

Pathomorphological Lesions in the Ovaries of Water Buffaloes

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ABSTRACT

Background: Buffalo breeding is common in many countries. Buffalo's milk is used in the production of mozzarella, yoghurt, ice cream, and various dairy desserts; meat is preferred in sausage production. The female buffaloes are bred to benefit from their milk and to obtain offspring. These animals, which are not suitable for feeding in barns, generally live in pastures, especially on wet land, and are very difficult to follow. Therefore, diseases occur randomly in slaughterhouses. Studies on genital system problems are very limited. Water buffaloes (*Bubalus bubalis*) are animals with economic value as many buffalo products are provided. In this respect, inspection and control of female genital disorders is fundamental to ensure good reproductive performance of female buffaloes. The aim of the study was to investigate pathomorphological lesions occurring in the ovaries of water buffaloes which were sent to slaughterhouses.

Materials, Methods & Results: A total of 198 ovaries of water buffaloes were collected from various slaughterhouses located in Adapazarı, İstanbul, Kocaeli, Samsun and Trabzon cities of Turkey. After macroscopic examination; tissue samples were fixed in 10 % buffered formalin, processed routinely and were stained with hematoxylin-eosin (HE). As a histochemical staining, Masson's trichrome staining was applied to characterize the lesions. Immunohistochemistry (IHC) was performed on 10 % neutral formalin fixed, paraffin-embedded, 4-6-µm-thick sections from ovaries using progesterone receptor (PR) with streptavidin-biotin complex peroxidase (StrepABC-P) method. Histopathologically, follicular cysts (n:147) and luteal cysts (n:22) were seen. While the cyst lumens were sometimes surrounded by granulosa and/or luteal cells, most of them were limited by the connective tissue capsule structure. This capsule structure was shown in blue by Masson's trichrome staining. Hemorrhage was observed in some cystic corpus luteum, diagnosed as corpus hemorrhagicum. In addition, cysts giving papillary extension into the lumen and inflammation of some ovaries were observed. Immunohistochemically, the staining with PR antibody in ovarian cysts showed no immunolabelling around the follicular cyst, while the nuclei of some of the luteal cells forming the luteal cyst had strongly nuclear positivity and slightly cytoplasmic positivity. In the biochemical examination of the fluids obtained from cystic ovaries (n: 37), the average of estradiol was 2.84 ng/mL (min: 0.01 ng/mL, max: 4.30 ng/mL) and progesterone average is 49.09 ng/mL (min: 1.88 ng/mL, max: 254.2 ng/mL).

Discussion: Ovarian cysts in buffaloes seem to be among the serious fertility problems as in cattle. Although the exact cause of ovarian cysts has not been determined yet, it is known that the hypothalamic-pituitary-gonadal (HPG) axis is disrupted in cyst formation in general. In this study, the mean estradiol value was within the standard range but close to the lower limit; progesterone value was above the limit. The increase in progesterone level was compatible with the pathogenesis of cyst genesis. Beside this result, staining with PR was positive in the luteal cells that formed the luteal cyst immunohistochemically. In addition, although the hemorrhages observed in the corpus luteum are considered physiological, it should not be forgotten that they can be vital if they rupture. All these results show us the animals sent for slaughtering mostly have serious genital problems threatening their fertility.

Keywords: buffalo, *Bubalus bubalis*, histopathology, immunohistochemistry, ovarian cysts, fertility.

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INTRODUCTION

Buffalo breeding (*Bubalus bubalis*), is increasing in many countries. Buffalo milk is usually used for ice cream, various desserts, cheese (mozzarella) and yoghurt production; while its meat is preferred for taste and color in sausage manufacturing. The female buffaloes are bred to benefit from their milk and to obtain offspring. In this regard, examination and control of female genital disorders is fundamental to ensure good reproductive performance of female buffaloes. Buffaloes that are sent to abattoir usually have low productivity economically, have problems in fertility and have issues in milk production. In order to maintain animal health, it is necessary to determine the problems occurring in their genital tracts [2,3].

In ovaries; primordial, primary and secondary follicles, antral, and pre-ovulatory follicles that have completed its development, corpus luteum (CL) formed at the site of the ovulated follicles and corpus albicans formed by the retraction of the corpus luteum are observed histologically [5].

Pathomorphologically, ovarian cysts (OC) can be seen in cattle and buffaloes. These have an economically important impact on fertility. Macroscopically, cysts can be subdivided into follicular and luteal cysts. Follicular cysts have a thin wall and follicular fluid which is anechogenic, while luteal cysts have a thicker wall and echogenic fluid. They can be also diagnosed on histopathological examination. Follicular cysts are lined by granulosa cells with an increased amount of follicular fluid and absence of an oocyte. They generally have a thin capsule formation. Luteal cysts 'have a large cavity lined by layers of luteal cells that are polyhedral with foamy, vacuolated, eosinophilic cytoplasm [22].

From this aspect, investigation of pathomorphological lesions seen in the ovaries are important in order to gain more economical income from female buffaloes. Therefore, histopathological examination of water buffaloes' genital organs is important in order to achieve this goal.

MATERIALS AND METHODS

Sample collection

A total of 198 ovaries of water buffaloes were collected from various slaughterhouses located in Adapazarı, İstanbul, Kocaeli, Samsun and Trabzon cities of Turkey.

Histopathological examination

Collected materials were fixed in 10 % neutral formalin and evaluated macroscopically in Department of Pathology, Faculty of Veterinary Medicine, University of Ankara. Tissues were processed routinely and embedded in paraffin, sectioned at 4-6 µm thickness and stained with haematoxylin-eosin¹ (HE). Masson's trichrome staining was applied to tissues with ovarian cysts in accordance with the procedure [9]. The prepared slides were examined under the light microscope².

Immunohistochemical examination

Immunohistochemistry (IHC) was performed on 10 % neutral formalin fixed, paraffin-embedded, 4-6-µm-thick sections from ovaries using progesterone receptor (PR) with streptavidin-biotin complex peroxidase (StrepABC-P) method according to UltraVision Large Detection System³. Briefly, the sections mounted on charged glass slides were deparaffinized and then rehydrated. After deparaffinization and rehydration, the sections were exposed to antigen retrieval, citrate buffer (pH 6.0) in pressure cooker for 20 min. Then all slides were incubated in 3% H₂O₂ for 15 min to block endogenous peroxidase activity. Anti-PR (Sc-820) (monoclonal, Santa Cruz, 1:100)⁴ antibody was applied as described by the manufacturer. As chromogen, 3 amino-9-ethylcarbazole (AEC)³ was used and the slides were counterstained with Gill haematoxylin¹. For negative control, primary antibodies were replaced by mouse IgG isotype serum. All findings were evaluated under light microscopy².

Biochemical analysis

Ovarian cyst fluids were collected with a syringe from the ovarian cysts (n: 37) observed as grossly cystic in the slaughterhouse and transferred to the laboratory with ice molds. The samples were analyzed for progesterone and estradiol using automatic biochemistry analyzer, electrochemiluminescence IAs (Roche Diagnostics E170)⁵. Normal follicular fluid values for buffaloes were evaluated according to the hormone values (Estradiol 0,84-26.47 ng/mL and progesterone 0.02-27.46 ng/mL) reported by Khan *et al.* [8].

RESULTS

Histopathologically, 147 follicular cysts and 22 luteal cysts were found. Both follicular and luteal cysts were noticed in 14 water buffaloes where 12

of them were seen at the same ovary and 2 of them were located at different ovaries. Some follicular cysts were lined by granulosa cells and becomes larger than normal follicles. Also, some have a thin wall that surround the cyst cavity. Luteal cysts were large cysts surrounded by either layers of luteal cells or connective tissue capsule structure. Both follicular and luteal cyst wall demonstrated blue cytoplasmic staining with Masson's trichrome staining (Figure 1). Some of both follicular and luteal cysts had fluidy exudate.

In one of the follicular cysts, papillary extension projecting into the lumen was evident. In the lumen of some cystic corpus luteum, hemorrhages were noticed. In some cases, luteal cells were degenerative

in CL. Besides, interstitial edema was prominent in the ovaries of 11 animals. One included mononuclear cell infiltration consisting of macrophages and plasma cells (Figure 2).

Immunohistochemically, the staining with PR antibody in ovarian cysts showed no immunolabelling around the follicular cyst, while the nuclei of some of the luteal cells forming the luteal cyst had strongly nuclear positivity and slightly cytoplasmic positivity (Figure 3).

In the biochemical examination of the fluids obtained from cystic ovaries (n:37), the average of estradiol was 2.84 ng/mL (min: 0.01 ng/mL, max: 4.30 ng/mL) and progesterone average was 49.09 ng/mL (min: 1.88 ng/mL, max: 254.2 ng/mL).

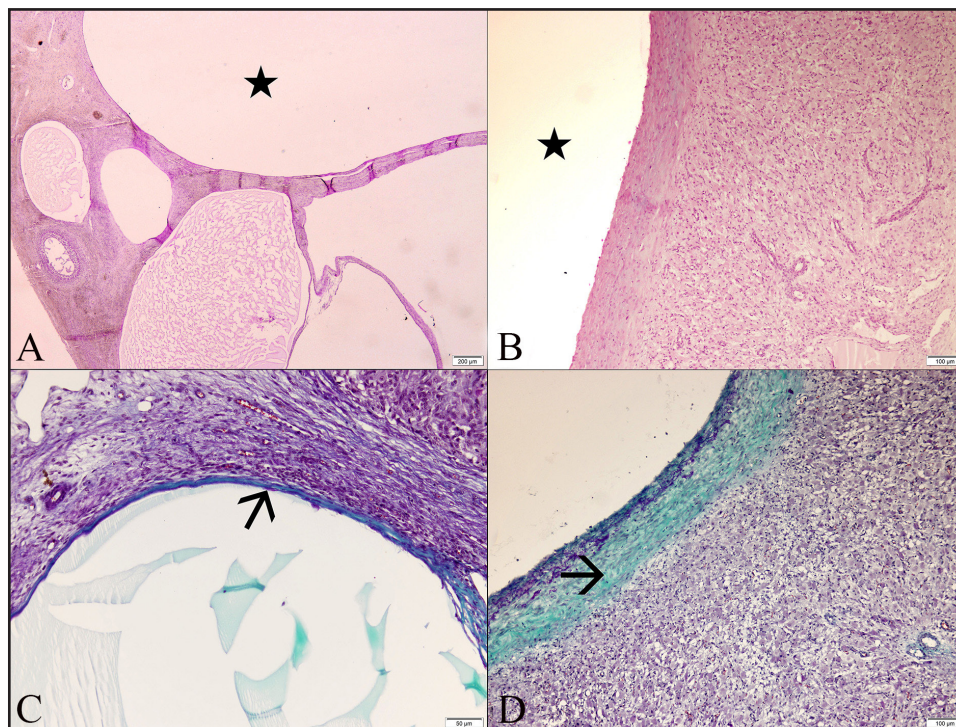


Figure 1. Histopathological features of follicular and luteal cysts structures. A- Follicular cyst lumen (star) lined by fibrous capsule [HE]. B- Luteal cyst lumen lined by fibrous capsule and luteal cells [HE]. C & D- Follicular and luteal cyst fibrous capsule demonstrated by blue cytoplasmic color (arrow) [Masson's trichrome staining].

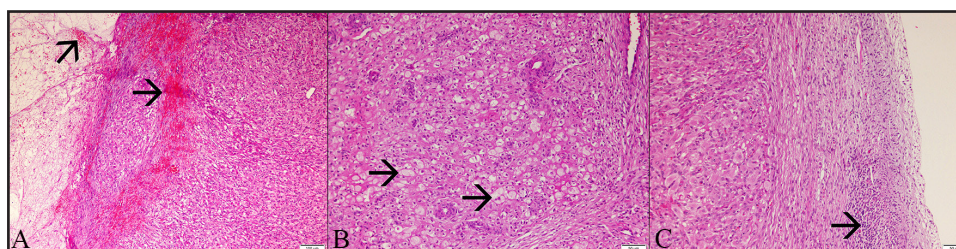


Figure 2. Histopathological features of corpus luteum. A- Hemorrhage (arrows) in cystic corpus luteum and between luteal cells [HE]. B- Severe degenerative luteal cells of corpus luteum [HE]. C- Mononuclear cell infiltration around of corpus luteum [HE].

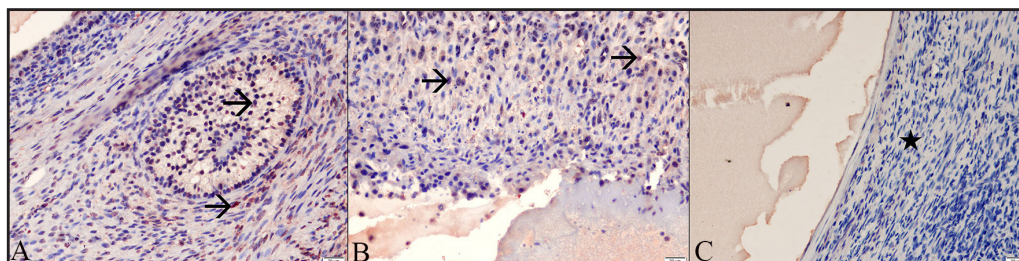


Figure 3. Ovarian immunohistostaining for PR, StrepABC-P. A- Expression of PR in granulosa and theca cells (arrows) in normal follicular stage. B- Strongly nuclear positivity and slightly cytoplasmic positivity for PR in luteal cells of luteal cyst. C- Negative staining of PR in follicular cyst cells.

DISCUSSION

In order to understand the fertility problems that cause economic losses, it is necessary to investigate the etiology and pathogenesis of ovarian cysts. Although the exact cause has not been determined yet, it is known that the hypothalamic-pituitary-gonadal (HPG) axis is disrupted in cyst formation in general. As a result of altered axis, abnormal LH release seems to be a reason that generates ovarian cysts in ruminants [13,22]. HPG axis dysfunction can occur due to multifactorial etiology, in which genetic, phenotypic and environmental factors are involved [7,15]. If we look at the mechanisms in more detail, it has been thought that the endocrine signaling pathway of steroid and gonadotropin receptors is disrupted in general and that specific proteins and genes may be responsible for these disruptions [10,17]. Another prominent problem for cattle is stress. Adrenocorticotrophic hormone (ACTH) stimulates the release of cortisol and progesterone. Increased progesterone secretion inhibits GnRH release. The positive feedback of estradiol on the hypothalamus and on pituitary disappears, ovulation does not occur and a cyst is formed [10]. Since the current study was on buffaloes, the effect of the stress factor could be very small. Intensive breeding is not done in buffaloes. It is a fact that they are not exposed to stress like cattle, as they are free-living animals in wetlands [3]. Other hypotheses are that insulin concentrations are effective in cyst formation [14,21]. Also, it was reported that the increase of mineralocorticoid secreted from zona glomerulosa of adrenal glands plays a main role in the formation of the follicular cyst [11]. So, in further studies hormonal pathway examinations should be performed in detail.

Based on published reports, normal follicular fluid values were determined as 0,84-26.47 ng/mL for estradiol and 0.02-27.46 ng/mL for progesterone [8]. In this study, the average of estradiol was 2.84 ng/mL and progesterone average was 49.08 ng/mL. Accordingly, the mean estradiol value was within the standard range but close to the lower limit; progesterone value was above the limit. The increase in progesterone level was compatible with the pathogenesis of cyst formation [4]. Khan *et al.* [8] examined three ovarian cyst fluids in their study in 2011 and respectively, 15.02 ng/mL, 3.76 ng/mL, 0.60 ng/mL for estradiol; they found 54.28 ng/mL, 62.00 ng/mL, 49.00 ng/mL for progesterone. These findings are in line with the current study and show that the hypothalamic-pituitary-gonadal (HPG) axis is impaired [4,22].

PR positive staining in luteal cells was similar with other studies [1,20]. Nuclear positivity has also been reported in granulosa cells around the normal and/or cystic follicle in studies on the ovary [12,18,20]. Because of ovulation disorder, the theca luteinizes and forms luteal cysts. So, they are also usually considered to be the later form of follicular cysts [16,22]. Immunohistochemically, the staining with PR antibody in ovarian cysts showed no immunolabelling around the follicular cyst. We attributed this to the formation of connective tissue around the follicle cavity in the stained case. On the other hand, it has been reported that follicular cysts produce less amount of progesterone compared to other cyst types [16].

In this study, 147 follicular cysts, 22 luteal cysts were noted. In previous studies, luteal cysts were less reported than follicular cysts [11,19]. But in the study done by Damodaran [6] the rate of luteal cysts was higher.

Another important point is corpus luteum cysts. These cysts are not considered pathological and are luteal structures starting from the corpus hemorrhagicum [16]. In this study, hemorrhage observed in some corpus luteum were interpreted as corpus hemorrhagicum, which is a part of the physiological process. But the presence of hemorrhage in the parenchyma, that is, between the luteal cells, indicated the pathological condition. On the other hand, if the hemorrhagic corpus luteum reaches large sizes and ruptures, it can cause major problems.

CONCLUSIONS

As a result, it was concluded that the water buffaloes sent to slaughterhouses commonly have serious genital problems threatening their fertility. So, in the aim of improving productivity and expanding the lifespan, routine clinical examination is a must as an antemortal prevention of disorders and diseases.

MANUFACTURERS

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³UltraVision Large Detection System-Thermo Sci. Waltham, MA, USA.

⁴Santa Cruz Biotechnology Inc. Heidelberg, Germany.

⁵Roche Diagnostics International AG. Rotkreuz, Switzerland.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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