https://doi.org/10.52973/rcfcv-e361794 Revista Científica, FCV-LUZ / Vol. XXXVI

Expression patterns of growth differentiation factor 8 protein in ovarian follicles and the corpus luteum of domestic cats

Abundancia de la proteína del factor de diferenciación del crecimiento 8 en los folículos ováricos y el cuerpo lúteo de gatos domésticos

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ABSTRACT

The Growth Differentiation Factor 8 is a member of the Transforming Growth Factor Beta family and plays a vital role in ovarian dynamics. The present study investigated: i) the expression patterns of Growth Differentiation Factor 8 protein in feline ovarian follicles and corpus luteum, and ii) the effect of reproductive cycle stages on ovarian GDF8 expression in domestic cats. Ovaries were collected from 28 healthy domestic female cats undergoing elective ovariohysterectomy and classified into follicular (n = 15) and luteal (n = 13) phase groups. Growth Differentiation Factor 8 protein expression was assessed via enzyme–linked immunosorbent assay (ELISA) and immunohistochemistry. In primordial follicles, Growth Differentiation Factor 8 immunoreactivity was restricted to the oocyte. In primary and secondary follicles, both oocytes and granulosa cells exhibited Growth Differentiation Factor 8 staining, while thecal cells remained negative. In antral follicles, Growth Differentiation Factor 8 was present in oocytes, granulosa cells, and follicular fluid, but absent in thecal cells. In the corpus luteum, Growth Differentiation Factor 8 immunoreactivity varied by developmental stage. Mild staining was observed in luteal cells during early development and maintenance. Severe immunoreactivity appeared in late developmental and maintenance stages, while moderate staining was present during regression. Non–steroidogenic cells showed no reactivity. No significant differences in ovarian Growth Differentiation Factor 8 expression were found between follicular and luteal phases, nor between maintenance and regression stages of the corpus luteum. Growth Differentiation Factor 8 may significantly regulate folliculogenesis and corpus luteum development in the feline ovary.

Key words: Cat; ovary; GDF8; folliculogenesis; corpus luteum

RESUMEN

El Factor de Diferenciación del Crecimiento 8, miembro de la familia del Factor de Crecimiento Transformante Beta, desempeña un papel crucial en la dinámica ovárica. El presente estudio tuvo como objetivos: i) caracterizar los patrones de expresión de la proteína del Factor de Diferenciación del Crecimiento 8en los folículos ováricos y en el cuerpo lúteo de gatos domésticos, y ii) evaluar el efecto de las diferentes fases del ciclo reproductivo sobre la expresión ovárica del Factor de Diferenciación del Crecimiento 8. Se recolectaron ovarios de 28 hembras domésticas sanas sometidas a ovariohisterectomía electiva, clasificándose en fase folicular (n = 15) y luteal (n = 13). La expresión del Factor de Diferenciación del Crecimiento 8 se analizó mediante ensayos inmunoabsorbentes ligados a enzimas (ELISA) e inmunohistoquímica. En los folículos primordiales, la inmunorreactividad del Factor de Diferenciación del Crecimiento 8 se limitó al ovocito. En los folículos primarios y secundarios, tanto ovocitos como células de la granulosa mostraron tinción positiva, mientras que las células tecales permanecieron negativas. En los folículos antrales, el Factor de Diferenciación del Crecimiento 8 estuvo presente en ovocitos, células de la granulosa y líquido folicular, pero ausente en las células tecales. En el cuerpo lúteo, la intensidad de la inmunorreactividad del Factor de Diferenciación del Crecimiento 8 varió según la etapa de desarrollo: leve durante el desarrollo temprano y la fase de mantenimiento, intensa en las etapas tardías de desarrollo y mantenimiento, y moderada durante la regresión; las células no esteroidogénicas no mostraron reactividad. No se observaron diferencias significativas en la expresión ovárica del Factor de Diferenciación del Crecimiento 8 entre las fases folicular y luteal, ni entre las etapas de mantenimiento y regresión del cuerpo lúteo. Estos resultados sugieren que el Factor de Diferenciación del Crecimiento 8 podría desempeñar un papel regulador clave en la foliculogénesis y en el desarrollo y función del cuerpo lúteo en el ovario felino.

Palabras clave: Gato; ovario; GDF8; foliculogénesis; cuerpo lúteo

Received: 11/09/2025 Accepted: 09/12/2025 Published: 26/12/2025

INTRODUCTION

The Growth Differentiation Factor 8 (GDF8), or myostatin, is a Transforming Growth Factor Beta (TGF–β) family member that inhibits protein synthesis in skeletal muscle. Its gene is highly conserved across species, with over 90% C–terminal sequence homology in cattle (*Bos taurus*), sheep (*Ovis aries*), rabbits (*Oryctolagus cuniculus),* dogs (*Canis lupus domesticus*), pigs (*Sus scrofa domesticus*), and primates [1]. GDF8 knockout mice (*Mus musculus*) ($GDF8^{-/-}$) show marked muscle hyperplasia and hypertrophy [1], and mutations in GDF8 cause the "double muscling" phenotype in cattle [2], dogs [3] and sheep [4].

Beyond its well–known role in muscle development, GDF8 is increasingly recognized for its functions in reproductive biology [5]. In the ovary, GDF8 is expressed in oocytes, granulosa cells, and follicular fluid [6, 7], where it binds activin receptors type IIA/IIB (ARIIA/B) and TGF–β receptor type I (ALK5), activating the Smad2/3–Smad4 signalling pathway [7]. Through this signalling cascade, GDF8 regulates the synthesis of estradiol–17β (E_2) and progesterone (P_4) by modulating the expression of key steroidogenic genes, including steroidogenic acute regulatory protein (StAR), aromatase, follicle–stimulating hormone receptor (FSHR), and luteinizing hormone receptor [8].

Additionally, GDF8 also protects oocytes by reducing intracellular reactive oxygen species [9]. Elevated or dysregulated GDF8 signalling has been associated with the polycystic ovarian syndrome [6] and poor ovarian response during controlled ovarian hyperstimulation in humans [10].

Abnormalities in GDF8 signalling can disrupt reproductive function; for instance, heterozygous $GDF8^{+/-}$ sows exhibit delayed puberty, compromised reproductive performance, and reduced litter size [11]. Moreover, homozygous $GDF8^{-/-}$ gilts were found to have increased uterine smooth muscle mass [12].

Ovarian GDF8 expression has been investigated in several species, including chickens (*Gallus gallus domesticus*) [13], humans [6], cattle [14], and buffalo (*Bubalus bubalis*) [15]. In the bovine ovary, GDF8 is expressed in thecal and granulosa cells of antral follicles, and in oocytes and granulosa cells of primordial, primary, and secondary follicles [14]. In buffalo, GDF8 was present in oocytes and granulosa cells of all follicles, with weaker staining in primordial follicles, large antral follicles, and thecal cells; oocytes showed stronger immunoreactivity than granulosa and cumulus cells [15]. In human ovarian follicles, GDF8 expression was observed in the oocytes of all follicular stages. Primary and antral follicles also exhibited GDF8 immunoreactivity in granulosa and thecal cells. Furthermore, GDF8 protein expression was detected in the corpus luteum (CL), suggesting a potential role in its development [6].

Although GDF8 has been extensively studied in various species, its role in cat (*Felis catus*) ovarian physiology remains poorly understood. This study aims to elucidate the localization of GDF8 protein within feline ovarian follicles and the CL, and to determine whether its immunoreactivity patterns differ across distinct developmental stages of these structures.

The present study aimed to determine: i) expression patterns of the GDF8 protein in the feline ovarian follicles and different stages of CL (development/maintenance and regression), and ii) the effect of the reproductive cycle period on ovarian GDF8 protein expression.

MATERIAL AND METHODS

Animals, ethics, surgery and samplings

The study was approved by the Hatay Mustafa Kemal University Animal Experiment Ethics Committee (22/12/2023, No. 2023/09-5). Twenty–eight healthy, non–pregnant, client–owned female domestic cats, aged 5–24 months and weighing (DS-300-DC, Densi, Türkiye) 2.5–4 kg, were included. All owners provided consent for the use of ovary and blood samples. None of the cats had mated with intact males. The study was conducted between January and August 2024. All cats fasted for at least 8 hours (h) and underwent general anaesthesia following preoperative blood tests.

Prior to surgery, Cephazolin (25 $mg \cdot kg^{-1}$, IV; Sefamax®) and Meloxicam (0.2 $mg \cdot kg^{-1}$, SC; Meloxicam®) were administered. Anaesthesia was induced with Xylazine (1 $mg \cdot kg^{-1}$, IM; Rompun®) and Ketamine (10 $mg \cdot kg^{-1}$, IM; Keta–Control®), followed by intubation and maintenance with Isoflurane (2%) in oxygen. Lactated Ringer's solution (5 $mL \cdot kg^{-1} \cdot h^{-1}$, IV; Poliflex®) was given intraoperatively. Cats were positioned in dorsal recumbency for midline ovariohysterectomy [16].

Postoperative Cephazolin (25 $mg \cdot kg^{-1}$, IM, BID for 5 days–d) was given, and sutures were checked on day (d) 3 and 7, then removed on d 10. Preoperative blood was collected from the cephalic vein into anticoagulant–free tubes for E_2 and P_4 analysis. Serum was separated (5 min, 3000 G) (Nüve, Nf200, Türkiye) and stored at -20°C (5507NE, Arcelik, Türkiye). Post–surgery, ovaries were halved—one part fixed in 10% buffered formalin for histology, the other stored at -20°C (5507NE, Arcelik, Türkiye) for GDF8 analysis [17].

Determination of the reproductive cycle of cats

The reproductive cycle was determined by ovarian morphology, serum E_2 and P_4 levels, and histopathology. Cats with E2 > 20 $pg \cdot mL^{-1}$ and 2–3 mm follicles were classified as follicular phase; those with P_4 > 1 $ng \cdot mL^{-1}$ and corpora lutea as luteal phase [18, 19]. E_2 and P_4 were measured using the Atellica IM analyzer (Siemens®, Germany) with electrochemiluminescence kits (E_2: 11.80–3000 $pg \cdot mL^{-1}$; P_4: 0.21–60 $ng \cdot mL^{-1}$) [20, 21]. Reproductive phase was also confirmed histologically [18, 22].

Histopathological and immunohistochemical analysis

Tissue samples were fixed in 10% buffered formalin, dehydrated through a graded ethanol series, cleared in Xylene, and subsequently embedded in paraffin. Sections (RM2125, Leica, United Stated) (4 μm) were stained with hematoxylin and eosin. The GDF8 detection used a mouse monoclonal anti–myostatin antibody (Abcam, ab201954, 1:100, 1 h, 45°C). Endogenous peroxidase was blocked with 3% H_2O_2/methanol, and antigen retrieval was performed in pH 6.0 buffer with Tween–20. Diaminobenzidine tetrahydrochloride was used as the chromogen, and Harris hematoxylin for counterstaining. Follicles were classified according to the criteria of Bristol–Gould and Woodruff, and Gozer *et al.* [18, 21], and CL staging was performed following the methodology

described by Amelkina *et al.* [22]. Immunostaining was scored - (0), + (weak, 1), ++ (mild, 2) and +++ (severe, 3) [20, 21]. All evaluations were performed by a single blinded observer.

Ovarian GDF8 measurement

Ovarian tissues stored at -20°C (5507 NE, Arcelik, Türkiye) were thawed and homogenized in PBS (1:10, w/v), then centrifuged (Nüve, Nf 800R, Türkiye) at 10000 G for 5 min at 4°C. Supernatants were collected, and total protein was measured using the BCA assay (Pierce™ BCA Kit) [23]. GDF8 levels were quantified with a commercial ELISA kit (Abcam, ab267656), read at 450 nm (Erba Manheim, Lisascan EM, Czech Republic), and expressed as $ng \cdot mg^{-1}$ protein. The assay's detection range was 0.819–200 $ng \cdot mL^{-1}$, with a sensitivity of 0.83 $ng \cdot mL^{-1}$ and intra/inter–assay CVs < 10% and < 12%, respectively.

Statistical analysis

Power analysis (G*Power 3.1.9.2) determined a sample size of 28 cats ($\alpha = 0.05$, power = 0.80, effect size = 1.10). Descriptive statistics were calculated, and normality and variance homogeneity were assessed with Shapiro–Wilk and Levene tests. GDF8 levels were compared by reproductive phase and CL presence using the Mann–Whitney U test. Immunohistochemical differences among follicle cell types were analyzed with Mann–Whitney U and Kruskal–Wallis tests, followed by Dunn's post–hoc test. Statistical analyses were performed using Stata 12/MP4, with significance set at $P<0.05$.

RESULTS AND DISCUSSION

Immunohistochemical findings

In primordial follicles, mild immunoreactivity was detected only in the oocyte. No immunoreactivity was observed in the granulosa cells of the primordial follicles (FIG.1a). In primary follicles, mild GDF8 immunoreactivity was detected in the oocytes and granulosa cells (FIG. 1b). In secondary follicles, oocytes and granulosa cells exhibited immunoreactivity for GDF8, while no staining was observed in the theca cells (FIG. 1c).

Immunohistochemical scoring analysis in follicle types

In primordial follicles, the oocyte exhibited higher immunoreactivity for GDF8 compared to granulosa cells ($P<0.001$). In primary follicles, there was no difference in GDF8 immunoreactivity between granulosa cells and the oocyte ($P=0.991$). Granulosa cells showed significantly higher GDF8 immunoreactivity than both oocytes and theca cells in secondary and antral follicles ($P<0.001$). In thecal cells, no immunoreactivity was observed in follicle types (TABLE I).

TABLE I
Cell–based immunohistochemical scoring analysis of different follicle types (Mean ± standard error of the mean [SEM])

Cells	Follicle Types			
	Primordial	Primary	Secondary	Antral
Granulosa	0.00 ± 0.00 [a]	0.91 ± 0.06	1.29 ± 0.13 [a]	1.50 ± 0.16 [a]
Oocyte	1.00 ± 0.00 [b]	0.91 ± 0.06	1.00 ± 0.00 [b]	1.00 ± 0.00 [b]
Thecal	–	–	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]
P–value	< 0.001*	0.991	< 0.001*	< 0.001*
Effect Size	3.425	0.075	5.342	2.877

*Different superscripts ([a, b, c]) in the same column indicate significant differences between cells in immunohistochemical scoring of follicle types ($P<0.001$)

In cat ovaries, GDF8 immunoreactivity was detected in the oocytes of all types of follicles. However, no immunoreactivity was detected in the granulosa cells of the primordial follicles (FIG. 1a). GDF8 exerts its cellular effect via activin receptor type IIA (ACRIIA), activin receptor type IIB (ACRIIB), and (ALK5 via Smad2/Smad3-Smad4 dependent pathway in granulosa cells [7].

Both GDF8 and its receptors were found in the human oocyte and granulosa cells [6]. In the cat ovary, ACRIIA, ACRIIB, Smad2, Smad3, and Smad4 were also located in the oocyte of primordial follicles [24].

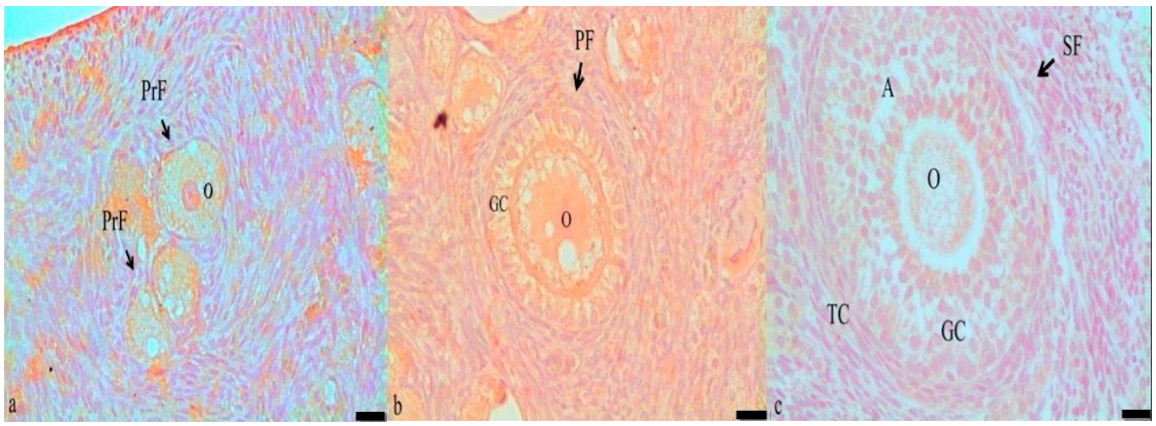

FIGURE 1. Immunoreactivity of GDF8 in primordial, primary and secondary follicles. (a) Primordial follicle, mild (+) immunoreactivity in the oocytes. No staining in the granulosa (–) cells (100x); (b) Primary follicle, mild (+) GDF8 expressions in the oocytes and granulosa cells (400x); (c) Secondary follicle, mild (+) GDF8 expressions in the oocytes and moderate (++) GDF8 expressions in granulosa cells. No staining in the thecal cells (400x). Abbreviations: PrF, primordial follicle; PF, primary follicle; SF, secondary follicle. OC, oocyte; GC, Granulosa cell; TC, thecal cell; A, Antrum

It has been stated that GDF8 improved the oocyte quality by scavenging the reactive oxygen species in the oocytes [9] inhibition of the GDF8 results in reduced antioxidant enzymes and increased intracellular free radicals. GDF8 inhibition also leads to down–regulation of GDF9 and BMP15 gene expressions in the cumulus–oocyte complex, delaying the *in vitro* maturation and reducing the fertilization and cleavage rates [25]. GDF8 may be a regulatory factor in the survival, development, and maturation of oocytes in domestic cats, as described previously [9, 25].

Fang *et al*. [26] noted that GDF8 inhibited StAR, which is a rate–limiting enzyme in steroid hormone synthesis and transports cholesterol from the cytoplasm to mitochondria to start steroidogenesis. Decreased level of StAR with increased aromatase is related to granulosa cell luteinization. In another study, GDF8 increases FSHR, thereby improving the sensitivity of the granulosa cells toward FSHR and decreasing the sensitivity of the granulosa cells against luteinizing hormone, hence lowering the P_4 synthesis in human granulosa cells. GDF8 is also related to an increase in the cytochrome P450 aromatase enzyme, leading to increased estrogen and further sensitivity of the granulosa cells to FSHR [8].

In antral follicles, GDF8 immunoreactivity ranged from mild to severe in the oocytes, granulosa cells, and cumulus oophorus, with no staining observed in the theca cells (FIG. 2a, 2b, 3a, 3b). Additionally, GDF8 was detected in the follicular fluid of antral follicles (FIG.3b).

Another study carried out on human granulosa cells by Chang *et al.* [8] reported that connective tissue growth factor (CTGF) was upregulated by GDF8, leading to a decrease in granulosa cell proliferation. In the present study, the immunoreactivity of GDF8 was mild or severe in the granulosa cells but absent in the thecal cells in the antral follicles of the cats (FIG. 2a, 2b), which indicates that GDF8 may be much more involved in the regulation of the function of the granulosa cells than thecal cells.

The presence of GDF8 immunoreactivity in the granulosa cells is consistent with that observed in humans [6], cattle [14], and buffalo [15].The current study also exhibited the immunoreactivity for GDF8 in the follicular fluid and cumulus oophorus complex in the ovarian follicles (FIG. 3b), which is similar to humans [7]. It has been reported that the presence of high levels of GDF8 in the follicular fluid led to low pregnancy rates in patients with polycystic ovarian syndrome [27].

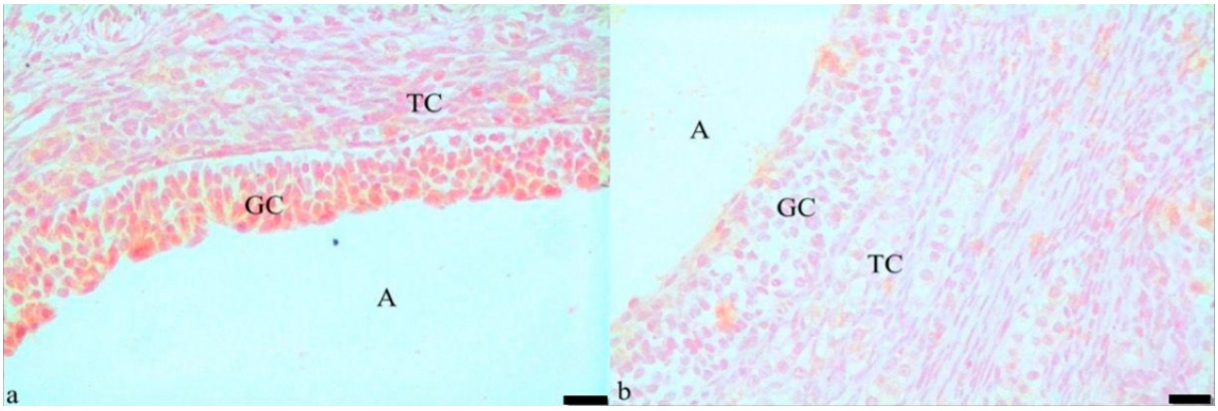

FIGURE 2. Immunoreactivity of GDF8 in granulosa and thecal cells ofantral follicles. (a) Antral Follicles, severe (+++) GDF8 expressions in granulosa cells; no staining (-) in the thecal cells (400×); (b) Antral Follicles, mild (+) GDF8 expressions in granulosa cells (400×); no staining (-) in the thecal cells. GC: Granulosa cell, TC: thecal cell, A: Antrum

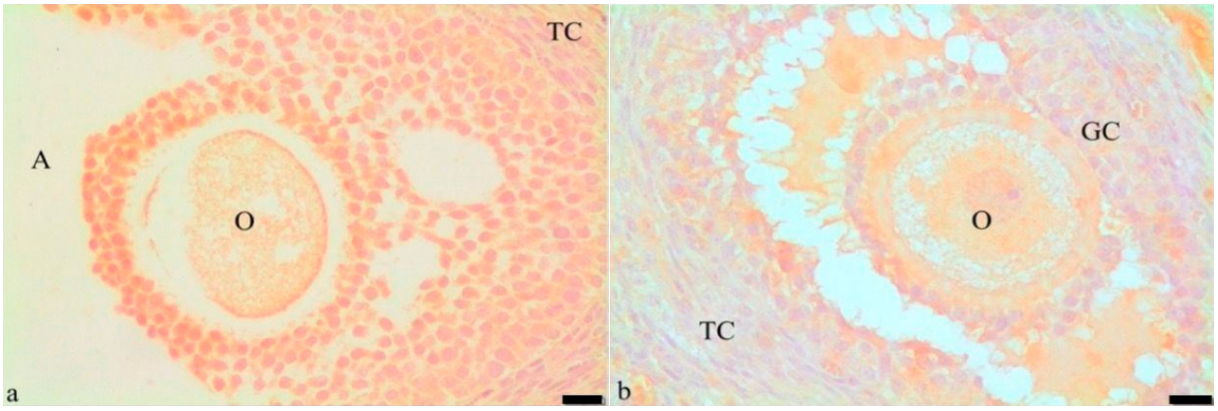

FIGURE 3. Immunoreactivity of GDF8 in cumulus oophorus and thecal cells of antral follicles. (a) Antral Follicles, severe (+++) GDF8 expressions in the cumulus oophorus; no staining in the thecal cells (400×); (b) Antral Follicles, mild (+) GDF8 expressions in the cumulus oophorus and follicular fluid; no staining in the thecal cells (400×). O: oocyte, GC: Granulosa cell, TC: thecal cell, A: Antrum

The present study indicated that mild immunoreactivity for the GDF8 was detected in some large antral follicles, similar to what has been reported in cattle [14]. This may result from the suppressive effect of follistatin on GDF8 expression. Follistatin is the major antagonist of GDF8 actions and displays its effect by binding to GDF8 [28].

Lower GDF8 expressions were observed in large oestrogen–active antral follicles with high levels of follistatin [14]. Likewise, uterine GDF8 expressions were lower in high levels of the estrogenic environment [29]. In addition, GDF8 receptors (ACRIIA, ACRIIB) and signalling pathway (Smad2/Smad3) disappear beyond the small antral follicles and are absent in large antral follicles in the feline ovary [24].

Hence, GDF8 is not expected to affect the large antral follicles in domestic cats. It can be inferred that the absence of GDF8 in the large follicles/dominant follicles may be associated with the ovulatory follicle selection process. Further studies must be conducted to examine GDF8 in the feline ovulatory follicle selection.

Growth Differentiation Factor 8 immunoreactivity ranged from mild to severe in different developmental stages of CL, with no staining observed in the non–steroidogenic cells (FIG. 4a, 4b, 4e). In the early developmental and maintenance stages of CL, mild immunoreactivity was observed in luteal cells (FIG. 4e).

In contrast, severe immunoreactivity was detected in the luteal cells of the late developmental and maintenance stage of CL (FIG. 4b). In the regression stage of CL, moderate immunoreactivity was observed in luteal cells (FIG. 4c, 4d).

Immunohistochemical scoring analysis in different developmental stages of corpus luteum

In CL, there were no differences in terms of immunoreactivity for GDF8 in different developmental stages of CL (TABLE II).

To date, there is only one study that investigates the role of GDF8 in CL Lin *et al.* [6] reported that GDF8 and its receptor are expressed in both large and small luteal cells within the human CL,

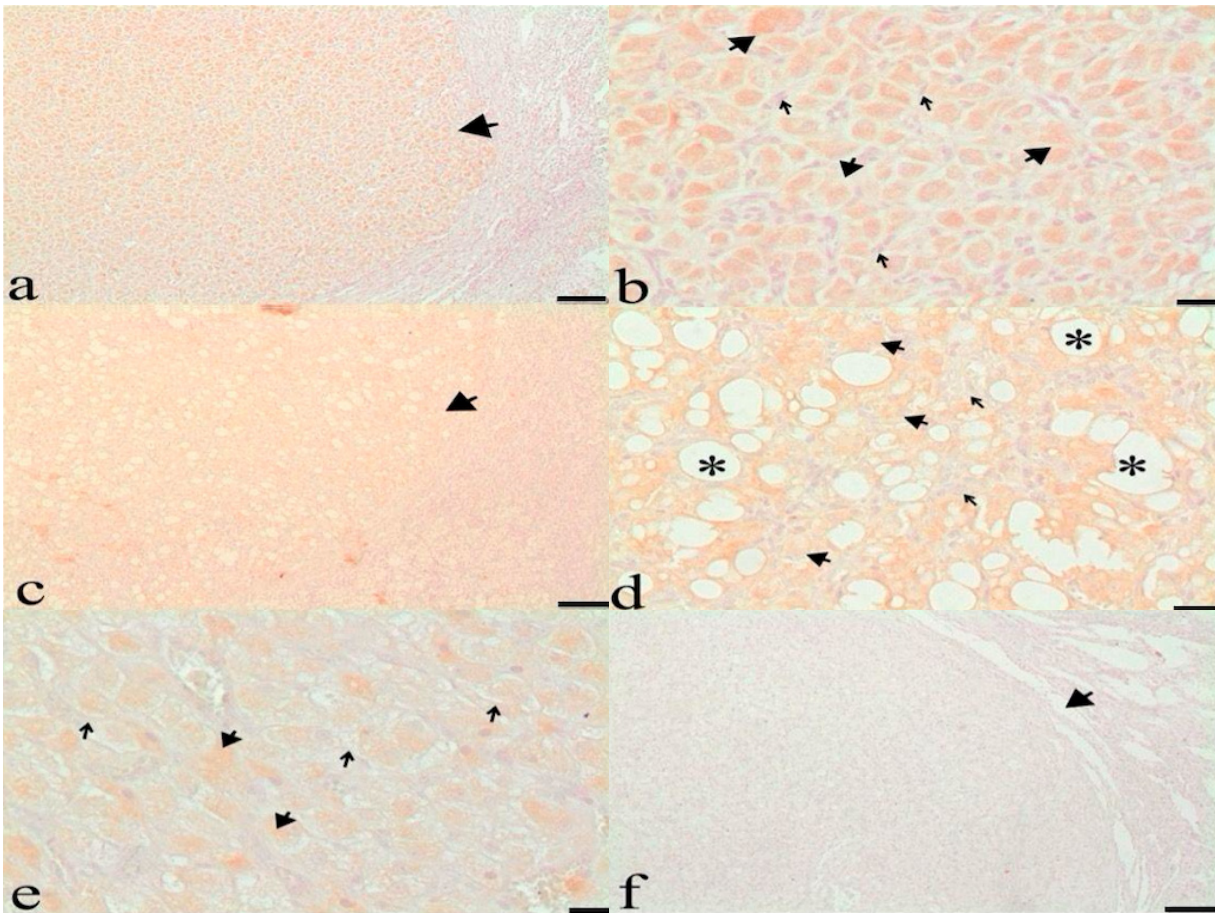

FIGURE 4. Immunoreactivity of GDF8 in different developmental stages of corpus luteum (CL). (a) late development and maintenance stage of the corpus luteum (100×). (b) late development and maintenance maintenance stage of the corpus luteum. The thick arrow shows severe (+++) GDF8 expression in luteal cells; thin arrow indicates the non–steroidogenic cells, with no immunoreactivity observed (400×). (c) regression stage of the corpus luteum (100×). (d) regression stage of the corpus luteum; the thick arrow shows moderate (++) GDF8 expression in luteal cells; thin arrow indicates the non–steroidogenic cells, with no immunoreactivity observed; * indicates type II vacuolization, which is indicative of a regressing corpus luteum (400×). (e) early, development and maintenance of the CL. The thick arrow shows mild (+) GDF8 expression in luteal cells; thin arrow indicates type I vacuolization, which is indicative of a maintenance stage of CL (400×). (f) Negative control, CL (100×)

TABLE II
Immunohistochemical scoring analysis according to corpus luteum stage (maintenance or regression) (Mean ± standard error of the mean [SEM])

Stage	Corpus Luteum			
	Mean ± SEM	Median	Min	Max
Maintenance	2.00 ± 0.21	2.00	1.00	3.00
Regression	2.00 ± 0.00	2.00	2.00	2.00
P–value		0.994		
Effect Size		0.168		

with notably higher expression in large luteal cells of the normal ovary. Consistent with present study's findings, they also observed that granulosa cells exhibited greater GDF8 expression than thecal cells. The present study detected mild immunoreactivity for GDF8 during the early developmental and maintenance stages of CL (FIG. 4e). This pattern may be attributed to the absence of GDF8 immunoreactivity seen in thecal cells of antral follicles, given that granulosa cells differentiate into large luteal cells. In contrast, thecal cells give rise to small luteal cells [30]. In the late developmental and maintenance stages of CL, strong GDF8 protein expression was observed (FIG. 4a, 4b). This may be attributed to the predominance of large luteal cells, originating from granulosa cells, during these stages of CL development. Small luteal cells predominate in the early developmental stage of CL in pseudopregnant cats. However, as CL matures, large luteal cells become the dominant cell type [31]. Therefore, changes in the GDF8 immunoreactivity may be associated with physiological changes in luteal cells.

Ovarian GDF8 concentrations

No statistical differences in ovarian GDF8 protein expression were found between the follicular and luteal groups (*P*=0.928; TABLE I). Similarly, there were no differences in GDF8 protein expression between the regression and maintenance stages of the CL (*P*=0.836; TABLE III).

This study investigated ovarian expression of GDF8 protein for the first time in domestic cats. The findings revealed that GDF8 protein expression in the ovary remained consistent across different reproductive phases (follicular and luteal) and various developmental stages of CL. Additionally, the results suggested that CL serves as a source of GDF8 in the feline ovary. The steroid hormones and gonadectomy status can influence GDF8 expression. For instance, Ciarmela *et al.* [29] demonstrated that ovariectomy increased GDF8 expression in the uterus, while estrogen replacement therapy reduced its expression in mice.

TABLE III
Ovarian GDF8 expressions (ng·mg^{-1} protein) in different periods of the reproductive cycle and in different developmental stages (maintenance and regression) of the corpus luteum (Mean ± standard error of the mean [SEM])

	Mean ± SEM	Median	Min	Max	*P*–value
Follicular (n = 15)	18.82 ± 5.33	11.57	3.36	81.99	0.928
Luteal (n = 13)	21.10 ± 6.90	10.61	2.18	81.63	
Maintenance (n = 7)	20.52 ± 8.54	12.33	2.18	68.08	0.836
Regression (n = 6)	21.77 ± 12.04	9.52	6.94	81.63	

Similarly, Wong *et al.* [32] reported peak GDF8 mRNA expression three days post–mating in hamsters (Cricetinae). Furthermore, Wallner *et al.* [33] observed a decrease in serum GDF8 concentrations following treatment with combined oral contraceptives (dienogest plus ethinylestradiol) in women. In contrast to these findings, the present study demonstrated no significant difference in ovarian GDF8 protein expression between the follicular and luteal phases. This discrepancy may be attributable to the methodology employed in this research. GDF8 protein was measured in whole ovarian tissue, encompassing multiple compartments such as follicles and CL. As a result, the composite nature of the tissue samples may have masked phase–specific variations, leading to similar protein expression levels across reproductive stages. Further studies using isolated luteal cell cultures or compartment–specific ovarian tissues are warranted to understand better the relationship between the reproductive cycle and GDF8 expression in the feline ovary.

This study has several limitations that should be considered. First, this study is strictly descriptive and investigates the immunoreactivity pattern of GDF8 detected by immunohistochemical method in the ovarian follicle and CL. Although this is, in general, an innovative approach, it remains unknown how GDF8 controls or governs steroidogenesis, folliculogenesis, and CL development or regression in cats. Second, we acknowledge that a limitation of presentstudy is the absence of species–specific validation of the GDF8 antibody in cats, such as validation via peptide–blocking assays in immunohistochemistry analysis. Nevertheless, despite the lack of such species–specific validation in the present study, it is important to note that the GDF8 protein is highly conserved across species.

Zhu *et al.* [15] demonstrated that the myostatin gene is well conserved among mammals, with water buffalo GDF8 sharing high sequence identity with other species 99, 96, 96, 94, 93, 93, and 92% identity with *Bos taurus, Ovis aries, Capra hircus, Sus scrofa, Felis catus, Equus caballus*, and *Homo sapiens*, respectively.

Additionally, the myostatin 5'–regulatory region sequence similarity between cats and humans is reported at 87.1% [34]. Based on this high degree of conservation, it can be reasonably assumed that the GDF8 antibody used in this study will likely recognize the feline GDF8 protein. However, the development and use of feline–specific antibodies and validated measurement methods for the GDF8 protein remain necessary to achieve more precise and validated results.

CONCLUSION

The present study demonstrated the presence of GDF8 protein expression in ovarian follicles and corpus luteum in feline ovaries for the first time. It also indicated ovarian GDF8 protein expressions in different reproductive periods and various developmental stages of the CL in domestic cats. The present study demonstrated that the feline ovary is the source of the GDF8, and ovarian follicles and CL exhibited GDF8 protein expression in cats.

ACKNOWLEDGEMENTS

This study was supported by Hatay Mustafa Kemal University Scientific Research Committee with Project number 24.GAP.007.

Conflict of interest

The authors declare that there was no conflict of interest.

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