NEOPLASTIC CONDITIONS OF OVARIES IN BUFFALOES- A ABATTOIR BASED STUDY

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ABSTRACT

An abattoir-based study was carried out to assess the neoplasms affecting the ovaries of buffaloes. A total of 200 pairs of ovaries from 3 to 12 years old she buffaloes carcasses were examined and collected for routine histopathological studies. Ovarian neoplasms were recorded in 12 animals accounting for 6% and in all the cases the involvement was unilateral. Epithelial tumours were recorded in 6 cases (three each in right and left). Histologically, all the epithelial tumours were adenomas and were further sub-classified as simple adenoma (3 cases), fibro-adenoma (2 cases) and a solitary case of papillary cystadenoma. Sex cord-stromal tumors were recorded in 4 cases that included a solitary case of granulosa cell tumour in the right ovary and luteoma in 3 cases (one in right and two in left). Among the tumours of mesenchymal origin, haemangioma was observed in 2 cases involving each of left and right ovary.

Keywords: *Bubalus bubalis*, buffaloes, epithelial, mesenchymal origin, sex cord tumours, ovary

INTRODUCTION

The ovaries and ovarian structures of buffaloes are inherently smaller as compared to cows and seasonal ovarian hypofunction and pathologies limit the breeding value of this important species. Ovarian abnormalities impair the development of ovarian follicles and corpus luteum, and subsequently estrous cycle as well as pregnancy (Jainudeen, 1986; Dobson and Kamonpatana, 1986) in buffaloes. Many abattoir based (Kumar and Singh, 1985; Khan et al., 1989; Khan, 1991; Sharma et al., 1993; Kumaresan and Ansari, 2002; Azab, 2005; Saxena et al., 2006; Narnaware et al., 2007; Ananda Raja and Srilatha, 2007; Azawi et al., 2008) and clinical studies (Mittal et al., 2010; Narsinha Rao and Sreemannarayana, 1982; Jeevanajyothi et al., 2003; Raju et al., 2007; Modi et al., 2011; Pande et al., 2016) have identified some ovarian pathology in the bubaline species, however their clinical description is still far from perfect. Clinical evaluations commonly utilize trans-rectal palpation as the means of various differentiating ovarian pathologies although its efficiency continues to be low even for the physiological structures that form on the

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ovarian surface structures namely, follicles and corpus luteum periodically (Sharifuddin, 1983; Qureshi *et al.*, 1992; Honparkhe *et al.*, 2004).

Among the various ovarian pathologies, neoplastic conditions have been reported as incidental findings during the abattoir-based studies (Narnaware et al., 2007; Purohit, 2014; Pande et al., 2016; Deeb et al., 2018). Based on the ovarian compartment and the cell type, neoplastic and associated conditions are classified as: epithelial tumors, germ cell tumors, sex cordstromal tumors e.g. granulosa cell tumors (GCT), mesenchymal tumors, and hematomas. Among the various types, sex cord tumors of the granulosacell type are the most common ovarian neoplasms in cattle and buffaloes (Ganorkar and Paikne, 1994; Ali et al., 2009; Purohit, 2014; Deeb et al., 2018) and occur in all age groups with no apparent breed predilection. These conditions may produce steroid hormones causing nymphomaniac or virilizing in affected animals.

The diagnosis of ovarian neoplastic conditions from live animals continues to be difficult and therapy is neither possible nor successful in regaining fertility in bilateral conditions. Recently, hormone assays (Banu et al., 2012) and transrectal ultrasonography (Terzano, 2012) have been utilized in identifying ovarian structures and reproductive functions in buffaloes, however; their use in identifying ovarian pathologies has been less documented. Ovarian neoplasms have been sparsely classified for most domestic animals (Mac Lachlan, 1987) and in the buffalo these tumours have been less frequently documented under clinical settings probably because of frequent slaughter of aged and sub fertile buffaloes (Purohit, 2014). Despite its rarity, neoplastic conditions should be recognized as ovarian pathologies

which can be responsible for a great number of long-standing infertility problems in the said species (Narnaware *et al.*, 2007). Although, there are several diagnostic techniques available, histopathological examination accompanied by histochemical staining is considered to be the best and most reliable method for appropriate diagnosis of ovarian neoplasms. The present report describes the prevalence and classification of ovarian neoplastic conditions in buffaloes slaughtered at abattoirs in Chennai region of Tamil Nadu, India.

MATERIALS AND METHODS

A total of 1000 buffaloes carcasses ranging from 3 to 12 years of age slaughtered as a source of meat at slaughterhouse located at Chennai, Tamil Nadu, India was examined in-situ for gross abnormalities of the ovaries. Both the ovaries from 200 buffaloes were collected irrespective of lesions in the left or right and subjected to detailed examinations for enlisting various conditions/ affections. The ovaries were opened by frontal incision extending from free border to attached border; exposed parenchyma was examined for change in colour, nature of the fluid and alterations, if any. The observations were recorded, and the affected organ was preserved in 10% neutral buffered formalin (NBF). The tissue samples were processed by routine paraffin-embedding technique and 4 to 5 µm thick sections were prepared and stained by routine Haematoxylin and Eosin (H & E) method for detailed histopathological studies. In selected cases, adjacent sections of tissues were stained using special staining techniques (Luna, 1968).

RESULTS AND DISCUSSIONS

Reproductive failure has been recognized in recent times as one of the most serious problems affecting our economy through dairy industry. Genital abnormalities cause negative impact in animal breeding either by causing infertility or sterility and thus inflict heavy economic losses to livestock farmers. Generally female animals are culled and sent to slaughterhouse either because of infertility/sterility or disease conditions. Hence, an abattoir provides information and material on the prevalence of various reproductive disorders and studying pathological lesions of buffalo reproductive organs (Dobson and Kamonpatana, 1986).

Among the various diseases of genital tract, ovarian pathologies are foremost important in affecting the reproductive potential of buffaloes. Clinically, most of the ovarian pathologies including the neoplastic conditions are occult and difficult to diagnose. Hence, ovarian tumors have been described in the buffalos mostly from studies based on specimens collected from abattoirs (Luktuke et al., 1973; Potekar et al., 1982; Ganorkar and Paikne, 1994; Narnaware et al., 2007; Pande et al., 2016; Deeb et al., 2018). In the present abattoirbased study, neoplastic conditions of the ovaries were recorded in 12 animals out of 200 buffaloes examined accounting for 6% and were recorded unilaterally. In a similar study, ovarian neoplasms were recorded to be 3% (Narnaware et al., 2007). The overall incidence of ovarian neoplasm in buffaloes was reported as 0.11% (Patel et al., 2007). In the present study, majority of the animals were aged above 5 years and the ovarian affections were more in the right ovary than the left ovary corresponded with the earlier findings of higher incidence among the aged animal (Meuten, 2017;

Deeb et al., 2018).

Classification of ovarian tumors assumes that these tumors arise from one of the three ovarian compartments: epithelium; germ cells, or ovarian stroma, including the sex chords (Mac Lachlan, 1987; Yener *et al.*, 2004). In the present study, epithelial tumours were recorded in 6 cases (three each in right and left), sex cord-stromal tumors in 4 cases that included a solitary case of granulosa cell tumour in the right ovary and luteoma in 3 cases (one each in right and left) and haemangioma, tumour of the mesenchymal origin was recorded in 2 cases involving the left and right ovary.

Epithelial tumours

Adenoma was noticed in 5 (2.5%) cases in the present study. Grossly, all the ovaries were apparently normal. Histologically, all the epithelial tumours showed features of adenomas and were further sub-classified as simple adenoma (2 cases), fibro-adenoma (2 cases) and a solitary case of papillary cystadenoma.

Microscopically, the neoplastic cells were arranged in the form of gland. The glands were lined by a single to multiple layers of cuboidal to columnar epithelial cells, many of which were ciliated (Figure 1). The neoplastic cells showed round to oval hyperchromatic nuclei and large prominent nucleoli. In 2 cases there was moderate proliferation of spindle shaped fibroblasts along with the glandular epithelial cells suggestive of fibroadenoma.

One (0.5%) case of cystadenoma was recorded in the right ovary during the present study. Grossly, the ovary was apparently normal. Microscopically, the growth was characterized by neoplastic epithelial cells arranged in papillary projections supported by fibro-vascular stroma

into the lumen with the tendency to form cysts. The lining epithelial cells were cuboidal to columnar with basally located moderately enlarged ovoid to vesicular hyperplastic nuclei and vacuolated cytoplasm (Figure 2). The lumen of these cysts contained mucinous substance, which was found to be PAS positive.

Epithelial tumors develop from the surface epithelium of the ovary, which is coelomic mesothelium. In domestic animals, these tumors have a predominantly serous character and present a uniformity regarding location, invasiveness, the mitotic index, and morphology. Benign and malignant forms are difficult to differentiate in most cases. Epithelial tumours are considered as uncommon in most domestic animals and had been rarely reported in buffalo. The vast majority of epithelial tumors arise from the surface epithelium, although rarely, they arise from the rete ovarii (Mac Lachlan, 1987). Epithelial tumors of the ovary are usually adenoma and adenocarcinoma. Based on histology, the adenomas may be either cystic or papillary type, named as cystadenoma and papillarycyst adenoma. Adenoma recorded in the present study is reported to occur rarely in buffaloes (Moulton, 1990). Histologic descriptions mention that such tumors consist of arboriform papillae that project into the cyst lumen (Mac Lachlan, 1987). Our findings of Cystadenoma corresponded with earlier studies on abattoir specimens in buffalo (Damodaran, 1956; Dwivedi and Singh CM. 1970; Dwivedi and Singh, 1971; Kumar and Singh, 1984), however clinical descriptions of such tumors are not available.

Sex cord-stromal tumors

Sex cord stromal tumors are derived from, or histologically resemble, the normal cellular constituents of the ovary other than the epithelium or germ cells. Ovarian sex cord stromal tumors are a heterogeneous group of benign or malignant tumors that develop from the dividing cell population that would normally produce cells which support and surround the oocytes, including the cells that produce ovarian hormones (Dwivedi and Singh, 1985; Mac Lachlan, 1987; Gerhenson and Wharton, 2013). Sex cord tumours arise from granulosa cells, theca cells (thecoma), luteal cells (luteoma) and interstitial endocrine cells and have the potential to produce oestrogens, testosterone, or progesterone. The exact classification of sex cord-stromal tumours depends on the predominant cell type and pattern (Mac Lachlan, 1987).

Granulosa cell tumour

Granulosa cell tumour was noticed in the right ovary of 1 (0.5%) case. The most common ovarian sex cord stromal tumor in the buffalo appears to be granulosa cell tumor (Dwivedi and Singh, 1975). The incidence of such tumor in Indian buffaloes was 0.71% (Raja and Srilatha, 2008) and 1.5% (Narnaware *et al.*, 2009). In a recent study, a higher incidence (18.4%) of granulosa cell tumour has been reported (Deeb *et al.*, 2018).

In the present study, granulosa cell tumour was recorded unilaterally affecting only one ovary. Although this number is insignificant to make any definite conclusions, similar observation of unilateral involvement has been made in all the 9 buffaloes that had granulosa cell tumour (Deeb et al., 2018). According to Carlton et al. (1995); Radostits et al. (2001) granulosa cell tumors are most often unilateral and may suppress the function of the contralateral ovary. In the present study, the left ovary was histologically normal and agreed to the observations of Deeb et al. (2018) who reported that the unilateral GCTs did not interfere with the function to the contralateral ovary.

Generally, granulosa cell tumour varies in size from relatively small, solid, yellow to white structures to large structures composed of multiple cysts, a single large cystic structure, or a combination of solid and cystic structures (Zinnebauer, 1961; McEntee 1990). In contrast, the ovary was apparently normal in the present study and agreed to the observations of Deeb *et al.* (2018) as GCTs appeared only microscopically without gross changes on ovaries affected either in shape, size, color, consistency and/or cut sections.

GCTs microscopically appeared in three growth patterns or forms; diffuse, trabecular, and follicular form, but the most common pattern was the follicular form according to Hunt et al. (1996). Follicular and diffuse patterns of growth of the tumor were also reported by McEntee (1990) as common GCTs growth pattern. El-Nesr et al. (2006) described four histopathological patterns of granulosa cell tumors in Balady cows whereas in a study conducted involving cows and bufflaoes Deeb et al. (2018) have recorded ten different histological patterns of granulosa cells. The authors added that the formation of Call Exner's bodies was the prominent structure to GCTs. In the present study, the tumor foci appeared in the form multicystic spaces separated by fibrous tissues that were lined cylindrical to polyhedral type of cells with centrally placed elongated nuclei and vacuolated cytoplasm (Figure 3). At a few places, there were tendency to form rosette pattern, however, the characteristic Call Exner's bodies were not noticed in the present study. In the earlier studies, Kitano et al. (1996); El-Nesr et al. (2006) mentioned that Call-Exner's bodies were numerous in the newly forming tumors in contrast to the large ones and these bodies may undergo hyalinosis in the form of multilavered basal laminae in some cases. Endocrine abnormalities have been associated

with sex cord stromal tumors (Mac Lachlan, 1987; Bovine Granulosa Cell Tumors- Newsletter, 2002.) although not documented for the buffalo species.

Luteoma

Luteoma is a neoplasm of ovarian interstitial cells. They have a smooth surface, firm consistency, solid, yellowish white to orange in color, may contain areas of hemorrhage and necrosis. Tumor cells are large, polyhedral, with large amount of eosinophilic cytoplasm. They contain lipid droplets of varying dimensions, it may present a yellow lipid pigment, resembling cells in the corpus luteum (Jones et al., 1996). In the present study, luteoma was recorded in 3 (1.5%) cases. Grossly, the ovary was apparently normal. Microscopically, the growth consisted of dense sheet of round, relatively uniform cells with welldefined cytoplasmic margin resembling lutein cells. These proliferating lutein cells were supported by thin fibrous tissue stroma. The neoplastic cells were large, polyhedral to oval with centrally placed large, round to oval hyperchromatic nuclei and prominent nucleoli. These cells contained moderate quantity of granular to eosinophilic cytoplasm with varying sized lipid droplets (Figure 4).

Luteoma is a neoplasm related to hormonal changes, cows and mares can be affected by imbalance in the plasma levels of sex hormones, such as estradiol, progesterone, and testosterone. Such hormonal imbalances can manifest themselves, clinically, in the form of nymphomania and masculinization, however not documented for the buffalo species (Jones *et al.*, 1996). This type of tumor is directly associated with the production of progesterone (Nasrin *et al.*, 2012).

Mesenchymal tumors

Mesenchymal tumors comprise

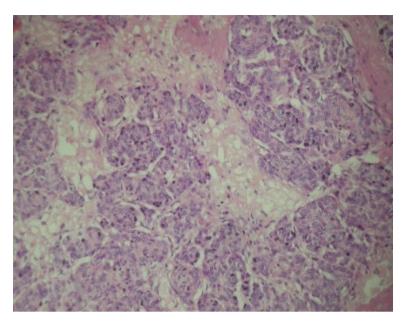


Figure 1. Adenoma showing proliferation of glandular epithelium in cortical area of the ovary. H & E x 200.

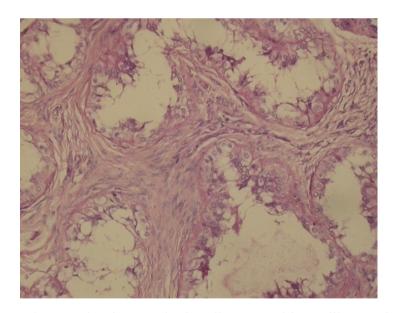


Figure 2. Papillary cystadenoma showing neoplastic cells arranged in papillary projections with varying sized cysts. H & E x 400.

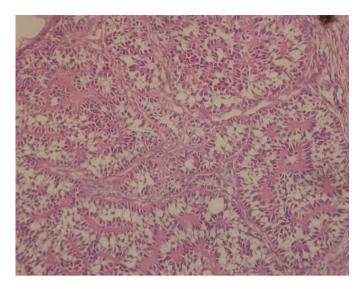
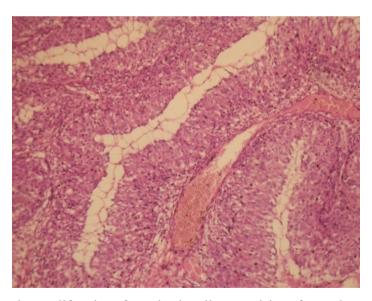


Figure 3. Granulosa cell tumour showing neoplastic cells arranged in tubular pattern. H & E x 200.



Figutre 4. Luteoma showing proliferation of neoplastic cells comprising of granular to eosinophilic cytoplasm with lipid droplets. H & E x 200.



Figure 5. Haemangioma showing multiple nodules of reddish-brown spongy tissue.

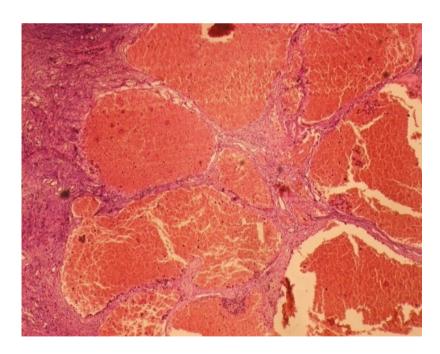


Figure 6. Cavernous haemangioma showing blood filled vascular spaces lined by single layer of flattened endothelial cells. H & E x200.

heterogeneous group of neoplasm that are not specific to the ovary and include fibromas, hemangiomas, leiomyomas, and their malignant counterparts (Mac Lachlan, 1987; Talerman, 2014). Two (1%) cases of cavernous haemangioma were recorded in this study, with one (0.5%) case each in left and right ovary.

Haemangioma

Grossly, the growth comprised of single or multiple masses of red-brown spongy tissue (Figure 5). Microscopically, multiple varying sized vesicular lumina lined by flattened endothelial cells aligned on thin collagenous septa were separated by fibrous connective tissue stroma. Organized thrombi with focal collection of haemosiderin pigment were also seen (Figure 6). The neoplastic endothelial cells had scanty cytoplasm and flattened dark nuclei of uniform size.

Hemangiomas and leiomyomas have a higher incidence. In buffaloes, the neoplasm of this category recorded includes haemangioma and has been sparsely reported earlier. Perusal through the available literature revealed a solitary care report on hemangioma in lung of buffalo (Dwivedi, 1969). Ovarian vascular hamartoma (OVH), one of the rare ovarian tumor-like anomaly has been reported in cows. OVH is a congenital, nonneoplastic tumor-like anomaly of vascular origin, characterized by an excessive focal overgrowth of mature endothelial cells with a disorderly arrangement and imperfect differentiation (Lee and Ladds, 1976; Benoit *et al.*, 2005), which can be confused with haemangioma.

Although haemangioma has been rarely reported in cows, however this neoplastic condition is observed commonly in the ovaries of mature and aged sows and other animals (Jubb *et al.*, 1993). The gross and histological feature of haemangioma

in the present study was suggestive of cavernous type.

CONCLUSION

The current study reports 12 cases of ovarian tumors in buffaloes, wherein the gross appearance of ovaries of all tumor bearing animals excepting haemangioma, were apparently normal. The study demonstrates importance of abattoir specimens and the usefulness of histopathological examination to determine the characteristics of each tumor and classify the neoplastic conditions. The present findings indicate that these occult changes might cause ovarian dysfunction leading to sterility and infertility.

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