.F. and L.L.; Literature review, P.M., S.C. and L.S.; Writing original draft preparation, M.Q., E.F. and L.L.; Formal analysis, M.Q. and G.E.; Writing-review and editing, P.M., S.C. and L.S.; Supervision, M.Q. and L.L. All the authors gave final approval to the manuscript and any revised version submitted.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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