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**COMPARATIVE STUDY ON LESIONS OF REPRODUCTIVE DISORDERS OF  
COWS AND FEMALE DROMEDARY CAMELS SLAUGHTERED AT ADDIS  
ABABA, ADAMA AND AKAKI ABATTOIRS WITH BACTERIAL ISOLATION**

**MSc THESIS**



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Parasitology MSc in Tropical Veterinary Pathology**

**JUNE, 2017  
BISHOFTU, ETHIOPIA**

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A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis  
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Science in Tropical Veterinary Pathology

By

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June, 2017  
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## ***DEDICATION***

*This thesis manuscript is dedicated to my father Mandefro Getahun and my mother Almaz Tadesse for nursing me with affection and love and for their exhaustive support in the success of my life.*

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Department of Pathology and Parasitology

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by **Aynalem Mandefro Getahun** entitled as **“Comparative study on lesions of reproductive disorders of cows and female dromedary camels slaughtered at Addis Ababa, Adama and Akaki abattoirs with bacterial isolation.”** It is recommended that it is accepted as fulfilling the thesis requirement for the degree of Masters of Science in Tropical Veterinary Pathology.

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## **STATEMENT OF AUTHOR**

First, I declare that this thesis is my original work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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## LIST OF ABBREVIATIONS

BCS	Body Condition Score
CL	Corpus Luteum
COD	Cystic Ovarian Disease
COF	Cystic Ovarian Follicle
FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
H and E	Hematoxylline and Eosin
LH	Luteinizing Hormone
MR-VP	Methyl Red And Vogues Proskauer Test
NAHDIC	National Animal Health Diagnostic Investigation Centre
NEB	Negative Energy Balance
OC	Ovarian Cyst
PCL	Persistence Corpus Luteum
PG	Prostaglandin
PGF2 $\alpha$	Prostaglandin F2 Alpha
PID	Pelvic Inflammatory Disease
PMN	Polymorpho Nuclear Cell
RBC	Red Blood Cell
RP	Retained Placenta
Spp	Species
TSI	Triple Sugar Iron
XLD	Xylose Lysine Deoxycholate

## ABSTRACT

A cross-sectional study was conducted from November 2016 to May 2017 on reproductive organs of cows and dromedary camels slaughtered at Akaki slaughter house, Addis Ababa and Adama municipal abattoirs to compare lesions and bacteria isolates. A total 280 reproductive organs (140 from cows and 140 from camels) were grossly inspected. Grossly visible lesions were documented and tissue samples with lesion were collected for histopathological lesion characterizations and bacteriological isolations. Various pathological lesions with different degrees of severity were observed in 36.4% (n=51) and 34.2% (n=48) of cows and dromedary camels, respectively. Age, species and body conditions of animals were not statistically associated with most of the disorders ( $P>0.05$ ). Comparatively, in cow the most frequently observed lesions were that of ovaries 16.4% while in camels it was that of uterus 21.4%. Grossly, endometritis were characterized by congestion in acute cases and congestions and thickening in chronic cases. Microscopically, endometrial glands degenerations, sloughing of epithelium, periglandular cuffing and infiltrations of inflammatory cell were some of characteristics change observed in endometritis. Grossly, the follicular cyst were large, some of them occupied the entire ovary and very thin walled. Microscopically, the follicular cysts were characterized by extremely thin granulosa layers. Most of endometritic tissues cultured for bacterial isolations were positive for either single and/or mixed bacterial infection. *Staphylococcus* species 28.5%, *Streptococci* species 19.6%, *Coynebacterium* species 8.9%, *Escherichia coli* 26.7%, *Salmonella* species 10.7% and *Klebsiella* species 5.35% were isolated from cows uteri, while in the dromedary camels, *Escherichia coli* 35.5%, *Staphylococcus* species 26.6%, *Streptococcus* species 13.3%, *Pseudomonas* species 6.6%, *Proteus* species 4.4%, *Salmonella* species 8.8% and *Klebsiella* species 4.4% were isolated. It could be concluded that reproductive organ lesion were important problem in both species of animals and these disorders might be causes of infertility and appearance of these females in abattoir. The role of each identified lesion on infertility needs further investigations.

**Keywords:** *Abattoir, Addis Ababa, Adama, Akaki, Bacteria, Cow, Dromedary camel, lesion, reproductive organ.*

## 1. INTRODUCTION

The productivity of animal largely depends on their reproductive performance. Regular and successful reproduction is the key to profitable large animal production (Arata, 2015). Therefore reproductive efficiency is an important facet for achieving maximum return from the animal (Khaton *et al.*, 2015). However, the physiological process of reproduction could get disrupted or hampered due to variety of factors including; nutritional deficiencies, specific and non-specific infections, hormonal abnormalities, immunological malfunctions, environmental stress and others too (Khanvilkar *et al.*, 2009). Thus, identification of reproductive tract diseases is important, especially when we deal with genetically superior animals (Ali *et al.*, 2010). Reproductive diseases are considered as an important contributor to the decline in fertility potential in the large farm animals. Generally, in large farm animals, the highest incidence of infertility resulted in decreased milk production, treatment costs, extra labour and increased rate of culling (Mohammed *et al.*, 2014).

The reproductive performance depends upon the normal structure and functions of genital organs of an animal (Siddiqui *et al.*, 2005). Pathological lesions of the female genital tract are believed to be the major reason for economic loss associated with infertility, culling and slaughtering of cows (Abalti *et al.*, 2006; Simenew *et al.*, 2011 and Mekibib *et al.*, 2013) and in dromedary camels (Shawky *et al.*, 2004 and Simenew *et al.*, 2015). In general, female animals are culled and sent to slaughterhouse either they are uneconomic to maintain or else they have diseases problems. Hence, abattoirs are a good source for studying pathological lesions of reproductive organs that are severe enough to cause infertility and even sterility (Thrusfield, 1995). Moreover, most female reproductive organ pathological lesions lack additional outward manifestation, examination of gross and microscopic lesions of genital tract play a central role in the identification of these problems. Therefore, most of these abnormalities can only be diagnosed when the animal is subjected to postmortem examination (Buregelt, 1997). Previously abattoir based studies on reproductive organs abnormalities of cows have been done in Bahir Dar

(Abalti *et al.*, 2006), in Addis Ababa (Gebrekidan *et al.*, 2009), in Sululta (Simenew *et al.*, 2011), in Hawassa and Tulla (Mekibib *et al.*, 2013) and in Jimma (Arata, 2015). According to the results of these previous studies, the reproductive organ abnormalities of cows based on the anatomical classification include; abnormalities of uterus, ovary, oviduct, cervix and vaginal were recorded. Ovariobursal adhesion, follicular cyst, luteal cyst, paraovarian cyst, ovarian hypoplasia, vaginitis, cervicitis, hydrosalpinx, pyosalpinx, hydrometra, endometritis, cervical ring hypoplasia and hypoplasia of the uterus were some of the characteristic pathological lesions recorded in most of the above studies. Apart from the above studies carried out in Ethiopia many studies (Chaudhari and Paul-Bokko, 2000; Tafti and Darahshiri, 2000 and Ali *et al.*, 2006) were also done in different parts of the world with almost similar findings with above mentioned lesions.

Information about female dromedary camels' reproductive organs lesions in Ethiopia is very sparse. Only single published research reports by Simenew *et al.* (2015) was available. However there are several reports of female dromedary camel reproductive organ pathological conditions from other camel keeping countries like Egypt (Shawky *et al.*, 2004), Saudi Arabia (Ali *et al.*, 2010; Ali *et al.*, 2011; Al-Afaleq *et al.*, 2012), Nigeria (Mahmoud *et al.*, 2011), Algeria (Mohammed *et al.*, 2014), Iraq (Wajid, 2015) and Pakistan (Mustafa *et al.*, 2016). According to the findings of these above studies different reproductive pathological lesion include (lesions of uterus, ovary, oviduct and cervico-vaginal) were recorded among which lesions of the uterus was the most frequent in almost all studies. Endometritis, cystic ovarian disease, ovarian hypoplasia, hydrobursitis, ovarian tumor, uterine cysts, vaginitis, cervicitis and hydrosalpinx are some of the characteristic lesions reported in the above studies. Simenew *et al.* (2015) also reported almost similar pathological lesion with the above studies.

The inappropriate use of broad spectrum antibiotics and corticosteroids for the treatment of reproductive disorders or in the management of retained placenta and other obstetrical procedures in camels and cows has led to increased bacterial contamination of the vagina and subsequent invasion of the uterine environment (Tibary and Anouassi, 2001; Gani *et al.*, 2008). Uterine infection is a significant cause of reproductive failure and infertility in

dromedary camel (Ali *et al.*, 2010). On the other hand uterine infection is associated with delayed uterine involution and poor fertility in cow (Hasan.*et al.*, 2015). The most common and economically important bacteria for uterine infection are *Actinomyces* spp, *Escherichia coli*, *Fusobacterium* spp, *Pasteurella* spp, *Pseudomonas* spp and *Staphylococcus* spp. (Erin *et al.*, 2005).

Reproductive inefficiency in animal due to observed pathological lesions of female genitalia cause huge economic loss to farmers. To circumvent these problems, isolation of bacteria from the uterus and histopathological investigations of each lesion are critical for the diagnosis and management of poor reproductive performance in animals. Most research reported previously were based on gross pathological lesion observation and done either on cows or female dromedary camels. Comparative study on reproductive organ pathological lesions, isolation and identifications of possible aerobic bacteria involved in uterine disorder were very few in general and some were not yet attempted in Ethiopia. Hence, the present study was conducted to narrow these information gaps.

In line with the above background information and justifications, the main objectives of the present study were to:

- Identify the types and frequencies of reproductive organ pathological lesion and compare between cows and female dromedary camel.
- Describe and characterize gross and microscopic lesions of the observed abnormalities.
- Isolate and identify aerobic bacteria from uterine lesion of cows and female dromedary camels.

## 2. LITERATURE REVIEW

### 2.1. Physiology of Reproduction in Female Dromedary Camel and Cow

Good understanding in physiology of reproduction is helpful in successfully managing animal production. Anatomies of reproductive tract in camels are similar to the cows; however, they differ primarily in the shape of the uterus and cervix (Norman *et al.*, 2009). Dromedary camel uterus is bicornuate with a developed uterine body, from which the two horns diverge and taper anteriorly to give a combined uterine shape intermediate between that of letters 'Y' and 'T'. The left horn is longer than the right horn (Umaru and Bello, 2013). The cervix of dromedary camel is very short ( $3.62 \text{ mm} \pm 1.32$ ) and resembles the size of the cervix of the cow but in contrast has four to five distinct rows of annular mucosal folds (Srikandakumar *et al.*, 2003).

Physiology of reproduction in cow is different from that of dromedary camel. Female dromedary camels reach puberty at three years of age but first mating is generally delayed at 4 years; first parturition generally occurs at five years. Management and the nutritional status can influence the onset of reproductive activity (Skidmore, 2003). Bovine females progress through a series of stages of reproduction; at about 11 months of age, on average, heifers typically reach puberty, or first estrus. After puberty, a female then exhibits continued estrous cycles at even intervals, normally every 18 to 24 days (Parish *et al.*, 2010). Camels are considered seasonal breeders; with peak sexual activity from November to February in the Northern hemisphere. Proper nutrition and a good management may help to override seasonal effects and allow the breeding to occur throughout the year (Monaco *et al.*, 2015). However, cows are polyestrous exhibit heat more than one time per year (Rich and Turman, 2014).

Cows are spontaneous ovulators, which means ovulation occurs at a certain time during the estrous cycle whether mating occurs or not (Parish *et al.*, 2010); however, camels are induced ovulators and normally ovulate only in response to mating; follicles tend to

grow, have a period of maturity during which are capable to ovulate, and then regress if ovulation is not induced (Skidmore *et al.*, 2013). The changes in the ovarian follicular dynamics in dromedary camels are described as a follicular wave pattern. Each follicular wave is divided into four phases namely: recruitment phase, growth phase, mature phase and regression phase (Tibary and Anouassi, 1997; Monaco *et al.*, 2015). The length of gestation in dromedary camel has been reported to be about 13 months on average 385 day. The pregnancy is almost 99% located in left uterine horn and prolonged calving interval in camels is ascribed to lengthy gestation period, limited breeding season and late post-partum estrus (Monaco *et al.*, 2015). However, length of gestation in cows is approximately 283 days, during gestation the period between fertilization and parturition or calving, a cow or heifer is pregnant and not cycling. The postpartum stage is the period of recovery after female calves, typically 40 days or more. To maintain an annual calving interval, a beef female has approximately 82 days and 40-50 days in dairy cow between calving and rebreeding (Parish *et al.*, 2010).

## **2.2.Major Reproductive Organs Pathology**

### *2.2.1. Disorders of ovary*

#### Cystic ovarian diseases

Cystic ovarian diseases are an important ovarian dysfunctions and major causes of reproductive failure in dairy cattle (Jeengar *et al.*, 2014). However, the term cystic ovaries does not always apply to *camelidae* because a large proportion of females develop some form of follicular cyst if not bred given time that ovulation in these species is induced (Tibary and Anouassi, 2000). It is generally accepted that disruption of the hypothalamo-pituitary-gonadal axis by endogenous and/or exogenous factors causes cyst formation (Khan, 2010). According to Carter *et al* (2008) cystic ovaries contain one or more persistent fluid-filled cavities larger than a ripe follicle. Ovarian cysts are described according to the structure involved and their appearance (Tibary and Anouassi, 2000).

Cysts are classified as follicular cysts, luteal cysts, cystic corpora lutea or hemorrhagic cysts according to their histological and physical characteristics. Follicular and luteal cysts are the only two true types of anovulatory cysts that are associated with an abnormal condition in animal. The ovulatory cystic CL is considered to be no pathological (Tibary and Anouassi, 1997).

### Ovarian follicular cysts

Ovarian follicular cysts are a follicular structure on ovary in the absence of luteal tissue, larger than normal follicular size that persists for a significant amount of time and affects the estrus cycle of the animal (Youngquist and Threlfall, 2007). Though we cannot determine the exact cause of ovarian follicular cysts, we can recognize that they develop when one or more follicles fail to ovulate and subsequently do not regress but maintain growth and steroidogenesis (Vanholder *et al.*, 2006). Cystic ovarian disease is the most common endocrine pathology to be found in dairy cows, and incidence is believed to vary from 1 to 30% depending on herd and breed conditions (Jeengar *et al.*, 2014).

According to Tibary and Anouassi (2000) follicular cysts are a normal evolution of the non ovulatory follicle the incidences vary from 30 to 40% of females in dromedary camel. Follicular cysts, when compared to other ovarian cystic conditions, are characterized by thin walls and produce very small amounts of progesterone. Occasionally, a persistent condition can lead to increased testosterone levels, causing some cows to exhibit masculine aggressive and sexual behavior. However, most cystic animal will remain in anestrous as long as the condition persists (Ball and Peters, 2004).

Macroscopically, the cysts occupy the periphery, the centrum or sometimes almost the entire ovary. They are single or multiple in one or both ovaries. In camel their diameters were ranged from 0.5-4.5 cm and their walls were thin, tense, transparent or semitransparent and had clear congested blood capillaries on their surfaces, especially in cysts of large diameter (Shawky *et al.*, 2004). Microscopically, the zona Granulosa disappears entirely, the theca follicular internal becomes fibrous and the graafian follicles

lose their original form. In some cases, the granulosa cells shrink, small cystic degeneration is observed mainly in the cortex of the ovary (Lacey and Rosenberg, 2010).

#### Luteinized cystic ovary disease

The Merck Veterinary Manual (Kahn, 2010) describes luteal cystic ovary disease as being characterized by enlarged ovaries with one or more cysts, the walls of which are thicker than those of follicular cysts because of a lining of luteal tissue. Luteal cysts develop when ovulation fails to occur and the theca undergoes luteinization (Schlafer and Donald, 2007). They are also often considered to be the later form of ovarian follicular cysts and therefore the causes pertaining to follicular cysts can also be considered the original causes of luteal cysts (Vanholder *et al.*, 2006). Luteal cysts in *camelidae* are usually single, thick-walled, grayish-yellow and smaller in size than follicular cysts are originated from luteinization of follicular cyst (Tibary and Anouassi, 2000). When compared to follicular cysts, luteinized cysts are more likely to persist over long periods of time and can lead to nymphomania in some animals (Peters *et al.*, 2009).

Macroscopically, they are smooth and rounded, with a spherical cavity that is lined by a layer of fibrous tissue surrounded by the luteinized cells (Schlafer and Donald, 2007). Microscopically, the wall of cysts formed from small theca-luteal cells and fat containing granulosa cells contained homogenous eosinophilic structureless substance mixed with some luteal cells in the lumens (Shawky *et al.*, 2004).

#### Cystic Corpora Lutea

Occasionally the corpus luteum does not regress normally even though the animal is not pregnant, however, if persists without the occurrence of pregnancy it is considered a cystic corpus luteum (Lashari *et al.*, 2012). The terms for cystic CLs can often be confused with those for luteal cysts, though the first is a normally functional structure and the latter is a pathological condition. The maintenance of CL is the result of precise interaction between pituitary and embryonic gonadotropins, as well as intraluteal

autocrine and paracrine signals that modulate the endocrine function of luteal cells. In addition the maintenance of CL in the absence of pregnancy may also originate because of metritis and similar effects are possible with pyometra and late embryonic mortality (Struve *et al.*, 2013).

According to Kahn (2010), cystic CL has a soft, mushy core area, due to presence of fluid from a degenerating blood clot. In addition, the cystic CL as well as the typical CL may or may not have an ovulation crown or papilla at its apex. Absence of this ovulation crown or papilla should not be considered diagnostic of the cystic condition because 10-20% of functional, normal CL fails to develop this feature. Persistent corpora lutea are rare in the female *Camelidae* and however, the condition has been suspected on the basis of prolonged elevated plasma progesterone levels in the absence of pregnancy (Tibary and Anouassi, 2000).

Macroscopically, in cow corpora lutea had an average diameter of 1.5 to 3.5 cm. Histopathological examinations showed that the cystic corpora lutea had a zone of fibrous connective tissue between the luteal tissue and the cystic cavity (Hatipoglu *et al.*, 2002). Incidence of cystic CLs ranges from 25.2% to 78.8% during diestrus and decreases with progression of the estrous cycle. Because cystic corpora lutea are found in cows that are normally cycling or pregnant, they are considered to be a normal stage or variation of CL development (Khan, 2010).

#### Para-Ovarian Cysts

These are fluid filled structures located in the broad ligament near the ovary or oviduct. Para-ovarian cysts are suspected to arise from persistent embryonal structures which are vestiges of wolffian ducts. Parovarian cysts are remnants of the mesonephric ducts that are occasionally found around the ovary and fallopian tubes, attached in the broad ligaments (Russo *et al.*, 2010). They contained a clear yellowish serous fluid and were either attached to the mesovarian ligament or mesosalpinx. Their widest diameters varied from 1 to 6 cm and their walls were commonly thin and transparent and usually round or oval

in shape in cows and cysts can be single or multiple, unilateral or bilateral, round or oval, measuring 0.5 to 5 cm in diameter in dromedary camels (Tibary and Anouassi, 1997). Palpation or ultrasonography can be used to detect the cyst in animal and they appear as fluid-filled anechoic structures and are usually round or oval in shape, occur as a single cystic structure, and range from 1-5 cm in diameter (Peter *et al.*, 2009). They may sometimes be mistaken from cystic ovary on rectal palpation because of closeness to the ovary (Khan, 2010). There are two different types of paraovarian cysts; those derived from the cranial mesonephric tubules are called epoophoron, while those from the caudal tubules are referred to as paroophoron. All paraovarian cysts are benign, with no negative effects on reproduction and fertility (Peter *et al.*, 2009).

#### Ovarian hydrobursitis

Ovarian hydrobursitis is a peculiar affection of the ovarian bursa characterized by the accumulation of variable amounts of fluid and encapsulation of ovary. Ovarian hydrobursitis is responsible for large number of long standing infertility problems in dromedary camels characterized by adhesion, fluid accumulation and encapsulation of the ovary (Tibary and Anouassi, 2001 and Ali *et al.*, 2011). This syndrome is manifested by early embryonic death, abortion, repeated breeding and refuses mating (Ali *et al.*, 2011). Inflammatory processes in and around the ovary, 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 (Peter *et al.*, 2009).

#### Ovarian bursal adhesion

Ovarian bursa adhesions are structures that occur as fibrous bands between the surface of the ovary and its bursa. According to Mekibib *et al.* (2013) the severity of the condition varies from the presence of a few very small strands of fibrous tissue to encapsulation of ovary. Though there is no definitive cause of adhesions, they are most likely the result of excessive follicular hemorrhaging during ovulation, trauma to the ovary or bursa caused by rectal examination, an infection from the uterus, or damage during calving (Ball and

Peters, 2004). Generally, ovary bursa adhesions do not cause reproductive problems in affected animal, unless, in severe cases, where the adhesion is so large that the fallopian tubes are blocked and fertilization of the ovum is prevented. Where the ovarian bursa is completely adhered to the ovary and sometimes the fallopian tube interference with ovulation, bursal and salphingeal occlusion predisposes to development of bursal cysts, hydrosalpinx and pyometra (Peter *et al.*, 2009).

### Ovarian hypoplasia

Hypoplastic ovary is essentially an underdeveloped ovary that does not function properly. The condition is characterized by incomplete development so that the ovary is lacking in primordial follicles (Shawky *et al.*, 2004). Hypoplasia can occur partially or completely, on one or both ovaries and like many other ovarian cysts and abnormalities, hypoplasia causes anestrus and therefore, the condition must be differentiated from the others (Peter *et al.*, 2009). According to Tibary and Anouassi (1997) absence of ovarian follicular activity is a frequent condition in camel it can be due to congenital or acquired ovarian hypoplasia. Ovarian activity is greatly affected by body condition, lactation and use of the animal. Ovarian activity is reduced in females just retired from racing and in females with a low body condition score. Ovarian hypoplasia due to genital or chromosomal abnormalities is also possible.

Despite the difficulties posed by smaller structures, the most effective method of diagnosis is through transrectal examination. The ovaries may feel like thin, narrow, firm cord-like structure (Peter *et al.*, 2009). Macroscopically, the ovaries were smaller in size, firmer in consistency and contained very small follicles on the surface of non affected part (Shawky *et al.*, 2004). Histopathologically, the follicles were few in number, not organized; blood vessels were congested in the center of the follicles and instead of forming follicles of different types and cells were simply aggregated in to focal area. Hyperplasia or excessive proliferation of stroma cells and fibroid connective tissue were also detected (Simenew *et al.*, 2015).

## Inflammatory Disorders of the Ovary

Inflammation of the ovary (oophoritis) and surrounding structures (perioophoritis) is caused due to harsh manipulation of the ovaries in attempts to rupture an ovulatory haemorrhagic follicle or due to ascending infections from uterus or arise from specific disease such as tuberculosis, brucellosis and campylobacterosis (Kunbhar *et al.*, 2003). Grossly it is characterized by loss of follicular activity because of the presence of adhesions between the ovarian surface and the surrounding tissues including the ovarian bursa, uterine tube and sometimes extending to include some intestinal loops (Tibary and Anouassi, 2000). Chronic localized inflammation of the ovary accompanied by abscess formation near the ovary has been observed in a few cases of *camelidae* (Tibary and Anouassi, 1997). Macroscopically, the ovary was discoloured and histologically, the ovarian medulla was congested and infiltrated by neutrophils, macrophages and lymphocytes, and the ovarian cortex revealed antral and atretic follicles (Mahmoud *et al.*, 2011).

### 2.2.2. Disorders of fallopian tube

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 (Rhaman *et al.*, 2012). The most dominant pathology of the uterine tube in camel is inflammation with occlusion or accumulation of fluid in the form of pyosalpinx or hydrosalpinx (Tibary and Anouassi, 1997). Other uterine tube pathologies found in the dromedary include mucosal cysts. Inflammatory changes can also be seen at the level of the uterine tubal junction (uterine tube papillae) with development of microabscesses (Tibary and Anouassi, 2000). Disorders of the oviducts include salpingitis, hydrosalpinx, pyosalpinx and adhesions (Rhaman *et al.*, 2012).

## Salpingitis

Salpingitis refers to infection and inflammation of the fallopian tubes caused by extension of endometritis or metritis. Grossly the fallopian tubes were found distended, elongated and tortuous forming many coils in the mesosalpinx but may not reveal any changes in consistency (Singh, 2009). Salpingitis is classified as chronic salpingitis, purulent salpingitis and tuberculosis salpingitis. Chronic salpingitis characterized by degeneration and desquamation of the mucosal epithelium and lymphocytic-plasmatic infiltrates with increase of connective tissue in the lamina propria. Macroscopic lesions consisted of oedematous appearance and thickening of oviduct, adhesions between mesosalpinx and perisalpingial tissues and accumulation of yellowish green pus within oviductal lumen. In purulent salpingitis, the lumen of oviduct was filled with extensive neutrophils accumulation and desquamated epithelial cells. Severe neutrophils accumulations and scattered lymphocytic-plasmacytic infiltrates throughout oviductal wall, and sometimes necrosis also detected (Hatipoglu *et al.*, 2002).

## Hydrosalpinx

Hydrosalpinx is an affection in which the fallopian tube is filled with inflammatory fluid and is the end result of pelvic infection. In this infection the tubes become inflamed, which even after treatment may be blocked due to presence of residual fluid inside. Continued fluid buildup over time dilates the tube more, resulting in hydrosalpinx of various sizes (Inskeen and Dailey, 2005). Grossly, the fallopian tubes were found distended, elongated and tortuous forming many coils in the mesosalpinx. Histologically, the wall was thin, translucent, with flattening of the lining epithelium and distended with large amount of clear fluid. A hydrosalpinx does not have healthy cilia, hence, embryos that find their way into the fallopian tube become trapped and may implant there resulting in a dangerous ectopic pregnancy that needs to be removed surgically (Shawky *et al.*, 2004).

## Pyosalpinx

Pyosalpinx refers to presence of pus in one fallopian tube. When both tubes are affected with the accumulation of pus inside, the term used is pyosalpinges. It is a consequence of PID which may be caused by streptococcus and staphylococcus infection. Infections may start from vagina, and progress up to the cervix, uterus, and to one or both fallopian tubes if not treated early. Majority of pyosalpinx cases revealed moderate to marked infiltration of neutrophils, mononuclear cells and hoisting mucosal and muscularis layers. Some cases revealed marked thickening of mucosal layer due to infiltration of neutrophils, macrophages, histocytes and fibrous tissue formation (Tsianos *et al.*, 2011).

## Pachysalpinx

Is affection characterized by the enlargement of the whole length of uterine tube which is kinked and distorted the normal shape (Shivhare *et al.*, 2012). The central lumen is completely filled with a connective tissue mass and the distinct tubal mucosal folds are absent. In a study on repeat breeding associated with fallopian tube affections 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 multilocular intramuscular cyst formation by the fusion of adjacent folds and denuded epithelial linings due to salpingitis and resultant tubal blockage (Inskeen and Dailey, 2005).

### 2.2.3. Disorders of uterus

The uterus of *camelidae* is the site of congenital or acquired pathologies. Amongst the most common congenital abnormalities of the uterus reported in *camelidae* are segmental aplasia, uterus unicornis and infantilism. The acquired abnormalities of the uterus are dominated by inflammatory and infectious conditions such as metritis and endometritis. Segmental aplasia represents the lack of development of parts of the tubular system of the genitalia. Occurrence of the aplasia in the posterior part of the tubular genitalia (from the

cervix to the hymen) is usually detected by the presence of an enlarged uterus due to fluid accumulation in the organ pyometra/mucometra (Tibary *et al.*, 2006).

However, uterine function is often compromised in cow by bacterial contamination of the uterine lumen after parturition; pathogenic bacteria frequently persist, causing uterine disease, a key cause of infertility (Sheldon and Dobson, 2004). There are different types or manifestations of uterine disease among postpartum dairy cows. Metritis, endometritis, mucometra and pyometra are the types of uterine diseases most commonly reported (Drillich *et al.*, 2006).

#### Endometritis and Metritis

To the pathologist, the general definitions of inflammation of the genital tract are simple. Inflammation limited to the endometrium is termed endometritis; involvement of the entire thickness of the uterine wall is metritis; of the serosa, perimetritis; and of the suspensory ligaments, parametritis (BonDurant, 1999; Kennedy and Miller, 1993). Metritis can be distinguished from endometritis; in the former, all layers of the uterine wall show evidence of inflammation such as edema, infiltration by leukocytes and myometrial degeneration. Endometritis is a superficial inflammation of the endometrium, extending no deeper than the stratum spongiosum with histological evidence of inflammation (BonDurant, 1999). During recovery from acute endometritis, there is fibrosis and leukocytosis, with depletion of endometrial glands and atrophy of the remainder with increased time to pregnancy (Bonnett *et al.*, 1993).

Metritis resulting in systemic signs of sickness, including fever, red-brown watery foul-smelling uterine discharge; inappetance elevated heart rate, and low production (Sheldon *et al.*, 2006). Clearly, metritis is a much more severe disease than endometritis, requiring a different therapeutic approach (Sheldon *et al.*, 2008). The main risk factors for uterine diseases are traumas occurred at first parturition, dystocia, twins, stillbirth, abortion, prolapsed uterus, retained placenta (RP), ketosis and hypocalcemia (Sheldon *et al.*, 2006).

Uterine diseases cause lesions in the endometrium disrupt endometrial function and impair embryo development. In addition these diseases decrease luteinizing hormone, first dominant follicle size and growth, and follicular ability to secrete estradiol therefore affecting ovulatory capacity. Uterine diseases such as metritis and endometritis are highly prevalent in high producing dairy cows (Sheldon *et al.*, 2008). Uterine infections are the most common acquired reproductive problems camel resulting in infertility. A very large incidence of the endometritis (45.9%) was reported in Saudi Arabia by Ali *et al.* (2010).

### Pyometra

Pyometra is characterized by a collection of purulent exudate of variable amount within the uterine lumen. This condition is most likely to develop in cows that have their first postpartum ovulation before bacterial contamination of the uterus has been eliminated (Foldi *et al.*, 2006). The condition is associated with corpus luteum activity in the ovary; it often persists longer than the expected duration of the luteal phase. It has been suggested that it is the presence of this structure, with its secretion of progesterone that results in endometritis developing into pyometra (Kennedy and Miller, 1993). Early ovulation after parturition and formation of an active corpus luteum may predispose to pyometra. On the other hand, the retention of the corpus luteum may be associated with failure of luteolysis (Sheldon *et al.*, 2006).

Although there is functional closure of the cervix, the lumen is not always completely closed and some pus may discharge through the cervix into the vaginal lumen. Pyometra with an open cervix and vaginal discharge is observed primarily in the postpartum period and is due to a postpartum complication (retained placenta, dystocia, uterine prolapse) resulting in delayed involution due to infection and accumulation of fluid. Closed cervix pyometra is the most prevalent in *camelidae* and is usually associated with cervical adhesions or prolonged progesterone therapy (Tibary and Anouassi, 2000). Ultrasonographically pyometra is characterized by the presence of a CL on an ovary, an accumulation of fluid of mixed echo density in the uterine lumen and distension of the uterus (Sheldon *et al.*, 2006).

## Hydrometra and Mucometra

This pathological condition are clinically called pseudo pregnancy before the advent of ultrasonography due to the lack of equipment that would permit a more precise diagnosis these uterine disorders are only differentiated by the physical characteristics of the fluid present in the uterus. Hydrometra or mucometra develops in untreated cases of cystic ovarian degeneration, hydrometric uterus containing gallons fluid associated retained corpus luteum was reported by several researchers in the past (Fathalla, 2000).

Mucometra are conditions characterized by an enlargement of the uterus due to accumulation of varying quantities of fluid a few milliliters to several gallons have been reported in dromedary camels (Tibary and Anouassi, 2000) and cows (Mekibib *et al.*, 2013). Administration of prostaglandin (PGF<sub>2</sub> alpha) causes luteolysis and expulsion of uterine contents within 3 to 4 days in animals which have luteal cyst, retained corpus luteum or a corpus luteum coexisting with a cyst. Mucometra occurs in female cattle as a result of adhesion or fibrosis. It is also seen in cattle with segmental aplasia or in heifers with persistent hymens. Mucometra has also been linked to chronic cystic ovarian disease (COD) but it is by no means characteristic of that condition (Fathalla, 2000).

### 2.2.4. *Disorders of cervix*

Examination of the cervix is very important for the evaluation of the infertile animal as well as for the evaluation of physiological states such as prepartum dilation and postpartum involution. Congenital anomalies of the cervix, due to abnormal development of the mesonephric and paramesonephric ducts, lead to the formation of cervical cyst or segmental aplasia. The most common abnormality of development of the paramesonephric ducts is the persistence of its medial walls giving rise to the formation of a double cervix (Tibary and Anouassi, 1997).

The most common acquired pathological conditions of the cervix are due to local inflammation (cervicitis) or injuries during parturition or gynecological manipulations.

Other acquired anomalies of the cervix include cervical adhesions or lacerations resulting from a complication of birth or excessive trauma during manipulation. Cervical cysts of varying size and shape are reported in female cattle. The cysts were found at the external orifice of the cervix having inspissated cervical mucus (Kumaresan and Ansari, 2002). Cervicitis is usually associated with uterine infections and vaginal mucopurulent discharge in *camelidae* and it should be differentiated from the normal hyperemia and slight irritation found immediately following breeding (Tibary and Anouassi, 2000).

Cervicitis also reported in cow by Mekibib *et al.* (2013) and dromedary camel Shawky *et al.* (2004). Macroscopically, the cervix was enlarged and the mucosa was edematous, congested and covered with yellowish or whitish viscous exudates. Histopathologically, congestion of blood vessels and desquamation of the lining epithelium with hyperplasia of cervical glands were detected. Focal areas of edema and hemorrhages with inflammatory cellular infiltration of cervical mucosa and submucosa mostly lymphocytes were noticed (Shawky *et al.*, 2004).

#### 2.2.5. Diseases of the vagina and vulva

Several abnormalities of the vagina and vestibulum have been reported including segmental aplasia, persistent hymen, vaginal constriction, and presence of vaginal septum. These anomalies should be suspected when there is difficulty in penile intromission. Inflammation of the vagina vaginitis is relatively common and associated with bad management of breeding or inadequate manipulation of the vaginal cavity. The vaginal mucosa appears bruised and hyperemic; sometimes there is a mucous secretion. Coitus can also induce traumatic injuries especially if the female is still young. Traumatic injuries of the vagina can lead to the formation of complete adhesions between the vaginal wall and development of pyometra. Vaginal prolapse also occurs in camels during pregnancy (Tibary and Anouassi, 2000).

Macroscopically, the vaginal mucosa was severely swollen and congested. Microscopically, congestion of blood vessels, desquamation of the lining epithelium and

edema of submucosa with inflammatory cellular infiltration mostly lymphocytes were detected. Moreover, focal aggregation of mononuclear inflammatory cells mainly lymphocytes was seen (Shawky *et al.*, 2004).

### **2.3.Common Bacterial Infection of Uterus**

Infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or release of bacterial toxins that lead to establishment of uterine disease (Janeway *et al.*, 2001). In pregnant animal, vulva, vestibule, vagina and cervix function as anatomical barriers that protect the uterus from bacterial contamination during pregnancy. Relaxation of the vulva and cervical dilation during parturition allow for the entrance of bacteria into the uterus (Sheldon *et al.*, 2004; Azawi, 2008). Necrotized caruncles, blood and cell debris provide a perfect media for bacteria to grow during the immediate postpartum period. Under normal circumstances, the process of uterine involution effectively expels debris and encourages endometrial regeneration, so that the percentage of cows in which bacterial infection remains present at 3 weeks postpartum should decline to 40%; however, in approximately 10 to 17% of postpartum animal, conditions favouring bacterial growth persist and eventually cause endometritis (Sheldon *et al.*, 2004).

The development of uterine disease depends on the immune response of the animal, as well as the species and number (load or challenge) of bacteria. The number of pathogenic bacteria in the uterus of postpartum cows may be great enough to overwhelm uterine defense mechanisms and cause life-threatening infections, although these are relatively uncommon (Sheldon and Dobson, 2004). Indeed, it is non-life-threatening uterine infections that are most common and associated with impaired reproductive performance (Sheldon *et al.*, 2006).

Uterine bacterial infections are important because they disrupt not only the function of the uterus, but also the ovary and the overarching higher control centre in the hypothalamus and pituitary. The inflammatory and immune response to uterine bacterial

infection compromises animal welfare as well as causing sub fertility and infertility. Understanding the mechanisms underlying the effect of uterine bacterial contamination and the associated immune response on bovine reproduction is an important challenge for reproductive biologists in the 21st century (Sheldon and Dobson, 2004).

Infectious organisms are also responsible for a myriad of diseases that directly or indirectly affect reproductive success in *camelidae*. Infections of the genital tract may lead to temporary or permanent infertility in the male and female (Tibary *et al.*, 2006). Establishment of uterine bacterial infection may depend in part on the endocrine environment; in particular, progesterone seems to suppress uterine immune defenses. Formation of the first corpus luteum after parturition and secretion of progesterone often precedes the onset of uterine disease (Sheldon and Dobson, 2004).

The most common bacteria isolated from the uterus of *camelids* with endometritis are *Escherichia coli*, *Streptococcus zooepidemicus*,  $\beta$ -haemolytic *Streptococci*, *Enterococcus*, coagulase negative *Staphylococcus*, *Proteus* spp., *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Arcanobacter pyogenes*. *Pseudomonas aeruginosa*, *Campylobacter fetus*, and *Tritrichomonas fetus* have been isolated from infertile camels and may be associated with venereal transmission and should be considered as possible causes in infertility or abortion outbreaks. *Aspergillus* spp. and *Mucor* spp. have been isolated from female dromedaries with endometritis (Tibary *et al.*, 2006).

## **2.4. Diagnosis of Reproductive Organ Disorders**

### *2.4.1. Clinical evaluation*

Uterine infection should be suspected in any animal with a history of repeat breeding, early embryonic death or abortion. The barren female with endometritis or metritis may have a history of recent abortion, retained placenta, dystocia or uterine or vaginal prolapse. Systemic signs are usually absent in cases of chronic endometritis. However, fever, depression and signs of toxic shock may accompany acute postpartum metritis

(Qureshi *et al.*, 2002). Examination of the perineum and vulva may reveal mucopurulent discharge. In some cases, dried flakes of vaginal discharge may be present at the base of the tail. In the postpartum female, a thick, pinkish or white postpartum discharge from the vagina is normal and may persist for up to 1 week after parturition. However, a profuse, watery or smelly discharge should be considered abnormal and a sign of postpartum metritis. Conformation of the vulva and perineum are very important in the evaluation of the barren female. An incompetent vulva or vestibulovaginal sphincter due to tears or laceration may be the primary cause of contamination of the vagina and uterus (Tibary *et al.*, 2006).

#### 2.4.2. *Ultrasonography*

Ultrasonography has been used extensively as a diagnostic tool in veterinary medicine. Most of the research has been focused on the presence, volume and nature of uterine luminal fluid. Examined postpartum female by per-rectum ultrasonography and concluded the volume of intrauterine fluid was significantly associated with impaired uterine involution and that the intrauterine fluid volume score was positively correlated with bacterial growth. It seems reasonable that a local inflammatory response within the endometrial would result in some degree of tissue thickening (Tibary *et al.*, 2006).

#### 2.4.3. *Endometrial cytology and uterine bacterial culture*

Uterine culture and cytology samples are preferably taken when the female is at peak follicular phase and the cervix is open. In large camels these procedures are performed using the same rectovaginal techniques used in the bovine (Tibary *et al.*, 2006). Numerous pathogens could be localized at the female reproductive tract, affecting the success of fertilization. Infectious diseases could provoke vulvitis, vaginitis, cervicitis, endometritis and it is interesting to diagnose these disorders. Usually, uterine inflammatory disorders begin with bacterial contamination into the uterine lumen, and continue with adhesion of pathogens to the mucosa, colonization or penetration of the epithelium, and/or release of endotoxins. Uterine inflammation, even in the absence of

active bacterial infection, may disrupt embryonic survival and provoke RBC syndrome (Janeway *et al.*, 2001). The endometrial bacteriological diagnosis is interesting to detect pathogens implicated in infertility. In cattle, especially due to the cervical anatomy, samples can be taken using a catheter connected to a syringe containing 30-60 ml of sterile saline. It is deposited into the uterus and then it is removed and cultured in such way clinical or subclinical endometritis could be diagnosed (Sheldon *et al.*, 2006).

Endometrial cytology is a practical technique to diagnose subclinical endometritis, when clinical signs are absent. The uterus flushing technique is by far the best, but has the disadvantage of being time consuming. For this technique, the uterus is flushed with a small quantity of sterile saline using either a foley catheter or a mare insemination pipette. The fluid is collected, fixed in 40% ethanol and centrifuged to concentrate cells. Smears are prepared from the sediment, air-dried and stained with Wright–Giemsa, Papanicolaou stain or Hematoxyline and Pollack’s trichrome. The degree of inflammation is assessed by evaluation of the amount and morphology of polymorphnuclear (PMNs) leucocytes (Tibary *et al.*, 2006).

#### 2.4.4. Uterine palpation

It is important to be able to diagnose the presence of uterine infection to facilitate timely and appropriate treatment and to quantify the severity of disease, which allows a prognosis to be given for subsequent fertility (Williams *et al.*, 2005). Uterine palpation per-rectum has been the most frequently used method to diagnose endometritis (Sheldon *et al.*, 2004; Sheldon *et al.*, 2006). Diagnosis of clinical endometritis by palpation per-rectum is a challenge because uterine size and palpable quality of content may vary between individuals and strongly depends on the stage of the postpartum period. It has been reported that diagnosis of clinical endometritis using palpation per-rectum is subjective, not effective and prone to error as it lacks standardization (Foldi *et al.*, 2006).

The definitive diagnosis of endometritis is made on the basis of histological examination of endometrial biopsies and these are predictive for subsequent fertility. However, the

technique is costly and time consuming, not clinically accessible in most situations, and may depress fertility (Bonnett *et al.*, 1993)

#### 2.4.5. Vaginoscopy

Vaginoscopy is an easy tool used to evaluate the vagina and cervix in postpartum cows and it should be employed as a routine diagnostic tool by veterinary practitioners especially if rectal palpation is the only other diagnostic modality being used (Barlund *et al.*, 2008). Vaginoscopy using a small mare tube speculum for camel may reveal vaginal discharge or inflammation. The cervix should be evaluated for signs of inflammation (cervicitis) and discharge. The normal cervix may be hyperemic and bleed immediately after mating (Tibary *et al.*, 2006).

### 2.5. Treatment of Reproductive Organ Disorders

The COD therapy appears to be simple however, regaining fertility, which often requires longer time due to perturbations in endocrinology and uterine pathology that follows in long standing cases. Many techniques and therapeutic strategies have been used to treat COD in domestic animal (Purohit, 2008). Some of the earliest treatments included ovariectomy, injection of ovarian extract, injection of CL extract, uterine infusions of antibiotics or antiseptics and injections of adrenaline chloride (Roberts, 1986). Many endocrine based treatments for cysts have been evaluated and the success of therapy in terms of disappearance of OC with different hormonal treatments is good, yet the establishment of pregnancy requires variable times due to formation of new OC and pathological alterations that occur in the uterus with long term persistence of OC (Purohit, 2008).

Uterine infections can lead to irreversible damage resulting in a total loss of fertility uterine lavage or flushing, intrauterine antibiotic infusion, systemic antibiotic treatments or a combination (Tibary and Anouassi, 2000). There is no clinical trial comparing the efficacy of different treatments of endometritis in *camelidae*. Most practitioners use

treatments proposed for the bovine or equine species (Tibary *et al.*, 2006). For cow a great variety of intrauterine antimicrobial agents (oxytetracycline: 4 to 6 g/day) and antiseptic chemicals (iodine solutions: 500 ml of 2% Lugol's iodine immediately after calving and again 6 hours later as a preventive measure), systemic antibiotics (penicillin or one of its synthetic analogues: 20,000 to 30,000 U/kg/cow), ceftiofur /third generation cephalosporin/ 2.2 mg/kg daily for 5 days) and supportive therapy (nonsteroidal anti-inflammatory drugs such as flunixin meglumine, fluid therapy in case of dehydration, therapy with calcium and energy supplements in case of depressed appetite), and hormone therapy (oxytocin: 20 to 40 U repeated every 3 to 6 hours within 48 to 72 hours after calving; prostaglandin F<sub>2α</sub> or its synthetic analogues) have been introduced in the field (Risco *et al.*, 2007).

The most common antibiotics used in camels are penicillin K  $1.5 \times 10^6$  U, Gentamicin sulfate, 500–1000 mg, Ticarcillin (3 g for dromedaries, particularly for *Pseudomonas* infections), Amikacin sulfate (2 g for camels infected with *Pseudomonas* spp and *Klebsiella* spp) and ceftiofur sodium 1 gram for camels. The third generation cephalosporin, ceftiofur, has a broad spectrum of action and is effective against both Gram-negative and Gram-positive bacteria. Antibiotics should be diluted in sterile water or saline solution, 60 ml for treatment should continue daily for 5–7 days (Tibary *et al.*, 2006).

## **2.6. Prevention of Female Reproductive Organ Disease**

Prevention of uterine infection requires sound individual and herd reproductive management; prebreeding examinations should be performed on all animals to avoid breeding animals that are too young or have no follicular activity (Tibary *et al.*, 2001). A complete gynecological evaluation should be performed before breeding on all females with a history of infertility, obstetrical problems or postpartum complications. This allows early detection and treatment of uterine infections as well as prevention of venereal transmission of organisms. The incidence of endometritis can be reduced by observing strict rules of hygiene during breeding and parturition (Tibary *et al.*, 2006).

Good management of sanitation, nutrition, population density and stress to prevent or reduce the incidence of these predisposing factors should be impeccable. Therefore prevention remains limited to general guidance on hygiene at calving. Adequate nutrition and the control of infectious diseases one of the pharmacological approaches to the prevention and treatment of metritis should be use routine systemic administration of ceftiofur but its effect on reproductive performance is not significantly different to that of no treatment (Risco and Hernandez, 2003).

## **2.7.Economic Importance of Reproductive Organ Disease**

Reduced reproductive performance is the most common cause of economic loss in dairy cattle industry at worldwide level. The goal of reproduction management is to animal become pregnant at a biologically optimal time and at an economically profitable interval after calving. The timing of examination of animals after parturition should allow for the normal process of involution, yet also provide sufficient time for treatment and response prior to the start of the breeding period (Thurmond *et al.*, 1990). Cystic ovarian disease is a cause of temporary infertility and one of the most common reproductive disorders in dairy cows with a reported incidence of 6 to 23% (Butler, 2003). COD is associated with a 6 to 11 day extend in the calving to first service gap and a 20 to 30 day increase in the calving to pregnancy interval above the standard. It is also known to increase the risk of culling. The economic loss resulting from the rate of ovarian cysts are mostly due to the effects on the cost of feed, average growth of calves, labor and medical prices (Gundling *et al.*, 2009).

Reproductive inefficiency in cattle can have devastating effects on economic success in dairy farms, where revenue is directly dependent upon reproduction or its associated effects on milk production. In dairy herds, the largest source of lost income as a result reproductive waste or lost pregnancies is more days open, resulting in fewer days at peak milk production (Thurmond *et al.*,1990). Economic losses can also be consider, both in times of the cost of keeping animal and the lost cash opportunity from fewer calves available to market (Sheldon and Dobson, 2004).

### **3. MATERIALS AND METHODS**

#### **3.1. Study Area and Study Population**

The study was conducted at Addis Ababa and Adama municipal abattoirs and Akaki slaughter house. Akaki slaughter house is located in the southern outskirts of Addis Ababa 20 Km away from the centre of Addis Ababa. Adama municipality abattoir is located at Adama town in Oromia regional state about 99 Km away from center of Addis Ababa. Addis Ababa municipality abattoir is located in Addis Ababa, the capital of Ethiopia. The study populations were female dromedary camels and cows slaughtered at these abattoirs. Three to seven camels were slaughtered at Akaki slaughter house, every day. Female dromedary camels were brought to this abattoir from Afar and Borana pastoral areas. All cows slaughtered at Addis Ababa and Adama municipality abattoir at the day of visit were included in the study. On average seven cross and local breed cows were slaughtered every day in each abattoir. Cows slaughtered at Addis Ababa and Adama municipality abattoirs were came from in and around Addis Ababa, Adama town, Harar town, Arsi and Bale zone. During the study period the total study animals included were 280 animals (140 female dromedary camels and 140 cows). All female dromedary camels were from Akaki slaughter house.

#### **3.2. Study Design and Sampling Method**

A cross sectional study design was conducted from November 2016 to May 2017. Age and body condition score of animal were gathered during ante mortem examination. Age of female dromedary camels was estimated by dental examination on the basis of their dental formulas and tartar deposition on the teeth (annex 1), as described by Schwartz and Dioli (1992). However, cow's age were estimated according to Puck and Soliame (2004) as indicated on (annex 2). We categorised age less than 5 years as young, 6-10 years as adult and 10 or more years as old animal according to Sghiri and Driancourt (1999). The animals' body condition score (BCS) was evaluated on a scale of 0 to 5 by

considering visual examination and fat cover palpation over the animal body (annex 3), following the description by Nicholson and Butterworth (1998). BCS were categorised into 3 groups; poor as  $\leq 2$ , medium as  $\leq 3$  and good as  $\geq 4$  according to Mohammed *et al.* (2014). Purposive sampling technique was used and all female camels and cows with any reproductive lesion after slaughter were sampled. Pregnant tracts were not included in the study.

### **3.3. Sample Collection and Transportation**

During postmortem examination the entire reproductive tract was carefully removed from the carcass, taken to one corner of the abattoir, and visually examined and thoroughly palpated. Each reproductive tract was opened along its longitudinal axis starting from the vagina down to the horns using sterile scissors and was observed for any abnormalities in color, odor, consistency and morphology (Feyissa and Bekana, 2000; Mekibib *et al.*, 2013). Those with obvious gross lesions were noted based on their appearance, type, location and frequency of occurrence (Jenberie *et al.*, 2012).

Tissues with grossly visible lesions were sampled for histopathological and bacteriological examinations. For histopathology, a tissue cut of 1-2 cm from lesion including some normal part was collected into a universal bottle containing 10% buffered formalin according to Talukder (2007) and then transported to NAHDIC for histopathological processing.

For bacteriological examination, pieces of tissue from active lesion at the boundary were collected aseptically using sterile forceps, scissors and scalpel blade and placed into screw capped universal bottles contain sterile saline water according to Quinn *et al.* (2004), labeled and transported to the veterinary microbiology laboratory of Addis Ababa University College of Veterinary Medicine and Agriculture in ice box containing ice packs for culturing.

### **3.4. Sample Processing**

#### *3.4.1. Histopathology procedures*

Tissue specimens were processed using standard procedures of tissue processing, described by Talukder (2007). Briefly, tissues were trimmed, fixed in 10% buffered neutral formalin, dehydrated in ascending grades of alcohol, cleared with xylene and impregnated with molten paraffin wax. Then tissues blocks were sectioned at a thickness of 4-5 micrometers, spread on warm water, mounted on frosted glass slides, deparaffinized by heat (incubated at 60°C) and xylene (three changes), followed by rehydration in descending grades of alcohol and stained with hematoxylin and eosin (the detail was depicted in annex 4) Stained slides were examined under microscope (at 10 x, 40x and 100x magnification) using phase contrast microscope (Nikon, Japan) and photomicrographs were taken.

#### *3.4.2. Bacteriological procedures*

All bacteriological procedures were performed according to Quinn *et al.* (2004) the detail is indicated on (annex 5). The tissue sample was cut into pieces with sterile scalpel blade and forceps then inoculated to brain heart infusion broth and aerobically incubated at 37°C for 24hrs. After 24hrs, tubes were observed for growth (turbidity) and a loop-full of sample was streaked parallel on sheep blood agar (7%) and MacConkey agars and incubated aerobically at 37°C for another 24hrs. The blood agar plates were checked for presence of growth, haemolysis (types), colony morphology, size and shape and the MacConkey agar plates were check for presence of growth, lactose fermentation or not fermenting and for colony morphology, size and shape. For primary identification, gram stain, catalase, oxidase and motility tests were conducted. Selective and differential media such as Mannitol salt agar, Edwards medium, Eosine methylene blue and Salmonella shigella agar were used for the suspected samples from the primary test results. After 24hrs of incubation, characteristic growth on selective medium was registered, a colony was then further inoculated in to nutrient broth for further

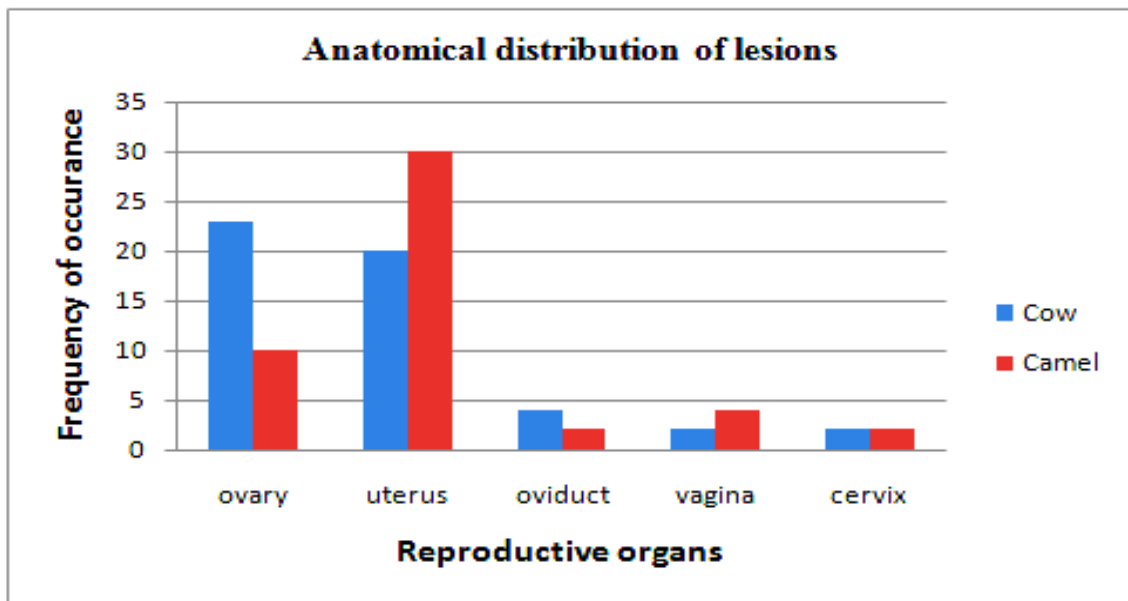
biochemical tests. In general Coagulase, Indole, Methyl red (MR), Vogues-Prousker (VP), Citrate, Urease, Lysine and Triple Sugar Iron (TSI) tests were performed as secondary biochemical tests.

### **3.5.Data Analysis**

The data generated in this study were recorded, checked and coded on spreadsheet of Microsoft Excel and STATA version 13 statistical software was used for descriptive analysis. Chi-square test was used to determine the presence of dependency among different variables (age, species and BCS) with pathological lesions of the reproductive organs. Uterine bacterial isolate were compared between species by using descriptive analysis. Gross as well as histopathological lesions and findings were described using qualitative methods.

#### 4. RESULTS

During the study period, a total of 280 reproductive tracts (140 from cows and 140 from female dromedary camels) were collected and examined. Various pathological lesions with different degrees of severity were observed in 34.2 % (n=48) and 36.4% (n=51) of dromedary camels and cows, respectively. Cows and dromedary camels with lesions were observed to have one or more pathological lesions. Comparatively, the most frequently observed lesions in dromedary camels were uterine abnormalities 21.4% followed by ovarian lesions 7.14%. However, in cows, ovarian lesions 16.4% were the most frequently observed reproductive lesions followed by uterine lesions 14.2%. Vaginal lesions 2.85% were observed more frequently in dromedary camels than cow 1.42%. The details of lesion types with frequencies were shown on table 1, 2 and 3 below.



**Figure 1:** Anatomical distribution of reproductive organ lesions in cow and dromedary camel

The association between frequency in occurrence of macroscopic lesions with species, age and body condition score were evaluated. There was no statistically significant

association ( $P>0.05$ ) between species, age and body condition score with frequency of occurrence of most reproductive pathological lesions (Table 1, 2, 3).

#### **4.1.Ovarian Abnormalities and Lesion Characterization**

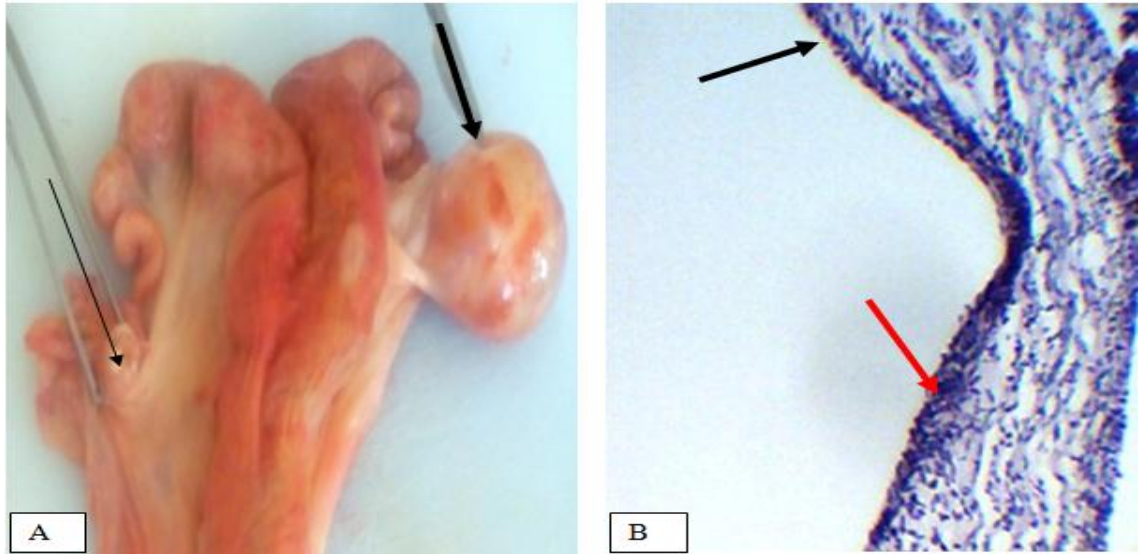
Different ovarian abnormalities were observed in 16.4% (n=23) and 7.14% (n=10) of examined cows and female dromedary camels respectively. Follicular cyst, luteal cyst, ovarian hypoplasia, paraovarian cyst, ovarian bursal adhesion, Oopharitis and hydrobursitis were lesions observed (Table 1). Ovarian bursal adhesion and Oopharitis were the lesions observed only in examined cows whereas; hydrobursitis was seen only in dromedary camel ovary.

##### *4.1.1. Follicular cysts*

Follicular cysts were seen in 4.2 % and 2.85% of examined cows and dromedary camels, respectively. The lesions were unilateral in both species. Three of them were on the right while the other three were on the left ovary in cows, but in dromedary camel only one was on the right and the rest three were on left ovary.

Macroscopically, most of the cysts were spherical in shape and were occupying ovarian cortex in both species. Their diameters were ranged from 30 mm to 55 mm and the walls of the cysts were thin, slightly opaque and had straw colored serous fluid. One follicular cyst was accompanied by ipsilateral ovarian hypoplasia and endometritis in a cow (fig 2A).

Microscopically, the follicular cysts were lined by few layers of granulosa cells, as large portion of the granulosa cells were degenerated and by pressure exerted follicular fluids. It was difficult to differentiate the theca interna from the theca externa as the cells were compressed by fluid pressure (fig 2B). Moreover, larger cysts resulted in pressure atrophy of the adjacent ovarian tissues result in the ovum and the surrounding cells completely degenerated or absent.



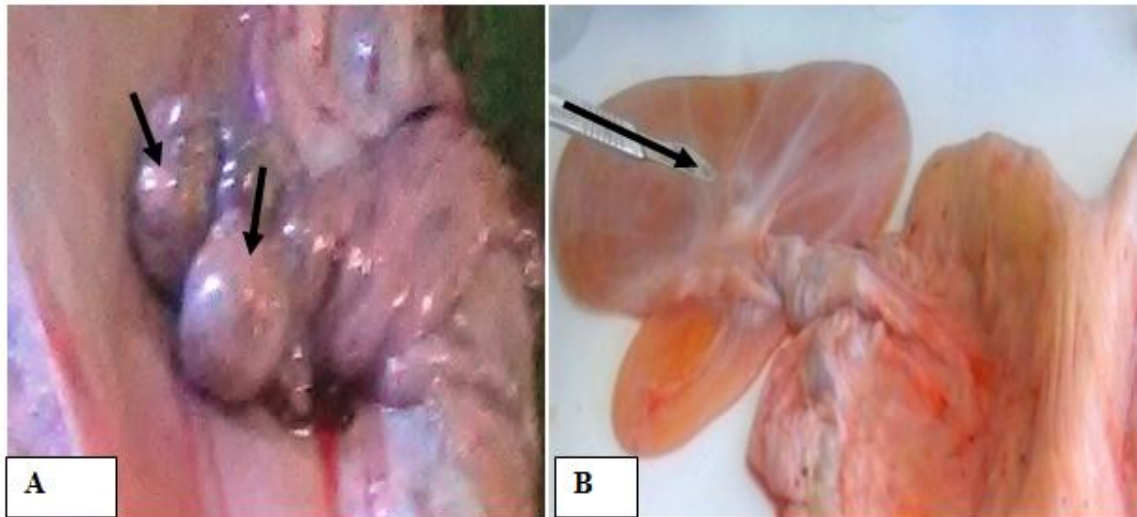
**Figure 2:** (A) gross appearance of follicular cyst (cow). Note that the cyst occupies the entire right ovary and very thin walled (thick arrow). The left ovary was hypoplastic (thin arrow), (B) Microscopic lesion of follicular cysts which was characterized by extremely thin granulosa cells (black arrow) due to follicular fluid pressure and degeneration of granulosa cell (red arrow). H and E stain X 40.

#### 4.1.2. Luteal cysts

Luteal cysts were observed in 2.14 % and 1.42 % of examined cows and dromedary camels, respectively. In cows, two cysts were on the right ovary and one on the left. In dromedary camels, all cysts were observed on the right ovary.

Macroscopically, the luteal cysts were thick walled, opaque and have meat like consistency. The wall of the cyst was lined by a whitish brown or yellow membrane. Furthermore, in cows, all the luteal cysts appeared as a single rounded mass on the surface of the ovary. In one camel multiple luteal cysts were observed. The diameter of the luteal cysts varies and ranged from 20- 25mm (fig 3A).

Microscopically, the wall of the luteal cysts was formed from thick layer of lutein cells and fat containing granulosa cells. They contained homogenous eosinophilic structureless substance mixed with some luteal cells in the lumens.



**Figure 3:** (A) macroscopic lesions luteal cyst. Note cyst was compacted and thick walled unlike the thin walled follicular cysts. (B) Macroscopic lesion of ovarian hydrobursitis, note straw colored fluid accumulation in ovarian bursa of camel

#### 4.1.3. *Paraovarian cysts and Hydrobursitis*

The frequency of occurrence of paraovarian cysts was all the same 1.4% both for female camels and cows examined. Macroscopically, the cysts were detected unilaterally only on the left ovary in both species. The cysts were located either in mesovarium or in mesosalpinx ligament. These cysts were 10-20 mm in diameter and transparent, having thin wall and contained clear watery fluid.

Ovarian hydrobursitis was observed in 0.71% of examined dromedary camel but, none of this lesion was observed in examined cow. The size of the affected bursa was about 5 x 6.4 cm and the affection was unilateral. Moreover, the bursa was having about 25 ml of accumulated watery and straw color fluid indicated in (fig 3B).

**Table 1:** Frequency (%) ovarian disorders and association of ovarian lesions with putative factors

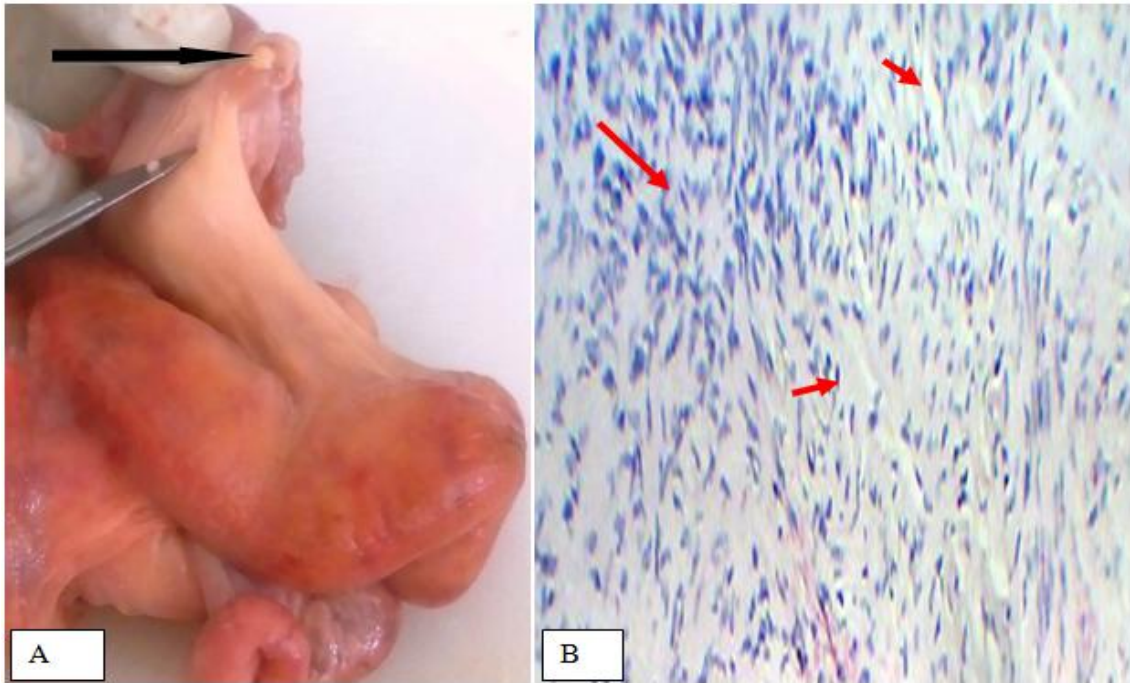
Variable		Follicular cyst	Luteal cyst	Paraovarian cyst	Hypoplasia	O.B adhesion	Oophrotis	O.H bursitis
Spp	Cow	6(4.28)	3 (2.14)	2 (1.42)	4(2.8)	7 (5)	1 (0.72)	0
	Camel	4 (2.85)	2 (1.42)	2 (1.42)	1(0.7)	0	0	1(0.7)
	$\chi^2$	0.4148	0.2036	0.000	1.8327	*	*	*
	P value	0.520	0.652	1.000	0.176	0.007	0.316	0.316
Age	Adult	7 (5.34)	2 (1.57)	3 (2.3)	3 (2.2)	3 (2.2)	0	0
	Young	1 (3.44)	1 (3.57)	1 (3.4)	1 (3.4)	1(3.4)	0	0
	Old	2 (1.66)	2 (1.66)	0	1 (0.8)	3 (2.5)	1 (0.83)	1(0.83)
	$\chi^2$	2.4600	0.5169	*	1.2677	0.130	*	*
	P value	0.292	0.772	0.195	0.531	0.937	0.512	0.512
BCS	Poor	1 (0.74)	2 (2.27)	0	4 (4.5)	2 (1.4)	0	1(1.1)
	Good	8 (9.0)	2 (1.48)	1 (0.74)	1 (0.7)	4 (2.8)	0	0
	Medium	1 (1.75)	1(1.75)	3 (5.26)	0	1(0.7)	1 (1.75)	0
	$\chi^2$	11.4720	0.1906	*	*	0.267	*	*
	P value	0.003	0.909	0.201	0.058	0.875	0.140	0.335

\* = 2 and more than 2 cells have expected counts less than 5 have no  $X^2$  value  
O.B adhesion (Ovario-bursal adhesion), O.H bursitis (Ovarian hydrobursitis)

#### 4.1.4. Ovarian hypoplasia/ Inactivity

The frequency of occurrence of ovarian hypoplasia was 2.85 % in cow. However, only 0.72% of dromedary camel was found with hypoplastic ovary. It was bilateral and the ovary was very small, oval in shape and measured 1 cm by 1 cm in camel. In cows all were unilateral with three of them on the right and one on the left ovary. The size varies from 1.3 cm by 1.1 cm to 0.78 cm by 1.0 cm.

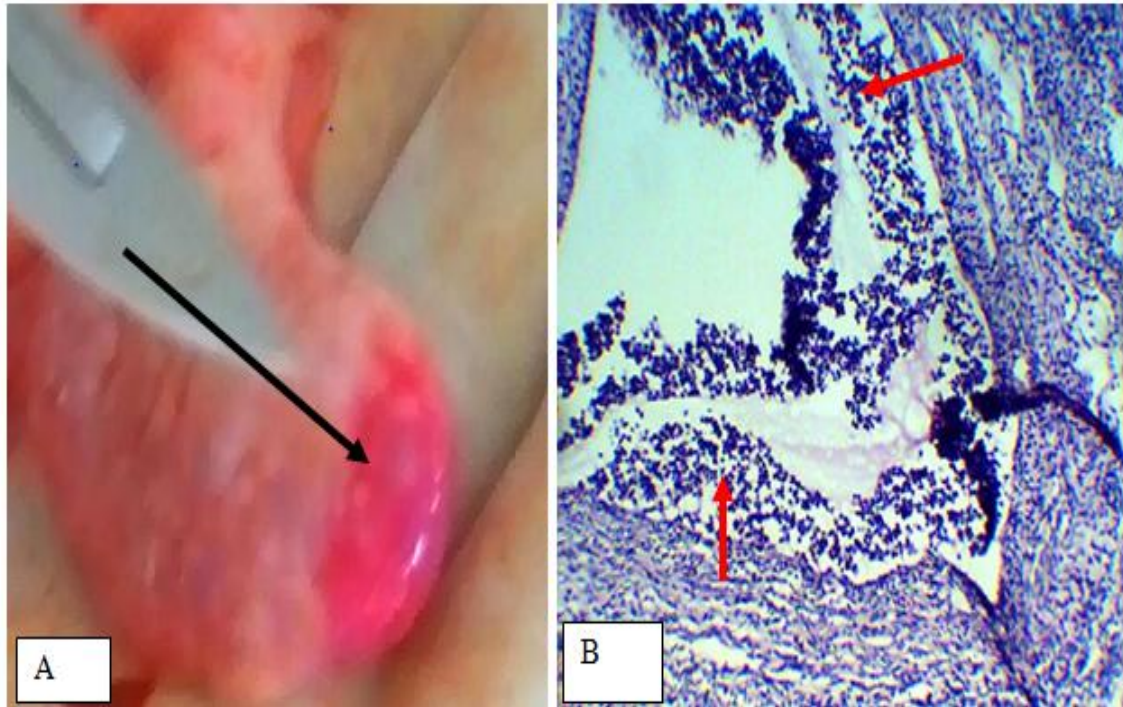
Macroscopically, the ovaries were smaller in size, firmer in consistency and contained very small follicles on the surface of non affected part. Microscopically, excessive fibrous connective tissue proliferation with complete absence of follicular or luteal developments was seen (fig 4).



**Figure 4:** (A) grossly small hypoplastic ovary of cow (B) Microscopic lesion of hypoplastic ovary. Note excessive fibrous connective tissues proliferation with complete absence of follicular or luteal development. H and E stain X 40

#### 4.1.5. Oophoritis

Oophoritis was observed only in a cow however, it was not seen in examined dromedary camels. This lesion might be incriminated in ovarian bursal adhesion as they accompanied each other. Macroscopically, the ovary was hyperemic and slightly swollen. Microscopically, the ovarian medulla was infiltrated by inflammatory cells (fig 5).



**Figure 5:** (A) grossly hyperemic and swollen camel ovary (B) excessive infiltrations of inflammatory cells into ovarian medullary regions (red arrow) with cellular necrosis leaving cystic structures filled with edema fluids. H and E stain X 40.

#### 4.1.6. Ovario-bursal adhesion

Ovario-bursal adhesion was the most frequent 5% ovarian lesion encountered in cows. However, none of this lesion was observed in dromedary camels. Except for a single adhesion which was bilateral, all examined lesions were unilateral. Four of the adhesions were on the right and two on the left ovary. In one case ovario-bursal adhesion was concomitantly occurred with oophoritis.

Macroscopically, the ovaries were found adhered to the bursa and surrounded by a layer of connective tissue. The severity of adhesions varied from case to case. In five cows the adhesions were mild with sparse strands of connective tissue between the ovary and bursa. In two, the adhesions were intense in which, the ovaries were completely encapsulated in thick fibrous connective tissues.

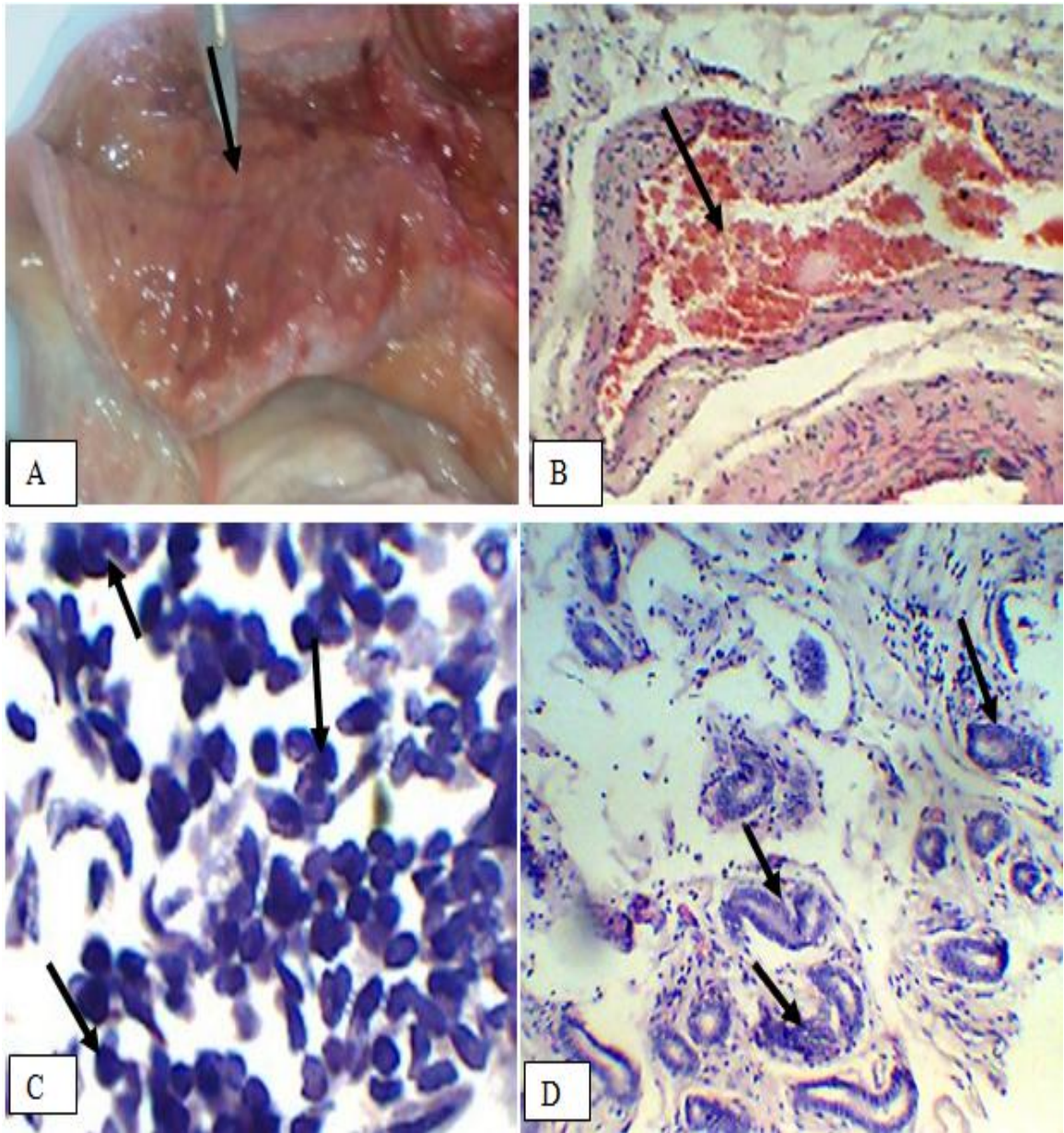
## **4.2. Uterine Abnormalities and Lesion Characterization**

Uterine lesions were observed at high frequency 21.4% (n=30) than other disorders in dromedary camel's. However, it was the second most frequent 14.2% (n=20) in cows. Grossly the severity of uterine lesion varies between species and it was more severe in dromedary camels than cow. Microscopically, all uterine lesions, with the exception of one, which found to be leiomyoma, were inflammatory in both species. Acute endometritis, chronic endometritis and catarrhal endometritis were common in both species. However, suppurative endometritis was observed only in cow's uteri (Details of lesions were indicated under specific topics below and the frequency of occurrence each of uterine lesions was indicated in table 2).

### *4.2.1. Acute endometritis*

Acute endometritis were more frequent in dromedary camels than cows (Table 2). Acute endometritis were accompanied by luteal cyst in two of the dromedary camel. Macroscopically, the affected uteri were enlarged and the mucosa was either severely congested (red brown) or was severe reddened (fig 6). Furthermore, thick blood tinged exudates was seen in the uterine lumen of three dromedary camels and one cow.

Microscopically, endometrial epitheliums were necrotized and sloughed in most cases with either congestion or hyperemia of endometrial blood vessels especially in basilar endometrial region. Moreover, polymorphonuclear cells, mainly of neutrophilis were infiltrating the endometrium. Excessive periglandular cuffing of cells and atrophy of endometrial glands were characteristics of acute endometritis.



**Figure 6:** (A) gross lesion of endometritis from camel. Note severely hyperemic blood tinged exudates in the upper corner. (B) Severely congested endometrial blood vessels (40X) (C) aggregation of inflammatory cells mainly of neutrophils (arrows). H and E stain 100X (D) misshapen and atrophied endometrial gland with periglandular inflammatory infiltrations (arrows) H and E 40X

#### 4.2.2. *Suppurative endometritis*

Suppurative endometritis were observed only in 1.42% of cows. Macroscopically, the endometrium was congested and covered with thick creamy white pus. The lesions were also further expanded to uterine horn and fallopian tube resulting pyosalphix.

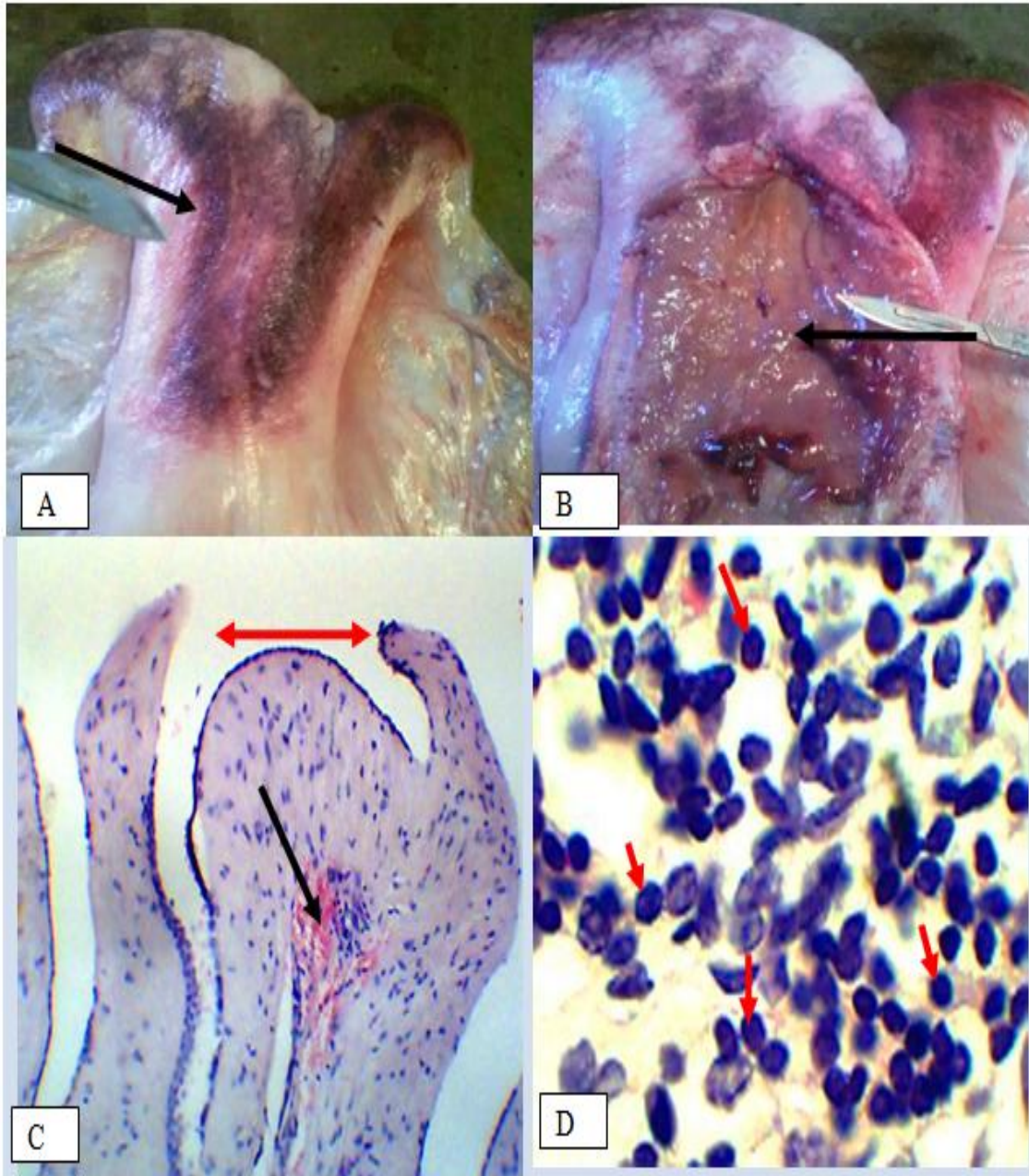
Microscopically, suppurative endometritis was characterized by infiltration of neutrophils into the endometrium with severe congestion of blood vessels and distortion of some endometrial glands

#### 4.2.3. *Chronic endometritis*

Chronic endometritis were more frequent in dromedary camels than cows (Table 2). In one cow with chronic endometritis there was concomitant luteal cyst, vaginitis and cervicitis.

Macroscopically, most of the uteri with chronic endometritis were thick, doughy, rigid and their mucosa was severely congested. In one dromedary camel with chronic endometritis the uterus was severely congested, with corrugation of the perimetrium and dark brown hemorrhage on external surface (fig 7 A and B).

Microscopically, the mesothelium of this uterus was hyperplastic with polypoid like projections and diffuse thickening of basal fibrous connective tissue. Most of the uteri affected by chronic endometritis were characterized by endometrial glandular degenerated, mononuclear inflammatory cells mainly of lymphocytes, macrophages and to less extent neutrophils infiltrations. In some there was hyperplasia of endometrial epithelium (fig 7C and D).



**Figure 7:** Chronic endometritis (camel spp). (A) Note severe congestion visible from outside. (B) Congested and thickened mucosa on incision (C) severely hyperplastic (papillomatous) mesothelium indicated by (double headed red arrow) and hemorrhage (black arrow) H and E 40x. (D) Heavy infiltration of inflammatory cells mainly of lymphocytes into the endometrium H and E 100x.

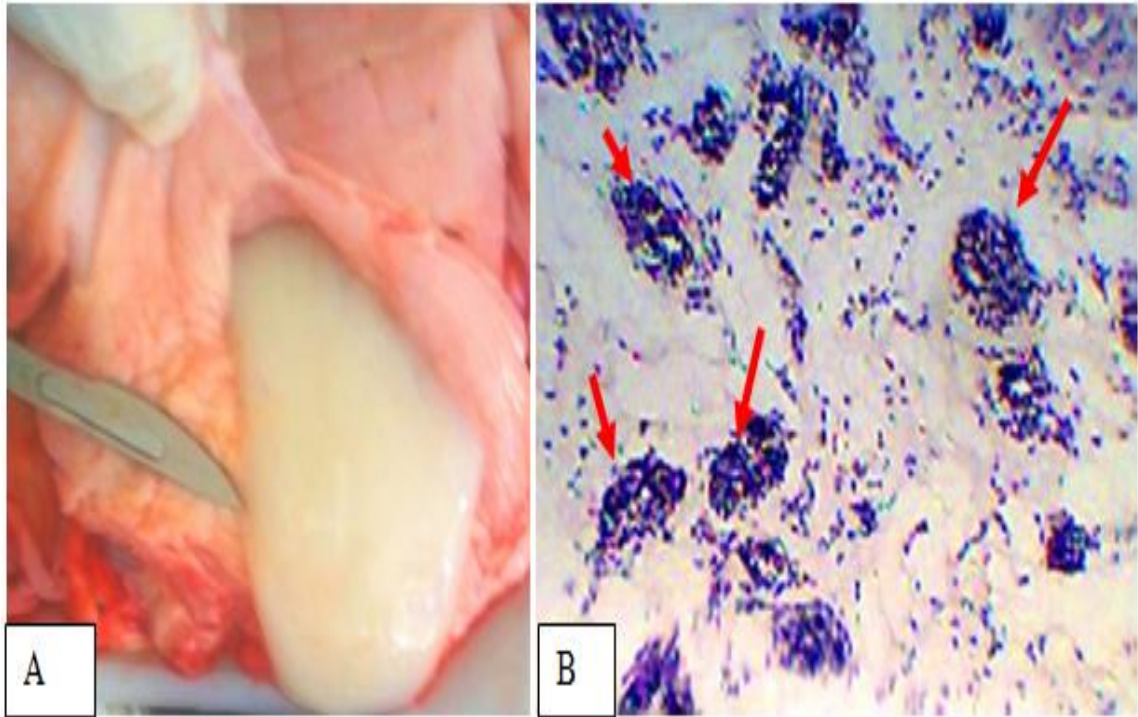
**Table 2:** Frequency (%) and association of uterine lesions with different variable

Variable		Acute endometritis	Chronic endometritis	Catarrhal endometritis	Suppurative endometritis	Uterine tumor
Species	Cow	9(6.42)	7 (5)	2 (1.42)	2 (1.42)	1(0.72)
	Camel	12(8.57)	16 (11.4)	2 (1.42)	0	0
	$\chi^2$	0.463	3.836	0.000	*	*
	P value	0.496	0.055	1.000	0.156	0.316
Age	Adult	11 (8.39)	13 (10.8)	0	2 (1.52)	0
	Young	2 (6.9)	5 (17.2)	1(3.44)	0	0
	Old	8 (6.66)	5 (3.81)	3(2.5)	0	1(0.83)
	$\chi^2$	0.2873	7.5861	*	*	*
	P value	0.866	0.023	0.156	0.318	0.512
BCS	Poor	7 (7.95)	7 (7.95)	3 (3.4)	2 (2.27)	1 (0.72)
	Good	9 (6.66)	11 (8.14)	1 (0.74)	0	0
	Medium	5 (8.77)	5 (8.77)	0	0	0
	$\chi^2$	0.2943	0.0322	*	*	*
	P value	0.863	0.984	0.155	0.111	0.335

\* = 2 and more than 2 cells have expected counts less than 5 have no X2 value

#### 4.2.4. Catarrhal endometritis

Catarrhal endometritis was observed in two dromedary camels and two cows. Macroscopically, the uterus was enlarged and its mucosa was slightly congested, edematous and was covered by thick mucoid exudates. In one camel the mucoid exudates further expanded to cervix and vaginal mucosa discharged to outside of the body (fig 8). The microscopic examination showed congestion of endometrial blood vessels, lymphocytic infiltration in the mucosa and submucosa with alternative areas of epithelial desquamation and hyperplasia of lining epithelium. Degenerations of endometrial glands were also seen.

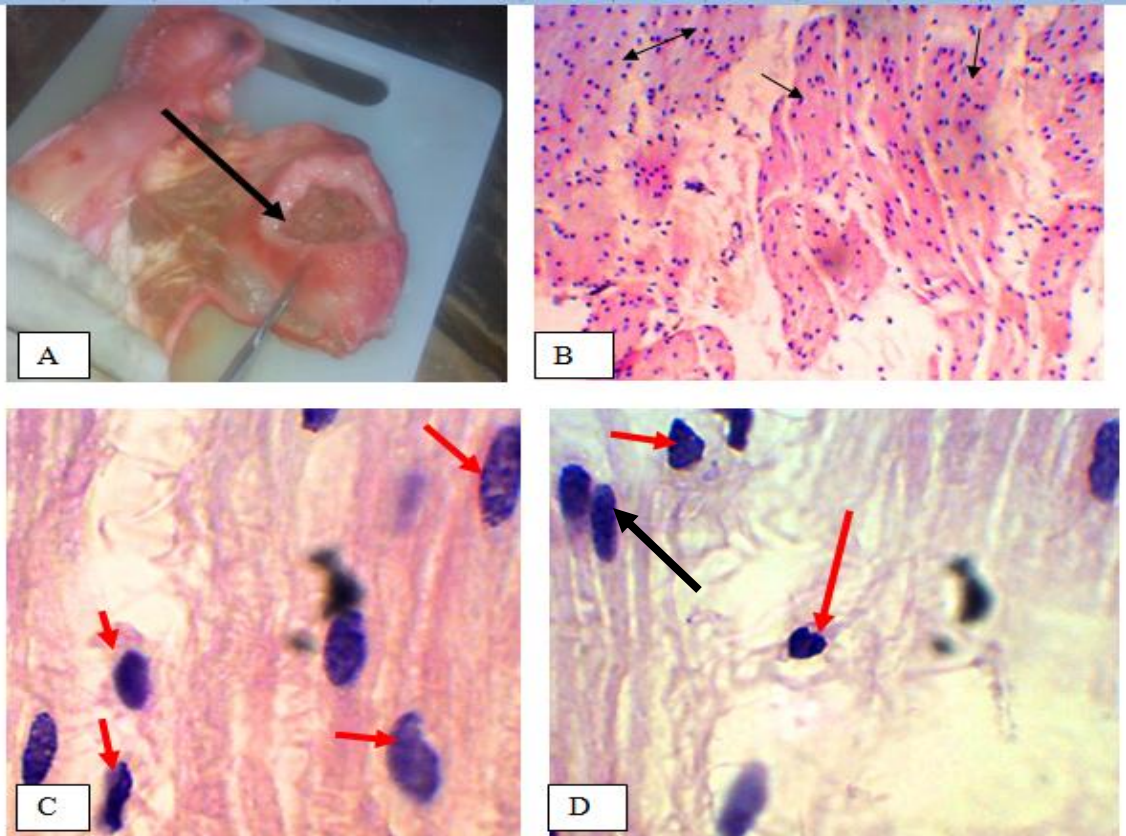


**Figure 8:** (A) gross lesions of catarrhal endometritis (from cow). Note the viscous mucoid exudates (B) microscopic lesions with endometrial gland degeneration, necrosis and infiltrations of inflammatory cells. H and E 40X

#### 4.2.5. Uterine tumor

The only observed uterine tumor was uterine leiomyoma. It was observed only in a cow and not in female camel. Macroscopically the neoplasm was firmly attached mass, and circular in shape with a diameter of 6 cm.

Microscopically the leiomyoma was comprised of smooth muscle cells and connective tissue components. Largely composed of composed of interlacing (interweaving) bundles of smooth muscle fibres with acidophilic cytoplasm and elongated and rounded blunt ending nuclei. The fibers were usually fusiform or stellate in shape, possessed large, ovoid to elongated nuclei and sometimes multiple nucleoli. There was slight pleomorphism, and little mitotic activity was seen (fig 9).



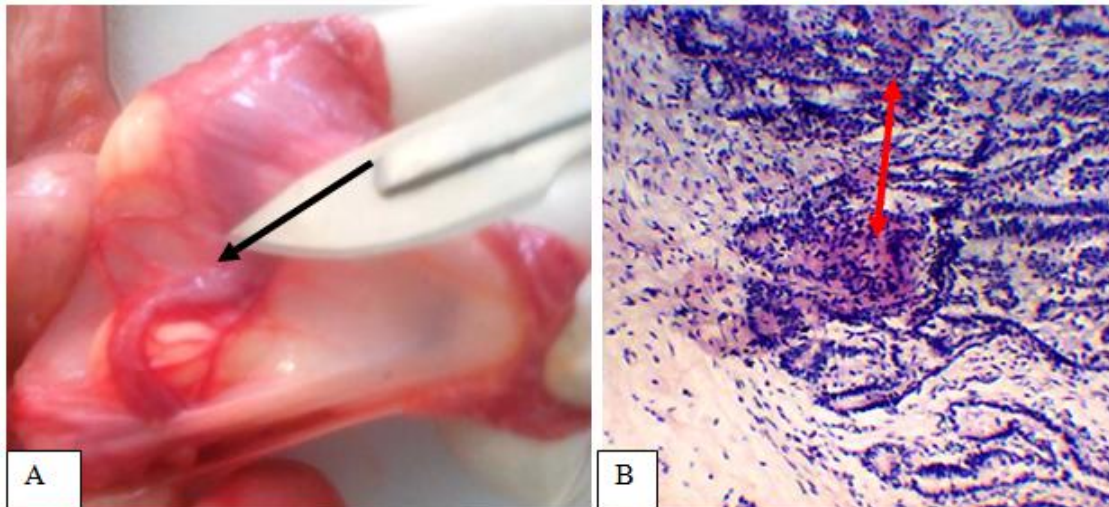
**Figure 9:** Leiomyoma of cow. (A) Firmly attached mass originated from body of uterus (B) Numerous nucleus indicative of neoplastic changes with bundles of smooth muscles running in various directions and interlaced with each other (10X). (C) Anisokaryosis and pleomorphism in the nucleus (higher magnification/100x). (D) Mitotic figure with the nuclear chromatin at two poles of a dividing cell and the cell unable to divide (red arrow) and a cell with two nuclei (black arrow) (X100).

### 4.3. Oviductal Lesions

#### 4.3.1. Hemosalphinx

Hemosalphinx was observed in one camel and two cows. In dromedary camels, it was unilateral and was observed in the right oviduct. However, it was bilateral in one cow and unilaterally in right oviduct in two cows. Macroscopically, in both species the oviduct was slightly enlarged in size and the mucosa was hyperemic and filled with blood.

Microscopically, hyperplasia of the lining epithelium and congestion of blood vessels with inflammatory cellular infiltration were noticed (fig 10).



**Figure 10:** Hemosalpinx of cow (A) Note the mucosa was hyperemic and the lumen filled with bloods. (B) Edema and heavy inflammatory cells infiltrated into the mucosa (red arrow) H and E stain X 10.

#### 4.3.2. *Pyosalpinx*

Pyosalpinx was detected in one camel and two cows. Macroscopically, the affection was bilateral in both species. Moreover, the oviduct was enlarged and distended with pus. In one cow pyosalpinx was accompanied with suppurative endometritis.

### 4.4. Cervico-Vaginal Lesion

#### 4.4.1. *Cervicitis and Vaginitis*

Cervicitis was seen in two cows and two camels. In general cervical lesions were observed with low prevalence than the rest of reproductive organ abnormalities in both species (Table 3). Macroscopically, the cervix was slightly enlarged, and the mucosa was congested, edematous and covered with whitish viscous exudates.

Vaginal lesion represented by vaginitis and vaginal lymphocytic myocytis accounted 2.85% of examined dromedary camels and it was with relatively higher frequency than the lesion observed in cows. However, vaginitis was the only lesion seen examined cow 1.42%. Macroscopically, in vaginitis the mucosa was slightly swollen and showed partial congestion and partial hyperemia on the other parts.

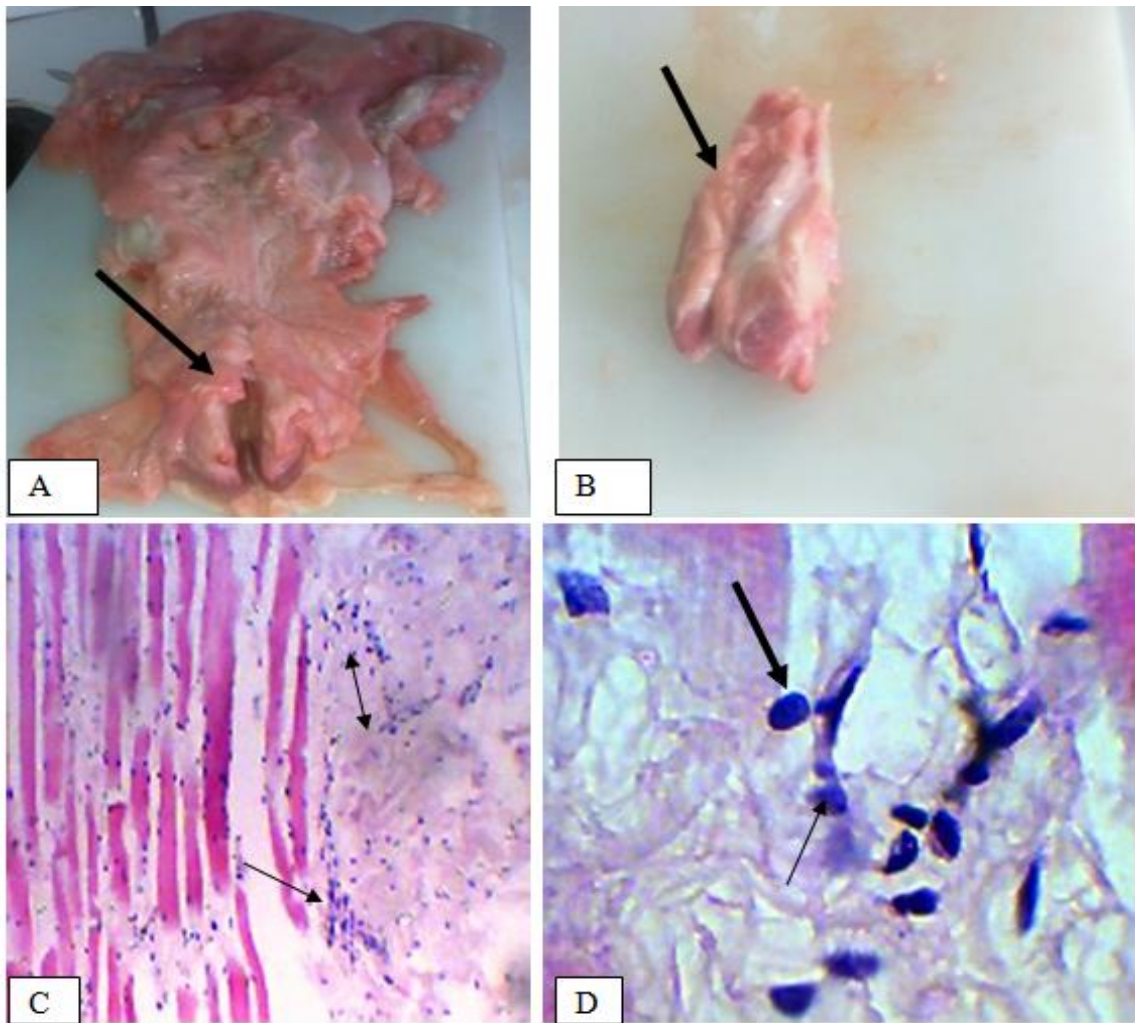
**Table 3:** Frequency (%) and association of oviductal and cervico-vaginal lesion with different variable

Variable		Hemosalphix	Pyosalphix	Cervicitis	Vaginitis	Vaginal myocytis
Species	Cow	2 (1.42)	2 (1.42)	2 (1.42)	2 (1.42)	0
	Camel	1 (0.72)	1 (0.72)	2 (1.42)	1 (0.72)	3 (2.14)
	$\chi^2$	0.3369	0.3369	0.0000	0.3369	*
	P value	0.562	0.562	1.000	0.562	0.082
Age	Adult	2 (1.52)	2 (1.52)	2 (1.52)	2 (1.52)	2 (1.52)
	Young	0	0	0	0	0
	Old	2 (1.66)	1 (0.83)	2 (1.66)	1 (0.83)	1 (0.83)
	$\chi^2$	*	*	*	*	*
	P value	0.788	0.728	0.788	0.728	0.728
BCS	Poor	2 (2.27)	1 (1.13)	1 (1.13)	1 (1.13)	2 (2.27)
	Good	2 (1.48)	1 (0.74)	2 (1.48)	1 (0.74)	1 (0.74)
	Medium	0	1(1.75)	1 (1.75)	1 (1.75)	0
	$\chi^2$	1.2741	0.3936	0.0990	5.6984	1.9547
	P value	0.529	0.821	0.952	0.058	0.376

#### 4.4.2. Vaginal lymphocytic myocytis

In dromedary camels a hard swelling mass which grossly looks like a kind of tumor (fig 10) but was proved lymphocytic myositis on microscopic examination was observed in 2.14% of camels. However, lesion was not seen in cow. In one of the three camels this lesion was accompanied by chronic endometritis.

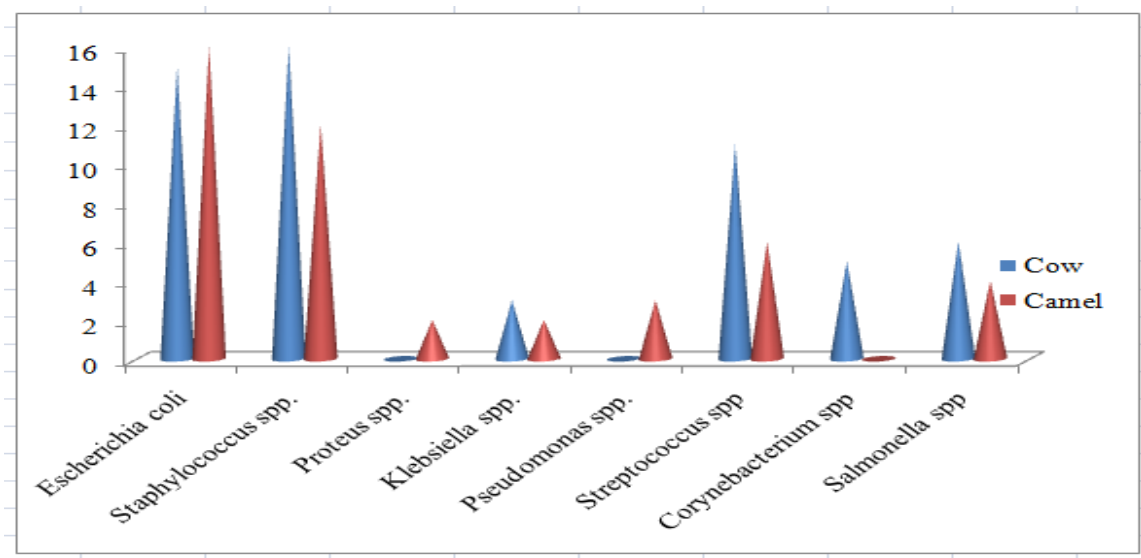
Macroscopically, in all the three camels there is single, and large sized (7-10 cm diameter) mass on the lateral side of the vagina just cranial to the lips of the vulvar commeasure and on palpation the mass was soft. Microscopically, these lesions were characterized by huge lymphocyte infiltrations into the smooth muscle fibers and some muscular degeneration. In some regions, the muscle fibers were necrotized and connective tissues were proliferated (fig 11).



**Figure 11:** Vaginal lymphocytic myositis of camel. (A) A cross section of swelling mass in the wall of the vagina (B) The tumor mass after removed from the wall (C) sloughed myocytes. H and E stain X40 (D) lymphocytes infiltration between myocyte. H and E stain X 100.

#### 4.5. Bacterial Isolates from Uterine lesions of Cows and Camels

Fifty uterine tissue samples (30 from camels and 20 from cows) with acute, chronic, catarrhal and suppurative endometritis were cultured aerobically for bacterial isolation of which 48 yielded bacterial isolates. Except for few bacteria which were isolated as pure culture, majority of the isolates were coupled with mixed bacteria. A total of 101 isolates were recovered, of which 56 were from cows and 45 from female dromedary camels. Of the 56 bacterial pathogens isolated from cows, *Staphylococcus* species 28.5%, *Streptococci* species 19.6%, *Corynebacterium* species 8.9%, *Escherichia coli* 26.78%, *Salmonella* species 10.7% and *Klebsiella* species 5.35% were the most frequent isolates, while in the dromedary camels, the most common isolates include *E. coli* 35.5%, *Staphylococcus* species 26.6 %, *Streptococcus* species 13.3%, *Pseudomonas* species 6.6 %, *Proteus* species 4.44%, *Salmonella* species 8.8% and *Klebsiella* species 4.44%. The common bacterial isolates identified from both species include *Staphylococcus* species, *Streptococcus* species, *Escherichia coli*, *Salmonella* species and *Klebsiella* species and these accounted more than 90% of isolated bacteria. Moreover, *Pseudomonas* species and *Proteus* species were isolated only from the camel uteri whereas, *Corynebacterium* species was only isolated from cows uterus.



**Figure 12:** Graphical presentation of bacteria isolated from uterine lesions.

## 5. DISCUSSION

Reproductive abnormalities play an important role in animal breeding either by causing infertility or sterility, and thus inflict heavy economic losses to the livestock owners. Many animals with reproductive problems and low milk production have been sold or sent to slaughterhouse. According to Hatipoglu *et al.* (2002) for minimization of these losses, the important disorder of genital organs and their incidence must be defined. There are no sufficient studies available concerning comparative reproductive disorders in female dromedary camels and cows to be compared with the results of the present study.

According to Dalling *et al.* (1988) camels were considered to be resistant to many disease conditions compared to other species. However, in the present study the occurrence of most reproductive disorders were similar between the two species. The current study support the report by Abbas and Agab (2002) and Gwida *et al.* (2011) who reported camels are quite as susceptible as other livestock to the common disease condition.

Unlike the report by Mshelia *et al.* (2012) who reported uterine lesion occur more commonly in cows 19.4% than dromedary camels 17.4%; the present study indicated that uterine lesions were a frequently observed lesion in dromedary camels 21.4% than cows 14.2%. The variation between studies might be attributed due to the differences in season, geographical environment, level of nutrition and health management of animals included in the study.

Among the observed reproductive lesions endometritis was the major uterine lesion observed in dromedary camels. This might be attributed to various factors one of which might be aggressive mating behavior of dromedary camel during the wrong phase of follicular development (Tibary *et al.*, 2001) Repeated insults of the uterus due to improper mating practices can also lead to inflammation and loss of the ability to resist infection (Tibary *et al.*, 2006). In addition, Ali *et al.* (2010) reported that postpartum

complications, unsanitary gynecological manipulations and errors including breeding with a young male, overuse of males and lack of verification of intromission during copulation can cause endometritis.

In present study occurrences of chronic endometritis varied within age group. Adult female dromedary camels and cows were positive to chronic endometritis than other age categories of animals this might be due to, long time exposure, increased frequency of mating, parturitions and repeated postpartum complications. Our findings were in accordance with Waheed *et al.* (2009) who reported that most findings of abnormal uterine conditions were in females more than 10 years of age.

Microscopic lesions observed in the investigated samples were those of the inflammatory uterine changes indicated by; endometrial glands degeneration, endometrial epithelial sloughing and hyperplasia of mesothelium cells in few cases, periglandular cuffing and infiltrations of inflammatory cells. These findings were similar to what was observed in earlier reports (Ahmadi *et al.*, 2005; Mshelia *et al.*, 2012 and Simenew *et al.*, 2015).

Uterine leiomyoma observed in present study was only seen in one of the examined cows. According to Sendag *et al.* (2008) the etiology of the uterine leiomyoma is not clearly known. The report of Kennedy and Miller (1993) on cellular make up of leiomyoma was in agreement with present findings in which the neoplastic cells of smooth muscle accompanied by varying quantities of connective tissue and lacks a glandular component. Also the macroscopic finding of uterine leiomyoma noted in present study was in line to the report of camel uterine leiomyoma by Shawky *et al.* (2004). Sendag *et al.* (2008); Timurkaan *et al.* (2009) and Arvind *et al.* (2012) reported microscopic findings of uterine leiomyoma that were in agreement with present reports but anatomically they report from cervical, vaginal and uterus and also that was from cows .

In the present study, the ovarian lesions of cows were almost twice than that noted in dromedary camels. This might be attributed to the high production potential of the cross breed cows that have had blood of Holstein Friesian cows that were highly selected for the production of milk. Opsomer *et al.* (2000) and Butler (2003) reported that high producing dairy cows that were exposed to negative energy balance (NEB) especially at early lactation was at risk of developing ovarian disorders. Also it is a well recognized fact that most of dairy cows in Ethiopia were owned by small holder farmers and would not be well managed.

Even though dromedary camels ordinarily are liable to develop follicular ovarian cysts in the absence of coitus given that ovulation in these species is induced by mating (Tibary and Anouassi, 1996); the current study, showed a higher frequency of follicular cysts in cows than in dromedary camels. This might be due to various influencing factors like level of milk production feeding, management and exercise affect the prevalence of cystic follicle in cattle (Herenda, 1987). Though the actual mechanism of development of cystic ovaries in dromedary camels is not completely known, the deficiency of LH surge may be considered as the main cause (Hegazy *et al.*, 2004).

The prevalence of follicular cyst varies within body condition score. Animal with good body condition develop follicular cyst at higher frequency than others. This might be due to, ovarian cysts are formed by hormonal disorder it doesn't affect animal health status. Our findings agree with earlier reports by Tibary and Anouassi (2001) who reported that camels with ovarian cysts had a general body condition fair to good. Moreover, similar finding in cattle was also reported by Abalti *et al.* (2006) for which the cysts were found in zebu cattle with body condition score of medium and fat.

Histopathological finding of follicular cysts; degeneration of surrounding theca and granulosa cells were seen in both species. This finding was comparable to previous reports of Hatipoglu *et al.* (2002), Shawky *et al.* (2004); Mahmoud *et al.* (2011) and Simenew *et al.* (2015).

The prevalence of luteal cysts in the present study was lower than that of follicular cysts in both species. This might be due the fact that luteal cysts originate from luteinization of follicular cyst, which occurred as a result of transformation of the granulosa cells into lutein cells (Shawky *et al.*, 2004). Moreover, they are often considered to be the later form of ovarian follicular cysts and therefore the causes pertaining to follicular cysts can also be considered the original causes of luteal cysts (Vanholder *et al.*, 2006). The macroscopic and histopathological finding of our study was in line with reports by Shawky *et al.* (2004) and Mahmoud *et al.* (2011).

Ovarian bursal adhesion in the present study was only seen cow this might be due to pregnancy complications. According to report by Roberts (1986) extreme adhesions have probably resulted from pregnancy complications that include retained fetal membranes and endometritis. Furthermore, this lesion can also result from hemorrhage due to harsh manipulation of the ovaries or attempts to rupture an ovulatory haemorrhagic follicle or also may be result from oophoritis and result in ovarian hydrobursitis and peritonitis (Tibary and Anouassi, 2001). The prevalence of ovarian bursal adhesion 5% in this study was in agreement with those of Abalti *et al.* (2006); Ali *et al.* (2006) and Mekibib *et al.* (2013) who reported 5.5%, 7.27% and 6.38%, respectively but it was higher than the report of Hatipoglu *et al.* (2002) 0.27%. The variation observed might be attributed to the difference in breed, management and level of nutrition.

Comparable cases of paraovarian cysts were recorded in examined female dromedary camels and cow's reproductive organs. According to Mahmoud *et al.* (2011) paraovarian cysts are suspected to arise from persistent embryonic structures which are vestiges of wolfian ducts. Paraovarian cyst does not interfere with reproductive performance until compression of the lumen of the oviduct occurs (Alam, 1984 and Peter *et al.*, 2009).

Ovarian hypoplasia was observed with low frequency in dromedary camels than examined cows. This might be the optimum breeding season in which the current study was carried out and during which most camels were cyclic. Monaco *et al.* (2015) justified that peak sexual activity of dromedary camel ranges from November to February.

Hypoplastic ovaries were characterized microscopically by complete absence of follicular or luteal development and excessive fibrous connective tissues proliferation. The gross and microscopic findings were in agreement with previous reports by Shawky *et al.* (2004) and Simenew *et al.* (2015).

In the present study oophoritis was observed only in cow with prevalence of 0.71%. This finding was in line with the report of camel oophoritis by Mahmoud *et al.* (2011) who reported 0.17%. According to Fathalla *et al.* (2000) oophoritis seems to be rare while perioophoritis is common pathological condition of bovine ovary. The prevalence of hydrobursitis in camel during this study 0.71% was approximately similar to that of Al-Afaleq *et al.* (2012) 1.95 %. However; it was lower than the report by Ali *et al.* (2011) and Mohammed *et al.* (2014) who reported 6.5 % and 4.3% respectively. The variation between studies might be attributed due to the differences in, geographical environment and health management of animals. The macroscopic finding of this lesion was in line with Mohammed *et al.* (2014). According to the report by Tibary and Anouassi (1997) the incidence of hydrobursitis was relatively higher in animals with a background of reproductive failure.

The frequency of oviduct affections in the present study was low in both species. The macroscopic finding of hemosalpinx which was characterized by thickening the wall of uterine tube due to filling with bloody discharge was in agreement with Azawi *et al.* (2008). However, the microscopic findings of this lesion were in accordance with that described by Fetaih (1991); Hatipoglu *et al.* (2002) and Shawky *et al.* (2004). In our finding pyosalpinx was accompanied with suppurative endometritis and this supports the report by Kennedy and Miller (1993) who reported pyosalpinx occurs following ascending infection. Moreover, Tibary and Anoussi (2000) considered that untreated uterine infections can lead to irreversible changes in oviducts and result in sterility.

Regarding to the detected pathological changes in the cervix, cervicitis was seen in two examined animals in both species. The low frequency of cervical affections may be due to good defense action of the mucous secreting epithelium of the cervix against bacterial

invasion (Jubb *et al.*, 1993). All observed cervicitis in the present study were associated with uterine affection or inflammation of endometrium and this finding was in line with the report by Shawky *et al.* (2004).

The pathological changes in the vagina of cows and camels were low compared to uterine and ovarian lesion in both species. This low frequency might be attributed to various factors. Among these protective effect of stratified squamous epithelium of vaginal mucosa which proliferates and matures under the influence of estrogen and become more resistant to infection is of great concern. In addition to the above factor local production of lactic acid which deposit into the epithelium is also considered (Jubb *et al.*, 1993). The relatively higher frequency of vaginal lesion in female dromedary camels 2.85% than in cow 1.42% might be due to traumatic injury during coitus especially in young females (Tibary and Anouassi, 2000). Furthermore, Ali *et al.* (2010) justified that the ethno-veterinary practiced by herdsmen using unusual substances, like dates, black seeds and salts might be irritant to the mucus membrane and leads to vaginal lesions in dromedary camels.

In the current study, it was observed that the bacteria isolated from dromedary camel's uteri were similar to those in cows. This might be associated with the camels husbandry practices in this part of the world, which allow them to graze together with other ruminants and mingling with them at watering points or market places, thereby creating conducive environment facilitating transmission of infectious pathogens circulating among livestock species cohabiting within the same ecologic zone (El-Yuguda *et al.*, 2010). The bacteria isolated from endometritis might be suspected as the main causes of endometritis in both species. This finding support the report by Tibary (2004) who reported resistance of the uterus to infection and its ability to rid itself of microorganisms was diminished in the presence of degenerative changes in the endometrium.

Although in the present study uterine infections were observed in dromedary camels and cows, the bacterial colonizing the uterine environment in cows was more than that of the *camelids*. In postpartum cow the occurrence of ovulation prior to the expulsion of

exudates and debris from the uterus has been shown to favor heavy growth of bacteria in the uterine environment which leads to the retention of the corpus luteum (CL) and consequent impairment of the ability of the uterus to secrete PGF<sub>2</sub>α (Kaneko *et al.*, 2013). Necrotized caruncles, blood and cell debris provide a perfect media for bacteria to grow during the immediate postpartum period (Sheeldon *et al.*, 2004). There is continuous bacterial clearance and recontamination of the uterine lumen for up to seven week's postpartum (Singh *et al.*, 2008); some bacteria still persist in the uterus triggering inflammatory responses and pathological changes. This delays uterine involution thereby lowering fertility (Williams *et al.*, 2005)

*Staphylococcus* species, *Streptococcus* species *Escherichia coli*, *Salmonella* species and *Klebsiella* species were the most common bacterial isolated from cow's and camels uterine lesions and accounted for 90.8% and 88.6 % of the total isolates respectively for the two animals species. These findings were similar to the report of Mshelia *et al.* (2012). Foldi *et al.* (2006) reported that most of the aforementioned bacteria attributed to most of the clinical reproductive disorders. *Proteus* species and *Pseudomonas* species were isolated only from uterine lesions of dromedary camels and *Corynebacterium* spp from that of cows'. Isolation of *Proteus* species from camel with endometritis in this study was in line with the report of Afaleq *et al.* (2012) and Simenew *et al.* (2015).

## 6. CONCLUSION AND RECOMMENDATIONS

Significant number of cows and dromedary camels slaughtered at abattoirs had one or more pathological abnormalities in reproductive organs. Uterine lesions were the major pathological disorders in dromedary camels while ovarian lesions were the most frequent in cows. It was observed that two cows with luteal cysts had acute endometritis and a camel with luteal cyst had chronic endometritis, vaginitis and cervicitis. Persistent luteal cysts can be prolonged source of progesterone which might lead to uterine infection as progesterone is immunosuppressive. The bacterial isolates might be considered important causes of uterine disorders in these animals. Also most of the pathological reproductive abnormalities detected in this study might be cause of infertility of these female animals and which brings them to be slaughtered at these abattoirs.

In line with this conclusion the following recommendations were forwarded

- Strong reproductive health managements that specially targeting the postpartum period, a critical period, should be implemented.
- Studies that involve significant number of abattoirs and female animals should be conducted to come with more figurative results.
- Studies that involve correlation between individual's reproductive lesions and hormonal disorders should be conducted to evaluate the infertility related to each lesion.
- Uterine cytobrush and uterine biopsy could be used to correlate bacterial isolates with uterine lesions.
- Trainings for concerned veterinarians and technicians on reproductive health and management should be implemented.
- Proper intervention mechanisms for the identified problem should be implemented.

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## 8. LIST OF ANNEXES

**Annex 1:** Camel age determination by dentition (Schwartz and Dioli, 1992).

Camel age is defined by changes of lower jaw teeth. Baby and permanent teeth need to be recognized correctly. Baby teeth are white, small, with a tiny teeth collar. Eruption of central deciduous incisors occurs at 10 days, laterals at 20 days and comers at 30-40 days. Three pairs of incisors and canine teeth are present by one year of age and teeth are attached together. Canines are shaped like the first incisors. All incisors are in deep wear, worn down to small irregularly shaped and loose stumps at four years. Central baby incisors are replaced by permanent incisors that look like 2 spoons have erupted. Baby 1<sup>st</sup> and 2<sup>nd</sup> molars are in deep wear as well as baby canines at five years. Second baby incisors are replaced by permanent teeth, looking like four spoons have erupted at six years. At seven years baby molars are replaced by permanent teeth; looking likes 6 spoons. Upper and lower canines are replaced by permanent.

All baby teeth are replaced by permanent teeth and central incisors and canines have erupted at eight years. At nine years central incisors are in wear. Central incisors are in progressive wear and spread into plain, shaped ellipse at ten years. Teeth at eleventh to twelve year's central and 2<sup>nd</sup> incisors are in deep wear, ellipse and 3<sup>rd</sup> incisors are in wear, separated from each other. All incisors are in deep wear, central incisors are triangular shaped and 2<sup>nd</sup> and 3<sup>rd</sup> teeth are in wear, shaped like an ellipse at fourteen to fifteen years. All incisors are worn, smooth and central incisors shaped square, 2<sup>nd</sup> incisors triangular and 3<sup>rd</sup> incisors ellipse. Separation between teeth became wider at sixteen to seventeen years. Teeth are separated from each other at fourteen to fifteen years. All teeth are worn and starts become gummy at seventy and over

## **Annex 2: Cow age determination by dentition (Puck and Soliame, 2004)**

It is possible to guess the age of animal by looking at its teeth. The time of eruption and the amount of wear are the major factors used to estimate age. The entire set of eight temporary incisors appears in the calf by one month of age. The first two central incisors are replaced with permanent teeth by two years of age. By three years the first intermediates (one of each side of the pincers) are fully developed. At four years the second set of intermediates are present. By the age of five years the animals usually has a full set of incisors with the corners fully developed. Wearing of the teeth starts to become quite noticeable by the age of five. Considerable wear is found at seven to ten years of age. By age twelve the arch in the animal's mouth has disappeared and the teeth become triangular. Progressive wearing to stubs is also quite noticeable.

## **Annex 3: Body condition scoring system (Nicholson and Butterworth, 1998).**

### **Body condition Score of 1 (very poor)**

- Individual short ribs have a thin covering of flesh.
- Bones of the chine, loin, and rump regions are prominent.
- Hook and pin bones protrude sharply, with a very thin covering of flesh and deep depressions between bones.
- Deep cavity under tail and around tail head (between pin bones)
- Bony structure protrudes sharply, and ligaments and vulva are prominent.

### **Body condition score 2 (poor)**

- Individual short ribs can be felt but are not prominent.
- Ends of ribs are sharp to the touch but have a thicker covering of flesh.
- Short ribs do not have as distinct an ‘overhanging shelf’ effect.
- Individual bone in the chine and rump regions are not visually distinct but are easily distinguished by touch.
- Hook and pin bones are prominent, but the depression between them is less severe.

- Area below tail head and between pin bones is somewhat depressed, but the bony structure has some covering of flesh.

### **Body condition scoring 3 (medium)**

- Ends of short ribs can be felt by applying slight pressure.
- Short ribs appear smooth and the overhanging shelf effect is not so noticeable.
- The backbone appears as a rounded ridge; firm pressure is necessary to feel individual bones.
- Hook and pin bones are rounded and smooth.
- Area between pin bones and around tail head appears smooth, without signs of fat deposit.

### **Body condition score 4 (good)**

- Individual short ribs are distinguishable only by firm palpation.
- Short ribs appear flat or rounded, with no overhanging shelf effect.
- Ridge formed by backbone in chine region is rounded and smooth.
- Loin and rump regions appear flat.
- Hooks are rounded and the span between them is flat.
- Area of tail head and pin bones is rounded, with evidence of fat deposit.

### **Body condition Score 5 (fat)**

- Bony structures of backbone, short ribs, and hook and pin bones are not apparent; subcutaneous fat deposit very evident.
- Tail head appears to be buried in fatty tissue.

#### **Annex 4: Histopathological procedures (Takulder, 2007)**

- Fixation of tissue by 10% neutral buffered formaldehyde
- Trimming part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).
- Tissue specimen processing: fixation of tissue by formalin, dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax.

Formalin-I 2hr → Formalin-II 2hr → 70% Alcohol 1hr → 95% Alcohol → 100% Alcohol-I 1hr → 100% Alcohol-II 2hrs → 100% Alcohol-III 2hrs → Xylene-I 1:30hrs → Xylene-II 1:30hrs → Xylene-III 1:30hrs → Paraffin-I 2hrs → Paraffin-II 3hrs.

- Embedding of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60C<sup>0</sup>) is poured and allowed to settle and solidify.
- Sectioning: sectioning of tissue in 3-5 micron thickness and put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put an incubator over night.
- Staining with H.E

#### **Hematoxyline eosine stain procedure**

- Deparaffinize slides in 2 changes of xylene for 5 minutes.
- Hydrate slides in 3 changes of 100% alcohol each for 3 minutes and 1 changes of 95% alcohol for a minute and 1 change of 70% alcohol for 3minutes
- Rinse in distilled water until ripples disappear from slides.
- Place in heamatoxyline (mayer's hematoxline) for 10-15 minutes
- Rinse in tap water until water runs clear
- Decolorize in 1% acid alcohol, 3-6 quick dips. Check differentiation microscopically: Nucleic should be distinct; cytoplasm should be uncolored.

- Rinse in tap water until ripples disappear from slides.
- Stain in eosin, 3 dips.
- Rinse in tap water until water runs clear.
- Dehydrate in 95% alcohol of 3 dips and 100% alcohol, 3 changes each for 3 minutes.
- Clear in 3 changes of xylene for 5 minutes each.
- Mount cover glass with Canada balsam.
- Examination of the prepared slides under the microscope.

**Annex 5:** Methods used to identify different bacteria (Quinn *et al.*, 2004).

**Blood agar (CM 0271, OXOID, Basingstoke, England)**

*Composition (g/l):* heart muscle, infusion from (solid) 2.0; pancreatic digest of casein 13; yeast extract 5.0; sodium chloride 5.0; agar 15.0.

*Direction:* suspend 40g powder in 1 litre of distilled water. Mix thoroughly and heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121C<sup>0</sup> for 15 minutes and cool the base to 45-50C<sup>0</sup> and add 5-7% sterile sheep blood. Colony growth on blood agar base and haemolysis formation was observed after streaking the purposed specimens.

**Mannitol Salt Agar (CM0085, OXOID, Basingstoke, England)**

*Principle:* mannitol salt agar base contains mannitol as a substrate and bromocresol purple indicator used to identify bacterium that can ferment mannitol salt.

*Procedure:* All the colonies that were collected through the necessary identification tests (catalase, O-F and coagulase test) were streaked on manitol salt agar which is selective media for members of *Staphylococci* and the bacterium were incubated at 37<sup>0</sup>C for about 24 hr. A positive result showing growth and a clear media change from red to yellow.

**Salmonella, Shigella Agar (S.S. Agar) (CM0099, OXOID, Basingstoke, England)**

*Composition (g/l):* Peptone 5.00; Lactose 10.00; Bile salts 8.50; Sodium citrate 10.00; Sodium thiosulphate 8.5; Ferric citrate 1.00; Brilliant green 0.00033; Neutral red 0.025 and bacteriological Agar 15.00.

*Preparation:* Suspend 63 grams in one liter of distilled water. Bring to the boil with frequent agitation, and allow simmering gently to dissolve the agar. Do not autoclave. Cool to about 50°C, mix, and pour into petridishes.

**Simmons Citrate Agar (M099, HIMEDIA, Mumbai, India)**

*Composition (g/l):* Ammonium dihydrogen phosphate 1.00; dipotassium phosphate 1.00; Sodium chloride 5.00; Sodium citrate 2.00; Magnesium Sulphate 0.20; bacteriological Agar 15.00; bromthymol blue 0.08. Final PH:  $6.8 \pm 0.2$  at 25°C, Distilled water 1 liter

*Preparation:* Suspend 24.28 grams of the medium in one liter of distilled water. Heat to boil until the medium dissolves completely. Dispense in tubes and sterilize in the autoclave at 121°C for 15 minutes. Cool the tubes in a slanted position so that the base is short (1-1.5 cm. deep)

**Triple Sugar Iron Agar (CM0277, OXOID, Basingstoke, England)**

*Composition (g/l):* Pancreatic digest 10.00; Disodium Phosphate 3.50; Sodium Chloride 5.00; Monopotassium Phosphate 1.50. Final pH:  $7.0 \pm 0.2$  at 25°C; Distilled water 1 liter

*Preparation:* Dissolve 20 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize at 121°C for 15 minutes.

### **Buffered peptone water (M614, HIMEDIA, Mumbai, India)**

*Composition (g/l):* Peptone Mixture 20.00; Lactose 10.00; Sucrose 10.00; Sodium Chloride 5.00; Beef Extract 3.00; Yeast Extract 3.00; Glucose 1.00; Ferrous Ammonium Citrate 0.30; Sodium thiosulphate 0.30; Phenol Red 0.024; Bacteriological Agar 12.00. Final pH:  $7.4 \pm 0.2$  at 25°C, Distilled water 1 litre

*Preparation:* Suspend 65 grams of the medium in one liter of distilled water. Bring to the boil to dissolve completely. Mix well and distribute in tubes. Sterilize by autoclaving at 121° C for 15 minutes and cool in

### **Tryptone Soya Broth (M011, HIMEDIA, Mumbai, India)**

*Composition (g/l):* Pancreatic digest of casein 17.00; enzymatic digest of soya bean 3.00; Sodium Chloride 5.00; Dipotassium hydrogen phosphate 2.50; Glucose 2.50. Final PH:  $7.3 \pm 0.2$  at 25°C, distilled water 1 litter

*Preparation:* Suspend 30 grams of the medium in one litter of distilled water. Mix well and heat slightly until complete dissolution of the medium if necessary. Dispense in tubes and sterilize by autoclaving at 121°C for 15 minutes. Larger quantities may require longer sterilization time, but the temperature should not be increased.

### **Gram staining**

*Principle:* Gram positive bacteria due to their thick peptidoglycan layer will retain the crystal violet complex even after it is subjected to decolourization with acetone or alcohol. Hence the counter stain Safranin has no action on gram positive cells. But in the case of gram negative, the thin peptidoglycan layer and more lipid contents in the cell wall will easily make them susceptible to the action of decolorizer and hence CVI complex is easily washed out and hence the gram negative cells will the colour of counter stain Safranin.

## **Procedures**

- Applying a primary stain (crystal violet) for 60 second to a heat-fixed smear of a bacterial culture. Then wash off with tap water.
- Addition of gram iodine which remain for 60 second. Then wash off with tap water.
- Rapid decolourization with ethanol or acetone for only 15-30 second.
- Then wash off with tape water.
- Counterstaining with safranin for 60 seconds.
- Then wash off with tap water and dried with blotting paper.

## **Catalase test**

*Principle:* the breakdown of 3% hydrogen peroxide in to oxygen and water is mediated by the enzyme catalase.

*Procedure:* a loop of bacterial growth is taken from nutrient agar medium. Then the bacterial culture is placed on a clean microscopic slide and a drop of 3% hydrogen peroxide is added. Organisms which produce the enzyme break down the hydrogen peroxide, and the resulting O<sub>2</sub> production produces bubbles in the reagent drop.

## **Oxidation/ Fermentation Test**

*Principle:* This test is used to determine the oxidative or fermentative metabolism of a carbohydrate by the bacterium in a semi-solid medium which contains glucose as a test sugar and bromothymol blue as a pH indicator.

*Procedure:* Take two test tubes of OF medium and heat in a beaker of boiling water immediately before use to remove the any dissolved oxygen. Cool the tubes rapidly under cold running water. Stab inoculates both tubes with the bacteria to be tested. On the top of one tube, cover with sterile paraffin oil to about 1 cm. Incubate at 37<sup>0</sup>C and examine in 24 hours and then daily for up to 14 days.

## *Result*

- Open tube Sealed tube → Unreactive Green Green,
- Oxidation → Yellow Green
- Fermentation → Yellow Yellow

## **Oxidase test**

*Principle:* Anaerobes are oxidase negative and thus can reduce the dye (tetramethyl-p-phenylene diamine dihydrochloride).

*Procedure:* A piece of filter paper is moistened in a Peri dish with 1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride. Streak the test bacterium firmly across the filter paper with a glass rod.

*Result:* positive → a dark purple colour along the streak line

## **Coagulase test**

*Principle:* pathogenic staphylococcus species reacts with coagulase reacting factor in plasma to form a complex, thrombin, and then converts fibrinogen to fibrin resulting in clotting of plasma.

*Procedure:* 0.5 ml of rabbit plasma was poured in to 10 mm test tube and equal amount of overnight grown presumptive Staphylococcus bacteria was added in the tube and mixed, then incubated at 37C<sup>0</sup>. The tests were read by slowly tilting the tube. A positive test results in a highly viscous clot formation in the plasma. Once a coagulum, no matter how small, has formed the test is considered positive (usually within 4 hours). A negative test results in the plasma remaining free flowing with no evidence of a clot, were incubated overnight before a test is called negative, but prolonged incubation (over 24 hours) may result in the dissolution of a formed clot.

## **Citrate test**

*Principle:* Citrate contains carbon. If an organism can use citrate as its only source of carbon the citrate in the media will be metabolized. Bromthymol blue is incorporated into the media as an indicator. Under alkaline conditions this indicator turns from green to blue. The utilization of citrate in the media releases alkaline bicarbonate ions that cause the media pH to increase above 7.4 causes the media blue.

*Procedure:* Take loopful of inoculum by touching the center of 3-5 representative colonies with inoculating loop and streak it onto the surface of a citrate slant. Incubate the tube aerobically at 37°C with cap left loosen for 22 hours. After 22 hrs incubation observe the tube for growth and color change.

*Result:* Positive test shows development of deep blue color or visible growth along with the streak. In some cases re-incubation is necessary for the development of a blue color.

## **Indole test**

*Principle:* Organisms those possess the enzyme tryptophanase can break down the amino acid tryptophan to indole. When indole reacts with para-dimethylaminobenzaldehyde (Kovac's reagent) a pink -colored complex is produced. Tryptophan is plentiful in most media, but growth on blood agar or chocolate agar produces the best effects.

*Procedure:* Take loopful of inoculum by touching the 3-5 representative colonies with inoculating loop from pure colonies and inoculate Tryptone soya broth tube. Incubate the tube at 37°C for 24 hours and cap left loosen to aerate the tube. After incubation, add 5-10 drops (0.5ml) of Kovac's reagent to the culture broth and agitate gently. Then observe the tube for color change within 5 minutes.

### **Urease test (Urea hydrolysis test)**

*Principle:* Organisms that possess the enzyme urease hydrolyze urea to form ammonia. The pH of the medium increases and the indicator, phenol red, turns from yellow to red.

*Procedure:* Streak the slant portion of a urea agar (Christensen's medium) slant, Cap loosely and Incubate at 35°C.

*Result:* Positive: Pink color, often starting at the slant. Rapid urea splitters such as *Proteus* produce a positive reaction in 1-2 hours. Slow splitters take 24-48 hours. Negative: the agar remains yellow

### **Triple sugar iron (TSI) test**

*Principle:* Bacteria that ferment any of the three sugars in the medium will produce by products which will change the color of the red pH-sensitive dye (phenol red). A bacterium that is a non-lactose fermenter and ferments glucose, initially causes a yellow slant/yellow bottom (acid/acid reaction) after 8 hours, but then converts to a red slant/yellow bottom after 24 hours (alkali/acid reaction). Where as if it ferments both lactose and glucose, it results in a yellow/yellow tube and remains that way due to the large amount of acid produced in the reaction.

*Procedure:* By sterile inoculating loop touching the centre of colony from isolated pure colony take loop full of inoculum. Streak the inoculum back and forth on TSI agar in tube along the surface of the slant. Incubate the tube with the cap loosened at 35 °C for 22 hours.

#### *Result*

- Yellow slant / Yellow butt (A/A)→ Lactose fermenters (E.coli, Klebsiella)
- Pink slant / Yellow butt (K/A)→Non lactose fermenters (Salmonella, Shigella).
- Pink slant / no color change (K/K)→Non fermenters
- Black color →H<sub>2</sub>S production→Proteus

## **Methyl red and Vogues Proskauer test**

*Principle:* Some organisms produce acid from the metabolism of glucose in a sufficient quantity to produce a pH of 4.4 in the media. These acids are not further metabolized and are said to be stable acids. At a pH of 4.4 or less the pH indicator methyl red is a bright cherry red. While also some organisms initially produce acid from glucose metabolism but further metabolize the acid produced to neutral end products, such as acetoin, and 2, 3-butanediol. Initially the pH may drop to 4.4 but the neutral end products raise the pH so the methyl red test will be negative. Acetoin and 2, 3-butanediol under alkaline conditions will react with alpha-naphthol (1-naphthol) to produce a mahogany red color.

*Procedure:* Take loopful of inoculum by touching the centre of 3-5 representative colonies with inoculating loop from the pure isolated colonies and inoculate MR-VP broth with inoculum, incubated 37 °C for 48 hours. Aseptically from incubated broth after 48 hrs transfer aliquot to two clean test tubes each with two ml of broth culture with sterile pipette. Add 5 drops of methyl red to one tube. The result read immediately. The tube didn't mix. Add 15 drops of Voges-proskauer reagent (5% alpha naphthanol) shake it and follow adding of 5 drops of Voges-proskauer reagent B (40% KOH) to the other tube containing transferred broth and shake the tube gently to aerate. Then observe tube for appearance of red color within 20 minutes.

### **Result**

MR → cherry red color,

VP → red color at the top of the medium