Original Research Article

Morphological pathology of bovine ovarian abnormalities in correlation to uterine changes

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ABSTRACT

Female genital tracts of six to eight years Baladi cows (n=30) were collected from Belevia abattoir of Beni-Suef province during the period from March 2016 to May 2016 to investigate ovarian abnormalities and uterine changes in cattle based on histopathology. Prevalence rates of granulosa cell tumors were 26.67% and 38.89% in right and left ovaries, respectively. Follicular cysts could be detected in both ovaries with percentages of 50.0% and 44.44%, respectively. The most predominant pathological lesions in ovarian medulla were hyalinosis of blood vessels and mononuclear cell infiltration. The main uterine pathological alterations were endometritis associated with degenerative changes and necrosis in the endometrial linings in most cases (n=25), endometriosis (n=13). Variable degrees of congestion from moderate (n=25) to highly congested (n=5) were elucidated. Perivascular cuffing (n=2) and perivascular fibrosis (n=3) of uterine blood vessels could be detected. Immunohistochemically, granulosa cell tumors were positive to vimentin and negative to inhibin.

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1. Introduction

Reproductive disorders are key determinants affecting fertility to a great extent and ultimately causing huge economic losses to livestock industry. The ovaries are unique organs that control estrous cycle, hormonal production, fertilization and the maintenance of the embryo until its arrival in the uterus. Pathological affections of the ovaries are common diseases in domestic mammals, especially cattle and buffaloes (McEntee, 1990; Azawi et al., 2008). The uterus, particularly the endometrium lining the uterus has important roles in normal reproductive cycles, implantation and placentation, and supporting a healthy fetus until parturition. Microbial infections of the uterus have a negative impact because they cause infertility, abortion, preterm labor and clinical disease (Wira et al., 2005; Jabbour et al., 2009; Sheldon et al., 2009; Mor and Cardenas, 2010). Many infections reach the genital tract via the cervix while others reach through circulation. Also, the endometrium with its innate and adaptive immunity has important role in countering the microbial invasion (Wira et al., 2005; Sheldon et al., 2009).

Major uterine pathologies reported in cattle and buffalo involve endometritis (subclinical and clinical), puerperal and septic metritis, pyometra, perimetritis, parametritis, hydrometra, mucometra and certain congenital anomalies (Hatipolgu et al., 2002; Ali et al., 2006; Saxena et al., 2006; Azwai et al., 2008b; El-Sakkar et al., 2008; Rhyaf, 2010; Modi et al., 2011). One of different uterine affections is sub clinical endometritis that plays an important role in the failure of reproduction in cattle as well as buffalo (Moghadam and Mamoei, 2004; Sheldon et al., 2008; Senosy and Hussein, 2013).

The aim of current study is to investigate and describe the histopathological alterations in ovaries in correlation to uterine changes. And also to investigate the expression of vimentin, and inhibin in bovine ovarian granulosa cell tumors.

2. Materials and methods

A total number of 30 cows from Belevia, Beni-Suef during the period from March to May 2016 were pathologically examined. Specimens were collected from recently slaughtered cow aged 6 to 8 years old, beladi breed, but without information on history and cause of slaughter. Tissue specimens were taken from right and left ovaries, and uterine body. These samples were fixed in formalin 10% for 48 hrs and then processed according to Bancroft and gamble (2012). Five microns tissue sections were mounted on clean glass slides and stained with eosin and haematoxylin (HE).

Five microns-ovarian sections were mounted on positive slides for immunohistochemistry for vimentin and inhibin expressions. Dewaxing, antigen retrieval and immunostaining were performed using mouse monoclonal antibody against vimentin and inhibin (Dako envision kit, St. Cruz, California) according to the protocol given by Buchwalow (2010). Control positive and control negative were used.

3. Results

Examination of stained embedded materials obtained from 30 paired ovaries and uteri collected from local abattoirs in Beni-Suef province revealed the followings:

1. Ovarian alterations

Pathological alterations of 30 ovaries of cows (right and left ovaries for each) allocated that as follows: Microscopic granulosa cell tumors were identified in 15/30 cows (8 right and 7 left) (50%). Different forms of granulosa cell tumor including diffuse form, insular form and micro follicular form were seen (Fig.1a-c). The diffuse form were commonly found in secondary and tertiary follicles. On the other hand, the other two forms were identified in the larger follicles. Corpus luteum was seen in a few animals consisting of GCTs in the same ovary or contralaterally.

The follicular cysts were found in 23 (15 right and 8 left) (76.66%) cows. Those cysts ranged from 3-5cm in diameter with clear fibrous wall encircling straw yellow fluid. Some cysts were active as they were lined by one or more granulosa cell layers (5 animals). Oppositely, the other 18 cows had cysts with no granulosa cells (inactive) (Fig.1d,e). Follicular atresia was commonly seen in most cases (Fig.1f). The main pathological lesion of ovarian medulla was congestion (Fig.1g) and hyalinosis of blood vessels with mononuclear cell infiltration in some animals (Fig.1h).

2- Uterine alterations

In 5 animals, lamina epithelialis of the endometrium showed more or less normal histological structures, while vacuolar degeneration
could be detected in 8 (26.66%) cows. Necrosis and desquamation of lamina epithelialis was found in 13 (43.33%) animals. Endometrial hyperplasia could be detected in 3 cases (Fig. 2a), while cystic glandular hyperplasia detected only in one case.

The lamina propria of the endometrium exhibited more or less normal structure in 5 cows. Meanwhile, mononuclear leucocytic infiltration (mainly lymphocytes, plasma cells and occasionally macrophages) was found in 18 cows (Fig. 2b). Only one case revealed the presence of massive neutrophilic infiltration. Mixed polymorphnuclear cells (P.M.Ns) and eosinophils together with mononuclear cells were found in 3 cases (10.0%) (Fig. 2c). True eosinophilic infiltration was found in 2 animals (Fig.2d).

Endometriosis was found in 13 (43.33%) cows (Fig. 2e), while the remaining animals showed variable pathological lesions consisted of degeneration and necrosis. Cystic dilatation associated with periglandular cuffing in 2 cases (Fig. 2f). Submucosal blood vessels had varying degrees of congestion ranged from slight congestion (n=25) to highly congested (n=5) (Fig. 2g). Some blood vessels underwent perivascular cuffing (n=2) (Fig. 2h) and perivascular fibrosis (n=3).

Main pathological lesions in the myometrium were vacuolar degeneration of myometrium (n=17) (Fig. 2i) and infiltration with mononuclear cells in a focal manner (n=5). Blood vessels of the same layer revealed the presence of congestion (Fig.2j) and vacuolar degeneration in the tunica media.

Vimentin was highly expressed intracytoplasmic in granulosa cells around Call-Exner bodies as a brown staining reaction (Fig. 1i). Granulosa cells rested on the basement membrane of the tumor foci were strangely positive. In contrast, inhibin was negative (Fig.1j).

4. Discussion

Reproductive infertility or sterility is considered the most serious problems affecting dairy cattle industry. Subfertility or infertility has a good prognosis than sterility as sterile animals are clinically, easily identified compared to those having transient form of reproductive disorders (Alam, 1984; Shivhare et al., 2012). Pathological conditions of the ovary as well as uterus, seriously interfere with normal functions of the entire reproductive tract, consequently, decreasing and affecting the reproductive potential of the animal (Ali et al., 2006).

The present study described the pathological findings of 30 cows of bovines aged 6-8 years. It was found that the main ovarian alterations are follicular cysts in 23 (76.66%) cows. The second alteration was granulosa cell tumors (GCTs) (15/30; 50.0%). Granulosa cell tumor is the type of ovarian sex cord stromal tumor seen in cows and other animal species (McEntee, 1990). It is usually benign (Moulton, 1978) with low grade of malignancy, but represented 5.0% of malignant ovarian tumors (Scully, 1977; Alexiadis et al., 2011). The prevalence varied from 28.57% (40/140) to 60.43% (84/139) in cows (McEntee, 1990; El-Nesr et al., 2006). In the later, GCT was detected in 2 out of 40 animals occupying the whole ovarian stroma in the form of multicystic spaces separated by fibrous septae. Bovine ovarian granulosa cell tumors were recorded at age ranging from new born to 19 years. Nymphomania, virilism or no clinical manifestations were reported in cases with GCTs (McEntee, 1990). The occurrence of granulosa cell tumors was greater during such age compared to younger and newly-born animals (Moulton, 1978; McEntee, 1990) revealing a higher prevalence in old cows (average 7 years). Oppositely, Nielsen and Kennedy (1990) reported that GCTs tend to be more predominant in younger animals and in newborn calves.

Different hypotheses of pathogenesis of granulosa cell tumors are supposed. One is dependent upon irregular granulosa cell proliferation and neoplasia due to the degeneration of follicular granulosa cells after losing of oocyte with diminished sex hormone secretion, especially estradiol-17beta (Vanderhyden et al., 2003; Cohen 2010). Furthermore, due to the location of granulosa cells in the ovary, they are waiting for a hormonal trigger, especially due to increased FSH concentrations which are oncogenic (Schumer and Cannistra, 2003). Grossly, in bovines, the size of the tumor varied from microscopic up to 40 kg (Bauman, 1935; Zinubaur, 1961; Bosu, 1977; El-Nesr et al., 2006, 2013). Different morphological patterns were recorded in several studies including macrofollicular and/or microfollicular patterns (diffuse, follicular, trabecular, or rosettes form) (McEntee, 1990; El-Nesr et al., 2006).

Microscopically, GCTs appeared in different growth patterns; diffuse, trabecular, and follicular form, with the most common pattern was the
Fig. 1. Ovarian alterations in examined cows. a) Focal form of granulosa cell tumor (GCT). Scale bar= 50µm. b) Insular form of granulosa cell tumor (GCT). Scale bar= 200µm. c) Micro-follicular form of granulosa cell tumor (GCT). Scale bar= 100µm. d) Active follicular cyst. Scale bar= 100µm. e) Inactive follicular cyst. Scale bar= 200µm. f) Follicular atresia. Scale bar= 200 µm. g) Congestion in medullary blood vessels. Scale bar= 200µm. h) Hyalinosis in medullary blood vessels. Scale bar= 100µm. i) Positive reaction against monoclonal antibody of vimentin. Scale bar= 50µm. j) Negative reaction against monoclonal antibody of inhibin. Scale bar= 50µm.
Fig. 2. Uterine alterations in examined cows. a) Endometrial hyperplasia. Scale bar= 100µm. b) Mononuclear leuckocytic infiltration (mainly lymphocytes, plasma cells and occasionally macrophages). Scale bar= 50µm. c) Mixed polymorphnuclear cells (P.M.Ns) and eosinophils together with mononuclear cells. Scale bar= 50µm. d) Eosinophilic infiltration. Scale bar= 20µm. e) Endometriosis. Scale bar= 100µm. f) Periglandular cuffing. Scale bar= 50µm. g) Submucosal blood vessels with varying degrees of congestion ranged from slight congestion to highly congested. Scale bar= 200µm. h) Submucosal blood vessels showing perivascular cuffing. Scale bar= 100µm. i) Vacuolar degeneration of myometrium. Scale bar= 50µm. j) Blood vessels of the myometrium revealed the presence of congestion. Scale bar= 200µm.
Table 1. Abnormal ovarian structures in relation to uterine changes in cows.

<table>
<thead>
<tr>
<th>Ovarian structures</th>
<th>No.</th>
<th>%</th>
<th>Uterine changes (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic follicles</td>
<td>23</td>
<td>67.66</td>
<td>degeneration and necrosis of mucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>congestion</td>
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<td></td>
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<td>endometritis</td>
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<td>endometriosis</td>
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<td></td>
<td></td>
<td></td>
<td>cystic glandular hyperplasia</td>
</tr>
<tr>
<td>Granulosa cell tumors</td>
<td>15</td>
<td>50</td>
<td>endometriosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>degeneration and necrosis</td>
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<td></td>
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<td>endometritis</td>
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No. referred to number of positive animals  % referred to the percentage of positive animals

follicular form (Chalvardjian and Derzko, 1982; McEntee, 1990, Jones et al., 1996). El-Nesr et al. (2013) stated that granulosa cell tumors were found in 40 animals out of 140 in abattoir samples in Beni-Suef, Egypt. The tumors were unilateral in 25 animals and bilateral in 13, distributing in the subcapsular area, cortex or in the ovarian hillus. Two cases had grossly tumor foci occupying the whole ovarian stroma and separated by fibro-elastic septae. Basically, different histopathologic patterns were seen; the first had uniform populations of granulosa cells surrounded with fibrous septa. The second consisted of granulosa cells arranged in clusters around eosinophilic material (Call-Exner bodies) forming rosette form. The third one, the tumor foci, appeared in the multicystic spaces separated by fibrous tissues that lined by granulosa cells. In some tumor masses, some cells were mitotically active with prominent nucleoli.

Call-Exner bodies were prevalent and numerous in newly-formed tumors but less frequent in adult types. Neoplastic granulosa cells were congruent to granulosa cells in growing follicles but they show hyperchromatic, oval to spherical nuclei, distinct nucleoli and scanty cytoplasm (McEntee, 1990).

In the current study, immunohistochemically, granulosa cell tumors were strangely positive to vimentin and negative to inhibin. Immunohistochemical techniques are considered valuable tools in the diagnosis of ovarian neoplasms especially granulosa cell tumors. Several markers are valuable in proving and diagnosis of such tumors. Vimentin is an important marker that highly expressed in granulosa cells of normal ovarian follicles and in GCTs. All neoplastic cells, irrespective of their specific growth patterns, contained both vimentin and desmoplakins.

Oppositely, cytokeratin is the lowest in granulosa cell tumors. Inhibin is a sensitive and specific immunohistochemical marker for GCTs. However, a negative immunostaining against inhibin does not exclude the diagnosis of granulosa cell tumour (Benjamin et al., 1983; Czernobilsky et al., 1985, Gitsch et al., 1991, Niekerk et al., 1993, Pelkey et al., 1998, Riccardi et al., 2007; Tamaskar, 2009).

Currently, the most important uterine lesions were variable involving mild inflammatory reactions that were reflected by degenerative changes and necrosis in epithelium of both endometrial and/or lining the subendometrial glands with moderate leucocytic infiltration, and endometriosis that suggesting estrogen production from this follicular cyst or granulosa cell tumors that stimulate the subendometrial glands to increase in number. It has been reported that GCT is associated with endometrial hyperplastic changes attributable to
stimulation of the endometrium by excessive estrogen production (Koukourakis et al., 2008). Such finding agreed with our results as endometriosis was recorded in some cases with granulosa cell tumors or cystic follicles. As a result of the increased hormonal activity of granulosa cell and overproduction of sex hormones (Anttonen, 2005), GCTs are associated with high plasma level of estrogen, progesterone and/or androgen (El-Nesr et al., 2006; AssisNeto et al., 2010).

In conclusion, the present investigation revealed that GCTs and follicular cysts were the most common ovarian abnormalities. Alternatively, endometriosis was the prevalent pathological uterine alteration in cows had GCTs or follicular cysts. Granulosa cell tumors were positively stained with anti-vimentin and negatively-stained with anti-inhibin.

References
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