A novel antimüllerian gene mutation in a woman with high antral follicle count and extremely low serum antimüllerian hormone levels

Laura Melado, M.D., Ph.D.,[a] Barbara Lawrenz, M.D., Ph.D.,[a,c] Jonalyn Edades, B.S.,[a] Ajay Kumar, Ph.D.,[b] and Human Fatemi, M.D., Ph.D.[a]

[a] ART Fertility Clinics, Abu Dhabi, United Arab Emirates; [b] Ansh Labs LLC, Medical Center Blvd, Webster, Iowa; and [c] Reproductive Unit, UZ Gent., Gent, Belgium

Objective: To report a case with a distinct difference between the ovarian reserve parameters of antimüllerian hormone (AMH) levels, antral follicle count (AFC), and follicle-stimulating hormone levels caused by a novel homozygous missense variant in the exon 1 of the *AMH* gene [NM_000479.4:c259G>A, p.(Val87Met)].
Design: Case report.
Setting: Tertiary referral in vitro fertilization clinic.
Patients: A 33-year-old woman, G4P4A0E0L4, with a BMI of 25.33 kg/m^2, high AFC, and repeated extremely low systemic AMH levels, was detected and measured using multiple enzyme-linked immunosorbent assays.
Interventions: Antimüllerian hormone analysis with multiple assays, whole exome sequencing through next generation sequencing to diagnose the missense variant, and inhibin B measurement.
Main Outcomes Measures: Genetic counseling and two subsequent ovarian stimulations for successful fertility preservation.
Results: Detection of the [NM_000479.4:c259G>A, p.(Val87Met)] variant in the AMH gene. Retrieval and cryopreservation of four euploid blastocysts and 26 metaphase II oocytes.
Conclusions: *AMH* gene mutations can lead to the absence of systemic AMH levels and might be discordant to other ovarian reserve markers like AFC, follicle-stimulating hormone, and inhibin B, without affecting the ovarian response to ovarian stimulation. Clinicians should not rely exclusively on AMH levels for ovarian stimulation. When severely reduced AMH levels are found in patients with high AFC, AMH variants should be suspected, and fertility treatments should be tailored adequately. (F S Rep® 2024;5:152–6. ©2024 by American Society for Reproductive Medicine.)
Key Words: Antimullerian hormone, antral follicle count, gene mutations, ovarian markers

Antimüllerian hormone (AMH) is a homodimeric glycoprotein produced and secreted by the granulosa cells of the preantral and antral ovarian follicles. It belongs to the transforming growth factor-β (TGF-β) family and is coded by a five-exon gene located on chromosome 19 (1). Within the ovary, AMH inhibits primordial follicle recruitment to follicle-stimulating hormone (FSH)–stimulated antral follicle development, like a follicular gatekeeper. This action limits follicle growth initiation and slows the depletion of the ovarian reserve (2, 3).

Antral follicle count (AFC) and AMH levels are considered the best markers for functional ovarian reserve assessment, showing a strong positive correlation between them (4). Both markers have shown similar fluctuations throughout the menstrual cycle (5, 6), explained by the fact that small growing follicles included in the AFC are the main contributors to serum AMH concentration (7). However, it is not uncommon to identify cases where AFC and AMH levels are discordant, even though both measurements are performed by highly trained professionals during the early follicular phase in the same center (8) and cannot be explained by technical limitations or assay variability (9). Furthermore, cases with very low levels of circulating AMH in women with high AFC have been published (10, 11). In the case described by Hoyos et al. (10), a genetic variant with impaired serum AMH immunoactivity was identified in a woman diagnosed with polycystic ovary syndrome (PCOS), yet no ovarian stimulation

Received May 11, 2023; revised and accepted January 30, 2024.
The data underlying this article cannot be shared publicly for the privacy of individuals who participated in the study. The data will be shared on reasonable request to the corresponding author.
Correspondence: Laura Melado, M.D., Ph.D., ART Fertility Clinics, Marina Village Villa B22–23, Abu Dhabi, 60202, United Arab Emirates (E-mail: laura.melado@artfertilityclinics.com).

F S Rep® Vol. 5, No. 2, June 2024 2666-3341

https://doi.org/10.1016/j.xfre.2024.01.006

was performed. Grbavac et al. (11) presented a case of undetectable AMH serum levels and high AFC who developed ovarian hyperstimulation syndrome (OHSS) during in vitro fertilization using a high dose of recombinant FSH; however, no genetic investigations were performed in this case. The finding of unexpectedly discrepant ovarian reserve parameters challenges the ovarian stimulation strategy with important clinical significance. Moreover, new fertility drugs have been developed and linked to AMH level only as a parameter to consider for dose decisions (12).

We present the case of a nonobese woman with severely reduced serum AMH levels and high AFC, its biochemical and molecular investigations, including a novel mutation in the *AMH* gene, fertility counseling, and family planning.

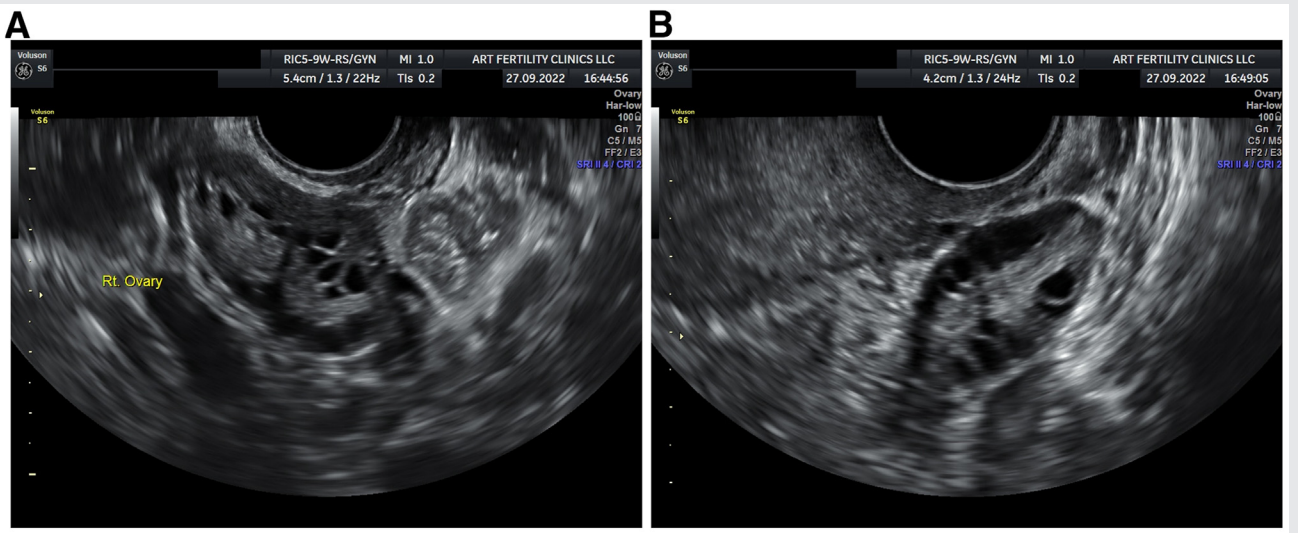
CASE REPORT

A 33-year-old woman, G4P4A0E0L4, BMI 25.33 kg/m^2, was referred to us in September 2022, seeking a second opinion after being diagnosed with premature ovarian insufficiency on the basis of her serum AMH levels. The AMH test result was <0.07 ng/mL (Beckman Coulter AMH generation II), performed in August 2022 and requested by her gynecologist in an external laboratory. Her past medical history was unremarkable: no known allergies, no smoking, and no parental consanguinity. She had menstrual irregularities since menarche, having spontaneous periods every 3–4 months, and polycystic ovarian morphology (PCOM) with an AFC of 20 per ovary (Fig. 1), although there were no signs of clinical or biochemical hyperandrogenism. She was married to her first-degree cousin and had four unassisted naturally conceived pregnancies followed by four normal vaginal deliveries without complications, all of them girls. The couple had a 7-month-old daughter who had been diagnosed with gross motor delay, hypotonia, and bilateral foot eversion.

Her serum hormone measurements are depicted in Table 1, along with her serum AMH levels measured with different enzyme-linked immunosorbent assay (ELISA) methods, which included antibodies mapped to different regions of the AMH molecule (N-terminus, C-terminus, and N and C-terminus). The patient had extremely reduced serum AMH levels detected for her age, irrespective of the assay methods used. Surprisingly, she presented a high circulating inhibin B concentration.

Because of consanguinity of the couple, and having a daughter with gross motor delay and hypotonia, a whole exome sequencing for the couple through next generation sequencing (Igenomix) was performed on DNA extracted from the patient and husband's blood. The couple was found to be carriers of a pathogenic mutation in gene *HBA* ($-\alpha$3.7) and a variant of uncertain significance in the *SC5D* gene [NM_006918.4:c.418G>A, p.(Gly140Ser)]. Pathogenic variants in the *SC5D* gene are associated with lathosterolosis with an autosomal recessive mode of inheritance. The couple was referred to a clinical geneticist for further evaluation of their daughter syndrome and the possible association with this variant of uncertain significance, which was not identified in the affected daughter. After discussing the results with the genetic counselor, the couple decided not to proceed with preimplantation genetic testing of Mendelian disorders (for this variant). Regarding the pathogenic mutation in gene *HBA*, a couple has a 25% risk of having offspring with a $-\alpha$3.7 variant in a homozygous state. However, this condition does not pose a risk for the severe form of α-thalassemia. Interestingly, the woman presented also a homozygous missense variant in exon 1 of the *AMH* gene [NM_000479.4:c259G>A, p.(Val87-Met)] that replaced the amino acid valine with methionine at

FIGURE 1



Transvaginal ultrasound images of patient's ovaries. (**A**) Right ovary. (**B**) Left ovary.

Melado. AMH *gene mutation in high AFC woman. F S Rep 2024.*

TABLE 1

Index woman serum hormone measurements.

Clinical and laboratory characteristics	Index woman
Basal E2 (pg/mL)	56.9
Basal FSH (mIU/mL)	7.66
Basal LH (mIU/mL)	23.48
Basal P4 (ng/mL)	0.05
TSH (μIU/mL)	1.39
PRL (μIU/mL)	127.30
DHEA-S (μg/dL)	152
TT (ng/dL)	37.5
Free T (calculated) (ng/dL)	0.005
Albumin (g/L)	42.8
SHBG (nmol/L)	105
Androstenedione (ng/mL)	1.08
AFC	40
Inhibin B (US InhB ELISA, Ansh Labs) (pg/mL)	165.5
AMH Gen II ELISA (Beckman Coulter) (ng/mL)	0.07
AMH pro-mature Elecsys ELISA (Roche) (ng/mL)	0.084
Pro-mature PicoAMH ELISA (Ansh Labs)	0.084
Pro-mature US-AMH ELISA (Ansh Labs) (ng/mL)	0.103
Mature-Mature AMH ELISA (Ansh Labs) (ng/mL)	0.047
PCOCheck AMH ELISA (Ansh Labs) (ng/mL)	0.122

Note: AFC = antral follicle count; AMH = antimüllerian hormone; DHEA-S = dehydroepiandrosterone Sulfate; E2 = estradiol; Free T = free testosterone; FSH = follicle-stimulating hormone; LH = luteinizing hormone; P4 = progesterone; PRL = prolactin (reference values: 86–324 μIU/mL); SHBG = sex hormone binding globulin; TSH = thyroid-stimulating hormone; TT = testosterone total (reference values: 8–60 ng/dL).

Melado. AMH *gene mutation in high AFC woman. F S Rep 2024.*

codon 87. Preimplantation genetic testing of Mendelian disorders was not indicated because the patient was homozygous for this variant and all her offspring will be carriers. The patient signed the informed consent related to the case to be reported in a medical publication.

On the basis of the genetic conditions and the wife's risk of possible early oocyte depletion, the couple decided to undergo two ovarian stimulation cycles for fertility preservation: one cycle for in vitro fertilization and intracytoplasmic sperm injection and one cycle for oocyte vitrification. First ovarian stimulation was performed with antagonist protocol using 225–150 IU of HP-HMG (Menopur, Ferring, Kiel, Germany) for 10 days and cetrorelix (Cetrotide, Merck, Darmstadt, Germany) for 7 days. As soon as three follicles reached ≥17 mm, dual trigger for final oocyte maturation was performed with 2,500 IU subcutaneous of hCG (Choriomon, IBSA, Lugano, Switzerland) and 0.3 mg subcutaneous of triptorelin (Decapeptyl, IPSEN, Signes, France). Serum oestradiol levels were 3,604 pg/mL and serum progesterone levels were 1.06 ng/mL on the day of trigger. Oocyte pick-up was performed under sedation 36 hours later, and 38 cumulus-oocyte-complexes were collected and 22 were metaphase II oocytes. On day 6, five blastocysts were biopsied for preimplantation genetic testing for aneuploidy, and four euploid embryos were vitrified. On the couple's request, after one menstrual cycle, a second ovarian stimulation was performed following the same protocol. Serum oestradiol levels were 4,411 pg/mL, and serum progesterone levels were 1.10 ng/mL on the day of the trigger. A total of 32 cumulus-oocyte complexes were retrieved, and 26 oocytes were vitrified. No signs or symptoms related to OHSS were observed during her follow-up after the oocyte retrievals.

DISCUSSION

Here, we present a case of a patient who was diagnosed mistakenly with premature ovarian insufficiency because of extremely low AMH levels. The patient presented extremely low serum AMH levels detected using multiple ELISA methods, high AFC, and normal levels of inhibin B and FSH. Further genetic analysis revealed a novel missense variant in exon 1 of the *AMH* gene [NM_000479.4:c259G>A, p.(Val87Met)], presented in homozygosis in this young fertile woman. The herein described variant had not been reported in the literature or population databases and was classified as a variant of uncertain significance according to the American College of Medical Genetics and Genomics guidelines (13). This missense variant severely impaired the circulating AMH immunodetection using different assay methods, yet further functional analysis was not performed to investigate the normal biologic function of the AMH variant. The patient successfully completed two cycles of ovarian stimulation for fertility preservation.

Mutations inactivating *AMH* or affecting the AMH receptor type 2 (AMHR2) are responsible for 90% of cases of persistent müllerian duct syndrome, defined as the persistence of müllerian derivates in otherwise healthy 46,XY men (1). It is transmitted as an autosomal recessive trait, with a high prevalence in consanguine families with known mutations. Although consanguinity is highly prevalent in the Middle East region, the index woman did not present parental consanguinity (14). Subjects with a single mutated allele or homozygous allele are phenotypically healthy (15). Women with homozygous mutations have a normal phenotype as well, but it has been suggested that they may undergo premature menopause because AMH normally inhibits primordial follicular recruitment (2) and does not fulfill this function because of the mutation.

Different *AMH* coding mutations have been described previously, some in patients with PCOS, coding different areas of the AMH molecule and affecting protein processing and/or bioactivity. Normally, granulosa cells primarily release AMH as a nonactive prohormone, which forms a stable active complex ($AMH_{N,C}$) after cleavage by proteinases (16). Circulating AMH is a mixture of AMH as a nonactive prohormone, which does not appear to activate AMHR2 but is important in AMH synthesis and extracellular transport, and $AMH_{N,C,}$ the mature bioactive form, which binds and activates the receptor (17). This processing exposes new antigenic sites, which may affect measurements using conventional ELISA methods (18). Blood samples from the patient were measured using six different AMH assay methods: Beckman Coulter Gen II assay, automated Roche Elecsys assay, Ansh Labs picoAMH assay, US-AMH assay,

Mature-Mature AMH assay, and the PCOCheckTM AMH assay. Antimüllerian hormone showed extremely low levels regardless of the assay performed, suggesting a lack of circulating AMH immunodetection using different commercially available assays. Interestingly, bioactive inhibin B (bio-InhB) levels were high (165.5 pg/mL). Inhibin B is produced by granulosa cells of antral follicles and is correlated with oocyte yield after ovarian stimulation (19). Hence, high bio-InhB levels were concordant with the AFC and corresponded to granulosa cell activity. It is important to differentiate between the immunoactivity and the bioactivity of the AMH protein. For that purpose, a functional analysis of the variant should be performed. Hoyos et al. (10) recently published a homozygous mutation in exon 5 in a woman with PCOS that modified the circulating AMH molecule immunoactivity, impairing AMH antibody-epitope recognition by a commercial picoAMH ELISA assay (10). In addition, Meng et al. (20) described the functional analysis of other variants in women with PCOS.

Although neither biochemical nor clinical signs of hyperandrogenism were presented, oligomenorrhea, AFC, bio-InhB, and the patient's elevated levels of luteinizing hormone were in line with possible PCOS (19, 21). Hence, the ovarian stimulation protocol and dosage were planned for an expected high responder. The ovarian response to stimulation confirmed a high number of growing follicles with adequate steroid production, obtaining a high number of mature oocytes in both stimulation cycles. Frequent monitoring was performed during and after ovarian stimulation, and no OHSS occurred. Although AMH level is a very useful tool for ovarian reserve assessment and ovarian stimulation, the case herein demonstrates that it should not be used as an independent ovarian reserve marker because the risk of severe OHSS and complications like ovarian torsion and bleeding may be high (11) and the wrong dosage of gonadotropins would have been administered. Moreover, the recently introduced fertility drug, follitropin delta, has been advised to be linked to systemic AMH only, which would have been unsafe for the current patient. Any discrepancies between laboratory findings and clinical condition should be cautiously considered during the ovarian stimulation planning.

In previous publications, including cases with discordant levels of AMH and AFC, index patients have been described and compared with typical cases of PCOS. However, it has been demonstrated in murine models that AMH prevents early depletion of the follicle pool in the ovary by inhibiting initial follicle recruitment (22). As shown in AMH-null mice, in the absence of AMH, more primordial follicles are recruited into the growing pool, and, as a result, ovaries are depleted of their primordial follicles significantly earlier (23). Although the same data have not been published yet in humans, it is important to keep in mind that subjects with homozygous mutated alleles for either AMH or AMHR2 may experience premature menopause, and close clinical observation of these patients should be visible. Adequate counseling including family planning and fertility preservation, should be provided to the patients.

CONCLUSION

A novel mutation in the *AMH* gene is described, together with fertility treatment and fertility preservation. The *AMH* gene mutations can significantly impair serum AMH levels and might be discordant to other ovarian reserve markers without affecting ovarian response during ovarian stimulation. Caution should be taken when undetectable or severely reduced serum AMH levels are found in patients with high AFC. *AMH* variants should be suspected, and fertility treatments should be tailored adequately.

Acknowledgments

The authors thank the Scientific Department at ART Fertility Clinics (UAE) for their support throughout the period of manuscript preparation.

CRediT Authorship Contribution Statement

Laura Melado: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Barbara Lawrenz:** Writing – review & editing, Validation, Supervision. **Jonalyn Edades:** Writing – review & editing, Resources, Project administration, Methodology, Data curation. **Ajay Kumar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Human Fatemi:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation, Conceptualization.

Declaration of Interests

L.M.V. has nothing to disclose. B.L. has nothing to disclose. J.E. has nothing to disclose. A.K. has nothing to disclose. H.F. has nothing to disclose.

REFERENCES

1. Picard JY, Josso N. Persistent müllerian duct syndrome: an update. Reprod Fertil Dev 2019;31:1240–5.
2. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of primordial follicle recruitment by anti-müllerian hormone in the mouse ovary1. Endocrinology 1999;140:5789–96.
3. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of anti-müllerian hormone in women. Hum Reprod Update 2014;20:370–85.
4. Fleming R, Seifer DB, Frattarelli JL, Ruman J. Assessing ovarian response: antral follicle count versus anti-Müllerian hormone. Reprod Biomed Online 2015;31:486–96.
5. Depmann M, van Disseldorp J, Broer SL, Eijkemans MJC, Laven JSE, Visser JA, et al. Fluctuations in anti-müllerian hormone levels throughout the menstrual cycle parallel fluctuations in the antral follicle count: a cohort study. Acta Obstet Gynecol Scand 2016;95:820–8.
6. Gnoth C, Roos J, Broomhead D, Schiffner J, Godehardt E, Freundl G, et al. Antimüllerian hormone levels and numbers and sizes of antral follicles in regularly menstruating women of reproductive age referenced to true ovulation day. Fertil Steril 2015;104:1535, 43.e4.
7. Melado L, Lawrenz B, Sibal J, Abu E, Coughlan C, Navarro AT, et al. Anti-müllerian hormone during natural cycle presents significant intra and inter-cycle variations when measured with fully automated assay. Front Endocrinol 2018;9:686.

8. Alebić MŠ, Stojanović N, Dewailly D. Discordance between serum anti-müllerian hormone concentrations and antral follicle counts: not only technical issues. Hum Reprod 2018;33:1141–8.
9. Nelson SM, Klein BM, Arce JC. Comparison of antimüllerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis patients at individual fertility clinics in two multicenter trials. Fertil Steril 2015;103:923–30.e1.
10. Hoyos LR, Visser JA, McLuskey A, Chazenbalk GD, Grogan TR, Dumesic DA. Loss of anti-müllerian hormone (AMH) immunoactivity due to a homozygous AMH gene variant rs10417628 in a woman with classical polycystic ovary syndrome (PCOS). Hum Reprod 2020;35:2294–302.
11. Grbavac I, Zec I, Ljiljak D, Rakoš Justament R, Bukovec Megla Ž, Kuna K. Undetectable serum levels of anti-müllerian hormone in women with ovarian hyperstimulation syndrome during in vitro fertilization and successful pregnancy outcome: case report. Acta Clin Croat 2018;57:177–80.
12. Nelson SM, Larsson P, Mannaerts BMJL, Nyboe Andersen A, Fauser BCJM. Anti-müllerian hormone variability and its implications for the number of oocytes retrieved following individualized dosing with follitropin delta. Clin Endocrinol (Oxf) 2019;90:719–26.
13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17:405–24.
14. Melado L, Lawrenz B, Loja R, Coughlan C, Altobelli G, Bayram A, et al. Female parental consanguinity is associated with a reduced ovarian reserve. Reprod Biomed Online 2022;44:659–66.
15. Picard JY, Cate RL, Racine C, Josso N. The persistent müllerian duct syndrome: an update based upon a personal experience of 157 cases. Sex Dev 2017;11:109–25.
16. Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, et al. Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. Cell 1986;45:685–98.
17. Gorsic LK, Kosova G, Werstein B, Sisk R, Legro RS, Hayes MG, et al. Pathogenic anti-müllerian hormone variants in polycystic ovary syndrome. J Clin Endocrinol Metab 2017;102:2862–72.
18. McLennan IS, Pankhurst MW. Is the understanding of AMH being confounded by study designs that do not adequately reflect that it is an atypical hormone? Hum Reprod 2017;32:14–7.
19. Danis RB, Sriprasert I, Ho JR, McGinnis LK, Kumar A, Stanczyk FZ. Association of bioavailable inhibin B and oocyte yield in controlled ovarian stimulation. FS Rep 2021;2:189–94.
20. Meng L, McLuskey A, Dunaif A, Visser JA. Functional analysis of rare anti-müllerian hormone protein-altering variants identified in women with PCOS. Mol Hum Reprod 2023;29:gaad011.
21. Feferkorn I, Ata B, Esteves SC, La Marca A, Paulson R, Blockeel C, et al. The HERA (Hyper-response Risk Assessment) Delphi consensus definition of hyper-responders for in-vitro fertilization. J Assist Reprod Genet 2023;40:1071–81.
22. Visser JA, Durlinger ALL, Peters IJJ, van den Heuvel ER, Rose UM, Kramer P, et al. Increased oocyte degeneration and follicular atresia during the estrous cycle in anti-müllerian hormone null mice. Endocrinology 2007; 148:2301–8.
23. Gruijters MJG, Visser JA, Durlinger ALL, Themmen APN. Anti-müllerian hormone and its role in ovarian function. Mol Cell Endocrinol 2003;211:85–90.