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# Ovarian and oviductal pathologies in the buffalo: Occurrence, diagnostic and therapeutic approaches

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#### ABSTRACT

Ovarian pathologies observed in the buffalo include developmental anomalies, inflammatory conditions and neoplasm's, the usual incidence of which has been observed to be low (0.1%-19.0%)in abattoir studies. Ovarian functional disorders are the most frequently observed clinical entities in buffalo and include conditions such as sub estrus, (7.4%-55.8%), persistent corpus luteum (0.54%) and ovarian cysts (0.5%-1.4%). The diagnostic approaches for ovarian hypofunction and ovarian pathologies include transrectal palpation and transrectal ultrasonography the efficiency of which continues to be suboptimal especially for unilateral small sized pathological conditions. Techniques such as laparoscopy have been utilized for visualization of buffalo ovaries however; their clinical use appears to be uncommon. The therapy of most ovarian pathologies except the functional disorders appears to be difficult. The prospects of surgical removal of ovaries in neoplastic ovarian pathologies are limited owing to the costs and loss of reproductive function under bilateral conditions. Oviductal pathologies in buffalo have been mentioned largely from abattoir studies and include salpingitis (0.2%-14.2%), hydrosalpinx (0.7%-14.2%), pyosalpinx (0.6%-11.9%), adhesions (1.5%-1.7%), congenital defects (0.2%) and oviductal blockage (1.2%-37.8%). The diagnosis of most oviductal pathologies in buffalo appears to be difficult except under conditions of gross enlargement. Under situations of bilateral involvement the therapy of most oviductal pathologies currently seems impractical as the reattainment of fertility appears to be difficult.

## **1. Introduction**

The ovaries and ovarian structures of buffaloes are inherently smaller compared to cows and seasonal ovarian hypofunction and ovarian pathologies limit the breeding value of this important species. Ovarian abnormalities impair the development of ovarian follicles and corpus luteum and subsequently estrous cycle and pregnancy<sup>[1,2]</sup> in buffaloes. Many abattoir<sup>[3–11]</sup> and clinical studies<sup>[12–15]</sup> have identified some ovarian pathology in the bubaline species however their clinical description is still far from perfect. Clinical evaluations commonly utilize transrectal palpation as the means of differentiating various ovarian pathologies although its efficiency continues to be low even for the physiological structures that form on the ovarian surface periodically (follicles and corpus luteum) [16–18]. More recently hormone assays[19–21] and trans-rectal ultrasonography[22–26] have been utilized in identifying ovarian structures and reproductive functions in buffaloes however; their use in identifying ovarian pathologies has been less documented. Ovarian pathologies have been sparsely classified for most domestic animals[27] and in the buffalo they are less frequently documented under clinical settings probably because of frequent slaughter of aged and sub fertile buffaloes[9] which probably suffer the most from pathological alterations in size, shape and function.

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Oviductal pathologies limit the transfer of male and female gametes and consequent embryos and pathologies such as salpingitis, hydrosalpinx, pyosalpinx and oviductal blockage have been mentioned mostly from abattoir studies in buffalo <sup>[28]</sup>. The diagnosis of oviductal pathology from live animals continues to be difficult and therapy is neither possible nor successful in regaining fertility in bilateral conditions. In this review, the ovarian and oviductal pathologies in the buffalo with respect to their occurrence, diagnostic and therapeutic approaches are mentioned.

#### 2. Ovarian pathologies

The classification of ovarian pathologies has been done under the following four sub groups. The description and occurrence for each sub–group is mentioned and in the end the diagnostic and therapeutic approaches are mentioned for each sub group.

#### 2.1. Ovarian developmental anomalies

Ovarian developmental anomalies are less frequent in the bubaline species and some of the anomalies have been described in clinical studies while others derive mention only in studies on material from the abattoir. Clinical descriptions for some conditions suffer from biased evaluations based on size dimensions of bubaline ovaries and not on actual detailed scientific analyses.

#### 2.1.1. Ovarian hypoplasia and aplasia

According to medical dictionary ovarian hypoplasia is incomplete or arrested development of ovarian tissue whereas aplasia is complete absence of ovaries. Hypoplasia is similar to aplasia but less severe and both the conditions are considered to be congenital. Ovarian hypoplasia associated with a deficiency of germ cells occurs infrequently in cattle<sup>[27]</sup>. This condition of the ovary is considered to be due to the failure of migration of primordial germ cells from the yolk sack to the developing gonad during embryonic stage<sup>[29]</sup>. Thus, the developing gonad becomes devoid of germinal epithelium which is the precursor of the follicular system. Both ovaries may be affected or sometimes only a single ovary or a part of the ovary may be affected. The ovary is small in size and is devoid of follicles or corpora lutea. In the Swedish Highland breed of cattle, hypoplasia is determined to have been caused by a single recessive autosomal gene<sup>[29]</sup>. Ovarian hypoplasia have been described in many clinical and abattoir studies in buffaloes (Table 1) however, the clinical descriptions are far from perfect as clinicians often consider smaller size of the ovaries as hypoplasia and the buffalo species has inherently lower ovarian dimensions compared to cattle<sup>[38]</sup>.

Sex chromosome abnormalities such as monosomy X was found to result in gonadal dysgenesis with poor development of both ovaries in buffalo<sup>[39,40]</sup>.

## 2.1.2. Freemartin

A freemartin is an infertile female (born co-twin to a male) that is imperfectly developed and sterile probably due to influence of the male hormones of the twin during development in the uterus. The sex determining region deriving from the male masculinizes the ovarian primordial cells of the undifferentiated female fetus and induces the secretion of androgens<sup>[41]</sup>. Freemartinism is distinct form of intersexuality which arises as a result of vascular anastomosis of the heterozygous fetuses in multiple pregnancies<sup>[42]</sup>. In freemartins, the ovaries of the female fetus usually fail to develop, and remain small and rudimentary. The genital tract is underdeveloped but vulva may be fairly normal with a prominent clitoris and large tuft of vulval hair. The frequency is dependent on heterosexual twinning in the population. Around 85%-92% of the female co-sibs have been reported as sterile freemartins in cattle [43,44].

Freemartin was recorded in a Murrah buffalo<sup>[45]</sup>. Studies on 42 buffaloes with reproductive problems in Italy revealed 10 freemartins determined by cytogenetic evaluations. Of the eight females affected 6 showed normal body conformation, vagina and clitoris while two showed some male traits (tight pelvis). The two males were normal<sup>[46]</sup>. In one study sex chromosome chimaerism was observed in triplets born to a Murrah buffalo<sup>[47]</sup>. Another study<sup>[48]</sup> evaluating heterosexual river buffalo quadruplet found that the female calves born co-twin to males were not freemartins. Cytogenetic evaluations of 119 female and 13 male buffaloes with reproductive problems revealed 18 females as freemartins and all females were sterile<sup>[40]</sup>. It thus appears that the general descriptions used for defining freemartins in cattle are not applicable to bubaline species and also freemartins are uncommon in buffalo.

#### 2.1.3. Hermaphrodites

Hermaphrodite is an individual that has both male and female reproductive organs. A hermaphrodite animal has congenital anatomical variation that confuses the diagnosis of sex. True hermaphroditism is a bisexual manifestation in which both ovarian and testicular tissues are present. The occurrence of true hermaphroditism is extremely rare in Indian buffaloes. Kaura<sup>[49]</sup> described a bovine hermaphrodite. In pseudo–hermaphrodites, gonads of one sex only are evident with external genitalia and secondary sexual characteristics resembling those of opposite sex. A male pseudo–hermaphrodite (genetic male with feminization of external genitalia) was reported in Indian buffalo<sup>[50]</sup>. Similarly 4 clinical cases of male pseudo–hermaphroditism in buffaloes were also described<sup>[51]</sup>.

## 2.1.4. White heifer disease

This inherited, congenital segmental aplasia of the tubular genitalia (paramesonephric duct), receives its name from the relatively high frequency in White Shorthorn heifers. However, it may occur in any breed of cattle. The aplasia can occur anywhere along the duct system but is most common in the cervical area. The remaining part of the uterine horn often gets filled up with secretions. A similar disease was described in buffalo specimens<sup>[50]</sup> and crossbred Jersey heifer<sup>[52]</sup>. The disease is associated with sub fertility in a large number of animals of the herd and considered an inheritable disease in cattle<sup>[53]</sup>. A buffalo freemartin with adhesions of both ovaries and mucus accumulation in right uterine horn cranial to the cervix was recorded in buffalo genitalia in Brazil<sup>[54]</sup>. A few cases of persistent hymen have been recorded in the buffalo<sup>[55-57]</sup> with expulsion of accumulated mucus on surgical excision of the persistent hymen[56,57] however other features common to white heifer disease were not mentioned in these studies.

#### 2.1.5. Diagnostic and therapeutic approaches

Diagnosis of most ovarian developmental anomalies requires specialized techniques. Hypoplasia in women is established on the basis of endocrinological evaluations and histologic investigations on biopsy specimens<sup>[58]</sup>. Ovarian hypoplasia was diagnosed in Swedish Highland cattle on the transrectal palpation of small sized furrowed or spindle shaped ovaries and lack of germ cells observed on histologic sections. More recent studies have utilized PCR assays <sup>[29]</sup>. However, the diagnosis of hypoplasia in live animals continues to be difficult because of poor clinical expression of this hereditary disorder in unilateral affection. Studies on buffaloes evaluating the histologic evidence of poor presence of germ cells are not available.

Sonographic diagnosis of hypoplasia has been recently mentioned for cows<sup>[59]</sup> however they are difficult to be applied clinically specially for buffaloes. Hypoplasia is considered to be inherited in cattle and difficult to be diagnosed clinically with absence of secondary sexual characters being the only clinical evidence in the presence of bilateral ovarian hypoplasia<sup>[58]</sup>. It appears that true germ cell weakness as observed in Swedish cattle has not been observed for buffalo.

It has been mentioned that freemartin's can be readily diagnosed in cattle from the breeding history and clinical examination where the length of the vagina is found to be 1/3 the normal length and a test tube can be inserted only up to a few centimeters deep into the vagina due to its under-development<sup>[41]</sup>. More recent studies indicate polymerase chain reaction for the diagnosis<sup>[60]</sup>. Cytogenetic evaluations are suggested for confirmation of the condition in infertile cows as freemartin is considered a chimera and some animals may not evidence the external features characteristic to freemartins<sup>[44]</sup>. The clinical expression of freemartins (altered genitals) as observed in cattle is not common in the buffalo species and cytogenetic evaluation seems necessary. Evaluation of blood cell chimaerism from 200 buffalo bulls revealed 3 young bulls with sex chromosome chimaerism<sup>[42]</sup>. The study concluded that compared to cattle chimaerism was uncommon in buffalo bulls<sup>[42]</sup>. However the fertility of chimaeric buffalo bulls was not affected. Studies on buffalo microsatellite markers evaluated and found that bovine sexing markers can also be used for evaluation of freemartin in buffalo<sup>[61]</sup>.

Hermaphrodites are evident clinically whereas white heifer disease is usually mentioned in abattoir specimens.

The therapy of ovarian hypoplasia in cattle is not suggested as the condition is inherited. Since small ovarian dimensions appear to have been interpreted as hypoplasia the therapy in buffaloes suggested is improved nutrition and management. The therapy of most other developmental anomalies is neither possible nor suggested.

#### 2.2. Ovarian inflammatory conditions

#### 2.2.1. Oophoritis and perioophoritis

Inflammation of the ovaries and surrounding structures is known as oophoritis and perioophoritis. The most common pathological condition of bovine ovary is perioophoritis<sup>[62]</sup> while oophoritis seems to be rare.

Perioophoritis and oophoritis were reported from abattoir studies on buffalo genitalia and their incidence varied from 0.3% to 8.3%<sup>[3]</sup>. A few of the more recent abattoir studies also described similar incidence in buffaloes (Table 1).

The etiology of inflammatory conditions of the ovaries usually lies in the ovarian manipulations, although infections from the uterus and infectious diseases like tuberculosis and brucellosis might also be involved<sup>[63]</sup>.

Severe bilateral inflammation causes sterility. In unilateral cases also, the prognosis is guarded. Whenever mesosalpinx or salpinx is involved in the inflammatory process, the situation is more serious. Trauma caused by improper manipulations during palpation, forced attempts to enucleate corpus luteum or to manually rupture cystic ovaries, is the most common cause of oophoritis. In tuberculosis when the reproductive tract is affected, the ovaries are also involved. Suppurative oophoritis may follow severe metritis and perimetritis.

Perioophoritis is usually chronic and often localized and seen as red fibrous and serosal tags attached to the surface of the ovary especially in heifers. Granulomatous perioophoritis may occur in peritoneal tuberculosis and in setariasis which may appear as small reddish nodules or tags. These infective guanulomas remain strictly localized to the surface of the ovary and do not penetrate its substance [11]. Grossly the ovarian surface is shaggy and often encapsulated with adhesions which interfere with ovulation.

## 2.2.2. Ovarian Abscess

Rare incidence of ovarian abscesses (0.85%) was recorded in Berari (Nagpuri) buffaloes<sup>[64]</sup>. The abscess usually develops subsequent to localization of focus of infection following ovarian inflammatory disease. An ovarian abscess is silent clinically and can be diagnosed with precision only by transrectal ultrasonography<sup>[65]</sup>.

## 2.2.3. Ovaro-bursal adhesions and encapsulation

The condition results from adhesion between the mesosalpinx and mesovarium, often including the flmbriae and ovary<sup>[9]</sup>. The extent of the adhesions vary with specimens, showing fine web–like strands in the depth of the bursa which do not involve the oviduct, to instances of complete envelopment of the ovary in a closely applied fibrous bursa<sup>[10,11]</sup>. Intermediate cases show fibrous strands of varying thickness which connect the fimbriae or bursa to the ovary. The condition is uncommon in heifers but its incidence increases with the age of the buffalo. Trauma from rough handling of the ovary and bursa by rectal manipulation or clinical expression of the corpus luteum and descending infections are common causes of this condition <sup>[13]</sup>.

Many previous abattoir evaluations revealed an incidence varying from 1.5%–18%<sup>[3,66–68]</sup> and nearly similar incidence was recorded in recent studies (Table 1). Clinical evaluations however, recorded an incidence of less than 3%<sup>[12,69]</sup> probably because of difficulty in clinical diagnosis. In one clinical study on 31 repeat breeding buffaloes no ovaro– bursal adhesions were recorded<sup>[70]</sup>.

The ovario bursal adhesion affects fertility mainly by interfering with tubal motility and leads to irregular returns to estrus. There is no satisfactory treatment for this condition. Some cases may be prevented by smooth handling of ovaries and preventing irrigation of uteri with large quantities of antiseptics. Prompt attention to cases of dystocia can reduce the incidence by preventing puerperal metritis.

## 2.2.4. Ovarian sclerosis

Sclerosis means hardening or inducation. Ovarian sclerosis appears to be a frequent gynecologic reason for which buffaloes are culled<sup>[71]</sup>. It is also one of the common ovarian abnormalities noticed in the abattoir specimens (Table 1).

The ovaries are usually very small and hard without any follicles. The etiology for ovarian sclerosis is poorly known. The pathogenesis of systemic sclerosis in human beings involves vascular, fibrotic, inflammatory, and immunologic processes<sup>[72]</sup> and probably similar mechanisms might be involved. Ovarian tuberculosis as recorded for cows<sup>[62]</sup> could be a possible reason for induration of ovaries. Severe debilitating diseases or inanition in buffaloes could possibly be involved in this condition.

The incidence of the condition has been described to vary between 1.69%–11.40% in various studies in buffaloes (Table 2).

## 2.2.5. Diagnostic and therapeutic approaches

Enlargement and hardening/thickening are key diagnostic transrectal palpable features for diagnosis of most ovarian inflammatory conditions including oophoritis and sclerosis<sup>[73]</sup> as most ovarian inflammatory conditions are clinically silent in most domestic animals including buffalo. The more sever type of ovaro-bursal adhesions can be diagnosed by transrectal palpation. In human females advanced diagnostic modalities such as ultrasonography <sup>[74]</sup>, computed tomography<sup>[75]</sup>, laparoscopy<sup>[76]</sup> and MRI<sup>[74]</sup> are routinely used in women with pelvic inflammatory disease with pelvic pain as the most striking clinical sign. Ultrasonographic evaluations can detect ovarian abscess and to some extent ovarian sclerosis <sup>[65]</sup> in cows, however similar descriptions are not available for the buffalo.

The heterogeneous hypoechogenic specks can be visualized within the ovarian boundary. Laparoscopic visualization of the buffalo ovaries has been mentioned<sup>[77–79]</sup> however their use for detection of ovarian pathologies is not described.

Therapeutic approaches suggested for ovarian inflammatory conditions in human females include the administration of antibiotics<sup>[80]</sup> and surgical excision in acute conditions such as ovarian abscess which are life threatening<sup>[91]</sup> however similar approaches are less likely in most domestic animals including buffalo because of poor clinical evidence and diagnosis. Buffaloes with ovarobursal adhesions and sclerosis when diagnosed are more likely to be culled<sup>[71]</sup>. An ovarian abscess may sometimes regress or rupture spontaneously or subsequently persist as sclerosis. Approaches for therapy of ovarian abscess can utilize the administration of broad spectrum antibiotics or trans-vaginal ultrasound guided aspiration of the contents. Such approaches are suggested only when the fertility of a precious animal is attempted to be regained.

## 2.3. Ovarian functional disorders

## 2.3.1. Sub estrum or silent heat

Lack of overt estrus expression is known as sub estrus or silent estrus. The condition is more prevalent in prepubertal buffalo heifers<sup>[82]</sup> and in adult buffaloes during summer <sup>[83]</sup> and the post partum period<sup>[84]</sup>. The etiology of this condition appears to lie in sub optimal secretion of estradiol by the mature follicles or higher threshold of estrogen for the neural mechanism controlling overt expression of estrus<sup>[82,85]</sup>. Other probable reasons could be a slow follicular growth<sup>[86]</sup> and low levels of circulating hormones and biochemicals<sup>[87]</sup>. Sub estrus animals have normal follicular development and ovulation without the overt manifestation of estrus<sup>[82]</sup>. One clinical study<sup>[15]</sup> recorded the incidence of sub estrus to be 7.85% in buffaloes. A large proportion (53%) of post partum buffaloes evidenced silent estrus<sup>[84]</sup>.

#### 2.3.2. Gestational estrus

Estrus exhibited during pregnancy is known as gestational estrus. In buffaloes, 6.05% incidence of gestational estrus was reported at an average 108±12 days of gestation at IVRI, Izatnagar India. Chauhan et al. [88] recorded a very high incidence of 20.3% gestational estrus among buffaloes in Punjab. One report mentioned that gestational estrus is shown in 6-18% of water buffalo<sup>[89]</sup>. Recent studies have shown that follicular growth and maturation continues during gestation in buffaloes<sup>[90]</sup> and thus probably the growing ovarian follicle secretes estrogens sufficient to manifest estrus and ovulation but insufficient to terminate pregnancy. Similarly it was previously mentioned that a moderate decrease in progesterone levels during pregnancy leads to maturation of dominant follicle and increased estradiol production sufficient to result in overt estrus but insufficient to terminate pregnancy is the cause of gestational estrus in a proportion of buffaloes<sup>[91]</sup>.

## 2.3.3. Persistent corpus luteum

Occasionally the corpus luteum does not regress normally even though the animal is not pregnant<sup>[92]</sup>. This is considered a persistent corpus luteum (PCL). The persistent CL continues to produce progesterone to prevent further follicular development, estrus and ovulation[92]. The maintenance of CL is the result of precise inter-action between pituitary and embryonic gonadotropins, as well as intraluteal autocrine and paracrine signals that modulate the endocrine function of luteal cells<sup>[93]</sup>. The maintenance of CL in the absence of pregnancy may originate because of metritis<sup>[94]</sup> and similar effects are possible with pyometra<sup>[92]</sup> and late embryonic mortality<sup>[95]</sup>. The mechanisms involving luteolysis are complex<sup>[93]</sup> are probably suspended under certain conditions and such animals continue to evidence anestrus in the presence or absence of uterine pathology [96]. The incidence of this condition in cows is known to vary from 2% to 11%[92,96].

In a few previous studies utilizing abattoir derived buffalo genitalia<sup>[5,12]</sup> the incidence of persistent CL was observed to vary between 0.19% to 9.12%. Similar incidence was recorded in more recent studies on abattoir derived buffalo genitalia (Table 1).

## 2.3.4. Ovulatory disturbances

Ovulatory disturbances include delayed ovulation and

anovulation. Ovulation is known to occur in buffalo 24– 48 h (mean 34 h) after the onset of estrus<sup>[97]</sup> or 10 to 14 h after the end of estrus<sup>[97,98]</sup>. Ovulation is known to occur when the follicle attains a diameter of 8.5 to 12.0 mm<sup>[23]</sup>. The size of the pre–ovulatory follicle has been shown to have a positive impact on the size of post ovulation CL and conception in buffaloes<sup>[24]</sup>. Due to smaller ovarian size and a smaller follicle diameter detecting ovulation by trans–rectal palpation in the buffalo seems difficult. Delayed ovulation/ Anovulation is one cause of pregnancy failures among buffaloes and often results in repeat breeding<sup>[70,99]</sup>.

Among Indian buffaloes ovulation failures were recorded to occur from 3.0% to 15.9%<sup>[99,100]</sup> and many of these buffaloes evidenced repeat breeding.

## 2.3.5. Ovarian cysts

Ovarian cyst refers to a condition in which there is presence of fluid filled structures on the ovary more than 2.5 cm in diameter without ovulation[101-103]. Ovarian cysts are uncommon in buffalo and most clinical studies record an incidence ranging from 0.5% to 1.48% whereas a few abattoir studies recorded a relatively higher incidence (Table 1). The incidence of ovarian cysts in one study on buffalo was 2.7% [32]. Both follicular and luteal cysts have been recorded in buffalo<sup>[104]</sup>. Unilateral or bilateral hemorrhagic cysts were recorded in previous studies on Egyptian buffaloes [101,105]. Buffaloes with ovarian cysts revealed significantly lower progesterone, T3 and T4 and significantly higher estradiol [105]. Bilateral ovarian cysts have been recorded in buffalo[106]. Biochemical evaluations of follicular fluid of follicular cysts revealed increased concentrations of nitric oxide, progesterone and cortisol and lower concentrations of ascorbic acid, insulin and glucose<sup>[107]</sup>. The clinical manifestations of ovarian cysts in buffalo are similar to cattle but signs are often less marked.

#### 2.3.6. Parovarian cysts

Parovarian cysts are remnants of the mesonephric ducts that are occasionally found around the ovary and fallopian tubes, attached in the broad ligaments of cows and buffaloes <sup>[10]</sup>. Tiny parovarian cysts of a few millimeter diameter are incidental findings in slaughtered buffaloes <sup>[11]</sup>. They are of little significance in relation to infertility.

The cysts may vary in size from 1 to 5 cm in diameter and are usually round or oval in shape. They may sometimes be mistaken for cystic ovary on rectal palpation because of closeness to the ovary.

Previous abattoir studies in buffaloes, recorded the incidence of parovarian cysts to vary from 0.43%-13.0%[64.66.68]. A nearly similar incidence was recorded in recent studies in buffaloes (Table 1).

## Table 1

Incidence of various ovarian pathologies in buffalo in different studies.

Type of study	Number of specimens	Ovarian hypoplasia	Oophoritis	Ovarian sclerosis	Oavrobursal adhesion	Sub estrus	Persistent CL	Ovarian cysts	Parovarian cysts	Reference
Clinical	16538	0.1%	_	_	-	0.54%	-	0.59%	0.19%	[13]
Abattoir	100	_	_	_	_	80.55%	_	2.77 %	3.12%	[30]
Clinical	451344	0.001%	_	_	_	55.79%	_	1.48%	_	[14]
Abattoir	221	0.45%	_	_	2.71%	_	_	2.25%	_	[31]
Abattoir	504	0.19%	0.59%	0.39%	-	_	_	1.78%	2.7%	[11]
Abattoir	706	3.09%	2.78%	2.47%	-	7.43%	3.09%	9.59%	3.4%	[9]
Abattoir	110	1.81%	_	_	_	_	_	_	_	[32]
Abattoir	340	0.29%	0.29%	_	0.88%	_	6.17%	2.64%	3.24%	[8]
Clinical	11209	13.19%	_	0.04%	_	_	_	0.07%	_	[15]
Abattoir	425	_	2.35%	_	1.41%	_	0.94%	_	1.65%	[33]
Abattoir	405	_	_	_	6.4%	_	1.2%	1.5%	4.4%	[10]
Abattoir	150	_	_	_	2.7%	_	_	8.0 5	4.7 5	[34]
Abattoir	505	_	_	_	2.8%	_	_	2.2%	7.9%	[35]
Abattoir	1080	_	-	_	19.04%	_	_	_	-	[36]
Abattoir	131	_	-	-	9.16%	-	-	0.7%	13.44%	[37]

#### 2.3.7. Diagnostic and therapeutic approaches

Most functional ovarian disorders are clinical problems and can be diagnosed by clinical methods such as transrectal palpation and transrectal ultrasonography. Transrectal palpation or transrectal ultrasonography on the day of estrus reveals normal ovulatory sized follicle and tonicity in the uterus without overt estrus in silent estrus buffaloes<sup>[15,108]</sup>. In making a diagnosis, the fact that many cases of the first and second postpartum ovulation in the ovarian cycle are quiet ovulation and that there are many cases in which careless stock keepers overlook external estrus signs should be noted<sup>[109]</sup> and care must be taken as to the differentiation between a retained corpus luteum and pregnancy.

The features of transrectal palpation of ovarian follicle development, growth and ovulation have been described previously<sup>[16,18]</sup>. Transrectal ultrasonography appears to be more precise in evaluating ovarian follicles and ovulation <sup>[18,24]</sup>. The evaluation of the development and demise of the corpus luteum can be performed by transrectal ultrasonography and validated by assay of progesterone hormone<sup>[16,19,20]</sup>. The diagnosis of persistence of the CL is based on its presence for more than 24 days in the absence or presence of uterine pathology. Thus repeated examinations are suggested. Ovulation can be detected by the repeated examination of an ovulatory size follicle. Ovarian cysts can be diagnosed by their size (<2.5 cm) and consistency (fluid filled) detected by transrectal palpation or transrectal ultrasonography. Parovarian cysts can be diagnosed similarly only when their size is more than 1.0 cm. The bubaline corpus luteum (CL) is smaller than that in cattle, often does not protrude markedly from the surface of the ovary and sometimes lacks a clear crown<sup>[97]</sup>. These characteristics make accurate identification of ovarian structures by transrectal palpation in buffalo more difficult than in cattle<sup>[110,111]</sup>. Ultrasonic imaging indicates that mature CL in buffalo range in size from 1.2 to 1.7 cm in diameter<sup>[112,113]</sup>. Compared to cattle, buffalo CL have no yellow coloration at any stage of development<sup>[114]</sup>.

The therapies of most functional ovarian disorders is similar to that described for cattle<sup>[63]</sup> and include prostaglandins for sub estrus<sup>[115]</sup> and persistent CL<sup>[1]</sup>, mineral, vitamin or hormonal supplementation for ovarian inactivity<sup>[82,108,109,116–120]</sup>, and GnRH and hCG for ovulatory disorders including ovarian cysts<sup>[70,121]</sup>. The therapy of parovarian cysts is neither possible nor required in small cysts.

#### 2.4. Ovarian neoplasm and associated conditions

Ovarian tumors have been described in the buffalo species mostly from studies on abattoir specimens<sup>[67,68,122–124]</sup>. 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<sup>[27]</sup>.

## 2.4.1. Epithelial tumors

Epithelial tumors are uncommon in most domestic animals including buffalo. The vast majority of epithelial tumors arise from the surface epithelium, although rarely, they arise from the rete ovarii[27]. Epithelial tumors of the ovary are usually cystic and papillary, thus the names cystadenoma and cystadenocarcinoma are frequently used[27]. Histologic descriptions mention that such tumors consist of arboriform papillae that project into the cyst lumen[27]. A few studies on abattoir specimens in buffalo recorded cystadenoma [122,124] however clinical descriptions of such tumors are not available.

## 2.4.2. Germ cell tumors

Germ cell tumors are composed of a number of histologically different tumor types derived from the primitive germ cells of the embryonic gonad<sup>[125]</sup>. The concept of germ cell tumors is based on i) the common histogenesis of these neoplasm's, ii) the relatively frequent presence of histologically different neoplastic elements within the same tumor mass and iii) the presence of histologically similar neoplasm's in extra gonadal locations<sup>[125]</sup>.

In the buffalo species many reports described the germ cell tumor teratoma<sup>[8,66–68,123,124]</sup> whereas other forms of germ cell tumors described for buffalo appear to be dermoids<sup>[66]</sup>. Both dermoids and teratoma are considered benign whereas dysgerminoma is another germ cell tumor which is considered malignant but not described for the buffalo. Dermoids are also known as mature teratoma<sup>[125]</sup>. Dermoids are cysts (1.25–6.25 cm) within the ovary with sebaceous glands and hair follicles and on excision evidence the presence of cluster of hair and other tissues<sup>[125]</sup>. The incidence of dermoids was only 1.44% for 1 725 abattoir derived genitals examined<sup>[122]</sup>. The incidence of dermoids in buffalo ovaries in a recent study was 0.71%<sup>[33]</sup>. The etiology of germ cell tumors continue to be poorly known. Rapid proliferation of germ cells is one postulated reason<sup>[27]</sup>.

## 2.4.3. 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<sup>[27]</sup>. 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<sup>[126]</sup> and include granulosa cell tumors, granulosa-theca cell tumors. Folliculoids are granulosa cell tumors in variegated forms with a tendency of these cells to arrange in small clusters (in histopathologic sections).

The most common ovarian sex cord stromal tumor in the buffalo appears to be granulosa cell tumor<sup>[66,127]</sup>. The incidence of such tumor in buffaloes was 0.71% in a recent study<sup>[33]</sup>. Folliculoids have also been recorded in the buffalo <sup>[127]</sup>. It was mentioned that folliculoids are probably anovulatory follicles

of aged buffaloes that persist and proliferate under the constant stimulus of gonadotrophins <sup>[127]</sup>. The incidence of trabecular, microfollicular and pseudo-adenomatous folliculoids was 36.0%, 44.0% and 20.0% respectively<sup>[146]</sup>. In a recent study the incidence of folliculoids was 0.24%<sup>[33]</sup>. Endocrine abnormalities have been associated with sex cord stromal tumors<sup>[27]</sup> although not documented for the buffalo species.

## 2.4.4. Mesenchymal tumors

Mesenchymal tumors comprise a heterogeneous group of neoplasm that are not specific to the ovary<sup>[128]</sup> and include fibromas, hemangiomas, leiomyomas, and their malignant counterparts<sup>[27]</sup>.

In buffaloes, the neoplasm's of this category recorded include haemangioma. Luktuke *et al* <sup>[129]</sup> observed proliferative or lymphosarcomatous growths in anoestrous ovaries of buffaloes. Fibroma was recorded in buffalo ovaries in a recent study<sup>[8]</sup>.

## 2.4.5. Hematoma

Ovarian vascular hematoma is a rare ovarian tumor like anomaly largely reported from abattoir studies in cows<sup>[130]</sup>. A vascular hematoma is a non neoplastic malformation that is present at birth and grows until puberty<sup>[130]</sup>. More recently a vascular hematoma was diagnosed in a clinical case of Holstein cow by unilateral enlargement of the ovary without follicles or CL. The tumor diagnosis was confirmed after its removal by right flank celiotomy<sup>[131]</sup>. Hematomas in the ovaries of Indian buffaloes were reported previously<sup>[66]</sup>. The incidence ranged from 0.26 % to 1.43 %.

## 2.4.6. Diagnostic and therapeutic approaches

Clinical signs of ovarian tumors in human females include abdominal pain, fatigue, leg swelling and associated symptoms<sup>[132]</sup> however; most bubaline ovarian tumors evidence few clinical signs such as infertility. Diagnostic approaches for ovarian tumors in human females are well developed and include techniques such as computed tomography, ultrasonography, MRI and laparoscopic visualization and biopsy<sup>[132]</sup>. Therapy of ovarian tumors in human females depends upon the type of tumor and includes surgical removal followed by chemotherapy[133,134]. Owing to economic reasons and loss of reproductive function in ovarian tumor affected buffaloes subsequent to bilateral removal of the ovaries, the diagnosis and therapy of ovarian tumors in buffaloes are limited. Plasma anti-Mullerian hormone was tested as a biomarker for detection of bovine granulosa-theca cell tumors in a recent study<sup>[135]</sup> with high accuracy, however the clinical application of such a test is limited.

## 3. Oviductal pathologies

occlusions and pachysalpinx<sup>[136]</sup>.

## 3.1. Salpingitis

Affections of the oviducts include congenital defects, salpingitis, hydrosalpinx, pyosalpinx and adhesions <sup>[28]</sup>. Another classification mentioned fallopian tube abnormalities to be classified into congenital and acquired<sup>[136]</sup>. Affections of the oviducts result in occlusion of the lumen preventing fertilization or creating an unfavorable environment for fertilization. A unilateral affection results in infertility, whereas a bilateral affection results in sterility. Affections of the oviducts have been diagnosed largely from abattoir studies. The overall incidence of affections of the oviduct varies from 10 to 29% <sup>[6,7,12,137,138]</sup>, of which salpingitis is the most common. A higher incidence of oviductal affections has been reported for buffaloes compared to cattle<sup>[38,64]</sup>.

In a recent review the congenital abnormalities mentioned for buffaloes included aplasia and accessory oviducts<sup>[136]</sup>. In segmental aplasia the oviducts have a blind end near the uterotubal junction<sup>[136]</sup>. The whole length of the oviduct is distended. In one report bilateral segmental aplasia in buffalo genitalia also evidenced hydrosalpinx<sup>[139]</sup>. In accessory uterine tubes there is duplication of the uterine tubes with two uterotubal junctions on one side<sup>[136]</sup>.

The acquired oviductal pathologies include salpingitis, hydrosalpinx, pyosalpinx, adhesions of the oviduct, oviductal

# Table 2 Insidence of evidential pathologies in huffeloos in a

Incidence of oviductal pathologies in buffaloes in various studies.

Salpingitis is inflammation of the fallopian tubes which may be the result of some infectious cause<sup>[140]</sup>. Salpingitis is usually caused by infections in the uterus, cervix or vagina. Buffaloes with salpingitis may be sterile depending on the severity of the condition<sup>[138]</sup>. Salpingitis develops due to upward infection from the uterus following abortion, retained fetal membranes, septic metritis and pyometra [27]. Tuberculosis is also one of the causes for oviductal lesions. Uterine irrigation with strong antiseptic solutions may escape into the oviducts and cause inflammation. A descending infection from the peritoneum can also occur. In an abattoir study on buffaloes, salpingitis was encountered in 4 cases (0.79%), which was 7.14 % of all the affected animals<sup>[141]</sup>. Grossly the salpinx were enlarged and thickened but did not reveal any changes in consistency. In human females the term Pelvic inflammatory disease (PID) is a common term used to denote diseases such as salpingitis, ovarian abscess and infection in the pelvic peritoneum. In contrast, salpingitis only refers to infection and inflammation of the fallopian tubes in animals [70,136]. The incidence of salpingitis in various studies varied from 0.20 to 14.29% (Table 2).

Salpingitis	Hydrosalpinx	Pyosalpinx	Adhesions	Congenital defects	Occlusion	Reference	
1.2%	0.7%	2.2%	1.5%	0.2%	1.5%	[11]	
3.2%	1.6%	0.8%	1.6%	_	18.0%	[142]	
1.4%	6.9%	2.9%	1.7%	0.2%	1.2%	[138]	
1.2%	_	_	-	_	2.08%	[31]	
0.20%	_	_	_	_	_	[14]	
_	14.28%	11.9%	_	_	_	[36]	
0.62%	_	_	_	_	_	[15]	
0.61%	_	_	_	_	_	[3]	
14.29%	_	_	_	_	_	[143]	
_	0.8%	_	_	_	37.8%	[35]	
_	1.47%	_	_	_	0.29%	[8]	
_	_	3.1%	_		_	[137]	

On histopathologic sections buffaloes with acute salpingitis evidence loss of cilia and focal desquamation of epithelium on the tips of some folds with leucocytic infiltration, histiocytes and fibrocytes could be observed [142,144]. Desquamation and degeneration are prominent in suppurative cases with adhesions in chronic cases [3].

## 3.2. Hydrosalpinx

Accumulation of fluid in the fallopian tube is known as

hydrosalpinx. The incidence of hydrosalpinx in buffaloes in different studies varied from 0.7% to 14.28% (Table 2). Bilateral hydrosalpinx was recorded in a buffalo<sup>[145]</sup>. The exact etiology of the condition continues to be poorly known although extension of inflammatory exudates from the uterus is considered one possible reason<sup>[146]</sup> and congenital serous secretions as another possible etiology<sup>[147]</sup>. Mastroianni <sup>[148]</sup> believed that the condition was the result of inflammation around the oviducts. The accumulated fluid creates a hostile environment that will prevent implantation of an embryo [136]. Dilatation of the affected tubes is common when the accumulations are large.

Grossly, the fallopian tubes are found distended, elongated and tortuous forming many coils in the mesosalpinx. Histologically, the wall is thin, translucent, and distended with large amount of clear fluid<sup>[3]</sup>. The ampullary region was more affected<sup>[142]</sup>. Degeneration and desquamation of the epithelial lining is common<sup>[142]</sup>. The mucosal folds are reduced and muscularis layer reveals hyalinization<sup>[142]</sup>. An oviduct with hydrosalpinx does not have healthy cilia; hence, embryos that find their way into the fallopian tube become trapped resulting in infertility in unilateral condition and sterility under bilateral conditions<sup>[136]</sup>.

## 3.3. Pyosalpinx

Pyosalpinx refers to presence of pus in the fallopian tube<sup>[136]</sup>. Infections may have their origins from the uterus or a result of prolonged salpingitis<sup>[137]</sup>. The common microbes isolated include *Arcanobacterium pyogenes*, *E. coli* and *Staphylococcus aureus*<sup>[137]</sup>. The accumulated pus hinders fertilization and might rarely result in escape of the pus in the peritoneum and consequent peritonitis. Histologically majority of cases reveal marked infiltration of neutrophils, mononuclear cells and hoisting mucosal and muscularis layers<sup>[142]</sup>. Some cases of pyosalpinx are associated with formation of sub mucosal cysts<sup>[142]</sup>.

## 3.4. Adhesions of the oviduct

Adhesions of the oviducts have been recorded in studies on buffalo (Table 2). The condition is perhaps the result of inflammation around the oviduct. Adhesions of the oviduct may develop to the surrounding bursa due to constant infection or following rough trans-rectal manipulations.

## 3.5. Occluded uterine tubes

Occlusion of the oviducts could be a result of stenosis of the oviduct or blockage of the lumen of the oviducts with small growths. Kessy and Noakes<sup>[149]</sup> found that occluded uterine tubes were macroscopically normal but the lumen was completely obstructed and the tunica mucosa was replaced by a mass of proliferative connective tissue with extensive cellular infiltration. The occlusion hinders the normal passage of gametes thus resulting in infertility. In one study on buffaloes unilateral tubal blockage was recorded in 8.40% genitalia whereas bilateral blockage was observed in 4.58% abattoir derived genitalia<sup>[143]</sup>.

## 3.6. Pachysalpinx

Pachysalpinx is an affection characterized by the enlargement of the whole length of uterine tube which are

kinked and distorted with normal shape and outline<sup>[136]</sup>. The central lumen is completely filled with a connective tissue mass and the distinct tubal mucosal folds are absent. Kavani *et al.* <sup>[150]</sup> in a study on repeat breeding associated with fallopian tube affections in cows and buffaloes found that salpingitis and pyosalpingitis causes atrophy /denudation of mucosal folds and moderate to massive fibrosis of tubular wall with multiple sub mucosal cyst formation in the ampullary region of the oviducts. Further they found multi–locular intramuscular cyst formation by the fusion of adjacent folds and denuded epithelial linings due to salpingitis and resultant tubal blockage.

## 3.7. Diagnostic and therapeutic approaches

Affections of the oviducts usually remain undetected probably because of their extremely small dimensions [27]. An expert ultrasound operator will be able to detect affections of the oviduct. Mild forms of salpingitis are difficult to diagnose and are usually detected after slaughter. In its severe form, the oviduct becomes thick, hard and swollen and it feels like a cord at trans-rectal palpation. When there is a large accumulation of fluid in the oviduct, it feels like a big cyst (hydrosalpinx) at palpation. Hydrosalpinx is characterized by the distension of the oviduct filled with amber colored fluid. The dilated oviducts may have a diameter of 30 mm<sup>[10]</sup>. Hyperplasia of mucosal epithelium is visible in histopathology. Ultrasonography can aid in the diagnosis of affections of the oviduct. These affections frequently pose threats to the normal passage of spermatozoa in the female tract preventing fertilization. It is therefore important to evaluate the patency of the oviducts. The common approach to evaluate the patency of the oviduct is to use the passage of air or Phenol-sulfo-naphthalein (PSP) dyes into the uterus using a Foley catheter fixed to the uterus. If the oviduct is open without any occlusions, the air or dye readily pass through the oviduct within 3-5 min and the dye may appear in the peritoneal cavity or in the urine [150-152]. Many other tests used in human females to evaluate the oviducts and surrounding structures including hysterosalpingography, sono-salpingography, laparoscopic chromo-perturbation, transvaginal falloposcopy and hystero-salpingo contrast sonogrpahy have been described recently<sup>[136]</sup> however, their use in most domestic animals including buffalo is not described.

Treatment may not be useful and prevention is more advantageous. If infection in the uterus is detected and treated early then it prevents the spread of the disease into the oviducts. If both oviducts are involved, then the animal will become completely sterile and has to be culled. Overall prognosis in affections of the oviduct has usually been unfavorable except in mild infections. Parentral as well as intrauterine infusion of broad–spectrum antibiotics may or may not be beneficial<sup>[27]</sup>. Gentle handling of the genitalia, proper treatment, control of uterine infections and sexual rest can reduce the incidence of affections of the oviduct. Oviductal surgeries described for human females such as salpingostomy and salpingectomy<sup>[136]</sup> are neither mentioned for most domestic animals including buffalo nor currently possible due to the pelvic location of the oviducts are mentioned .

## **Conflict of interest statement**

We declare that we have no conflict of interest.

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