Histopathological, microbiological and biochemical studies on uteri and ovaries of infertile slaughtered buffaloes in Dakahlia Governorate

By

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SUMMARY

Seventy uterine and ovarian samples were collected from adult female buffaloes, slaughtered in different slaughter houses in Dakahlia Province. The animals were suffering infertility. The specimens were examined bacteriologically, biochemically and pathologically. Ten blood samples (5 from normal and 5 from diseased buffaloes) were collected from the jugular vein, before slaughtering. The serum was separated and used for blood chemistry. The surface of uteri and ovaries were seared by a hot spatula and opened under complete aseptic precaution then a loopfull of the uterine content was directly cultured onto nutrient agar, MacConkey agar and blood agar. All the inoculated plates were incubated at 73°C for 24 h. The suspected colonies were examined culturally. Specimens were collected from the uteri and ovaries and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared, stained by H & E and examined microscopically.

The most prevalent microorganisms were E. coli (10%), Klebsiella oxytoca (2.85%), Proteus mirabilis (2.85%), Staph. aureus (5.7%) and Strept. pyogens (7.1%). Uterine adhesion, endometritis and cystic ovaries were encountered. The uterus showed degenerative changes in the uterine glands with periglandular mononuclear cell infiltration and edema besides congestion and vasculitis. The examined ovaries showed either thick walled follicular cysts or leutin cysts with luteinized granulosa cells, hyperplastic theca-interna and theca-externa besides atretic follicles and hyperplastic lining of other follicles. The blood vessels were congested and showed thickened walls of the coiled arterioles. The estrogen was significantly increased, meanwhile the progesterone, T₃ and t₄ hormones were significantly decreased.

It could be concluded that the affection of the uterus and ovaries leads to infertility associated with hormonal disturbances. E. coli was the highest to isolate, but Klebsiella oxytoca and Proteus merabilis were the lowest.

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INTRODUCTION

Buffalo is considered one of the most important multipurpose farm animals. The infertility problems are widely spread among this species causing excess reduction in their production. The ovarian lesions appeared to be the main cause of infertility among buffaloes (Wahba et al. 1995). The uterine disorders are contributing factors that impair the fertility of animals. The most common form of the uterine disorders is endometritis (Khan, 1994).

This study was planned to isolate the involved bacterial pathogens describe the associated histopathological lesions and evaluate of the ovarian and thyroid hormones in buffaloes having cystic ovary together with endometritis. Moreover, the antibiograms of some isolated pathogens were considered.

MATERIAL AND METHODS

Seventy uterine and ovarian samples were collected from slaughtered adult female buffaloes with a history of infertility the samples were collected from Mit-Ghamr, Kom-El Nour, Mit-Yaeesh and Atmeda abattoirs during the period from August, 2005 till November 2005 in Dakahlia Governorate.

The surface of uterus and ovaries were seared by a hot spatula and opened under complete aseptic precaution then a loopfull of the uterine content was directly cultured onto nutrient agar, MacConkey agar and blood agar. All the inoculated plates were incubated at 37°C for 24 h. The suspected colonies were examined culturally biochemically as described by Carter (1984). Drug sensitivity test was done for all the bacterial isolates according to Cruickshank et al. (1975).

Ten blood samples were collected from the jugular vein (before slaughtering) of 5 buffaloes suffering from cystic ovary with endometritis and other 5 healthy buffaloes. Serum was obtained by centrifugation at 3000 rpm for 20 min, stored at –20°C and used for hormonal assay. Serum progesterone was estimated by Radioimmunoassay (RIA) according to Kubasik et al. (1984), estrogen according to Xing et al. (1983) and thyroxin (T₄) together with triiodothyronine (T₃) according to Abraham (1981).

Specimens were collected from the uterus and ovaries and immediately fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin (Bancroft and Gamble, 2002) and examined microscopically.
Statistical Analysis:
The obtained results were statistically analyzed using Student t-test as described by Petrie and Watson (1999).

RESULTS AND DISCUSSION
1-Microbiological
The uterine and ovarian samples, from 70 slaughtered buffaloes, yielded 20 isolates (28.5%) as shown in table (1). The most prevalent organisms were E. coli (10%), Klebsiella oxytoca (2.85%), Proteus mirabilis (2.85%), Staph.aureus (5.7%) and Strept. pyogenes (7.1%). These findings are in partial agreement with those obtained by El-Sawaf et al (1961) and Fadel (2000) who recorded isolation rates of 25% and 20.60% respectively. These results are not in concurrence with Shouman et al (1977), Son-Changho et al (1998) and Barakat et al (2005) who recorded isolation rates of 7%, 15.68% and 14% respectively.

Table (2) shows the results of

<table>
<thead>
<tr>
<th>Tested antibiotic</th>
<th>E. coli (7)</th>
<th>Kl. oxy (2)</th>
<th>Pr. m (2)</th>
<th>Staph. aureus (4)</th>
<th>St. py (5)</th>
<th>Total (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>30</td>
<td>4</td>
<td>57.1</td>
<td>1</td>
<td>50</td>
<td>13</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>5</td>
<td>5</td>
<td>71.4</td>
<td>2</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>Erythromicin</td>
<td>15</td>
<td>1</td>
<td>14.3</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Florofincol</td>
<td>30</td>
<td>3</td>
<td>42.9</td>
<td>1</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>6</td>
<td>85.7</td>
<td>2</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>Oxytetracyclin</td>
<td>30</td>
<td>1</td>
<td>14.3</td>
<td>1</td>
<td>50</td>
<td>6</td>
</tr>
</tbody>
</table>

Table (1): The bacterial isolates from the 70 examined samples.

Table (2): Sensitivity test of the bacterial isolate.
pyogens. The total isolated microorganisms were highly sensitive to florafincol (75%), Enrofloxacin (70%), amoxycillin (65%), gentamycin (65%), erythromycin (35%) and oxytetracycline (30%). These results are in a partial agreement with Awad and El-Hariri (1980) and Pradhan et al (1999). They reported that enrofloxacin, gentamycin and chloramphenicol were highly effective against the isolates from bovine endometritis. On the other hand the obtained results disagree with Hussein et al (1993) who stated that tetracycline and ampicillin are the most effective in treating repeat breeder syndrome.

**Hormonal:**

Table (3) indicates that the estrogen level was significantly high meanwhile the progesterone and both the triiodothyronine (T₃) and thyroxin (T₄) were significantly low in the buffaloes suffering both cystic ovary and endometritis. These results agree with Cristofori et al (1986), Saleh et al (2000) and Mohamed and Hussein (2004) in the camel and buffaloes. Douthwaite and Dobson (2000) mentioned that the serum progesterone level was lower in cows with follicular cyst than in cows with leutein cysts. These results may be attributed to less lu-

teinization of the follicular cyst with low progesterone secretion (Zeitoun, 2003). The estrogen level in this study was high in buffaloes suffering from cystic ovary with endometritis. This goes along with the results of Mabrouk (1989) and Homeida et al (1991) who found a close correlation between the follicle size and estrogen level. Homeida et al (1988) observed that the estrogen concentration was parallel to follicle size. They suggested that the follicle secretes estrogen. Similar results were reported by Hamilton et al (1995) who observed a higher concentration of estrogen in cow with cystic ovary than those with ovulatory cycles. The current work showed that the levels of the T₄ and T₃ were low in the serum of buffaloes suffering from cystic ovary with endometritis. These results are in agreement with those of Metwelly et al (2004) who reported that the levels of both the T₄ and T₃ were the lowest in cases of cystic ovary associated with endometritis in she camel. Abdo et al (1969) reported that the low thyroid function plays a role in the occurrence of cystic ovaries.
Macroscopically, some examined uteri showed endometritis and others showed uterine adhesions. The inflamed endometrium was swollen and covered with turbid exudate. Some cases showed grayish endometrium with rough surface and irregularly dark red patches. Similar lesions were described by Hegazy et al. (1998) and Moustafa et al. (2002).

Almost all the examined ovaries were cystic, in one or both ovaries. Unilateral or bilateral hemorrhagic cysts were found (Fig. 1). Similar findings were previously noticed by Selim et al. (1998) in the right ovary only. Some ovaries revealed an-encapsulated, white and firm mass (fibroma). Jones et al. (1997) reported that fibroma was the more common ovarian neoplasms in cows.

**Histopathological examination of uteri:**

Microscopically, the endometrium of the affected buffaloes showed mononuclear cell infiltration (Fig. 2), in addition to severe congestion in the lamina propria (Fig. 3). Similar results were described by El-Mashad (1998), and Refat and Badr (2006). Variable degenerative changes were found in the uterine glands (Fig. 4). Almost similar results were reported by Hegazy et al. (1979), Amer et al. (2001) and Kubar and Jalakas (2002). Moreover, periglandular mononuclear cell infiltration and edema were found (Fig. 5). Similar

<table>
<thead>
<tr>
<th>Ovarian finding</th>
<th>Number of buffaloes</th>
<th>Progesterone (mg/ml)</th>
<th>Estrogen (Pg/ml)</th>
<th>T₃ (ng/ml)</th>
<th>T₄ (ng/ml)</th>
<th>T₃ : T₄ (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy ovary and uterus</td>
<td>5</td>
<td>1.98 ± 0.13</td>
<td>62.72 ± 6.31</td>
<td>2.36 ± 0.11</td>
<td>7.13 ± 0.05</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>Cystic ovary and endometritis</td>
<td>5</td>
<td>1.31 ± 0.22*</td>
<td>81.42 ± 3.51*</td>
<td>1.85 ± 0.19*</td>
<td>4.26 ± 1.13*</td>
<td>0.42 ± 0.02*</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05
results were described by Hegazy et al. (1979 & 1998) and El-Mashad (1998). The basilar uterine glands and adjacent stroma invaded the myometrium (adenomyosis) along the blood vessels (Fig. 6). Such lesion is triggered by the excessive estrogen production by the cystic follicles (Jones et al., 1997). The elevated estrogen level led to estrogen–mediated endometrial hyperplasia. The deep layer of the lamina showed thickened walls of the spiral arterioles, besides edema (Fig. 7). Similar results were described by Mahdy (1988) and El-Mashad (1998). The variation in intensity of the uterine inflammatory changes was attributed to the host resistance, environment, virulence of microorganisms and hormonal effect Amer (1998) and Abd El-Wahab (1991).

Histopathological examination of ovaries:

Microscopically, the examined affected ovaries showed follicular cyst with thick walls (Figs. 8 & 9). Similar findings were previously described (Al-Dahash and David, 1977; Hussein and Hassieb, 1989; Selim et al., 1998 and Amer et al., 2001). Other cases showed lutein cysts with hyperplastic theca interna and externa (Fig. 10). They developed from the most mature follicles due to disturbed secretion and release of the gonadotropin, besides hypothyroidism and L.H deficiency (Abdo et al., 1969 and Omar et al., 1982). Atretic follicles and degenerated lutein cysts were detected (Figs. 11 & 12). Similar results were described by Hussein and Hassieb (1989) and Wahba (1998). An ovary of aged buffalo showed fibroma consisting of interlacing bundles of fibrous connective tissue without any malignancy (Fig. 13). Other case showed fibroma with spindle shaped nuclei arranged in wavy bundles (Fig. 14). Similar tumor in uterus was described by Abd El-Mottaleb and Badr (2007).

It could be concluded that the endometrial lesions are among the most common causes of infertility. Moreover, the ovarian damage contributed to the problem.
Fig. (1): Ovaries of aged buffalo-cow showing hemorrhagic follicles and cysts (arrows).

Fig. (2): Uterus of buffalo-cow showing mononuclear cell infiltration (arrows) in the inner lamina propria (H & E., x 100).
Fig. (3): Uterus of buffalo-cow showing severe congestion in the lamina propria (C)

Fig. (4): Uterus of buffalo-cow showing variable degenerative changes in the uterine glands. (H & E., x 400).
Fig. (5): Uterus of buffalo-cow showing periglandular mononuclear cell infiltration (m) and edema (arrows) (H & E., x 100).

Fig. (6): Uterus of buffalo-cow showing adenomyosis (arrows) (H & E., x 100).
Fig. (7): Uterus of buffalo-cow showing thickened walls of spiral arterioles (v) in the depth of lamina propria besides edema (arrows) (H & E., x 100).

Fig. (8): Ovary of aged buffalo-cow showing follicular cyst (arrow) with thickened wall (H & E x 4).
Fig. (9): A high power of Fig. (8) to show the thickened wall of a follicular cyst (H & E., x 200).

Fig. (10): Ovary of aged buffalo-cow showing lutein cyst with hyperplastic theca interna (I) and theca externa (E) (H & E., x 100).
Fig. (11): Ovary of aged buffalo-cow showing atretic follicles (H & E, x 100).

Fig. (12): Ovary of buffalo-cow showing degenerated lutein cyst (H & E, x 200).
Fig. (13): Ovary of aged buffalo-cow showing fibroma Consisting of interlacing bundles of fibrous connective tissue (H & E., x 100).

Fig. (14): Ovary of aged buffalo-cow showing fibroma with spindle shaped nuclei arranged in wavy bundles (H & E., x 200).
REFERENCES


Hussein, F.M.; Rezk, M.S.H. and


Pradhan, R.C.; Barik, A.K.; Ray,


Son-Changho-KungKang-Byong


دراسات هستوضاثولوجية وميكروبولوجية وبيوكيميائية على أرحام ومبايض أثاث
الجاموس العقيم المحبوب بمحافظة الدقهلية

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قسم الكيمياء الحيوية معمل بيطري الزقازيق - معهد بحوث صحة الحيوان

المتخصص العربي

في هذا البحث تم تجميع (70) عينة من أرحام ومبايض أثاث الجاموس البالغة العقيمة
المذبوحة بمجازر عسير - محافظة الدقهلية كذلك تم أخذ 5 عينات دم من حيوانات سليمة و5
عينات دم من حيوانات مصابحة وصل سرير لفحص البيوكيميائي. كذلك تم أخذ مسحة من
الأرحام والمبايض للفحص البيكيرولوجي وكذلك أخذ عينات من الأرحام والمبايض المصابة
واثباثها في الحال في فور مالين 10% للفحص الهستوباثولوجي.

تم عزل ميكروب القولون الفصوى بنسبة 15% والكليبيسيلا أوكس توسا 2.85%
والبروفيس مارابلس 62.85% والمكور منقوثي الذهبي وميكروب المكور السبحي 7.1%
وفحص الأرحام والمبايض بالعين المجردة لاحظ وجود وجود الت적이ات لبعض الأرحام والمبايض
بالعين المجردة لاحظ وجود تكييس لبعض المبايض وتلف ببعضها.

وبالفحص الهستوباثولوجي لاحظت نتائج إضمحلالية ببعض الأرحام وتغلل خلوي
وأودينا بالإضافة لأحتقان الأوعية الدموية ومتلازمة جذورها. كذلك تغلظ جدران أكياس المبايض
وتلفت مبايض أخرى وفحص البيوكيميائي لاحظ زيادة الأستروجين بينما لاحظ نقص
البروجسترون وهرمونات الغدة الدرقية ويوضح من ذلك أن العقيم مصحوب بوجود إصابة
بكتيرية وتفاعلات هرمونية وباثولوجية.

المتخصص:

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