CVJ CLUJ VETERINARY JOURNAL

Article

Analysis of gonadocorticoids profile in canine patients diagnosed with ovarian cysts

Zoltán-Miklós GÁL [1], Ana Hîruța [2,*], Alexandru-Raul POP [1,†)], Alexandra IRIMIE [3], Ioan Ștefan GROZA [1]

[1] Faculty of Veterinary Medicine, Reproduction Department, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, 400372, Cluj, Romania
[2] Faculty of Veterinary Medicine, Pathology Department, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, 400372, Cluj, Romania
[3] Faculty of Veterinary Medicine, Anatomy Department, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, 400372, Cluj, Romania.
* Correspondence: ana.hiruta@usamvcluj.ro
†) These authors contributed equally to this work.

Abstract: Veterinarians can obtain a more comprehensive understanding of the progression of ovarian cystic pathology in dogs by assessing hormonal levels. This study aimed to examine the gonadocorticoid profile of dogs diagnosed with ovarian cysts and compare these levels with those of healthy female dogs. The study included 96 female dogs split into two groups: a control group of healthy females and a study group of females with ovarian cysts larger than 4.5–5 mm in diameter and in diestrus or anestrus phase, determined by progesterone measurements. The control group had no ovarian pathologies and matched the estrus cycle phase of the study group. Ovarian cysts were diagnosed using the Esaote MyLab X5 ultrasound machine, while sex hormone levels (testosterone, progesterone, and estradiol) were measured with the Biomerieux MiniVidas analyzer. The analyzer utilizes the Enzyme Linked Fluorescent Assay (ELFA) technique for hormonal assessments. The study group (ovarian cysts) had significantly higher estradiol levels (21.83 pg/ml) compared to the control group (10.70 pg/ml). However, there was no significant difference in progesterone levels between the two groups. The study group females showed significantly higher estradiol levels in both the anestrus phase ($p = 0.0105$) and the diestrus phase ($p = 0.0105$). There was also a significant difference in estradiol levels between control group females in diestrus and anestrus, with significantly higher levels observed in the anestrus phase ($p = 0.0105$). In summary, female dogs with ovarian cysts had higher estradiol levels in both estrus and anestrus phases compared to the control group. The authors recommend assessing hormonal profiles in dogs with ovarian cysts for better treatment planning.

Keywords: canine ovarian cysts; estradiol; progesterone; gonadocorticoids

Received: 05.08.2024
Accepted: 10.08.204
Published: 12.09.2024

DOI: 10.52331/vsa8e911

1. Introduction

Female canines are a monoestrous, non-seasonal, polytocous species, with an average interestrus period of 6 months, divided into 4 phases. The 4 phases of the sexual cycle for this species are: proestrus, with a duration of approximately 7-10 days, followed by estrus lasting 5-10 days, diestrus lasting 20-90 days, and anestrus, with a variable duration of 15-150 days [1,2].

In canine species, ovarian cysts are clinically significant as they are a major source of hyperestrogenism in bitches, potentially leading to prolonged estrus [3,4]. These follicular cysts are endocrinologically active, secreting estradiol and progesterone. Persistent follicular cysts are believed to induce hyperestrogenism in bitches with estrus extending up to three months [2,3].

The ovarian cysts are classified into the following types: follicular cysts, luteal cysts, cystic corpus luteum, cystic rete ovarii and cysts of the subsurface epithelium [5,6].

Functional ovarian cysts manifest as single or multiple fluid-filled structures of varying sizes, which can be unilateral or bilateral in bitches aged 6-8 years. The pathogenesis is not well understood, but possible causes include insufficient LH surge, changes in gonadotropin receptors within the follicles, and growth factors [6]. Inadequate LH peaks to

trigger ovulation of dominant follicles or insufficient receptor responsiveness can lead to ovarian cyst formation. Some researchers suggest that the inhibition of aromatase, an enzyme critical for the conversion of androgens to estrogens, may be an intraovarian disruption linked to the pathophysiology of polycystic ovary syndrome [7].

Clinically, follicular cysts must be differentiated from other ovarian cystic pathologies, including rete ovarii hyperplasia, superficial epithelial cysts, and ovarian neoplasms. Although the life expectancy of different dog breeds varies, there is an increased incidence of ovarian cysts in dogs over 6-8 years of age [6].

The primary endocrine lesion causing anovulation is the central failure to produce timely GnRH pulses and an LH surge, which are essential for final follicular growth and ovulation, or peripheral molecular homeostatic imbalances within the follicles that impair the ovary's response to the preovulatory LH surge. The former hypothesis explains the development of multiple follicular cysts, while the latter is more applicable to the occurrence of solitary follicular cysts [5].

As large cysts lose their structure and the arrangement of cellular layers changes under the pressure exerted by the cystic fluid, identifying the origin of the cyst becomes challenging. Follicular and luteal cysts are the two main types of functional ovarian cysts. Additionally, non-follicular cysts often arise from surface epithelium, underlying cells, or the mesonephric tubules of the ovary [8].

In humans, the etiology of polycystic ovarian syndrome (PCOS) has a strong genetic component. Genes such as CAPN10, the Cytochrome P450 family, the insulin gene, AR, FTO, and FSHR have been implicated [9]. Similarly, in dairy cows, variations in the bovine PCOS-related DENND1A gene have also been identified [10].

The prolonged presence of steroid hormones in the blood, which underlie the formation of ovarian cysts, not only predisposes the organism to uterine pathologies but also affects the ovulation rate, leading to a reduction in the size of the follicles. Histopathologically, the predominant uterine changes include cystic endometrial hyperplasia, periglandular fibrosis, lymphoplasmacytic endometritis, and adenomyosis, with incidences of 19.7%, 14.5%, 4.0%, and respectively 2.6% [11].

By evaluating the hormonal levels, veterinarians can gain a deeper understanding of how this pathology progresses in dogs. The aim of this research was to analyze the gonadocorticoids profile of canine patients diagnosed with ovarian cysts and compare the values with healthy canine females.

2. Materials and Methods

2.1. Study design

The study included a total number of 96 canine females. The dogs were segregated into two following two groups in accordance with the results of the clinical and paraclinical evaluation: control group for the females which were clinically healthy and study group for the females which were diagnosed with ovarian cysts. The study group inclusion criteria required the presence of ovarian cysts with sizes bigger than 4.5–5 mm in diameter, and the subjects needed to be in either the diestrus or anestrus phase. The sexual cycle phase was determined through routine progesterone measurement. For the control group, the inclusion criteria were the absence of pathological ovarian structures and being in the same estrus cycle phase (diestrus or anestrus) as the study group.

The diagnosis of females with ovarian cysts, as well as the collection of samples for endocrine profiling of these patients, was conducted at the Faculty of Veterinary Medicine in Cluj-Napoca within the Department of Reproduction, Obstetrics, and Reproductive Pathology (small animal clinic), as well as at the specialized veterinary practice Quantas Repro Vet in Cluj-Napoca.

The clinical history of patients suspected of having ovarian cysts primarily included infertility (repeated mating without successful conception), irregular heat cycles (prolonged estrus with shortened anestrus periods), pregnancy checks, routine gynecological examinations, essential vaginal hemorrhages, as well as other reproductive tract pathologies such as cystic endometrial hyperplasia, pyometra, mammary gland tumors, or vaginal tumors.

The imaging diagnosis of ovarian cystic formations was performed using a stationary Esaote MyLab X5 ultrasound machine with a microconvex probe. Subsequently, the cystic structures were measured using the ultrasound machine's software. In some cases, the diagnosis was confirmed through macroscopic examination of the ovaries, for the patients following ovariohysterectomy for therapeutic purposes or routine ovariohysterectomy (at the owner ´s request). It is noteworthy that in these females, clinical signs were absent, and preoperative clinical examination revealed that the patients were clinically healthy.

2.2 Sample Collection

Blood samples were collected from all patients for routine blood work. The blood samples were centrifuged at 5000 rpm for 10 minutes and the serum was stored at −18° C until the evaluations were done. The Biomerieux MiniVidas analyzer was used to measure sex hormones such as testosterone, progesterone, and estradiol. This automatic analyzer uses the Enzyme Linked Fluorescent Assay (ELFA) technique for hormonal assays.

All owners provided informed written consent, granting permission for sample collection and its use in research, as well as for the anonymous publication of the results.

2.3. Statistical analysis

The obtained 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

From the total of 96 canine females, 48 were included in the control group (healthy females) and 48 in the study group (ovarian cysts). Within the control group, 26 females were in the diestrus phase and 22 were in the anestrus phase. In the study group, 23 females were in the diestrus phase and 25 were in the anestrus phase.

The values resulting from the statistical analysis are presented in Table 1. The results for the testosterone levels were not included since all the females had values < 0.05 ng/ml, except for one female from the study group which had 0.09 ng/ml. The females from the study group (ovarian cysts) had significantly higher estradiol values ($p < 0.0001$) with an arithmetic mean of 21.83 pg/ml (95% CI: 14.65–25.37), compared with the control group, which had a median of 10.70 pg/ml (95% CI: 9.86–12.30). For the progesterone values comparison between the control group (median: 7 ng/ml) and the study group (median: 2.96 ng/ml), there wasn't a statistically significant difference ($p = 0.6735$), hence no additional comparisons involving progesterone were done. Estradiol values in diestrus and anestrus were further compared between and within the study and control groups.

Table 1. Statistical analysis results for the estradiol and progesterone levels in the control and study groups

Hormones	Estral phase	n	Mean ± SD	Median	Min.	Max.	Confidence Interval for Mean 95%
Estradiol study group	**Diestrus**	23	23.75 ± 13.41	23.17	9	55.86	17.95–29.55
	Anestrus	25	24.79 ± 20.10	21.19	9	95.40	16.49–33.09
	Overall	48	24.29 ± 17.05	21.83	9	95.40	19.34–29.25
Estradiol control group	**Diestrus**	26	10.51 ± 2.01	9.63	8.21	15.5	9.69–11.32
	Anestrus	22	13.24 ± 3.50	12.95	8.47	21.08	11.69–14.79
	Overall	48	11.76 ± 3.08	10.7	8.21	21.08	10.86–12.66
Progesteron study group	**Diestrus**	23	36.75 ± 23.69	38.26	4.07	80.00	26.50–47.00
	Anestrus	25	1.38 ± 0.92	0.95	0.25	2.98	1.00–1.77
	Overall	48	39.6 ± 22.78	2.96	0.25	80.00	11.32–25.34
Progesteron control group	**Diestrus**	26	37.57 ± 19.76	37.19	3.08	80.00	29.59–45.56
	Anestrus	22	1.22 ± 0.77	1.05	0.21	2.57	0.88–1.56
	Overall	48	20.91 ± 23.30	7.00	0.21	80.00	14.15–27.68

Legend: SD - standard deviation; n – number of individuals. The estradiol values are expressed pg/ml. Progesteron levels are expressed as ng/ml.

For the comparison between the estradiol values in the anestrum phase between the control group and the study group, it was noticed that there was a statistically significant difference. The females from the study group had a significantly higher (p = 0.0105) estradiol value in the anestrum phase with a median of 21.19 pg/ml (95% CI: 13.44–26.09), compared with the control group which had a median level of 12.95 pg/ml (95% CI: 10.46–15.72).

For the comparison between the estradiol values in the diestrum phase between the control group and the study group, it was noticed that there was a statistically significant difference. The females from the study group had a significantly higher (p = 0.0105) estradiol value in the diestrum phase with a median of 23.17 pg/ml (95% CI: 11.75–28.77), compared with the control group which had a median level of 9.63 pg/ml (95% CI: 9.02–10.92).

For the comparison of estradiol values between the control group females in diestrus and anestrus, it was noticed that there was a statistically significant difference. The females in the anestrus phase had a significantly higher (p = 0.0105) estradiol value with a median of 12.95 pg/ml (95% CI: 10.46–15.72), compared with the female in the diestrus phase which had a median level of 9.63 pg/ml (95% CI: 9.02–10.92).

For the comparison of estradiol values between the study group females (ovarian cysts) in diestrus and anestrus, it was noticed that there wasn't a statistically significant difference (p = 0.8607). The females in the anestrus phase had a median value for estradiol of 21.19 pg/ml (95% CI: 13.44–26.09), compared with the females in the diestrus phase which had a median level of 23.17 pg/ml (95% CI: 11.74–28.77).

4. Discussion

Female dogs exhibiting estrus behavior with a proestrus or estrus period extending beyond 30 days should undergo ovarian imaging using ultrasonography to determine the presence of follicular cysts. Follicular cysts are typically large (8-12 mm in diameter), with thin walls and containing an anechoic fluid. These cysts must be carefully differentiated from normal follicles, cavitary corpora lutea, and parovarian cysts. The consequences of untreated follicular cysts are unclear; however, they may lead in most cases to pyometra and neoplasms such as mammary gland tumors and vaginal tumors, in addition to that in some cases, bone marrow suppression may occur due to persistently elevated estrogen levels. Consequently, in most cases, attempts are made to induce luteinization using HCG (500 IU per bitch) administered three times. In cases unresponsive to treatment, progesterone administration was attempted to be used to achieve cyst regression (though progesterone increases the risk of pyometra in a uterus influenced by estrogen) or an ovariectomy may be performed [12].

In examining the prevalence of cystic structures among 400 specimens, one study found a diverse distribution of cyst types. Follicular cysts were identified in 41 specimens (10.3%), corpus luteum cysts were present in 9 specimens, representing 2.3%, more than 360 specimens, or over 90%, had rete ovarii cysts and cysts of subsurface epithelial structures were found in 20 specimens, comprising 5.0% of the total. [13]

The endocrine potential of each ovarian cyst can be determined by analyzing the concentrations of estradiol-17ß and progesterone in the cystic fluid. In a study, the levels of estradiol-17ß in cystic fluid ranged from 2.0 to 568,000.0 pg/ml (median 545.0 pg/ml), while progesterone concentrations ranged from 0.1 to 20,138.0 ng/ml (mean 31.0 ng/ml). Additionally, hormone levels vary among different types of cysts present on the same ovary [14].

A study involving a retrospective examination of ovaries removed during ovariohysterectomy performed for the treatment of pyometra over a 12-month period revealed that 20.7% (17/82) of bitches had follicular cysts. The presence of ovarian cysts showed no significant association with cystic endometrial hyperplasia in Fisher's exact test (P = .78). Approximately 35.3% of bitches with ovarian cysts also had pyometra, while 58.8% had pyometra without cystic endometrial changes. Only 5.9% of females developed ovarian cysts without uterine pathology [15]. Although hyperestrogenism is less common in bitches with follicular cysts, the development of the cystic endometrial hyperplasia-pyometra complex is frequent, leading to polyuria and polydipsia, mucoid or purulent vaginal discharge, vomiting, abdominal distension, abdominal stress, or pain [16]. Clinical signs associated with hyperestrogenism syndrome include pancytopenia, anemia, granulocytopenia, thrombocytopenia, or hemorrhagic vulvar discharge. In chronic cases, females may develop typical bilateral symmetrical alopecia, lichenification, hyperkeratosis, and bone marrow suppression [17].

In females, testosterone is primarily produced by the ovaries and the adrenal glands. However, as this study suggests, the ovaries do not produce significant amounts of testosterone in females with ovarian cysts or in healthy females.

The pathogenesis, diagnosis, and treatment of cystic ovarian diseases in bitches remain unclear. Therefore, it is crucial to establish an early diagnosis and implement appropriate treatment strategies promptly to prevent disease progression [6].

5. Conclusions

In conclusion, estradiol levels were significantly elevated in female dogs with ovarian cysts during both the diestrus and anestrus phases compared to the control group. In female dogs without ovarian cysts, estradiol levels were notably higher during the anestrus phase than in the diestrus phase, likely due to increased ovarian responsiveness to gonadotrophins in late anestrus.

The ovaries do not typically produce significant amounts of testosterone in females with ovarian cysts or in healthy females.

The presence of ovarian cysts does not alter significantly serum progesterone levels during either the diestrus or anestrus phases. Progesterone mainly regulates the estrous cycle in this species and is a reliable marker for identifying the estrous period in female dogs, even when cystic formations are present. This is corroborated by the observation that active corpora lutea can coexist with cysts in canines, particularly during the diestrus phase. The authors recommend hormonal profile determination in canine patients with ovarian cysts, for a better understanding regarding the evolution of this pathology and for a more accurate treatment plan. This proactive approach not only aims to improve the health and well-being of the affected dogs but also enhances the overall outcomes of treatment strategies.

Author Contributions: Conceptualization, Z.-M.G. and I.Ș.G.; methodology, A.-R.P., A.H. and A.I.; statistical analysis, Z.-M.G.; resources, A.-R.P.; data curation, A.H.; writing—original draft preparation, Z.-M.G., A.H. and A.I.; writing—review and editing, A.I.; supervision, I.Ș.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was founded by the Discipline of Animal Reproduction at the Faculty of Veterinary Medicine Cluj-Napoca.

Acknowledgments: The authors extend their gratitude to the Discipline of Animal Reproduction at the Faculty of Veterinary Medicine Cluj-Napoca, and the private practice reproduction referral clinic Quantas Repro Vet SRL in Cluj-Napoca for their help in collecting the samples.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Concannon, P.W. Reproductive Cycles of the Domestic Bitch. *Animal Reproduction Science* **2011**, *124*, 200–210, doi:10.1016/j.anireprosci.2010.08.028.
2. Groza, I.Ş.; Bogdan, L.M.; Cătană, R. *Ginecologie, andrologie şi obstetrică veterinară: compendiu*; Editura Academiei Române: Bucureşti, 2006; ISBN 978-973-27-1445-4.
3. Johnston, S.D.; Kustritz, M.V.R.; Olson, P.N.S. *Canine and Feline Theriogenology*; W.B. Saunders: Philadelphia London New York, 2001; ISBN 978-0-7216-5607-6.
4. Arlt, S.; Spankowsky, S.; Heuwieser, W. Follicular Cysts and Prolonged Oestrus in a Female Dog after Administration of a Deslorelin Implant. *New Zealand Veterinary Journal* **2011**, *59*, 87–91, doi:10.1080/00480169.2011.552858.
5. Knauf, Y.; Köhler, K.; Knauf, S.; Wehrend, A. Histological Classification of Canine Ovarian Cyst Types with Reference to Medical History. *J Vet Sci* **2018**, *19*, 725, doi:10.4142/jvs.2018.19.6.725.
6. Sasidharan, J.K.; Patra, M.K.; Singh, L.K.; Saxena, A.C.; De., U.K.; Singh, V.; Mathesh, K.; Kumar, H.; Krishnaswamy, N. Ovarian Cysts in the Bitch: An Update. *Topics in Companion Animal Medicine* **2021**, *43*, 100511, doi:10.1016/j.tcam.2021.100511.
7. Corbin, C.J.; Trant, J.M.; Walters, K.W.; Conley, A.J. Changes in Testosterone Metabolism Associated with the Evolution of Placental and Gonadal Isozymes of Porcine Aromatase Cytochrome P4501. *Endocrinology* **1999**, *140*, 5202–5210, doi:10.1210/endo.140.11.7140.

8. Payan-Carreira, R.; Pires, M.A. Ovarian Cysts in Dogs´ Practice. In *Advances in Medicine and Biology*; Nova Science Publishers Inc, 2016; Vol. 94, pp. 65–88.
9. Ajmal, N.; Khan, S.Z.; Shaikh, R. Polycystic Ovary Syndrome (PCOS) and Genetic Predisposition: A Review Article. *European Journal of Obstetrics & Gynecology and Reproductive Biology: X* **2019**, *3*, 100060, doi:10.1016/j.eurox.2019.100060.
10. Zheng, J.; Deng, T.; Jiang, E.; Li, J.; Wijayanti, D.; Wang, Y.; Ding, X.; Lan, X. Genetic Variations of Bovine PCOS-Related DENND1A Gene Identified in GWAS Significantly Affect Female Reproductive Traits. *Gene* **2021**, *802*, 145867, doi:10.1016/j.gene.2021.145867.
11. Maya-Pulgarin, D.; Gonzalez-Dominguez, M.S.; Aranzazu-Taborda, D.; Mendoza, N.; Maldonado-Estrada, J.G. Histopathologic Findings in Uteri and Ovaries Collected from Clinically Healthy Dogs at Elective Ovariohysterectomy: A Cross-Sectional Study. *J Vet Sci* **2017**, *18*, 407, doi:10.4142/jvs.2017.18.3.407.
12. *BSAVA Manual of Canine and Feline Reproduction and Neonatology*; England, G.C.W., Heimendahl, A. von, British Small Animal Veterinary Association, Eds.; 2nd ed.; British Small Animal Veterinary Association: Quedgeley, Gloucester [England], 2010; ISBN 978-1-905319-19-0.
13. Arlt, S.; Haimerl, P. Cystic Ovaries and Ovarian Neoplasia in the Female Dog – a Systematic Review. *Reprod Domestic Animals* **2016**, *51*, 3–11, doi:10.1111/rda.12781.
14. Knauf, Y.; Bostedt, H.; Failing, K.; Knauf, S.; Wehrend, A. Gross Pathology and Endocrinology of Ovarian Cysts in Bitches. *Reprod Domestic Animals* **2014**, *49*, 463–468, doi:10.1111/rda.12311.
15. De Bosschere, H.; Ducatelle, R.; Vermeirsch, H.; Van Den Broeck, W.; Coryn, M. Cystic Endometrial Hyperplasia- Pyometra Complex in the Bitch: Should the Two Entities Be Disconnected? *Theriogenology* **2001**, *55*, 1509–1519, doi:10.1016/S0093-691X(01)00498-8.
16. Ortega-Pacheco, A.; Gutiérrez-Blanco, E.; Jiménez-Coello, M. Common Lesions in the Female Reproductive Tract of Dogs and Cats. *Veterinary Clinics of North America: Small Animal Practice* **2012**, *42*, 547–559, doi:10.1016/j.cvsm.2012.01.011.
17. Schlafer, D.H.; Miller, R.B. Pathology of the Ovary (Nondevelopmental Lesions). In *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*; Elsevier Saunders: Philadelphia, 2007; Vol. 3, pp. 431–563.