

International Journal of Agriculture Extension and Social Development

Volume 9; SP-Issue 8; August 2026; Page No. 39-42

Received: 05-06-2026
Accepted: 08-07-2026

Indexed Journal
Peer Reviewed Journal

Ovarian biomarkers: An immunohistochemical perspective

¹ Nidhi Gupta, ² R Vaish, ³ P Jain, ⁴ RK Barhaiya, ⁵ S Tekam and ⁶ P Wasnik

¹ Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

² Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

³ Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

⁴ Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

⁵ Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

⁶ Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

DOI: <https://doi.org/10.33545/26180723.2026.v9.i8Sa.4039>

Corresponding Author: Nidhi Gupta

Abstract

Biomarkers help to improve the diagnosis of diseases and selective therapeutic interventions and prognosis. Immunohistochemistry is an important tool for detection of biomarkers and plays a vital role for a rapid morphologic diagnosis by localizing antigens in tissue sections. Immunostaining for cell markers represents a way to “talk with cells”. In recent years, immunohistochemical detection of biomarkers in ovarian histological and histopathological studies in both humans and animals has become a critical area of investigation. Immunohistochemical analyses of the ovary are typically divided into two principal categories: one emphasizing structural biomarkers, primarily using intermediate filament protein antibodies (Perez-Martinez *et al.*, 2001), and the other focusing on functional biomarkers, which target hormone receptors and hormone-associated proteins (Akahira *et al.*, 2002) ^[1]. Follicular atresia has been classified into four stages (atretic I-IV) based on Granulosa Cell (GC) layer organization and histomorphological characteristics (Tech *et al.*, 2018) ^[11]. Progressive follicular atresia is characterized by pyknosis and karyorrhexis of granulosa cells, accompanied by reduced expression of inhibin- α , Anti-Müllerian Hormone (AMH). AMH expression is observed throughout most stages of granulosa cell development, whereas inhibin- α is predominantly expressed in mature follicles. These immunohistochemical markers provide valuable information regarding follicular status and may serve as useful diagnostic indicators for ovarian pathologies, including granulosa cell tumors and cystic ovarian disorders.

Keywords: Biomarker, granulosa cell tumor, immunohistochemistry, ovary

Introduction

Bringing a new life into the world would be impossible without the female genital system. The ovary exhibits a complex architecture and diverse morphological variations at both macroscopic and microscopic levels. Early diagnosis of ovarian disorders is particularly important in domestic animals, as these conditions can significantly impair fertility and fecundity, leading to notable economic consequences in these species. A biomarker is defined as a characteristic, often a specific protein and/or structural entity that can be objectively measured and evaluated to indicate normal biological processes, pathogenic states, or pharmacologic responses to therapeutic interventions. Study of the biomarkers; help to improve the diagnosis of diseases and selective therapeutic interventions and prognosis.

Immunohistochemistry is an important tool for detection of biomarkers. There are many immunohistochemical reports on ovary, which are divided into two prominent groups, one focused on the structural aspect (structural biomarkers) using intermediate filament protein antibodies (Perez-Martinez *et al.*, 2001) ^[8] and the other focused on the functional aspect (functional biomarkers) using hormone protein like Anti-Müllerian hormone and Inhibin α or hormone receptor (estrogen and progesterone) antibodies (Akahira *et al.*, 2002) ^[1]. The expressions of cytokeratin and vimentin proteins are useful in differentiating the normal and neoplastic bovine ovaries along with differentiation of primary ovarian tumours from metastatic tumours. There is an association between the formation of ovarian cysts and alterations in the expression of cytoskeletal proteins (De

Sant'Ana *et al.*, 2015)^[4]. In addition to this, the distinct patterns of immunoreactivity of different granulosa cell stages play an important role in diagnostic assistance of various follicle conditions, including cystic ovaries and Granulosa Cell Tumours (GCT). Determination of Estrogen Receptor (ER) subtypes expression may improve response to hormonal therapy by tailoring the use of selective estrogen receptor modulators with different ER affinity.

Structure of ovary

The ovary is anatomically divided into the cortex and medulla. The cortical surface is initially covered by cuboidal epithelium during development, which transitions to squamous epithelium with advancing age. The surface epithelium is closely related to the lining epithelium of the Müllerian duct. The cortical parenchyma consists of follicles, interstitial cells and a collagenous connective tissue stroma. In the ovary of rodents, bitches and queens, the cortical stroma contains cords of polyhedral interstitial endocrine cells. In the ovary of bitches cortical tubules are also prominent; these are narrow channels lined by cuboidal epithelium that, in some sites, are continuous with the surface epithelium. The medulla contains loose collagenous matrix with large arteries, veins, lymphatics and nerves embedded in it. The rete ovarii present in the medulla has cords of cells which are homologous to the rete testis.

In most of mammalian ovary developing follicles are located within the cortex. However, in the mare, the ovarian anatomy is reversed, with follicular development occurring in the region corresponding to the medulla. Ovulation takes place at a concavity known as the ovulation fossa.

Follicular development

Development of the ovarian follicle is a sequential process which is primarily directed by the influence of gonadotrophins and can be variable between species. The sequence of follicular development is; primordial follicle, primary follicle, secondary follicle and tertiary follicles (Eurell and Frappier, 2006)^[5].

Primordial follicles are resting follicle having primary oocyte in the centre with single layer of flattened follicular cells around it. They are evenly distributed in ruminants and sow and occur in cluster in carnivores. Primary follicles are growing follicles still contain a primary oocyte surrounded by single layer of cuboidal follicular cells. In secondary ovarian follicles follicular (granulosa) cells proliferate (membrane granulosa) but are separated from the oocyte by a thick basement membrane called the zona pellucida. The organized stromal cells around the follicles are called theca cells. Secondary follicles start develops spaces between granulosa cells that coalesce to eventually form a large space called the follicular antrum. The granulosa cells secrete PAS positive material into these spaces. The stromal cells surrounding the follicle form two layers, the theca interna and the theca externa. Graafian (tertiary, mature) follicles represent large preovulatory follicles that protrude from the ovarian surface. Upon formation of the follicular antrum, the oocyte is surrounded by a remnant of granulosa cells termed the cumulus oophorus, with the cells immediately adjacent to the oocyte arranged radially (corona radiata). Corpus luteum contained large polygonal granulosa luteal cells and smaller theca luteal cells. The

basal lamina that previously separated the granulosa cells from theca cells has depolymerized during luteinisation.

Tech *et al.* (2018)^[11] categorized atretic follicle into four types: atretic-I, atretic-II, atretic-III and atretic-IV (Table 1). The categorization was based on the layer of GC and histomorphology. Atretic I (early stage)-atretic III (late stage) are continuously regressing stages of follicles. Atretic IV is a special type of follicle and found in all older cows (> 10 years age). The atretic IV follicle is characterized by a strand or island of vacuolated GC without proliferation of theca cells. The individual foci of GC were arranged in rosette patterns with eosinophilic secretion in between, resembling the Call-Exner body from Granulosa cell tumour. In the latter, ova were occasionally found in between these structures. The foci of dystrophic calcifications are also commonly found within the atretic follicle IV.

Granulosa cell phenotypic heterogeneity

Granulosa cells display different phenotypes within the follicle, depending on their location. The mural granulosa cells, antral granulosa cells, and cumulus granulosa cells each have distinguishing features that are likely determined by their proximity to the oocyte and theca cells and by the paracrine substances that they produce.

Mural granulosa cells within the antral follicle exhibit the highest steroidogenic activity. In preovulatory follicles, these mural granulosa cells also express the greatest levels of luteinizing hormone receptors. Granulosa cells adjacent to the antral cavity show lower expression of steroidogenic enzymes. Those in the middle region demonstrate greater mitotic activity compared to both antral and mural granulosa cells. Cumulus cells lack aromatase expression and display substantially lower LH responsiveness than their mural counterparts, while exhibiting higher expression of anti-Müllerian hormone.

Table 1: Classification and characterization of granulosa cells (GC) in various stages of bovine follicles

Stages of follicle	No. of GC layers	No. of GC	Morphology of GC	Inner theca Layers	Zona Pellucida
Primordial	1	<10	Flat	0	Nil
Primary	1-1.5	10-40	Cuboidal	0	Nil
Secondary	2-10	41 to >250	Cuboidal	0-3	Present
Graafian	>10	>250	Cuboidal	>3	Present
Atretic I	>10	Various	Pyknotic	>3 (detached)	Nil
Atretic II	3-10	Various	Pyknotic	>3 (detached)	Nil
Atretic III	<3	Various	Pyknotic	>3 (detached)	Nil
Atretic IV	>3	Various	Secretion resembles to Call-Exner body	0	Present

Functional biomarkers of ovary

1. Anti-Müllerian Hormone (AMH)

Anti-Müllerian Hormone (AMH), also known as Mullerian Inhibiting Substance (MIS) is a homodimeric disulfide-linked glycoprotein of the Transforming Growth Factor- β (TGF- β) family with 553-575 amino acids produced by

Granulosa Cells (GC) of the ovary (Baarends *et al.*, 1995) [2]. It is virtually undetectable but increases gradually until puberty and remains relatively stable through the reproductive period. Although Anti-Müllerian Hormone (AMH) has a long historical background, its presence was only recognized after the mid-20th century. Initially, it was understood that testicular tissue synthesizes testosterone, the hormone responsible for the development of male external genitalia, and also produces a factor that induces regression of the Müllerian ducts in rabbit fetuses. In late fetal development, AMH is secreted by ovarian granulosa cells in females, at a stage when the Müllerian ducts have lost their responsiveness to the hormone. AMH is primarily produced by the pre-antral and small antral follicles, and correlates with the number of primordial follicles at the gonadotrophin-independent stage of follicular development. AMH is a more direct measure of ovarian status compared with other endocrine reproductive hormones. It is the marker for the fertility competence of many farm animal species and also a good predictor of superovulation in cattle, sheep, and mares. The evaluation of AMH may also be useful in the diagnosis or the monitoring therapy of granulosa cells ovarian tumours.

AMH is expressed in almost all bovine GC with the exception of some atretic IV follicles, which showed either negative or weak staining. Intriguingly, unlike humans (Rey *et al.*, 2000) [9], AMH is also expressed in bovine theca cells (Table 2). This explained the reason why bovine plasma concentrations of AMH are surprisingly higher than in human and mouse. Almeida *et al.* (2018) [3] reported the immunohistochemical localization of AMH in the porcine ovary, demonstrating that, as in most species, AMH first appears during follicular development in the fusiform granulosa cells of recruited primordial follicles and persists in granulosa cells through the antral stage. At the pre-ovulatory stage, AMH staining intensity markedly increases, and both protein and gene expression extend beyond granulosa cells to include theca cells. Uniquely in the porcine ovary, AMH expression continues post-ovulation in the luteal cells of the corpus luteum.

2. Inhibin alpha (Inhibin α)

Inhibin is a dimeric 32-kD glycoprotein hormone that participates in the regulation of the pituitary-gonadal feedback system. Inhibin secreted in the ovary consist of an α -subunit linked to one of two β -subunits. In inhibin A an α -subunit is linked to a β -A subunit; in inhibin B an α -subunit is linked to a β -B subunit. The monoclonal antibody in general use for immunohistochemistry recognizes inhibin A. Inhibin is a sensitive and relatively specific marker of sex cord-stromal tumours of the ovary, and its main use in gynecologic pathology is in the differential diagnosis of such tumours. Luteinized stromal cells that are sometimes present around carcinomas can express inhibin, which can lead to the erroneous interpretation that a carcinoma expresses inhibin. In general, inhibin expression in carcinomas is encountered uncommonly, and when it is, the pattern is only exceptionally strong and diffuse. The immunolocalization of inhibin alpha is inversely proportional to immunolocalization of AE1/AE3 in developing ovarian follicles as they showed opposite immunolocalization patterns during folliculogenesis. Unlike

in humans and rats (Smith *et al.*, 1991) [10], the bovine corpus luteum lacks immunoreactivity to inhibin alpha (Table 2), which consistent with previous studies that failed to detect mRNA for the alpha and β subunits of inhibin in the corpora lutea of bovine and ovine. Thus, the major inhibin alpha source in bovine is from granulosa cells and theca cells of larger follicles.

Table 2: Immunophenotypic profile of Granulosa Cells (GC) during different stages of folliculogenesis in normal bovine ovaries (Tech *et al.*, 2018) [11]

Ovarian structure	AMH	Inhibin alpha
Oocytes	-	-
Primordial follicle	+	-
Primary follicle	++	-
Secondary follicle	+++	-
Graafian follicle	++++	++
Atretic follicle I	++++	++
Atretic follicle II	+++	-
Atretic follicle III	+++	-
Atretic follicle IV	±	-
Corpus luteum	++	-
Theca cells	+	+

(-, negative; +, <10% of cells stained; ++, 10%-30% of cells stained; +++, 30%-60% of cells stained; +++, >60% of cells stained)

3. Estrogen Receptors (ER)

Estrogen functions are mediated primarily by cellular receptors, including members of the nuclear receptor family-Estrogen Receptors (ER) alpha and beta-and membrane Estrogen Receptors (mERs), such as the G Protein-Coupled Receptor 30 (GPR30). The complexity of estrogen physiology reflects its signaling mechanisms, which have evolved over more than 500 million years. Initially, estrogen's actions were thought to be mediated by a single receptor (Jensen and Jacobson, 1962) [6]. However, Kuiper *et al.* (1996) [7] identified a second receptor with high homology, leading to the renaming of the original receptor as ER α and the new receptor as ER β . A third estrogen receptor, the GPR30/G Protein-Coupled Estrogen Receptor 1 (GPER), was subsequently discovered and characterized by Thomas *et al.* (2005) [12]. ER α is mainly located at thecal cells and the ovarian stroma in the corpus luteum and surface epithelium of the ovary. The main locations of ER β are granulosa cells. Expression of ER β is observed early during ovarian development and continued to be expressed throughout follicular formation and also during the early stages of follicular growth. ER β also identified in germ cells. At the large preantral stage of follicular growth, expression of ER α is also consistently observed in granulosa cells. The ratio of ER β and/or ER β to ER α decreases during ovarian carcinogenesis, suggesting that the loss of ER β expression may be associated with malignant transformation. The absence of ER β expression in ovarian tumors may serve as a hallmark of malignancy. Assessing the expression of estrogen receptor subtypes could enhance hormonal therapy outcomes by enabling the tailored use of selective estrogen receptor modulators with varying receptor affinities in selected patients. Additionally, ER β levels may serve as a valuable prognostic marker to guide the necessity and choice of adjuvant treatment in early-stage ovarian cancer.

Progesteron Receptors (PR)

Progesteron receptors are reported in the granulosa cells of preovulatory follicles. The presence of PRs in these follicles in the transition from the preovulatory to periovulatory periods may be crucial for successful ovulation. The blockage of progesterone action at the level of the ovary substantially decreases ovulation rates in hamsters and rats. Estrogen is the primary regulator of PR gene expression in uterine epithelial cells, but not in the ovary. On the other hand, estrogen action is important for preparing granulosa cells for gonadotropin induction of PR gene expression. Progesterone-dependent actions and associated biology in diverse tissues and tumours are mediated by two PR isoforms, PR-A and PR-B. Changes in the relative abundance of the progesterone receptor isoforms are associated with female reproductive tract cancers including ovarian cancer. To assess the importance of the PGR isoform ratio in the maintenance of the reproductive tract, Wetendorf *et al.* (2020) ^[13] generated mice that over express PGRA or PGRB in all PGR-positive tissues. Few PGRA-over expressing mice developed reproductive tract tumours, whereas all PGRB-over expressing mice developed ovarian neoplasms that were derived from ovarian luteal cells.

Conclusion

The establishment of an immunohistochemical panel in the normal ovary is essential for the differential diagnosis of ovarian tumors according to their cell of origin. Generally, panels of markers provide greater diagnostic accuracy than individual markers alone, as no single marker is entirely specific or sensitive for any tumor type. Furthermore, unexpected positive or negative staining reactions may occur; therefore, immunohistochemical findings should always be interpreted in the context of clinical presentation, gross pathology, and microscopic features.

References

1. Akahira J, Suzuki T, Ito K, Kaneko C, Darnel AD, Moriya T, *et al.* Differential expression of progesterone receptor isoforms A and B in the normal ovary and in benign, borderline and malignant ovarian tumours. *Jpn J Cancer Res.* 2002;93:807-815.
2. Akihara Y, Shimoyama Y, Kawasako K, Komine M, Hirayama K, Terasawa A, *et al.* Histological and immunohistochemical evaluation of canine ovary. *Reprod Domest Anim.* 2007;42:495-501.
3. Almeida FRCL, Costermans NGJ, Soede NM, Bunschoten A, Keijer J, Kemp B, *et al.* Presence of anti-Müllerian hormone (AMH) during follicular development in the porcine ovary. *PLoS One.* 2018;13(7):1-7.
4. Baarends WM, Uilenbroek JT, Kramer P, Hoogerbrugge JW, van Leeuwen EC, Themmen AP, *et al.* Anti-Müllerian hormone and anti-Müllerian hormone type II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development, the estrous cycle, and gonadotropin-induced follicle growth. *Endocrinology.* 1995;136(11):4951-4962. DOI:10.1210/endo.136.11.7588229.
5. DeSant'Ana FJF, Reis JLL, Araújo RLS, Gimeno EJ, Ortega HH. Cytoskeletal proteins in the follicular wall of normal and cystic ovaries of sows. *Pesq Vet Bras.* 2015;35:119-124.
6. Eurell JAC, Frappier BL. Dellmann's Textbook of Veterinary Histology. 6th ed. Iowa: Blackwell Publishing; 2006. p. 37-38.
7. Jensen EV, Jacobson HI. Basic guides to the mechanism of estrogen action. *Recent Prog Horm Res.* 1962;18:1-5.
8. Kuiper GG, Enmark E, Peltö-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A.* 1996;93:5925-5930.
9. Perez-Martinez C, Garcia-Fernandez RA, Escudero A, Ferreras MC, Garcia-Iglesias MJ. Expression of cytokeratins and vimentin in normal and neoplastic tissue from the bovine female reproductive tract. *J Comp Pathol.* 2001;124:70-78.
10. Rey R, Sabourin JC, Venara M, Long WQ, Jaubert F, Zeller WP, *et al.* Anti-Müllerian hormone is a specific marker of Sertoli and granulosa-cell origin in gonadal tumours. *Hum Pathol.* 2000;31(10):1202-1208.
11. Smith KB, Millar MR, McNeilly AS, Illingworth PJ, Fraser HM, Baird DT. Immunocytochemical localization of inhibin alpha-subunit in the human corpus luteum. *J Endocrinol.* 1991;129(1):155-160.
12. Teh APP, Izzati UZ, Mori K, Fuke N, Hirai T, Kitahara G. Histological and immunohistochemical evaluation of granulosa cells during different stages of folliculogenesis in bovine ovaries. *Reprod Domest Anim.* 2018;53:1-13.
13. Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology.* 2005;146:624-632.
14. Wetendorf M, Li R, Wu S, Liu J, Creighton CJ, Wang T, *et al.* Constitutive expression of progesterone receptor isoforms promotes the development of hormone-dependent ovarian neoplasms. *Sci Signal.* 2020;13(652):1-9.