ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20230381

Decreased growth differentiation factor 9, bone morphogenetic protein 15, and forkhead box O3a expressions in the ovary via ulipristal acetate

Damla Gül Fındık[1*], Gülnur Take Kaplanoğlu[2], Gökçe Nur Arık[2], Nagwa Bugumaa Abubaker Alemari[2]

SUMMARY

OBJECTIVE: Folliculogenesis is a complex process involving various ovarian paracrine factors. During folliculogenesis, vitamin D3 and progesterone are significant for the proper development of follicles. This study aimed to investigate the effects of vitamin D3 and selective progesterone receptor modulator ulipristal acetate on ovarian paracrine factors.

METHODS: In the study, 18 female Wistar-albino rats were randomly divided into three groups: control group (saline administration, n=6), vitamin D3 group (300 ng/day vitamin D3 oral administration, n=6), and UPA group (3 mg/kg/day ulipristal acetate oral administration, n=6). Ovarian tissue was analyzed by histochemistry and immunohistochemistry. For quantification of immunohistochemistry, the mean intensities of growth differentiation factor 9, bone morphogenetic protein 15, and forkhead box O3a expressions were measured by Image J and MATLAB. Blood samples were collected for the analysis of serum anti-Müllerian hormone levels by ELISA.

RESULTS: Atretic follicles and hemorrhagic cystic structures were observed in the UPA group. After immunohistochemistry via folliculogenesis assessment markers, growth differentiation factor 9, bone morphogenetic protein 15, and cytoplasmic forkhead box O3a expressions decreased in the UPA group ($p<0.05$). Anti-Müllerian hormone level did not differ significantly between the experimental groups ($p>0.05$).

CONCLUSION: Ulipristal acetate negatively affects folliculogenesis via ovarian paracrine factors. The recommended dietary vitamin D3 supplementation in healthy cases did not cause a significant change.

KEYWORDS: Ovarian follicles. Immunohistochemistry. Progesterone. Vitamin D.

INTRODUCTION

Folliculogenesis occurs in the ovaries which is a crucial organ of the female reproductive system. This mechanism is a complex process involving various growth factors and signaling molecules. Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are the factors involved in the development of the preantral follicle from the primary follicle[1]. These two factors not only improve the developmental competence of the oocyte but also act directly on the granulosa cells. GDF9 and BMP15 have critical effects on granulosa cell proliferation, differentiation, apoptosis, and cumulus expansion[2]. Forkhead box O3a (FOXO3a) is involved in various processes such as cell proliferation, apoptosis, differentiation, and metabolism. Activation of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) signaling pathway inhibits these transcription factors. Akt hyperphosphorylation results in the nuclear export of FOXO3a and primordial follicle activation[3]. Active FOXO3a causes infertility with insufficient oocyte and follicular development leading to anovulation. It has also been shown that inhibition of Akt leads to FOXO3a-dependent apoptosis[4]. Another factor, anti-Müllerian hormone (AMH), is an inhibitory regulator of primary and preantral follicle development[1].

Progesterone is one of the hormones involved in the control of ovulation. It is synthesized from the corpus luteum and follicle. The progesterone receptor (PR) is a nuclear receptor and is expressed in the granulosa cells of the Graffian follicle. Another progesterone-binding protein, PGR membrane component 1 (PGRMC1), is synthesized in the granulosa cells of developing follicles. PGRMC1 controls the antiapoptotic and antimitotic effects of progesterone on granulosa cells[5]. Progesterone regulates follicle

[1]Bilecik Şeyh Edebali University, Faculty of Medicine, Department of Histology and Embryology – Bilecik, Turkey.
[2]Gazi University, Faculty of Medicine, Department of Histology and Embryology – Ankara, Turkey.
*Corresponding author: damla.findik@bilecik.edu.tr

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: Scientific Research Project of Gazi University (TGA-2021-6954).
Received on May 05, 2023. Accepted on May 20, 2023.

growth through PI3K/Akt and mitogen-activated protein kinase (MAPK) signaling pathways[6]. According to previous research, progesterone at periovulation concentration was shown to stimulate primary follicle development[5]. UPA is a 19-norprogesterone derivative and a selective PR modulator. Selective PR modulators act as antagonists when the progesterone level increases, preventing the LH peak and ovulation. UPA inhibits ovulation possibly by suppressing the expression of PR-dependent genes critical for the process. Antiproliferative and apoptotic effects are the researched effects of UPA[7]. However, there are limited studies on the effect of folliculogenesis. According to a study conducted in 2000, the single dose administration of UPA in the mid-follicular phase suppressed lead follicle growth. They presented that high dose causes luteinized unruptured follicles[8]. In the literature, UPA effects on folliculogenesis markers are not elucidated. Effects via these factors are significant to understand the underlying mechanism.

Vitamin D3 (VitD3) is converted from 7-dehydrocholesterol in the skin under UV light. VitD3 regulates cellular functions via the VitD3 receptor (VitD3R). VitD3R binds to the vitamin response element region in DNA. These binding sites are involved in the regulation of many genes[9]. VitD3R is expressed in many organs, including the ovary. Previous studies have reported that VitD3 plays a significant role in the functions of the ovaries, including follicular development. VitD3 supplementation increases preantral follicle survival, antral follicle growth, and survival[10]. In VitD3-deficient rats, follicular development is stalled[11]. From the analyzed data, it is determined that VitD3 has a fundamental role in ovarian functions. However, VitD3 and folliculogenesis-associated mechanism is still not elucidated. It requires comprehensive research regarding the mechanism of action on ovarian paracrine signals[12]. In the literature, it was reported that 600,000 IU VitD3 single-dose administration led to an increment of BMP15 and GDF9 levels in follicular fluid[13]. The relationship between VitD3 and AMH according to a recent meta-analysis is controversial[14].

Ovarian paracrine factors originating from different compartments in the ovary play roles in follicular developmental stages. Considering progesterone and VitD3 significance for follicular development, we investigated the effects of recommended dietary VitD3 supplementation and emergency contraceptive UPA on ovarian factors. To contribute to the literature, the variations of GDF9, BMP15, FOXO3a, and AMH by UPA and VitD3 are analyzed. The observed changes and inferences of each group are given from a statistical perspective.

METHODS

Animals

We used 18 adult female Wistar albino rats (180–240 g, 6–8 weeks). The rats were housed in cages under a 12 h light/12 h dark cycle at a temperature of 22±2°C with free access to food and water. The experimental procedures were approved by the Ethics Committee of Gazi University (G.Ü.ET-20.055), and the study was conducted at Gazi University Experimental Research Center.

Experimental design

The rats were randomly divided into three groups:

Control group (n = 6): 0.05 mL/rat/day saline (peroral) per day,

VitD3 group (n = 6): 3 weeks oral VitD3 (300 ng/day) administration[15],

UPA group (n = 6): 5 weeks oral UPA (3 mg/kg/day) administration[16].

Saline, VitD3, and UPA gavage administrations for each group continued once a day and at a certain time (10.00–11.00 a.m.) throughout the week continuously. UPA and VitD3 doses were prepared to be nontoxic in accordance with the literature. As VitD3 is given to healthy rats, the application time is shorter to avoid toxic effects in rats[15,16]. VitD3 300 ng/day for a 200 g body-weighted rat is equivalent to 15.81 µg/day for a 60 kg human[17]. The recommended dietary allowance of VitD3 for adults through to 70 years is 600 IU daily (15 µg)[18]. UPA 3 mg/kg/day for a 200 g body-weighted rat is equivalent to 31.62 mg/day UPA for a 60 kg human[17]. UPA's daily dose in adults as an emergency contraceptive is 30 mg[19]. After the experiments, rats were anesthetized. Rats were euthanized by intracardiac blood collection.

Histological examination

Ovarian tissue samples were fixed in neutral buffered 10% formalin for 48 h. Samples were dehydrated through an ascending alcohol series, cleared with xylene, and embedded in paraffin. Paraffin-embedded ovarian samples were then sliced into 5 µm sections, rehydrated, and stained with hematoxylin and eosin. Ovarian sections were examined under a bright-field microscope. Images were acquired using Leica DCM 4000 (Germany).

Immunohistochemistry

For immunohistochemical analyses, the sections were deparaffinized, rehydrated, and incubated with pH 6.0 citrate buffer. Endogenous peroxidase activity was denatured with hydrogen peroxide. After Ultra V block (Thermo Scientific), the sections

were incubated with primary antibody at a dilution of 1:200 in BMP15 (E-AB-62302, Elab, USA), 1:100 in GDF9 (bs4720R, Bioss, USA), and 1:100 in FOXO3A (bs-1548R, Bioss, USA) for overnight. The sections were then washed and incubated with the biotinylated secondary antibodies (Thermo Scientific). Immunoreactive signals were detected using streptavidin-HRP (Thermo Scientific) and diaminobenzidine (Thermo Scientific). Sections were also counterstained with hematoxylin. Images were acquired using Leica DCM 4000 (Germany). For quantification of IHC staining, the mean immunoreactivity intensity for an ovarian section was measured. After color deconvolution via ImageJ software (ImageJ), images are inverted. The mean density was measured with the MATLAB software (MATLAB). Six ovaries from each group and five areas from each ovary were evaluated[20].

ELISA

Rats were euthanized by intracardiac blood collection after experiments. Serum AMH levels were measured by ELISA in these blood samples. The blood samples were centrifuged at 4,500 rpm for 15 min. The obtained serum samples were stored at -20°C before analysis. Serum AMH levels were determined using the Rat AMH ELISA kit (E0456Ra, BTLAB, China) following the manufacturer's kit procedures.

Statistical analysis

Statistical analysis was performed using an SPSS program (IBM SPSS Statistics 20). The Kruskal-Wallis method with the Dunn-Bonferroni post-hoc test was used for statistical analysis of immunoreactivity density among nonparametric groups. ANOVA test was used for statistical analysis of serum AMH levels. A p-value less than 0.05 was considered statistically significant.

RESULTS

Histomorphological findings

In the histological examination of the control group, cortex and medulla structures were observed. Normal follicles were detected through all developmental stages in the cortex (Figures 1A, B). Follicles at various developmental stages in the VitD3 group were normal in the cortex (Figures 1C, D). In the UPA group, hemorrhagic cyst structures were observed (Figure 1E). Follicles in their normal developmental stage have become atretic (Figure 1F). The number of these atretic follicles relatively increased in the UPA group compared with the other groups.

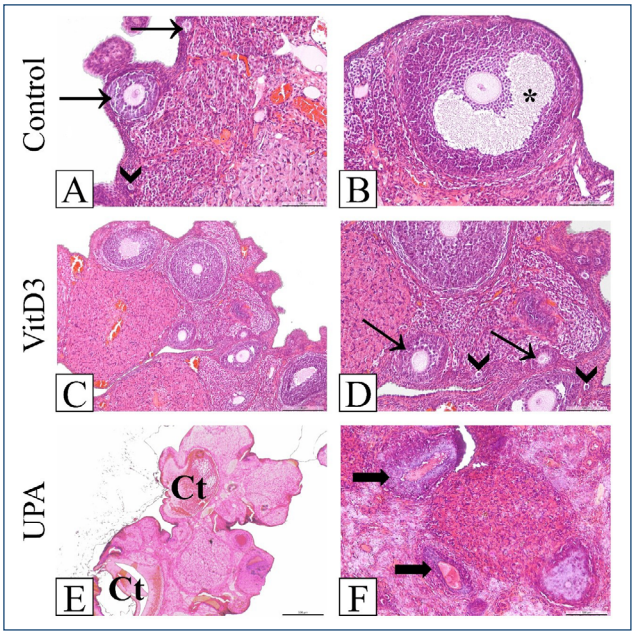

Figure 1. Hematoxylin & eosin stained sections belonging to the groups. Control group: **(A)** Primordial follicle (black arrowhead) and preantral follicles (black thin arrow). Scale bar: 100 µm. **(B)** Antral follicle (*). Scale bar: 100 µm. Vitamin D3 group: **(C)** Normal follicles at various developmental stages in the cortex. Scale bar: 200 µm. **(D)** Primordial follicle (black arrowhead) and preantral follicle (black thin arrow). Scale bar: 100 µm. UPA group: **(E)** Hemorrhagic cyst (Ct) structures. Scale bar: 500 µm. (F) Atretic follicles (black thick arrow). Scale bar: 100 µm.

Immunohistochemical results

Immunohistochemical studies were performed to evaluate the expression of BMP15, GDF9, and FOXO3a in the ovary. BMP15 expression was predominantly localized in the oocyte cytoplasm and granulosa cells of follicles. BMP15 expression was also detected in the corpus luteum. It was noticed that BMP15 expression decreased in the corpus luteums of the UPA group (Figure 2A). The mean pixel density of BMP15 throughout the ovary decreased in the UPA group compared with the other groups ($p<0.05$) (Figure 2D). The GDF9 expression was mainly detected in the oocyte cytoplasm. The GDF9 immunoreactivity was also detected in the corpus luteum structures. The GDF9 immunoreactivity decreased in the corpus luteums and preantral and antral follicles of the UPA group (Figure 2B). The mean pixel density of GDF9 decreased throughout the ovary in the UPA group compared with the other groups ($p<0.05$) (Figure 2D). FOXO3a expression was predominantly detected in the oocyte cytoplasm of the preantral follicles. It was observed that its expression decreased in antral follicles. UPA group shows weak FOXO3a immunoreaction in the antral and preantral follicles (Figure 2C). The mean pixel density of FOXO3a decreased throughout the ovary in the UPA group compared with the VitD3 group ($p<0.05$) (Figure 2D).

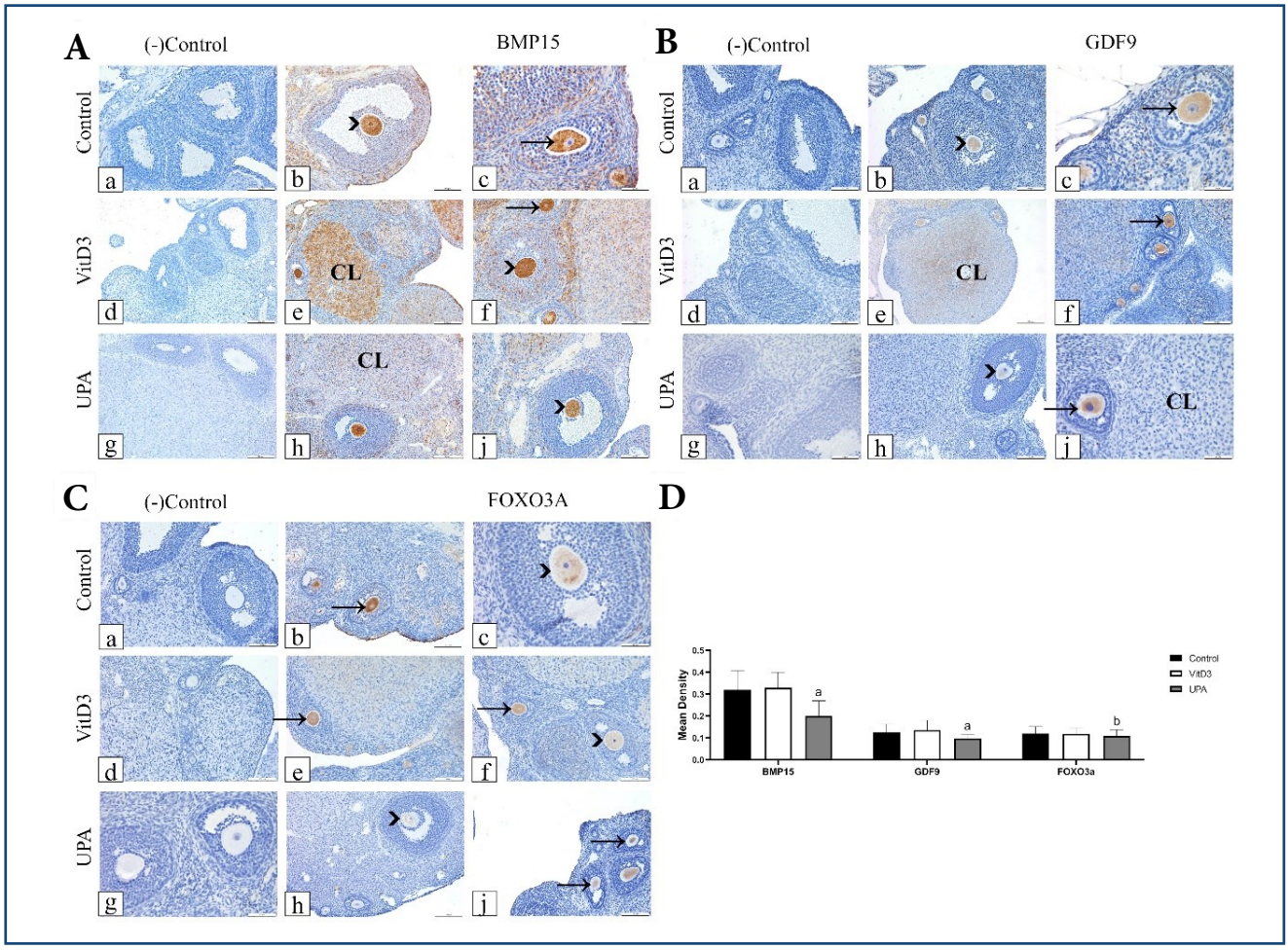

Figure 2. Bone morphogenetic protein 15, growth differentiation factor 9, and forkhead box O3a immunohistochemical staining of the groups and mean pixel density assessment. The first panels of immunohistochemical stains present negative controls **(a,d,g)**. Immunoreactivity is represented in the ooplasm of preantral follicle with a black arrow and antral follicle with a black arrowhead. **(A)** Bone morphogenetic protein 15 immunohistochemical staining; **(b)** bone morphogenetic protein 15 expression in the oocyte cytoplasm (black arrowhead). **(c)** Predominant bone morphogenetic protein 15 expression is localized in the oocyte cytoplasm (black arrow) and granulosa cells. **(e)** Vitamin D3 group shows strong immunoreaction in the corpus luteum. **(f)** Bone morphogenetic protein 15 expression in the oocyte cytoplasm (black arrowhead, black arrow). (h) UPA group shows weak immunoreaction in the corpus luteum. **(j)** Bone morphogenetic protein 15 expression in the oocyte cytoplasm (black arrowhead). Scale bars: Panel **(c)**—50 μm, panels **(d,e)**—200 μm, and others—100 μm. (B) Growth differentiation factor 9 immunohistochemical stain. **(b)** Growth differentiation factor 9 expression in the oocyte cytoplasm (black arrowhead). **(c)** Predominant growth differentiation factor 9 expression is localized in the oocyte cytoplasm (black arrow). **(e)** Growth differentiation factor 9 immunoreaction in the corpus luteum of the vitamin D3 group. **(f)** Growth differentiation factor 9 expression in the oocyte cytoplasm (black arrow). (h) UPA group shows weak immunoreaction in the antral follicle (black arrowhead). **(j)** UPA group shows weak immunoreaction in the preantral follicle (black arrow). Expression of growth differentiation factor 9 in corpus luteums decreased in this group. Scale bars: Control **(c,g,j)**—50 μm, **(e)**—200 μm, and others—100 μm. **(C)** Forkhead box O3a immunohistochemical stain. **(b)** Control group shows strong immunoreaction in the preantral follicle (black arrow). **(c)** Control group shows weaker immunoreaction in the antral follicle (black arrowhead) compared with preantral follicles. **(e,f)** Forkhead box O3a expression in the oocyte cytoplasm (black arrowhead, black arrow). (h) UPA group shows weak immunoreaction in the antral follicle (black arrowhead). **(j)** UPA group shows weak immunoreaction in the preantral follicles (black arrow). Scale bars: Panels **(c,f,g)**—50 μm and others—100 μm. **(D)** Immunoreactivity assessment via mean pixel density of the groups throughout the ovary; Kruskal-Wallis test and post-hoc Dunn-Bonferroni. Bone morphogenetic protein 15, growth differentiation factor 9, and forkhead box O3a mean pixel density decreased in the UPA group. **(a)** significant difference compared with the control and vitamin D3 groups. **(b)** Significant difference compared with the vitamin D3 group. Statistical significance: $p<0.05$.

Serum anti-Müllerian hormone levels

After the evaluation of serum AMH levels with ELISA, there was no statistically significant difference between the groups ($p>0.05$) (Table 1).

DISCUSSION

Studies on follicular development will provide comprehensive data on female reproductive life and facilitate the development of new therapeutic approaches against reproductive

Table 1. Comparison of serum anti-Müllerian hormone levels (ng/mL).

AMH (ng/mL)					
Groups	**Mean**		**Std. deviation**		**Std. error**
Control	6.245		4.14		1.85129
VitD3	4.963		2.072		0.92645
UPA	5.574		2.762		1.23535
Source of variation	**Sum of squares**	**Degree of freedom**	**Mean square**	**F**	**p-value**
Between groups	4.111	2	2.055	0.212	0.812
Within groups	116.233	12	9.686		
Total	120.344	14			

One-way ANOVA test. Statistical significance: p<0.05.

aging[1]. In the scope of this study, the effects of UPA and VitD3 on ovarian paracrine factors were examined immunohistochemically and biochemically. Progesterone affects granulosa cells via the PGRMC1 receptor, and its concentration correlates with follicle development. Komatsu et al. showed that when the variation of the progesterone concentration level was imitated, periovulation concentration stimulated primary follicle development[5]. According to a study conducted in 2000, Stratton et al. found that the single dose administration of antiprogestin UPA in the mid-follicular phase suppressed follicular growth[8]. In our study, we observed atretic follicles in the UPA group. By considering related studies, it was deduced that the specified situation presumably is related to the inhibition of required progesterone concentration by UPA for proper follicle development. In 2009, Tamura et al. demonstrated that mifepristone, a PR antagonist, caused luteal cysts and large corpus luteum structures at doses of 20 mg/kg or more in rats[21]. Large hemorrhagic cyst structures were also detected in our UPA group.

BMP15 is involved in normal follicular development as well as being mitogenic for somatic cells. Another crucial factor for preantral follicle development is GDF9[22]. In 2018, Xu et al. showed that VitD3 causes increased expression of BMP and GDF9 in preantral follicles[10]. There is no information in the literature about UPA effects on folliculogenesis markers. In our study, GDF9 and BMP15 expressions did not show a significant difference with recommended dietary VitD3 dose (approximately 15 µg/day for an adult human) in healthy cases[18]. UPA administration with an emergency contraceptive dose (approximately 30 mg) suppressed these expressions[19].

The phosphorylated FOXO3a by PI3K-Akt is an inactive form and localized in the cytoplasm. Its active non-phosphorylated form is localized in the nucleus and induces apoptosis[23]. In this study, decreased cytoplasmic FOXO3a expression in the UPA group represents inactive form and it is parallel with atretic follicle findings. We conclude that UPA causes atresia, possibly by inducing FOXO3a-dependent apoptosis. In the literature, it was concluded that activated FOXO3a reduces the expression of BMP15[24]. In our study, decreased expressions of BMP15 and inactive FOXO3a in the UPA group were consistent with the association of these factors.

The relationship between vitamin D and AMH in the literature is controversial[14]. In our study, recommended dietary VitD3 supplementation did not change serum AMH levels. In 2017, Dennis et al. indicated that the amount of AMH produced per follicle is only a minor determinant of circulating AMH level[25]. In this study, the absence of a significant change in serum AMH values between the groups was attributed to this deduction.

CONCLUSION

The effects of UPA and VitD3 on ovarian paracrine factors were studied in this work by considering studies addressing the corresponding topic. During histological analyses, UPA decreased inactive form of FOXO3a expression and caused follicular atresia. The recommended dietary VitD3 supplementation in healthy cases did not cause significant changes in GDF9 and BMP15 expressions. However, UPA suppressed these expressions. It was concluded that UPA negatively affects folliculogenesis via ovarian paracrine factors.

ACKNOWLEDGMENTS

This study was supported by the Scientific Research Project of Gazi University (TGA-2021-6954).

AUTHORS' CONTRIBUTIONS

DGF: Data curation, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. **GTK:** Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **GNA:** Data curation, Methodology, Project administration. **NBAA:** Data curation, Project administration.

REFERENCES

1. Oktem O, Urman B. Understanding follicle growth in vivo. Hum Reprod. 2010;25(12):2944-54. https://doi.org/10.1093/humrep/deq275
2. Sanfins A, Rodrigues P, Albertini DF. GDF-9 and BMP-15 direct the follicle symphony. J Assist Reprod Genet. 2018;35(10):1741-50. https://doi.org/10.1007/s10815-018-1268-4
3. John GB, Gallardo TD, Shirley LJ, Castrillon DH. Foxo3 is a PI3K-dependent molecular switch controlling the initiation of oocyte growth. Dev Biol. 2008;321(1):197-204. https://doi.org/10.1016/j.ydbio.2008.06.017
4. Das TP, Suman S, Alatassi H, Ankem MK, Damodaran C. Inhibition of AKT promotes FOXO3a-dependent apoptosis in prostate cancer. Cell Death Dis. 2016;7(2):e2111. https://doi.org/10.1038/cddis.2015.403
5. Komatsu K, Masubuchi S. The concentration-dependent effect of progesterone on follicle growth in the mouse ovary. J Reprod Dev. 2017;63(3):271-7. https://doi.org/10.1262/jrd.2016-154
6. Long H, Yu W, Yu S, Yin M, Wu L, Chen Q, et al. Progesterone affects clinic oocyte yields by coordinating with follicle stimulating hormone via PI3K/AKT and MAPK pathways. J Adv Res. 2021;33:189-99. https://doi.org/10.1016/j.jare.2021.02.008
7. Ghonim M, Magdy R, Sabbour M, Ghonim M, Nabhan A. A systematic review and meta-analysis of ulipristal acetate for symptomatic uterine fibroids. Int J Gynaecol Obstet. 2019;146(2):141-8. https://doi.org/10.1002/ijgo.12868
8. Stratton P, Hartog B, Hajizadeh N, Piquion J, Sutherland D, Merino M, et al. A single mid-follicular dose of CDB-2914, a new antiprogestin, inhibits folliculogenesis and endometrial differentiation in normally cycling women. Hum Reprod. 2000;15(5):1092-9. https://doi.org/10.1093/humrep/15.5.1092
9. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev. 2016;96(1):365-408. https://doi.org/10.1152/physrev.00014.2015
10. Xu J, Lawson MS, Xu F, Du Y, Tkachenko OY, Bishop CV, et al. Vitamin D3 regulates follicular development and intrafollicular vitamin D biosynthesis and signaling in the primate ovary. Front Physiol. 2018;9:1600. https://doi.org/10.3389/fphys.2018.01600
11. Dicken CL, Israel DD, Davis JB, Sun Y, Shu J, Hardin J, et al. Peripubertal vitamin D(3) deficiency delays puberty and disrupts the estrous cycle in adult female mice. Biol Reprod. 2012;87(2):51. https://doi.org/10.1095/biolreprod.111.096511
12. Findik DG. Vitamin D effects on folliculogenesis via ovarian paracrine factors. Eskisehir Med J. 2022;3(3):316-21.
13. Paffoni A, Somigliana E, Sarais V, Ferrari S, Reschini M, Makieva S, et al. Effect of vitamin D supplementation on assisted reproduction technology (ART) outcomes and underlying biological mechanisms: protocol of a randomized clinical controlled trial. The "supplementation of vitamin D and reproductive outcome" (SUNDRO) study. BMC Pregnancy Childbirth. 2019;19(1):395. https://doi.org/10.1186/s12884-019-2538-6
14. Moridi I, Chen A, Tal O, Tal R. The association between vitamin D and anti-Müllerian hormone: a systematic review and meta-analysis. Nutrients. 2020;12(6):1567. https://doi.org/10.3390/nu12061567
15. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev. 1998;78(4):1193-231. https://doi.org/10.1152/physrev.1998.78.4.1193
16. Pohl O, Harvey PW, McKeag S, Boley SE, Gotteland JP. Carcinogenicity and chronic rodent toxicity of the selective progesterone receptor modulator ulipristal acetate. Curr Drug Saf. 2013;8(2):77-97. https://doi.org/10.2174/15748863112079990012
17. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27-31. https://doi.org/10.4103/0976-0105.177703
18. Chang SW, Lee HC. Vitamin D and health - the missing vitamin in humans. Pediatr Neonatol. 2019;60(3):237-44. https://doi.org/10.1016/j.pedneo.2019.04.007
19. Dinis-Oliveira RJ. Pharmacokinetics, toxicological and clinical aspects of ulipristal acetate: insights into the mechanisms implicated in the hepatic toxicity. Drug Metab Rev. 2021;53(3):375-83. https://doi.org/10.1080/03602532.2021.1917599
20. Park MJ, Ahn JW, Kim KH, Bang J, Kim SC, Jeong JY, et al. Prediction of ovarian aging using ovarian expression of BMP15, GDF9, and C-KIT. Exp Biol Med (Maywood). 2020;245(8):711-9. https://doi.org/10.1177/1535370220915826
21. Tamura T, Yokoi R, Okuhara Y, Harada C, Terashima Y, Hayashi M, et al. Collaborative work on evaluation of ovarian toxicity. 2) Two- or four-week repeated dose studies and fertility study of mifepristone in female rats. J Toxicol Sci. 2009;34 Suppl 1:SP31-42. https://doi.org/10.2131/jts.34.s31
22. Karagül Mİ, Aktaş S, Coşkun Yılmaz B, Yılmaz M, Orekici Temel G. GDF9 and BMP15 expressions and fine structure changes during folliculogenesis in polycystic ovary syndrome. Balkan Med J. 2018;35(1):43-54. https://doi.org/10.4274/balkanmedj.2016.1110
23. Cui C, Han S, Yin H, Luo B, Shen X, Yang F, et al. FOXO3 is expressed in ovarian tissues and acts as an apoptosis initiator in granulosa cells of chickens. Biomed Res Int. 2019;2019:6902906. https://doi.org/10.1155/2019/6902906
24. Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF-9 and BMP-15 in ovarian function. Mol Reprod Dev. 2011;78(1):9-21. https://doi.org/10.1002/mrd.21265
25. Dennis NA, Houghton LA, Pankhurst MW, Harper MJ, McLennan IS. Acute supplementation with high dose vitamin D3 increases serum anti-Müllerian hormone in young women. Nutrients. 2017;9(7):719. https://doi.org/10.3390/nu9070719