# PATHOLOGICAL CHANGES IN THE REPRODUCTIVE ORGANS, LESION CHARACTERIZATION AND BACTERIAL ISOLATION FROM COWS SLAUGHTERED AT ADDIS ABABA AND ADAMA MUNICIPALITY ABATTOIRS

MSc THESIS

ADDIS ABABA UNIVERSITY, COLLEGE OF AGRICULTURE, DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

MSC IN TROPICAL VETERINARY PATHOLOGY

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JUNE, 2016

BISHOFTU, ETHIOPIA

# PATHOLOGICAL CHANGES IN THE REPRODUCTIVE ORGANS, LESION CHARACTERIZATION AND BACTERIAL ISOLATION FROM COWS SLAUGHTERED AT ADDIS ABABA AND ADAMA MUNICIPALITY ABATTOIRS

A Thesis submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University, in partial fulfilment of the requirements for the degree of Masters of Since in Veterinary Pathology

By:

Banteyegegn Tamirat

June, 2016

Bishoftu, Ethiopia

## DEDICATION

I dedicate this thesis manuscript for Fidel Castro and the Cuban people for helping me starting from elementary up to culmination of first degree and enter the sphere of science.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AI | Artificial insemination |
| BCS | Body condition score |
| BVD | Bovine viral diarrhea |
| CL | Corpus luteum |
| COD | Cystic ovarian disease |
| COF | Cystic ovarian follicles |
| CSA | Central statics Agency |
| DIM- | Days in milk |
| H.E | Hemathoxilline-Eosin |
| LH | Luteinizing hormone |
| NEB | Negative energy balance |
| OEC | Oviduct epithelial cell |
| PCL | Persistent Corpus luteum |
| PD | Pregnancy diagnosis |
| PGF2 | Prostaglandin $F_{2\alpha}$ |
| PID | Pelvic inflammatory disease |
| TSI | Triple sugar Iron |
| VMTH | Veterinary medicine teaching hospital |

**STETMENT OF AUTHOUR**

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 fulfilment of the requirement for an MSc degree at Addis Ababa University, College of veterinary medicine and agriculture and in 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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Date of submission: June 16

**ABSTRACT**

A cross-sectional study was conducted on reproductive organs of cows slaughtered in Addis Ababa and Adama municipality abattoir with objective of characterize the pathological lesions and isolate and identify possible aerobic bacteria from lesion. Out of the 209 genital tracts examined 12.9% (n=27) were pregnant and excluded from study. From total 182 reproductive organs examined 55 (30.2%) have one or more visible gross lesions. The most common abnormalities encountered were follicular cysts (2.20%), Luteal cysts (1.6 %,) Par ovarian cysts (3.3%), ovarian hypoplasia (2.2%), ovarian bursa adhesion (2.7 %), ovarian hemorige (1.6%), endometritis (18.7%), Pyometra (2.2%), Mucometra (1.1%) and Hydrometra (1.6%) with endometritis being the most frequent abnormality. Age, breeds, body conditions (BCS) and origin of animals were not statistically associated with the disorders (P>0.05). Endometritis were the major disorder recorded in this studies accounting for 34 (18.7 %) and all 34 endometritic ureine tissue were positive either single and/or mixed bacteria. *E. coli* 17 (50%), *S.aureus* 18(52.9%), S. *hicus* 1(2.9%) *S. intermedius* 2(5.9%), *C.N.S.* 12(35.3%), *Streptococcus spp.* 24 (70.6%) *Proteus bulgaris spp* 5(14.7%), and *C. fundi* 1(2.9%) were the bacteria isolated. It could be concluded that reproductive tract abnormalities were important diseases in the study areas which could have considerable impact on the reproductive performance of cows and in fact could be the sole reason for coming of these cows to abattoir. Another thing that worth mentioning was that significant (12.9%) of number of pregnant dairy cows were slaughtered which in fact should not have been happened.

***Key words**: Abattoir, cow, endometritis, Ethiopia, lesion, reproductive disorder*

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of –pathology and parasitology

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TITLE: PATHOLOGICAL CHANGES IN THE REPRODUCTIVE ORGANS, LESION CHARACTERIZETION AND BACTERIAL ISOLATION FROM COWS SLAUGHTERD AT ADDIS ABEBA AND ADAMA MUNICIPALITY ABATTOIRS.

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As members of the Examining Board of the final MSc open defence, we certify that we have read and evaluated the Dissertation prepared by Banteyegegn Tamirat PATHOLOGICAL CHANGES IN REPRODUCTIVE ORGANS, LESION CHARACTERIZATION AND BACTERIAL ISOLATION FROM COWS SLAUGHTERED AT ADDIS ABABA AND ADMA MUNICIPALITY ABATTOIRS and recommend that it be accepted as fulfilling the thesis/dissertation requirement for the MSc degree of pathology.

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## 1. INTRODUCTION

Livestock is an integral part of agriculture, and cattle provides important source of animal protein, Most of the third world countries are located in the tropics and production of livestock resources is very important to their economies (Assey *et al.,* 1998). The overall cost of keeping cattle in terms of costs associated with the health care, nutrition and reproduction management, however, has not matched to their contribution to the livelihood and the economy of the people in the region. As in many countries, livestock, particularly cattle play multiple roles in Ethiopia being a source of milk, meat and hide (Mekonnen *et al*., 1989). However, the animal productivity is found to be lower than it should be (Assey *et al.,* 1998).

The total cattle population of Ethiopia is estimated to be 55 million, out of this total cattle population, the female cattle constitute about 55.2 percent (CSA, 2010). Diseases and abnormalities of the female genital tract are believed to be the major reason for economic loss associated with infertility, culling and slaughtering of cows (Singleton and Dobson, 1995; Ashenafi, 2004; Abalti *et al.,* 2006).

As female cattle are source of milk and future herd expansion, their slaughter due to adverse economic problems, reduced reproductive efficiency or when they have disease require attention (Thrusfield, 1995). Therefore slaughter houses have been used as the preferred source for studying the pathological lesions of female reproductive tracts of cattle, which determine the causes of infertility.

Since most reproductive tract problems lack additional outward manifestation, hence, examination of gross and microscopic lesions of genital tract play a central role in the identification of these problems. Most of these abnormalities can only be diagnosed when the animal is subjected to post-mortem examination (Buregelt, 1997). Though, in different regions of Ethiopia, studies have been conducted on reproductive abnormalities of cows based on abattoir material; in Addis Ababa (Gebrekidan *et al.,* 2009), in Sululta (Simenew *et al*., 2011), in Tigray (Zerihun, 2001), in Jimma (Amare, 2002), in Nekemte (Samuel, 2002) in Asela

(Endalew, 2001), in Bahir Dar (Abalti *et al.,* 2006) and in Awassa (Ashenafi, 2004). However, more systematic work has to be done to assess the problem in depth.

Fertility is a very complex process and the final outcome is the result of a close and well-orchestrated interaction between hypothalamus, pituitary ovary and uterus. The complexity of this process indicates that any factors which interfere with the functioning of one or more of the organs involved will also influence the overall fertility outcome. The reduced fertility, observed in modern high yielding dairy female cattle, is most likely due to alterations at several consecutive steps in the reproductive process (Lucy, 2001).

One calf per year is the reproductive objective in dairy cattle production. It means that cows must get pregnant after AI, maintain the pregnancy, have parturition after 270 days later and wait for a period of 40-50 days to be successfully inseminated again. Nevertheless, this is not always attained and cows must be inseminated during several consecutive cycles (Bage *et al*., 2002).

Pathological conditions of ovary seriously interfere with normal functions of the entire reproductive tract, consequently, influencing the reproductive potential of the animal. Cystic ovarian disease (COD) occurs in over one million dairy cows per year based on the 1997 Agricultural Census (Garverick, 1999).

There are several types of cysts that can be found on the ovaries of the female cattle, which can have a significant impact on the reproductive efficiency of the animal. The cystic structures that were studied included follicular cysts, luteal cysts, and cystic corpora luteal. (Lacey and Rosenberg, 2010).

Cystic ovarian follicles (COF) are an important cause of sub fertility in dairy cattle, since they extend the calving interval (Fourichon *et al*., 2000). Treatment costs of COF result in economic loss for the dairy farmer. In most of the literature, COF are referred to as Cystic Ovarian Disease (COD).

Postpartum uterine infections results from uterine contamination with bacteria during parturition. The prevalence of uterine infections varies considerably among studies. Uterine 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.

The development of uterine disease depends on the immune response of the cow, as well as the species and number (load or challenge) of bacteria. The postpartum uterus has a disrupted surface epithelium in contact with fluid and tissue debris that can support bacterial growth. A variety of species of bacteria, both Gram-positive and Gram-negative aerobes and anaerobes, can be isolated from the early postpartum uterus. Most of these are environmental contaminants that are gradually eliminated during the first 6 weeks postpartum. A normal postpartum cow resolves uterine infection by rapid involution of the uterus and cervix, discharge of uterine content, and mobilization of natural host defences, including mucus, antibodies and phagocytic cells. Clinical signs of uterine infection vary with the virulence of the causative organisms and the presence of factors that predispose to the disease (Azawi, 2008).

The most common and economically important bacteria for uterine infection are *Actinomyces* spp., *Escherichia coli, Fusobacterium* spp., *Pasteurella* spp*., Pseudomonas* spp., *Streptococcus*. And *Staphylococcus* spp (Erin *et al.,* 2005). The Uterine diseases can be classified as metritis, clinical Endometritis and subclinical Endometritis (Sheldon *et al.,* 2006).

These diseases are highly prevalent in high producing dairy cows and have been associated with decreased pregnancy per AI, extended interval to pregnancy, increased culling, and economic losses ( Sheldon and Dobson, 2004; Gilbert *et al.,* 2005). Metritis affects about 20.0% of lactating dairy cows, with the incidence ranging from 8 to > 40% in some farms (Galvão *et al.,* 2009; Hammon *et al.,* 2006; Huzzey *et al*., 2007). Clinical Endometritis also affects about 20.0% of lactating dairy cows, with the prevalence ranging from 5.0 to >30% in some herds (Galvão *et al.,* 2009; LeBlanc *et al.*,2002; McDougall *et al*., 2007).

Subclinical Endometritis is the most prevalent of all uterine diseases; it affects ~ 30% of lactating dairy Cows, with the prevalence ranging from 11 to >70% in some herds (Barlund *et al*., 2008; Galvão *et al.,* 2009; Gilbert *et al*., 2005; Hammon *et al*., 2006; Kasimanickam *et al*., 2004).

Pathological Studies on reproductive organs of Zebu Cow on intact reproductive organs of 110 cows of non-descriptive breeds and ages revealed 7.27% ovarian adhesions, 1.81% Par ovarian cysts, 0.9% Cystic corpus luteum, 1.81% Teratomas, 2.72% Cystic ovaries and 6.36% uterine pyometra (Ali *et al*., 2006; Kunbhar *et al.,* 2003).

Most of the work so far done on reproductive tract pathology of dairy cows in Ethiopia were based on gross lesions, and similar trends were exist in the present study area. Thus still there were a gap on histopathologic lesions characterization and bacteria isolation from these lesions.

Therefore, the objectives of this study were:

- Investigate reproductive pathologic disorders of dairy cows with gross and microscopic lesions characterization.
- To isolate and identify aerobic bacteria from lesions of reproductive organs disorders.

## 2. LITERETUR REVIEW

### 2.1. Anatomy and Physiology of Reproductive Organ of Female Cattle

A good understanding of anatomy and physiology of female cattle is helpful in successfully managing reproduction. Successful reproduction on modern dairy farms requires an understanding of reproductive processes of the dairy cow and a working knowledge of the anatomy or parts of a cow's reproductive tract. The uterus, oviducts, and ovaries are attached to a ligament and suspended in the pelvic area of female cattle. This suspension allows these organs to move freely in the pelvic canal and into the body cavity, providing space to accommodate a growing fetal calf (Rich and Turman, 2014).

The ovary is the primary reproductive organ of the female. In a dairy female cattle, each ovary is approximately 1.5 inches long and 3 -4 inch in diameter .The ovaries are suspended from the broad ligament near the end of the oviduct and lien ear the tips of the curved uterine horns. It is responsible for two basic functions production of the female gamete, the egg by a process called ovogenesis and production of two primary reproductive hormones, estrogen and progesterone. The ovary contains several thousand tiny structures called primary follicles. Each follicle consists of a germ cell surrounded by a layer of cells. This germ cell has the potential to mature into an egg if the follicle completes development (Rich and Turman, 2014).

The oviducts of the bovine are fairly simple organs, whose wall is made up of three layers consisting of an outer connective tissue layer, a middle muscular layer and an inner mucosal layer. The inner mucosal layer consists of the lamina propria and the epithelium (Yaniz *et al.*, 2000). The lamina propria is built of loose connective tissue while the epithelium is a simple columnar epithelium, containing two types of cells, secretory and ciliated (Abe & Oikawa, 1993).

The oviducts play an important role before fertilization, transporting the oocyte from the ovulated follicle in the ovary through the infundibulum and the ampulla to the site of fertilization. Also, the oviducts are implicated in events such as sperm transport, storage

and capacitation, all events that require preservation of the motility, viability and fertilizing ability of spermatozoa (Parrish *et al*., 1989b; Lefebvre *et al*., 1995).

In most mammals it is divided anatomically into three parts: 1) the uterus tubal junction, that connects the oviduct to the uterus; 2) the isthmus, the region associated with the storage of spermatozoa before ovulation and where spermatozoa bind to the oviduct epithelial cells (OEC) on their way to meet the oocyte and;3) the ampulla ,where fertilization takes place Spermatozoa from most mammals can reside in the oviduct from a few hours up to a maximum of 5-7 days (Hunter, 2005). By contrast, the embryo spends only a few days (2-5) in the oviduct, which also varies depending on the species, in cows 2-4days (Crisman *et al.,* 1980).

The uterus consists of a body and two horns it is attached to the broad ligament and suspended within the pelvic cavity and posterior portion of the body cavity. The body of the uterus is adjacent to the cervix. In a non-pregnant state, it extends less than 2 inches before it divides into two separate horns. The uterine body is the major site of semen deposition during AI. If the tip of the inseminating rod is inserted too far into the uterus, semen is deposited in only one of the uterine horns. The uterus has many functions its walls are composed of several layers of muscle which aid in transport of sperm to the oviduct following insemination and in expulsion of the calf at birth. Certain glands within the walls of the uterus secrete fluid, uterine milk, which provides nutrients to the developing embryo before and after its attachment to the uterine (Rich and Turman, 2014).

The cervix is a thick walled organ forming a connection between the vagina and uterus which softens and relaxes to allow a passage way for sperm at mating and expulsion of the fetus at the time of birth. During pregnancy, the cervix is filled with a thick mucus secretion known as the cervical plug, which protects the uterus from infections entering from the vagina. The cervical plug is expelled and the cervical opening begins to dilate in the days prior to calving (Rich and Turman, 2014).

The vagina serves as a receptacle for the male's penis during service. In the cow, the semen is deposited in the vagina near the cervix during natural mating with the bull. When artificial insemination is used, an insemination instrument is threaded through the vagina and cervix and semen is deposited at the uterine side of the cervix. Urine is discharged

from the urinary bladder through the urethra, which opens into the base of the vagina. The region behind the urethral opening is called the vestibule and is a common passageway for both the urinary and reproductive systems. The external opening of the vagina is called the vulva (Rich and Turman, 2014).

## 2.2. Major Reproductive Organs Disorders

### *2.2.1 Ovarian disorders*

Investigations of ovarian disorders of the female cattle were initially stimulated for rather different reasons. In food animals such as cattle, disorders of the ovary become economically significant when they adversely affect fertility and fecundity in these species. The disorders that affect significant numbers of female cattle's in a herd are particularly important (Youngquist and Threlfall, 2007).

#### 2.2.1.1 Ovarian cyst

In the past years ovarian cyst were defined as fluid filled or hard structures of 2.5 cm or more in diameter persisting on the ovarian surface for 10 or more days. Over time new definitions have been suggested, there is still lack of consensus and clearly defined definition (Peter, 2004).

The ovarian Cyst is an important ovarian dysfunction and one of the major causes of reproductive problems in the dairy cattle. It is characterized mainly by anoestrous which takes to a lengthening of the reproductive intervals. The cysts are developed when a flaw happens in the ovulation and the follicles increase in size, beyond the ovulatory diameter and persist in the ovary interrupting the normal estral cycles. It is generally accepted that cystic follicles develop due to a dysfunction of the hypothalamic-pituitary-ovarian axis. This dysfunction has a multi factorial exogenous and endogenous etiology in which genetic, phenotypic and environmental factors are involved (Peter, 2004).

The most widely accepted hypothesis explaining the formation of a cyst is that LH release from the hypothalamus-pituitary is altered: the pre-ovulatory LH-surge is either absent,

insufficient in magnitude or occurs at the wrong time during dominant follicle maturation, which leads to cyst formation (Hamilton *et al*., 1995). This aberrant LH release does not seem to be caused by a lower GnRH content of the hypothalamus, nor by reduced GnRH receptor numbers or LH content in the pituitary. Cystic ovarian degeneration is misleading in that the ovary containing the cyst has not degenerated. In fact, both ovaries are still capable of supporting follicular development in the presence of a cyst (Hamilton *et al*., 1995**).**

There are three types of cysts that occur on the bovine ovary: follicular cysts, luteal cysts and cystic corpora luteal (CL). Follicular and luteal cysts are the only two true types of cysts that are associated with an abnormal condition in female cattle. Cystic CL are considered to be no pathological (Kesler and Garverick, 1982; Garverick, 1999).

Follicular and luteal cysts vary in size and their effects also vary according to their number and degree of luteinisation. Many unluteinised follicles tend to lead to nymphomania with frequent, irregular heats, whereas female cattle with a few extensively luteinised cysts may become anoestrous. The presentation and clinical problems are very different in one case, there is too much estrogen (follicular cysts) and in the other case there is too much progesterone (cystic CL). Female cattle with long-term cysts may show virilism. In addition to the pathologic follicular and luteal cysts, there are the non-pathologic cystic corpora luteal. These are normal structures that follow a normal ovulation but have a fluid-filled central cavity 7-10 mm in diameter. On rectal palpation they feel like normal corpora luteal but more fluctuant and soft. They do not alter the oestrous cycle duration and when conception occurs, it can be maintained to term (Garverick, 1999**)**.

Follicular cysts

Follicular Cysts are follicular structures of 2.5 cm or larger that persist for a variable period in the absence of a corpus luteum" (Youngquist and Threlfall, 2007). Follicular cysts present on one or both ovaries in the absence of any luteal tissue and that clearly interfere with normal ovarian cycle.

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

steroid genesis (Vanholder *et al*., 2006). It has also been determined that follicular cysts are an ovulatory structures so, as long as they persist, cows will remain infertile (Youngquist and Threlfall, 2007). Limited evidence exists female cattle's can even become pregnant when they have follicular cysts (Assey and Colleagues, 1997).

Using data collected in the abattoir, reported that a pregnant East African zebu cow had multiple ovarian cysts on both ovaries and a single CL. The incidence is believed to vary from 1 to 30% depending on herd and breed conditions and Holsteins are the most susceptible to develop a cystic condition compared to other breeds, and the most likely time of diagnosis is 30-60 days after parturition in high-yielding dairy cows (Gordon, 1996).

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 cows will remain in anestrous as long as the condition persists (Ball and Peters, 2004).

A high milk yield may promote the development of COF through the metabolic adaptations that occur to sustain the animal's high level of production. During the early postpartum period, the high yielding dairy cow has an energy deficit (negative energy balance (NEB)) due to the imbalance between energy intake through feed and energy expenditure through milk yield. This NEB is a risk factor for ovarian dysfunctions (Opsomer *et al*., 2000), 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 size, they are normally as large as somewhere between that of small graafian follicules. 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 of the ovary such degeneration is observed mainly in the cortex of the ovary (Lacey and Rosenberg, 2010).

Luteal cyst

The luteal cysts had been described as enlargement of ovaries with one or more cysts, which is characterized by thicker walls than those of follicular cysts, because of a lining of luteal tissue (Ball and Peters, 2004). Call these cysts luteinized cystic follicles, describing them as cysts with thicker walls that produce high levels of progesterone. In appearance, 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). On ultrasonography, they have a gray rim or "gray echogenic patches along the inner cyst wall or within the Antrim of the cyst" Luteal cysts are considered an ovulatory cysts and are associated with infertility and Mucometra in cattle" (Foley, 1996). 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 (Ball and Peters, 2004).

They are also often considered to be the later form of ovarian follicular cysts (Vanholder *et al*., 2006). Therefore the causes pertaining to follicular cysts can also be considered the original causes of luteal cysts. The luteal cyst occurs when the cells of the follicular cyst Granulosa and theca become luteinized and start producing progesterone (Peter *et al*., 2009). Luteal cyst incidence increases with age and most often affects female cattle's with high milk production (Foley, 1996).

Corpus luteum

Occasionally the corpus luteum does not regress normally even though the animal is not pregnant. This is considered a persistent corpus luteum (PCL). The persistent CL continues to produce progesterone to prevent further follicular development, estrus and ovulation. The maintenance of CL is the result of precise inter-action between pituitary and embryonic gonadotropins, as well as intra luteal autocrine and paracrine signals that modulate the endocrine function of luteal cells (Tomac, 2011). The maintenance of CL in the absence of pregnancy may originate because of metritis (Struve, 2013) 'and similar effects are possible with pyometra (Lashari and Tasawar, 2012), and late embryonic mortality, (Russo *et al*., 2010).

Par ovarian Cysts

Par ovarian cysts are cystic structures that do not occur in the ovaries themselves, but rather in the broad ligament close to the ovaries and the uterine tubes. Palpation or ultrasonography can be used to detect them, 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). There are two different types of par ovarian cysts; those derived from the cranial mesonephric tubules are called epophoron, while those from the caudal tubules are referred to as paroophoron. All par ovarian cysts are benign, with no negative effects on reproduction and fertility, (Peter *et al.,* 2009).

2.2.1.2. Ovarian hypoplasia

Ovarian Hypoplasia The hypo plastic ovary is essentially an underdeveloped ovary that does not function properly. The condition is characterized by incomplete development, or ovarian digenesis, so that the ovary is lacking in primordial follicles. Hypoplasia can occur partially or completely, on one or both ovaries. "In heifers, hypo plastic ovaries may be small such that they are difficult to locate by Trans rectal palpation. The ovaries may feel like thin, narrow, firm cord-like structures" (Peter *et al*., 2009). This condition of the ovary is considered to be due to the failure of migration of primordial germ cells from the yolk sack to the developing gonad during embryonic stage.

2.2.1.3. Ovary-bursa Adhesions

Ovary -bursa adhesions are structures that occur as fibrous bands between the surface of the ovary and the ovarian bursa. Their severity varies from the presence of a few very small strands of fibrous tissue". Though there is no definitive cause of adhesions, they are most likely the result of excessive follicular haemorrhaging 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).

Ovary -bursa adhesions generally do not cause reproductive problems in affected female cattle's, unless, in severe cases, where the adhesion is so large that the fallopian tubes are blocked and fertilization of the ovum is prevented. Additionally, in extreme cases, adhesions may extend to the opening of the ovarian bursa, resulting in a very narrow opening that may affect fertility. (Peter *et al.,* 2009**).**

2.2.1.4. Ovarian haemorrhage

The ovarian hemorige is that which follows manual inoculation of corpora Luteal in cattle. The blood loss may vary from 0.5 litre to several litres and Couse death. This haemorrhagic is more profuse in pregnant cows and those cows with pyometra than in normal cycling cattle. Relatively little hemorige follows manual rapture of follicular cysts especially if they are thin walled. In some cases organisation of the clot leads to fibrous bursal adhesion which may interfere with the function of the ovary and oviduct (Jubb K.V.F 1991).

2.2.1.5. Tumours

Female cattle genital tract tumours will be treated in each of the known anatomical segments: ovarian tumours; oviduct tumours; uterine tumours; cervical tumours; and vaginal and vulvar tumours. Ovarian tumours are relatively frequent in animals, especially in female cattle, while the tumours of the other anatomical segments of the female genital system oviduct, uterus, cervix, vagina and vulva have a significantly lower incidence.
In cows ovarian tumours are usually bilateral and are derived from the gonadal stroma, being of granular cell type. (Kennedy and Miller, 1993). The majority of authors have adopted a simple, practical classification, correlated with the tumour evolution based on the histological structure of the ovary, taking into consideration the pluri potency of cell components and their histogenesis. Classification of primary ovarian tumours assumes that these tumours arise from one of three ovarian components: epithelium, either of the ovarian surface, rate ovarian; germ cells or; ovarian stroma, including the sex cords, which probably contribute cells to ovarian follicles and thus to the endocrine apparatus of the ovary (Moulton, 1978).

Epithelia tumours

Epithelial tumours of the ovary are usually cystic and papillary, thus, the names cyst adenoma and cyst adenocarcinoma are frequently used. Clear or yellow/brown fluid is

present in many cysts. Solid areas are also present in most tumours. Histological, these tumours consist of arboriform papillae that project into the cyst lumen. The papillae consist of a connective tissue stalk that is lined by single or multiple layers of cuboidal or columnar epithelial cells that may or may not be ciliated. The wall of the cyst typically is also lined by epithelium, and the lumen of the cyst may contain proteinaceous material (Nielson *et al*., 1976).

Granulose- Cell Tumours

Granulose-theca cell tumours are made of both type of follicular cells, and are the most common variety of ovarian tumours in cattle, even though they are rare (P<0.5%). This type of tumour arises from the sex cord stromal tissue within the ovary and may be relatively small, solid, and yellow to white or large, filled with cysts of varying sizes and weight 11.9-12.3 kg, granulosa-theca cell tumours are most commonly benign and surgical removal of the affected ovary is generally curative but may be malignant and often hormonally active. (Peter *et al.,* 2009).

Thecoma or theca cell tumour

Exclusively composed of theca cells, which are fusiform well delimited, arranged in bundles. Nuclei are ovoid, elongated or fusiform; the cytoplasm is pale, with lipid drops. Usually, Thecoma is a benign tumour with expansive growth, without metastases (Nielsen *et al., 1976*).

2.2.1.6. Opharitis and perioophoritis

Inflammation of the ovaries and surrounding structures is known as Opharitis and perioophoritis, respectively. The most common pathological condition of female cattle ovary is perioophoritis while Oophoritis seems to be rare. Trauma caused by improper manipulations during palpation, forced attempts to enucleate corpus luteum or to manually rupture cystic ovaries, although infections from the uterus and infectious diseases like tuberculosis and brucellosis might also be involved (Kunbhr and Samo, 2003).

*2.2.2. Disorders of falopian tube*

The Fallopian tubes are amazing because they not only collect the eggs from the surface of the ovary but they provide a hook-up spot for eggs and sperm that is simply perfect for fertilization. They provide the early nursery for the new embryo for the first five days of its life, while gently transporting it to the waiting uterus for implantation. The fallopian tube is not just a passive pipe or a conduit, but an active organ with its separate locations performing separate functions. Starting from the ovarian end (fimbria) and proceeding toward the uterus. (Madhu *et al*., 2012).

The incidence of pathological conditions of different segments of female cattle genitalia has been reported to widely vary and tubal affections are opined to be more common in buffaloes than in cows (Alam, 1984). Grossly, the fallopian tubes were found distended, elongated and tortuous forming many coils in the mesosalpinx Histologically, the wall was thin, translucent, and distended with large amount of clear fluid .the Ampullary region was more affected (Khasatiya, 1997).

2.2.2.1. Salpingaitis

Salpingitis is inflammation of the fallopian tubes which may be for some infectious cause Pelvic inflammatory disease (PID) or diseases of the female upper genital tract include disease like Salpingitis Endometritis, Opharitis, myometritis, parametritis and infection in the pelvic peritoneum. In contrast, Salpingitis only refers to infection and inflammation of the fallopian tubes in animals (Singh, 2009).

Salpingitis 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 propia (Hatipoglu *et al.,* 2002). Grossly, the fallopian tubes were found distended, elongated and tortuous forming many coils in the mesosalpinx Histologically, the wall was thin, translucent, and distended with large amount of clear fluid .The Ampullary region was more affected (Khasatiya, 1997).

2.2.2.2. Hydrosalpinix

The hydrosalpinx is an affection in which the fallopian tube is filled with inflammatory fluid and is the end result of pelvic infection. This abnormalities is caused by tubal infection such as pelvic inflammatory disease (PID). In this infection the tubes become inflamed, which even after treatment may be blocked due to presence of residual fluid inside. Continued fluid build-up over time dilates the tube more, resulting in hydro-salpinx of various sizes. Grossly, the fallopian tubes were found distended, elongated and tortuous forming many coils in the mesosalpinx. Histologically, the wall was thin, translucent, and distended with large amount of clear fluid .the Ampullary region was more affected. 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 (Madhu *et al*, 2012).

2.2.2.3. Pyosalpinx

A 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. Pyosalpinx is a consequence of pelvic inflammatory diseases (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**)**.

*2.2.3. Disorders of uterus*

Uterine diseases are important because they cause infertility, abortion, pre-term labour and clinical disease (MOR and Cardenas, 2010). The incidence of uterine disease varies across individual herds, but on average, 20–40% of dairy cattle's develop acute clinical uterine disease within a week of calving that persists in 20% of animals as Endometritis, and approximately 30% of cows suffer from subclinical Endometritis (Sheldon *et al.,* 2009). In

addition, clinical Endometritis is shown to persist beyond 60 days post-partum in approximately 25% cows that previously had purulent cervico-vaginal discharge and occurs in over 10% of animals who were previously found to be healthy (Gautam *et al.,* 2010).

Purulent or fetid secretions manually obtained from the vagina are usually considered to be the most important symptoms of postpartum uterine inflammation. Accordingly, these symptoms were considered as key in the diagnosis of the uterine condition in our trial. Post-partum uterine diseases of animals are also of considerable economic importance. 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).

2.2.3.1. Metritis

Metritis (puerperal Metritis) is inflammation of the uterus 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). It occurs only in the first two weeks after calving, primarily in the first 7 days. Contamination of the uterus with potentially pathogenic bacteria occurs after calving in almost all cattle, so the development of Metritis depends largely on immune function in the early postpartum period (Hammon *et al.,* 2006).

Among female cattle's with Metritis, *E. coli* and a variety of anaerobic bacteria are common isolates. The largest risk factor for Metritis is retained placenta, but other conditions that may impair feed intake (Urton *et al.,* 2005) Grossly Metritis is characterized by the presence of an abnormally enlarged uterus, a fetid watery red-brownish uterine discharge associated with signs of systemic illness, and fever (>103) within 21 days in milk Animals without systemic signs but with an enlarged uterus and a purulent uterine discharge within 21 DIM may be Metritis classified as having clinical Endometritis and subclinical Endometritis (Sheldon *et al.,* 2006).

2.2.3.2. Clinical Endometritis

Clinical Endometritis is characterized by the presence of purulent (>50% pus) uterine discharge detectable in the vagina 21 days or more after parturition, or mucopurulent

(approximately 50% pus, 50% mucus) discharge detectable in the vagina after 26 days or a cervical diameter >7.5 cm after 20 days in milk (DIM) (LeBlanc *et al*., 2002).

The most substantial effects of this disease are an increase in the number of days to conception, increased services per conception and an increased risk of culling (LeBlanc *et al.,* 2002).

2.2.3.3. Sub clinical Endometritis

Subclinical Endometritis is defined by >18% Neutrophils in uterine cytology samples collected 21–33 days after calving, or >10% Neutrophils at 34–47 days. Subclinical Endometritis has been defined as the presence of inflammatory cells within the uterine lumen but without signs of clinical Endometritis (Foldi *et al.,* 2006).

2.2.3.4. Pyometra

It 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). 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. Ultrasound graphically 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.,* 2006b).

2.2.3.5. Hydrometra and Mucometra

Hydrometra and Mucometra, 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. In bovines Hydrometra or Mucometra develops in untreated cases of cystic ovarian degeneration, hydrometric uterus containing gallons fluid associated retained corpus luteum was in cows reported several researchers in the past. Administration of prostaglandin (PGF2 alpha) causes luteolysis and expulsion off 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*

The affection of cervix included congenital abnormalities, double cervix, double external os with a band of tissue situated dorso-ventrally at the external os uteri, and complete absence of cervix in female cattle, Cervical stenosis and incomplete closure and bending of cervix were also found but was rare. Cervical cysts of varying size and shape are reported in female cattle generally the cysts were found at the external orifice of the cervix having inspissated cervical mucus, (Kumarsan and Ansari, 2002).

### *2.2.5. Disorder of Vulvovagina*

Infectious pustular Vulvovaginitis of cows is caused by bovine herpes virus and is transmitted by natural service, nasogenital contact, or mechanically by insects such as flies. It is characterized by vaginal lesions. Affected cows show signs of vaginal discomfort (raised tail, frequent urination) and have numerous, round, white, raised lesions of the vestibular mucosa. Within a short time, these lesions progress to pustules and erosions or ulcers. Mucopurulent discharge may be prominent, even in pregnant animals in which pregnancy is uninterrupted. The histologic lesion consists of necrosis of vestibular and vaginal epithelium, with intranuclear inclusion bodies typical of herpes virus infection. The virus may be secreted in the semen of infected bulls (which have similar lesions of the penis and prepuce). Intrauterine inoculation of the virus produces necrotizing Endometritis and cervicitis (Robert and Gilbert, 2014).

## 2.3. Bacterial Infection of the Uterus

In pregnant cows, the 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). Therefore, bacterial contamination of the uterus postpartum is common. It has been demonstrated that 33% of dairy cows had positive bacterial cultures during the first week after calving and by the second week. Number of positive cases had increased to 44% (Fredriksson et al., 1985). More recent work, perhaps using more sensitive culturing techniques, has shown that the number of dairy cows with a uterine infection during the first 2 weeks postpartum is almost 80 to 100%, despite an uneventful calving (Sheldon et al., 2004; Foldi et al., 2006).

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 cows, conditions favoring bacterial growth persist and eventually cause Endometritis (Sheldon *et al.,* 2004).

One should differentiate between uterine contamination and uterine infection. The uterus of postpartum female cattle is usually contaminated with a range of bacteria, but this is not consistently associated with clinical disease. 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.

Normal postpartum female cattle resolves uterine infection by rapid involution of the uterus and cervix, discharge of uterine content, and mobilization of natural host defenses, including mucus, antibodies and phagocytic cells (Hussain and Daniel, 1992). Metabolic disorders, some traditional practice by farmers and herd semen by inserting the hand or implements in the vagina of female cattle to stimulate milk let down, as well as, unhygienic conditions under which animals are allowed to calve, can diminish uterine tonus.

The bacterial agents commonly isolated from the uterus of postpartum female cattle are *E. coli, Streptococci* spp, *Arcanobacterium pyogenic*, *Bacillus licheniformis, Prevotella* spp and *Fusobacterium necrophorum*. The most common pathogenic species are *E. coli*,

*Arcanobacterium pyogenic*, *Fusobacterium necrophorum* and *Prevotella* species (Foldi *et al.,* 2006).

Some bacteria, including *A.pyogenes*, *Necrophorum* and *Prevotella* spp., act synergistically to enhance the severity of uterine disease (Singh *et al.,* 2008; Bonnett *et al.,* 1991). Each of these species produces substances to enhance bacterial growth. Fusobacterium necrophorum actively invades uterine tissues and produces a leucocidal toxin that inhibits phagocytosis (Singh *et al.,* 2008; Sheldon, 2004). *Arcanobacterium pyogenes*, protected by the leucocidal toxin, in turn provides catalase and a growth factor which supports the proliferation of F. necrophorum It has been reported that persistent infection with *Arcanobacterium pyogenes* after 21 days postpartum will reduce conception rates at the first postpartum service Studies to evaluate the appearance and (Singh *et al.,* 2008).

odor of vaginal mucus have shown that *Arcanobacterium pyogenes, Proteus* species and *F. necrophorum* are associated with purulent or mucopurulent discharge evident in the vaginal mucus while *Arcanobacterium pyogenes*, *E. coli*, and non-hemolytic Streptococci are associated with foul smelling exudates (Williams *et al*., 2005).

## 2.4. Diagnosis of reproductive organ disorders in female cattle

### *2.4. 1. Uterine palpation*

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 (Gilbert, 1992; Foldi *et al*., 2006).

### *2.4.2. 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 (LeBlanc *et al*., 2002; Barlund *et al.,* 2008).

### *2.4.3. 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 cattle 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 (Mateus *et al*., 2002).

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

Numerous pathogens could be localized at the female reproductive tract, affecting the success of fertilization. Infectious diseases could provoke vulvitis, vaginitis, cervicitis or 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 (Carlos *et al.,* 2012).

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. Clinical or subclinical Endometritis could be diagnosed. The sample can also be taken by cytology brushes (65 cm, 4 mm) protected by a sterile metal tube (50cm, 5 mm). Endometrial cytology is a practical technique to diagnose subclinical Endometritis, when clinical signs are absent. The number of Neutrophils indicates the type and grade of endometrial inflammation (Carlos *et al.,* 2012).

## 2.5. Treatment of Reproductive Organ Disorders in Female Cattle

Many techniques and therapeutic strategies have been used to treat COD in dairy cattle. 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. There is only an empirical statement by (Roberts, 1986). That refutes ovariectomy as a treatment option. He states that "spaying will correct nymphomania but removing only one ovary if it is affected with cysts is useless, since the remaining ovary will promptly develop cysts (Roberts, 1986).

The first widely accepted treatment for COD is still used today. As early as 1874, manual rupture of the cysts has been advocated as treatment for COD. A great variety of intrauterine antimicrobial agents (ox tetracycline: 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), ceftioufur /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 F2α or its synthetic analogues) have been introduced in the field (Risco *et al.,* 2007).

## 2.6. Prevention of Female Cattle Reproductive Organ Disease

Prevention of uterine infection requires a good management of sanitation, nutrition, population density, 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 (Hartigan, 1980). 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 ceftioufur 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 in Female Cattle

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 a cow and the lost cash opportunity from fewer calves available to market. (Sheldon and Dobson, 2004; Gilbert *et al.,* 2005).

## 3. MATERIAL AND METHODS

### 3.1. Study Area and Study Population

The Study Was conducted in Addis Ababa and Adama municipality abattoirs in which the abattoirs slaughter sheep, goat and cattle and supply meat for consumers. About 5-8 local and cross breed female cattle slaughtered every day in each abattoirs. The study populations were cows slaughtered at the two abattoirs and the study units were cows slaughtered at the day of visit. The study was conducted on total of 209 female cattle (119 from Addis Ababa and 90 from Adama abattoir).

### 3.2. Ante Mortem Examination

General physical and clinical examinations were conducted before slaughters the female cattle's, giving attention on sign of reproductive system such as vulva for any gross lesion, sign of vaginal discharge and pregnancy Diagnosis were performed acording to (Roberts,1986). the body condition (BCS) were concidered as poor,good and fat acording to (Nicohlson and Butterworth ,1998) (Annex 1) and the age of animals were estimated acording to (Puck *et al.*,2004) for feruther information it was indicated in (Annex 2).

### 3.3. Postmortem Examination

After slaughter the reproductive organs were removed from the animal inspected and palpeted one by one for any gross pathological lesions and disorders befor any incision.(Assey *et al*., 1998; Garcia, 1998). The gross lesions examination were performed according to veterinary medicine teaching hospital (VMTH, 2009). Which included lesion distribution, texture, consistency, sheep, size and colour.

Tubular part of reproductive tract was disected by giving dorso-longitudinal cut and exmined.The vagina was the firest part of the tract to be opened and examined and the observation were recuerded.The cervix was disected dorso-longituidinally from os-

internum to os-externum and exmined for prolaps of cervical rings and any contentes insid. Uterus and fallopian tubes were opened by midline incision and examined for inflamation,presence of pus and other changes.The ovaries were examined externally and internally for the presence of cystes and other abnormalities. (kunbhar *et al.,* 2003).

### 3.4. Study Design

Cross sectional study design with purposive sampling techniques were used. All cows with any reproductive organ disorder were sampled from November 2015 to April 2016 for pathological disorders and associated bacterial causes. Origin, breed, Age and Body condition (BCS) of cows were considered as variable of interest.

### 3.5. Sample collection and transportation

Abattoirs visits was arranged 2 days in each abattoirs in every week for sample collection and the other days of the weeks have been used for laboratory sample processing. Tissues with lesion were transported to the laboratory in icebox and giving a good attention to avoid any damage of tissue for further Histopathological examination as well as bacteriological examination. The lesion part of the tissue is cut to the size of 2-3 cm and put in the universal bottle containing 10% buffered formalin which stabilizes the tissue to prevent any possible decomposition. (Talukder, 2007).

For bacteriological examination the surface of the tissue with the lesion were decontaminated by moderate hot application using flame of fire and then collected separately in sterile universal bottle containing sterile saline solution, labelled and transported to the laboratory in icebox (Quinn *et al*, 2004).

## 3.5. Sample Processing

### *3.5.1. Histopathological procedure*

Tissue processing was conducted following the procedure described by (Talukder, 2007) as indicated on (Annex 3).Tissue were trimmed, fixed in 10% buffered neutral formalin, dehydrate in ascending grade of ethyl alcohol, alcohols, clear with xylene and impregnating with paraffin wax the tissue is infiltrated with the embedding agent, almost always paraffin. Then, applied tissue section at 5 μm thickness spread on warm water bath and attaching the tissue to glass slide. The slide was incubated in incubator at $60^0$c to avoid paraffin wax. The sectioned tissues were deparaffinised in three changes of xylene, rehydrated in descending grades of alcohol and stained with Routine Stain HE (Haematoxylin-Eosin). Finally examined under microscope using 10 x 20x, and 40x magnification and photomicrographs are considered.

### *3.5.2. Isolation and identification of bacteria*

The sample were cultured on different general and selective media for isolation and identification of bacteria. Briefly the tissue were minced by using sterile blades and scissors, inoculated in to brain heart infusion broth and were incubated aerobically at 37ºC for 24 h. Growth were evaluated by turbidity and a loop full of fluid was streaked on both blood agar and MacConkey agar and were incubated at 370c aerobically for 24hrs After 24hrs both blood and MacConkey agar were observed for any growth (Quinn *et al., (2004).*

For primary identification, colony size, shape, color, hemolytic characteristics, Grams reaction and catalase production were used. Each culture was subjected to gram staining to determine their shape, and gram reaction. Catalase test using 3% Hydrogen per oxide (H2o2) was performed to identify catalase positive and catalase negative bacteria. Mannitol Salt Agar and purple base agar with 1% maltose were used to differentiate staphylococcus species and incubated at 37°C and examined after 24–48 hours for mannitol and maltose fermentation respectively.

Tube coagulase test using rabbit plasma was used to identify the coagulase positive and coagulase negative staphylococcus species. Enterobactericae species were identified using Oxidase test, Indole test after addition few drops of kovacs reagent and motility test, TSI (Triple Sugar Iron) to detect sugar fermentation, MacConkey agar for lactose fermentation and colony characteristics and Simmons citrate agar to differentiate bacteria based on citrate utilization. For confirmation the suspected colonies were picked up and sub cultured onto the selective media for identification by microscopic examination and further biochemical tests were conducted according to Quinn *et al.,* (2004) as indicated on (Annex 4).

### 3.6. Data analysis

The data was recorded, Checked and coded on Microsoft Excel spreadsheet (Microsoft Corporation) and Intercooled STATA (stat version 11) for descriptive analysis was used, Chi- square test and binary logistic regression, at 95% confidence level. Chi-square was used to determine presence of dependency between different variables and infection and pathological abnormalities of reproductive organs.

# 4. RESULTS

Out of 209 cows examine 27 were pregnant and were not included in the gross lesion characterization. The overall abattoir prevalence of reproductive organ abnormalities in cows of the two study abattoirs was 30.2% (55/182 ) and of the 10 different abnormalities observed, based on the anatomical classification, uterine abnormalities were prevalent ones accounting 43(23.6%) followed by ovarian abnormalities 25 (13.6%).

## 4.1. Gross pathological reproductive organs lesions and characterization

### *4.1.1. Ovarian abnormalities and associated lesions*

#### 4.1.1.1. Follicular Cyst

Follicular Cysts were seen in 4 cases (2.2 *%)*. Of four cysts one was on the left ovary and the rest three were on the right ovaries. The cystic follicles varied in size from 25 mm-30mm in diameter and were filled with sticky, watery transparent fluid. The walls of these cysts were thin and cyst were extremely pale (Fig 1, A).

Microscopically, the follicular cysts were lined by 1-2 layers of granulosa cells and the layer was extremely thin, In some cases, degeneration were seen in the granulosa cells and in other cases granulosa cells and theca cells exhibited degenerative changes No ovum were seen in cystic lumen in all cases (fig 1, B), and in theca externa were observed congested blood vessels (Fig 1, C).

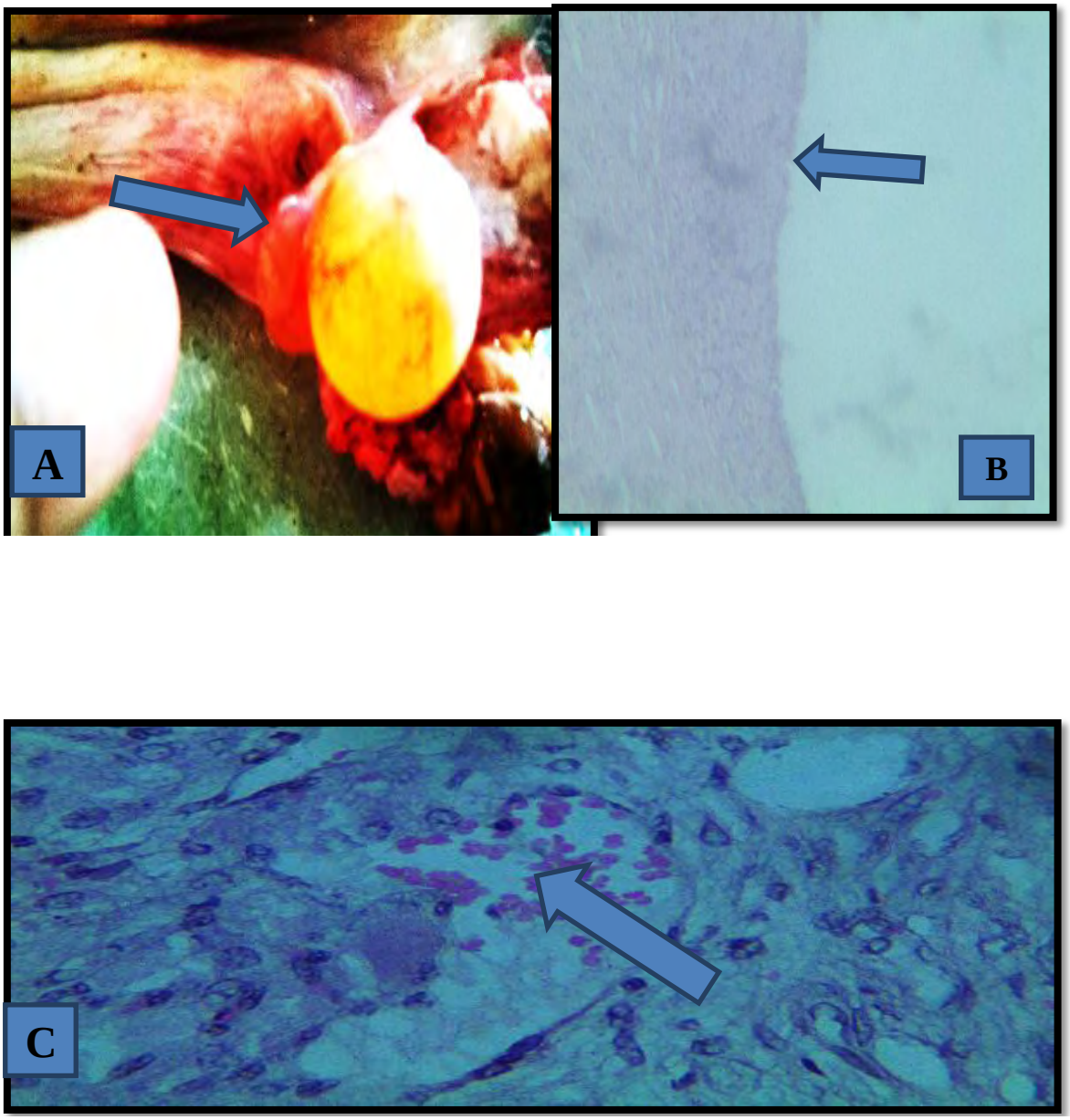


**Figure 1:** Follicular cysts. Note follicular cyst distended by fluid (arrow) and thin wall (A) which was microscopically characterized by extremely thin granulose cells (B). Note also the congested blood vessel in theca externa of the cysts (C) (40x magnification) (H&E stain).

4.1.1.2. Luteal cysts

Luteal cysts were seen in three cases (1.6%), two of which were on right ovary and one was on left ovary. The luteal cysts were large cysts, smooth and rounded, and the wall observed thick (Fig 2).

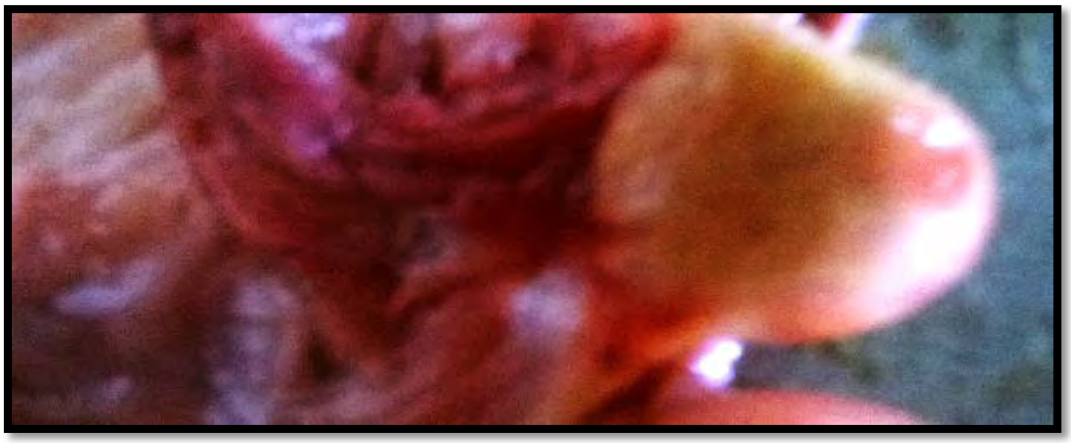

**Figure 2:** Luteal cyst. Note the large, thick walled cyst. When compared to follicular cysts (fig 1a) this cyst was yellow in colour, and not transparent (A) (arrow).

4.1.1.3. Par ovarian cysts

Par ovarian cysts were seen in 6 (3.3%) cases 1 on the right (0.5%) and 5 (2.7%) at left side Grossly these cysts were small and clear fluid field and measured 4-7 mm in size. Most of them were derived from the cranial mesonephric tubules (epophoron) and from the caudal tubules (paroophoron) and were attached to the mesovarium and round in shape (fig. 3).

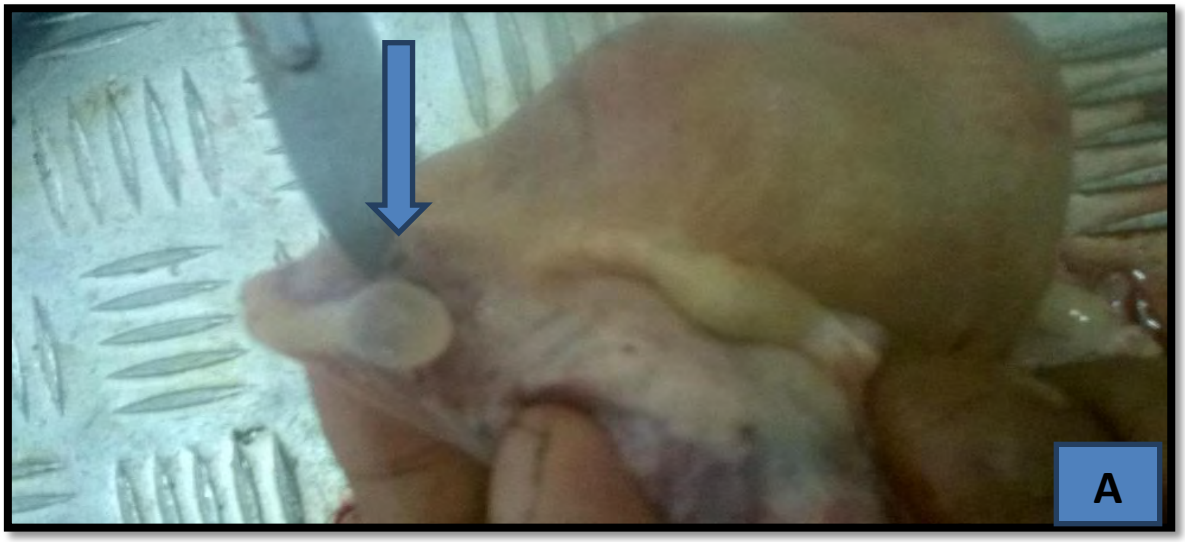


**Figure 3:** Paraovarian cyst on the mesonephric duct remenant (epophoron) (gross) (A) (arrow).

4.1.1.4. Ovarian hypoplasia

Ovarian hypoplasia was diagnosed in 4 (2.2%) cows of which two were unilateral and one was bilateral. Two were right ovarian hypoplasia, and one was left ovary hypoplasia (0.054%) and one bilateral (0.054%). Grossly, the ovaries were very smalls, oval in shape, measured 1.5-1.8cm in length and the surfaces of the affected ovaries were smooth, the follicles were difficult to observe and palpate externally (Fig. 4)

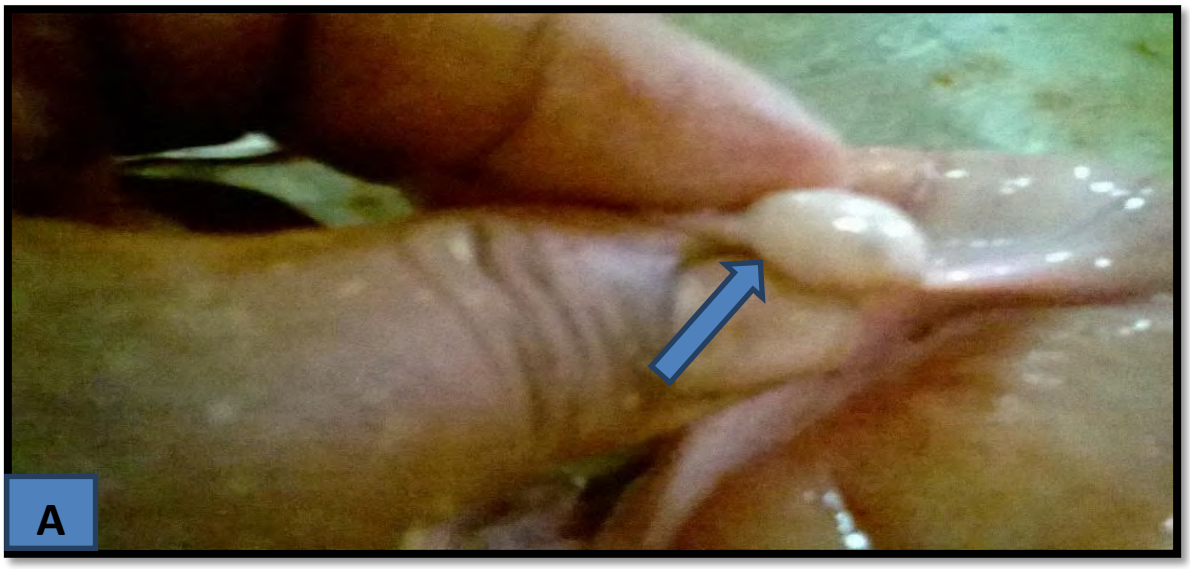


**Figure 4:** Hyoplastic ovary. Note very small ovary without visible follicles (A) (arrow).

4.1.1.5. Ovario-bursal adhesions

Adhesions were found in 5 (2.7%) of which one case was bilateral and four were unilateral. Two of the adhesions were on right ovaries, two on the left. The adhesions was characterized by fibrous strands connecting the ovary's with the mesosalpinx, extending as far as right and left half of the ovary's (fig.5).

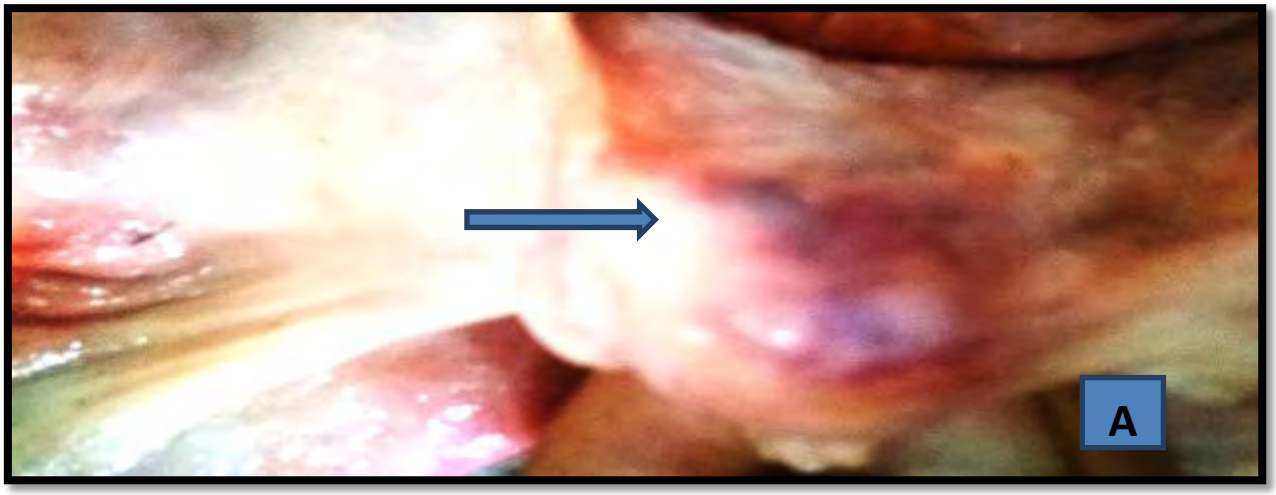


**Figure 5:** ovarian bursa adhesion. Note the ovary is totally encapsulated by fibrous tissue and adhered to the bursa (A) (arrow).

4.1.1.6. Ovarian haemorrhage

The ovarian hemorige as ovarian abnormalities were observed and recorded in 3 (1.6%) cases in which 2 on the right and 1 at left side. These ovaries were congested and enlarged in size (35-40mm) also after sectioning accumulated blood was visible.

*4.1.2. Uterine abnormalities and lesion characterization*

4.1.2.1. Endometrial lesion

The prevalence of endometritis was 18.7% (34). Grossly the affected uteri were congested and the congestion covered some region of endometrium in some cases and, generalized other cases. Some of the Endometritis were characterized by severe haemorrhages especially of pin point haemorrhages distributed over the entry part of endometrium and the muscularis layer and as well as serosa parts of the uterus, including both horns and the uterine body ( Fig 6 ).

Histologically, the Endometritis were characterized by sever congestion of blood vessels, infiltration by polymorphonucleal cells in most cases and by monomorphic nuclear cells and fibrosis in few cases. In all acute cases (infect in most cases) there were hyperaemia,

infiltration by neutrophils in endometrium and per glandular infiltration. The details of microscopic lesions were indicated as figure foot notes in the following microscopic figures.

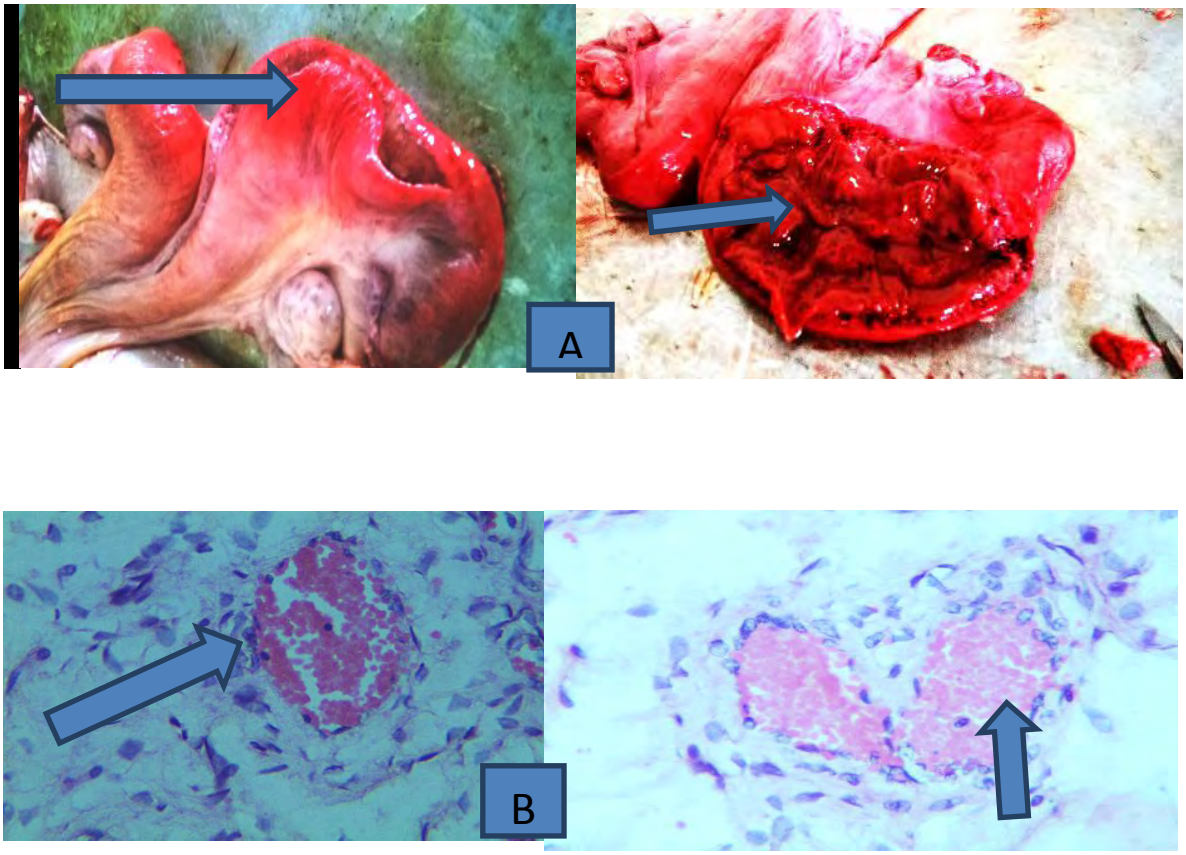


**Figure 6:** Haemorrhagic endometritis. Note the severely reddened uterus from outside (short arrow) and on cut surface uterine horns (large arrow) (A). Microscopic lesion of fig.6 B. Note the blood vessels were distended and filled by red blood cells (40xmagnification) (H&E stain) ( large and short arrows).

In some endometritis/metritis the uterine glands were severely hyperplasic and the inflammatory infiltration were severely dense (fig 7 A&B)

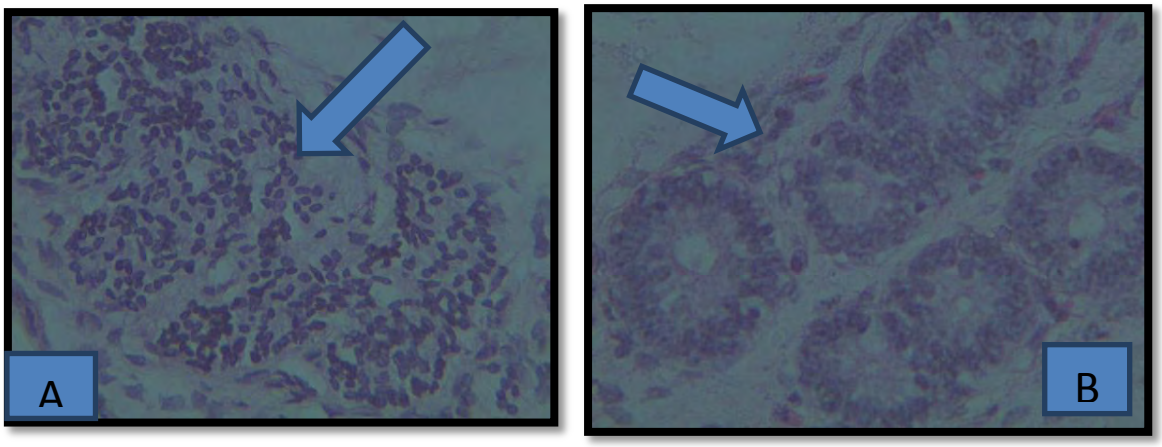


**Figure 7:** Suppurative endometritis with dense infiltration by neutrophils (A) (arrow) and sever uterine gland hyperplasia (B) (arrow) (40x magnification) (H&E stain).

In most chronic endometria the characteristic microscopic lesion were sub endometrial gland hypoplasia (fig 8 A), Adenomyosis, which means sub endometrial gland moved deep into myometrium (fig 8 B), and sever fibrosis (fig8 C).

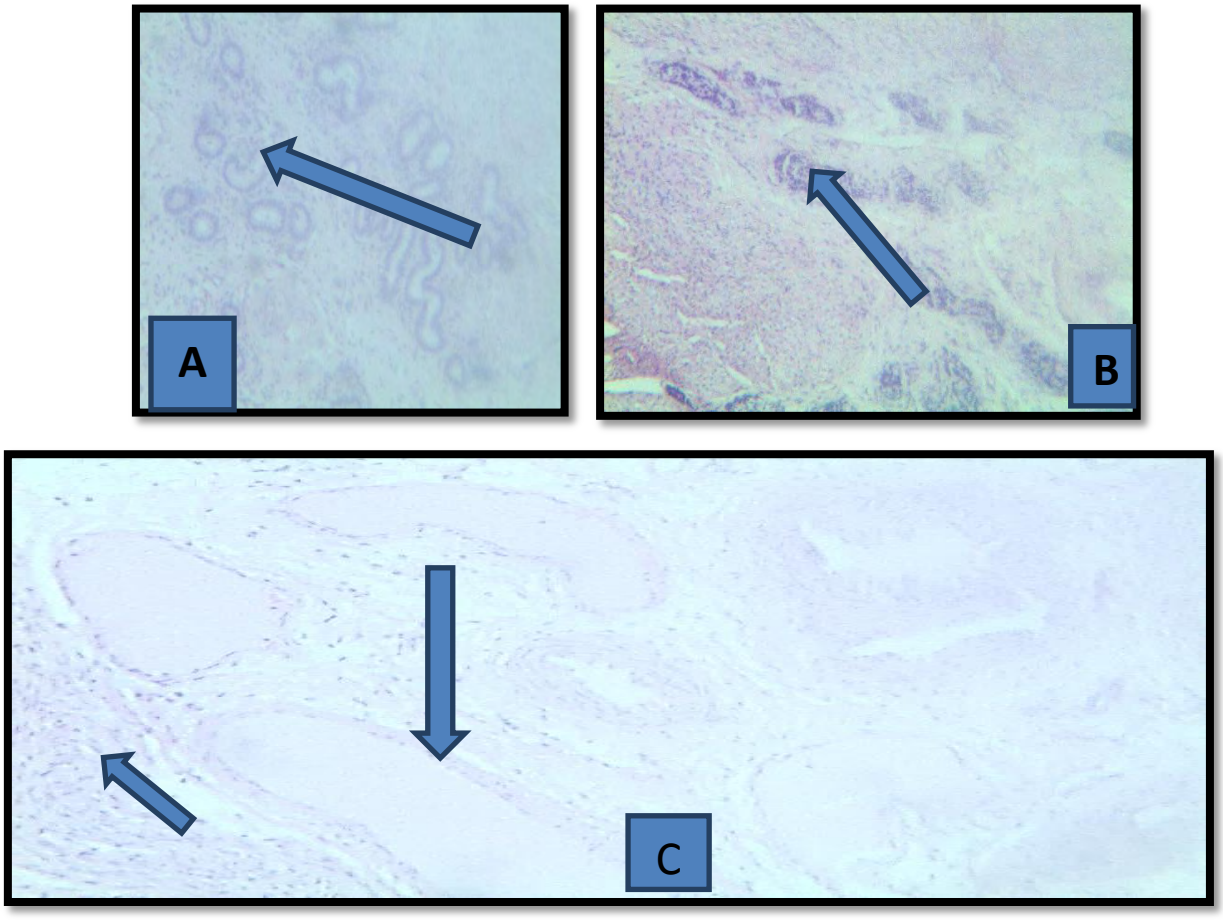

**Figure 8:** Chronic Endometritis. Note sever hypoplasia of sub endometrial glands and fibrosis of interstitial space (A) (arrow). Adenomyosis in which the presence endometrial glands between the muscle bundle at myometrium (B)( arrow) and presence of excess collagen fiber (short arrow) and blood vessels distended by oedematous uniformly staining material (Large) (C) (40x magnification) (E&H).

4.1.2.2. Pyometra

Pyometra was found in four cases 2.2%. Grossly it was characterized by a collection of thick purulent exudate of variable amount within the uterine lumen and greyish in colour without significant and appreciable distension of the uterus (Fig 9 A) microscopically the infiltration of polymorfonclear cells (neutrophils) and in some chronic cases the infiltration of macrophages and monocytes were seen in endometrium (Fig 9 B) (40xmagnification) (H&E).

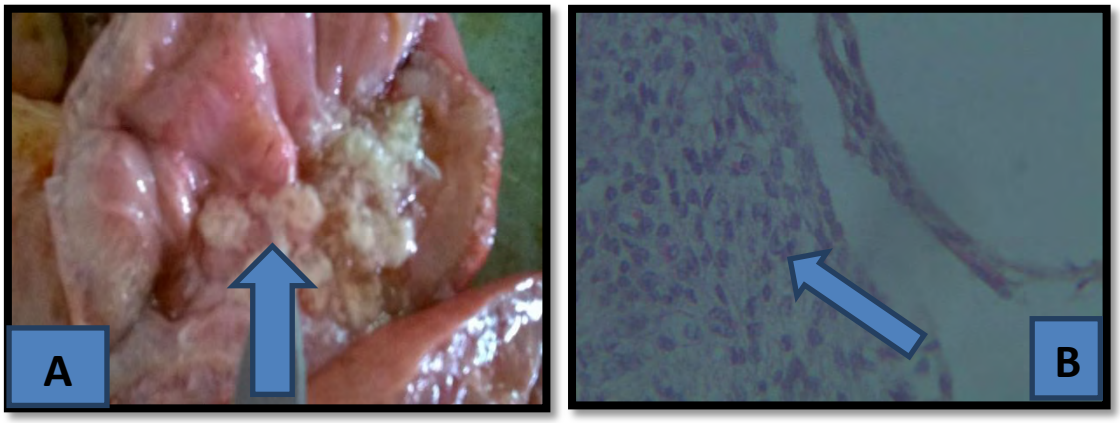


**Figure 9:** Accumulation of grey pus (pyometra) in the uterine horns (A) (arrow). Infiltration of polymorfonclear and macrophages and monocytes were seen in endometrium (B) (arrow).

4.1.2.3. Hydrometra

Three case (1.6%) of Hydrometra were encountered during the study. Uterus was thin-walled due to accumulation of clear and watery fluid (about 150-200 ml) in the lumen of corpus uteri and both uterine horns with stenosis of the cervical lumen. This lesion was accompanied by atrophy of curricula (Fig 10 A).

4.1.2.4. Mucometra

Mucometra were found in 2 (1.1%) cases, accumulations of 70-100 ml mucinous fluid were detected in the lumen in corpus and corn uteri in addition that in one case this mucous were extended to region of cervix (Fig 10 B).

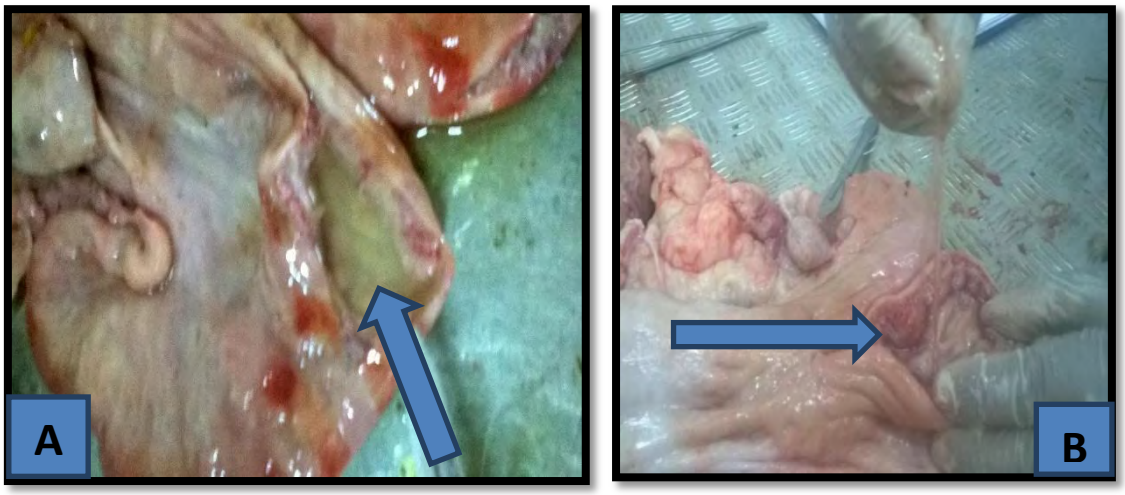


**Figure 10:** Hydrometra with clear and watery fluid in the uterine horns (arrow) (A) Mucometra with clear copious mucinous fluid in uterine lumen (arrow) (C)

Oviducts, vagina and cervix were examined carefully. No abnormality wear detected.

**Table 1:.**Gross female genital organs Abnormalities encountered in slaughter houses.

| | **Recorded Abnormalities** | **Number of lesions** | **Percentage %** |
|---|---|---|---|
| Ovarian | Follicular cysts | 4 | 2.2% |
| Abnormalities | Luteal cysts | 3 | 1.6% |
| | Par ovarian cysts | 6 | 3.3% |
| | Ovarian hypoplasia | 4 | 2.2% |
| | Ovarian bursa adhesion | 5 | 2.7% |
| | Ovarian hemorige | 3 | 1.6% |
| Uterine | Endometritis | 34 | 18.7 % |
| Abnormalities | Pyometra | 4 | 2.2% |
| | Mucometra | 2 | 1.1 % |
| | Hydrometra | 3 | 1.6% |
| **Total** | 10 | 73 | 37.2% |

**Table 2:** Type**,** Position and frequency of occurrence of ovarian cysts

| **cyst type** | **Position** | **No of affected** | **Percentage** |
|---|---|---|---|
| Follicular | Right | 3 | 1.6% |
| | Left | 1 | 0.5% |
| | Bilateral | no | --------- |
| Luteal cyst | Right | 2 | 1.1% |
| | Left | 1 | 0.5% |
| | Bilateral | no | --------- |
| Par ovarian cyst | Right | 1 | 0.5% |
| | Left | 5 | 2.7% |
| | Bilateral | 0 | -------- |

**Table 3:** Distribution of cystic ovaries, ovarian hypoplasia, ovarian hemorige and ovarian bursa adhesion on sides of ovary.

| **Abnormalities** | **Right** | **Left** | **Bilateral** | **Total** |
|---|---|---|---|---|
| Cystic ovaries | 6 | 7 | 0 | 13 |
| Ovarian hypoplasia | 2 | 1 | 1 | 4 |
| Ovaro bursa adhesion | 2 | 2 | 1 | 5 |
| Ovarian hemorige | 2 | 1 | 0 | 3 |

**Table 4:** Abattoirs Prevalence of Reproductive tract abnormalities in cow (n=182) related with different variables.

| Variable | Observation | Abnormalities number | Prevalence | $X^2$ | P. value |
|---|---|---|---|---|---|
| Abattoir location | | | | | |
| Addis Ababa | 117 | 37 | 31.62% | 0.31 | 0.580 |
| Adama | 65 | 18 | 27.7% | | |
| Origin of cattle | | | | | |
| Addis Ababa | 101 | 33 | 32.7% | | |
| Adama | 10 | 3 | 30% | 0.30 | 0.587 |
| Harer | 16 | 4 | 25% | | |
| Arise-Bale | 55 | 15 | 27.2% | | |
| Breed | | | | | |
| Cross | 111 | 35 | 31.5% | 0.23 | 0.630 |
| Local | 71 | 20 | 28.7 % | | |
| Age | | | | | |
| Yung | 59 | 19 | 32.2 % | 0.87 | 0.354 |
| Adult | 76 | 25 | 32.9% | | |
| Old | 47 | 11 | 34.4 % | | |
| BSC | | | | | |
| Poor | 12 | 2 | 16.7% | 1.26 | 0.264 |
| Medium | 127 | 38 | 29.9% | | |
| Good | 43 | 15 | 34.9% | | |

### 4.2. Bacterial Isolation

The bacteria isolation were conducted on 34 (18.7%) uterus with Endometritis. Totally 80 bacterial isolates were detected. *Escherichia coli* 17(50 %), *S. aurous* 18(52.9), *S. hicus* 1(2.9%), *S. intermiduium* 2 (5.9%), CNS 12 (35.3%), streptococcus 24 (24%), *P.Bulgaris* 5 (14.7%), *C. Frundi* 1 (2.9%) and in 30 samples mixed bacterial were isolated (Table 5).

**Table 5**: Uterine bacterial isolation

| **No** | **Bacterial isolates** | **No of isolates** | **Percentages (%)** |
|---|---|---|---|
| 1 | Streptococcus species | 24 | 70.6% |
| 2 | Staphylococcus species | | |
| | -*S.aureus* | 18 | 52.9% |
| | -*S.hicus* | 1 | 2.9% |
| | -*S.intermedum* | 2 | 5.9% |
| | -CNS | 12 | 35.3% |
| 3 | *Proteous vulgaris* | 5 | 14.7% |
| 4 | *E. coli* | 17 | 50% |
| 5 | *C. fundi* | 1 | 2.9% |

## 5. DISCUTION

Reproductive organ disease of cow are important problems affecting the reproductive performance. Study of the pathological disorders and infection of the reproductive disease is very important in evaluation of the reproductive efficiency since most reproductive tract problems lack additional outward manifestation, hence, examination of gross and microscopic lesions; and bacterial infection of genital tract play a central role in the identification of the problems. Most of these abnormalities can only be diagnosed when the animal is subjected to post-mortem examination (Buregelt, 1997).

Different study have been conducted in different parts of Ethiopia on reproductive abnormalities of cows based on abattoir material Gebrekidan *et al.,* (2009), in Addis Ababa Simenew *et al*, (2011), in Sululta, Amare, (2002), in Tigray and Abalti *et al.,* (2006) in Bahir Dar. All this studies were based on gross lesions and in most microscopical and bacterial cutures were not conducted.

In present study different variables were considered but none were found to statistically related to occurrence of reproductive disorders ($p>0.05$) .This was in-line with Mekibib *et al* (2013) who reported no statistical association of reproductive abnormalities of cows slaughtered at Hawassa municipality abattoir and Tula abattoir.

Out of the 182 female genital tracts examined, various abnormalities with different degrees of severity were observed in 55 (30.2%) cases. The overall prevalence of macroscopic genital abnormalities found in the present study were in agrmeent with the various previous studies of Gebrekidan *et al*, (2009), Mekibib *et al* (2013) and Abalti *et al* (2006) with prevalence of 31.5%, 36.8% and 39.5% respectively in different parts of Ethiopia. However, the reproductive organ abnormalities which recorded in our study were higher than the report of Smenew *et al* (2011) who recorded 134 (22.3%) abnormalities and Abdul *et al.* (2013) reported 21 (13.13%) from Ethiopia and Yemen , respectively. Our finding was lower than reports of Kumbhar *et al* (2013) in pakistan 65%. This variation could be attributed to the difference in breed, management, geographical environment and level of nutrition.

Based on the anatomical classification in the present study, uterine and ovarian abnormalities were prevalent. The ovarian abnormalities 25 (13.6%) found in this research is similar with Gebrekidan *et al* (2009) who reported 15.3% whereas uterine abnormalities determined by the same author was 10.7% which is lower than 43(23.6%) in the current finding.

The prevalence of follicular cysts in this studies 2.2% was lower than the result recorded by Kumbhar *et al* (2013) 10.8% of follicular cysts, Mekibib *et al* (2013) in Hawasa municipality 15(4.35%) and in –line with Hatipoglu *et al* (2002) 1.88 %. The difference could be due to difference breed, age, level of milk production, feeding, management and exercise are factors, suggested as influencing the prevalence of cystic ovaries in cattle (Herenda, 1987).

The incidence of luteal cysts in the present study 1.6 % was lower than Kumbhar *et al* (2013) 7.7%. However the current finding was higher than the report of Azawi (2008) 1(0.2%).

The incidence of par-ovarian cysts in this study 3.3% was higher than the result reported by Hatipoglu *et al* (2002) 0.75%. Ali *et al* (2006) and Abalti *et al* (2006) which reported 1.81% 1.5%, respectively. The findings of the current study was lower than the report of Kunbhar (2003) 15.4%. The difference could be due to difference breed, age, level of milk production, sample size, sample size, feeding, management and exercise are factors, suggested as influencing the prevalence of cystic ovaries in cattle (Herenda, 1987).

The incidence of ovarian hypoplasia in the present study was 2.2% and this was in agreement with that of Abalti *et al* (2006) in Bahirdar 2.9%.

The incidence of ovarian bursa adhesion in present study was 2.7%. It was lower than reported by Abalti *et al* (2006) and Ali *et al* (2006) 5.5% and 7.27%, respectively but the current finding were higher than the report of Hatipoglu *et al* (2002) 0.27%. The Ovario-bursal adhesions were associated with adhesion of the uterus and oviducts to the broad ligaments, endometritis, cervicitis and vaginitis. Although the exact mechanism by which

adhesion develops is unclear, extensive adhesions have probably resulted from ascending infection arising from pregnancy complications that include retained fetal membrane and endometritis (Assey *et al*., 1998). Mild adhesion could result from non-infectious conditions such as physical trauma as a result of manipulation or enuclation of cysts (Herenda, 1987; Assey *et al*., 1998).

The incidence of pyometra in this study was 2.2% and the current finding was lower than a report by Ali *et al (2006*) Pakistan 6.36%. The result of the current finding was in-line with a report by Abdul *et al* (2013) Yemen 1.8%.

The incidence of hydrometra and mucometra were found 1.6% and 1.1% respectively, the findings in the current study were lower than the result reported by Kumbhar *et al* (2013) 12.3%, but was higher than the finding of Azawi *et al* (2008) 0.2%.

The incidence of endometritis in the current studies was 18.7% which was higher than Abalti *et al* (2006) and Mekibib *et al* (2013) who report 3.9% and 4.93% respectively. However it was lower than that of Hatipoglu *et al* (2002) 38.5 %. This difference could possibly be attributed to the differences in breed and management employed, especially of hygiene as most of the cows were exposed to uterine infection during postpartum periods.

The microscopic finding of chronic and acute endometritis were similar to the report of Talib and Faraidoon, ( *2014*) in Iraq who reported acute endometritis with infiltration of PMN (polymorphonuclear cells) in the sub mucosal and among the uterine glands and in case of chronic endometritis appeared infiltration of mononuclear inflammatory cells represented by plasma cells, macrophage in sub mucosal layers with proliferation of fibrin, as well as presence of necrotic debris inside the lumen of uterine glands.

A uterine bacterial infection is the most commonly acquired reproductive problems and are reported as the most important cause of infertilities in female cattle (Mhelia *et al*., 2014). Mhelia *et al* (2014) during his comparative studies of bacteria isolation from cows slaughtered isolated 69 bacteria's 43 (53.8%) of which were *E. coli*, 11 (13.8%) *S. pyoge*ns, 9 (11.3%) *Proteus spp* and 6 (7.5%) other Staphylococcal spp. Similarly, in present study 80 bacteria were isolated of which *Escherichia coli* 17(50%), *streptococcus* 24 (70.6%), *P.Bulgaris* 5 (14.7%), *C.Frundi* 1 (2.9%), *S.aureus* 18(52.9%), *S.hicus* 1

(2.9%), *S.intermedum* 2 (5.9%). and about 30 cases of mixed bacterial infection were recorded. Certain bacteria that were identifies in this study were not (C. *fundi*) by other authors.

## 6. CONCLUTION AND RECOMMENDATIONS

Different pathological abnormalities of reproductive organs of cow were recorded and gross lesion of affected organs were characterized and processed in the laboratory for Histopathological characterization and bacterial isolation. The study used those cows which slaughtered in Addis Ababa and Adama abattoirs. Endometritis 34(18.7%) and par-ovarian cysts 6(0.32%) were major reproductive organ problems recorded in current study. About 5 types of bacteria genera were identified from endometritis in which staphylococcus species and *E.coli* were the major bacterial isolates. It could be concluded that these disorders could be the cause of slaughtering for these cows. As well the bacteria isolated might be cause of Endometritis, one the most encountered during this study. However we didn't established causal relationship in our study. Significant number of pregnant animals were also encountered slaughtered in abattoirs.

In line with this conclusion the following recommendation were forwarded

- Efforts and collaboration have to be done to minimize loss of pregnant and fertile female cows. Legislation bodies with collaboration of ministry of agriculture and; ministry of Livestock and Fishery must establish the legislation which prohibit slaughtering of fertile female cattle.
- Further study on cause of reproductive organs disorders, isolation and identification of both aerobic and anaerobic bacteria, viruses and fungi, should be conducted.
- Trainings for those concerned veterinarians and technicians on reproductive health and management of dairy cattle should have to be implemented.

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## 8. APPENDICES

**Annex 1:** Body condition scoring system

***Score of 1 (very poor body condition)***

- 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 body condition)***

- 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 bones in the chine, lion, 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 (good body condition)***

- 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 (fat)***

- 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.

***Score of 5 (very 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 2:** Age determination by dentition

It is possible to guess the age of cow by looking at its teeth. The milk incisors (cutting teeth) are replaced by permanent incisors at fairly regular intervals; age of a cow can be estimated quit accurately until approximately 4yers old. After this age we can only look at the were of the chewing surfs on the permanent incisors now. The ridges on top of the teeth which form a zig-zag line gradually become worn down until the surface is smooth (puck *et al*.,) for further information it was indicated in Annex 2.

**Annex 3**: Histopathological procedures (Takulder, 2007)

1. Fixation of tissue by 10% neutral buffered formaldehyde

2. 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).
3. 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.
4. Embedding of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60c$^{o}$) is poured and allowed to settle and solidify.
5. 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 overnight.
6. Staining: Haematoxylin eosin stain procedure
    a. Deparaffinise slides in 2 changes of xylene for 5minutes.
    b. Hydrate slides in 3 changes of 100% alcohol each for 3minutes and 1 changes of 95% alcohol for a minute and 1 change of 70% alcohol for 3minutes
    c. Rinse in distilled water until ripples disappear from slides.
    d. Place in heamatoxyline (mayer's hematoxline) for 10-15 minutes
    e. Rinse in tap water until water runs clear
    f. Decolorize in 1% acid alcohol, 3-6 quick dips. Check differentiation microscopically: Nucleic should be distinct; cytoplasm should be uncoloured.
    g. Rinse in tap water until ripples disappear from slides.
    h. Stain in eosin, 3 dips.
    i. Rinse in tap water until water runs clear.

ANNEX 4: Methods used to identify different bacteria (Quinn et al., 2002).
Blood agar base

*Composition* (g/l): hear muscle, infusion from (solid) 2.0; pancreatic digest of casein13; yeast extract 5.0; sodium chloride 5.0; agar 15.0. Direction: Suspend 40g of 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 121$^{o}$c for 15 minutes and cool the base to 45-50$^{o}$c and 5-7% sterile sheep blood. Colony growth on blood agar base and haemolysis formation were observed.

Gram staining reagent

*Procedure:*

- 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 iodide which remain for 60 second. Then wash off with tape 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 bloating 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 cell placed on a clean microscopic slide and a drop of 3% hydrogen peroxide is added. An effectiveness of oxygen gas, within a few seconds, indicates a positive reaction.

Coagulase test

Tube coagulase test was used for identification of coagulase positive and coagulase negative staphylococcus species based on the reaction of 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 37$^{o}$C. 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. Depending on the formation of clot for positive reaction S. *aureus* and *S. intermidius* and *S. hyicus* showed positive result otherwise considered as CNS.

Mannitol Salt Agar

*Procedure:* Mannitol Salt Agar media was prepared, then all gram positive bacteria were inoculated in to the medium and the result was recorded after 24 hours incubation period at 37 degree Celsius. *Staphylococci* species produced yellow colony and yellow medium that indicates mannitol fermentation other bacterial species could not ferment mannitol.

Manitol salt agar

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 37oc for about 24 hr. A positive result showing growth and a clear media change from red to yellow.

Purple agar base

*Principle:* Purple agar base contains maltose as a substrate and bromocresol purple indicator used to identify bacterium that can ferment maltose (1% maltose sugar).

*Procedure:* Purple agar base was pepared and the bacterium was inoculated and placed in the incubator at 37oc for 24 hour. A yellow color (acid) is a positive reaction for fermentation of the carbohydrate incorporated into the medium. Bubbles in the inverted fermentation vials are an indication of gas production.

Buffered peptone water

*Composition (g/l)*: Pancreatic digest 10.00; Disodium Phosphate 3.50; Sodium Chloride 5.00; Monopotassium Phosphate 1.50. Final pH: 7.0 ± 0.2 at 25ºC; 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.

Salmonella, Shigella Agar (S.S. Agar)

*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; Bacteriological Agar 15.00. Final pH, Distilled water 1 liter

*Preparation*: Suspend 63 grams in one liter of distilled water. Bring to the boil with frequent agitation, and allow to simmer gently to dissolve the agar. DO NOT AUTOCLAVE. Cool to about 50ºC, mix, and pour into petridishes.

Tryptone Soya Broth

*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 ± 0.2 at 25ºC, Distilled water 1 litter

*Preparation:* Suspend 30 grams of the medium in one litter of distilled water. Mix well. 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.

Simmons Citrate Agar

*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 ± 0.2 at 25ºC, Distilled water 1 liter

*Preparation:* Suspend 24.28 grams of the medium in one litter of distilled water. Heat to boiling till the dissolve the medium completely. Dispense in tubes and sterilize in the autoclave at 121ºC 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

*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 ± 0.2 at 25ºC, 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 121º C for 15 minutes and cool in

Methyl Red-Vogues Proskauer Medium

*Composition (g/l)*: Peptone mixture 7.00 (Peptic digest of animal tissue 5.00); Dextrose 5.00; Dipotassium Phosphate 5.00. Final pH: 7.5 ± 0.2 at 25ºC, Distilled water 1 litre

*Preparation:* Suspend 15 grams of the medium in one litter of distilled water. Heat to dissolve and distribute into tubes in 1 ml amount and sterilize by autoclaving at 121. Dispense in tubes in 10ml amounts and sterilize at 121$^{o}$C for 15 minutes. Reagents required Alpha-Naphthanol (5%) (1-Naphihol 6gm; Ethanol, 96% (volume fraction) 100ml, Potassium hydroxide solution (40%) (Potassium hydroxide 40gm; distilled water 100ml) *Preparation* dissolve the potassium hydroxide in the distilled water. Methyl Red alcoholic indicator solution (Dissolve 0.1g of methyl red powder in 300ml 95% ethanol and 200ml distilled water), 1% solution of 2, 3, 5-Triphenyl Tetrazolium Chloride (Dissolve 500mg of triphenylTetrazolium chloride in dehydrated alcohol tomake100ml)
Indole test

*Principle*: Organisms those possess the enzyme tryptophanase can break down the amino acid tryptophan to indole. When indole reacts with para-dimethylaminobenzaldehye (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.

Citrate utilization 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 cause 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 35°C with cap left loosen for 22 hours. After 22 hrs incubation observe the tube for growth and color change.

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 ºC for 22 hours.

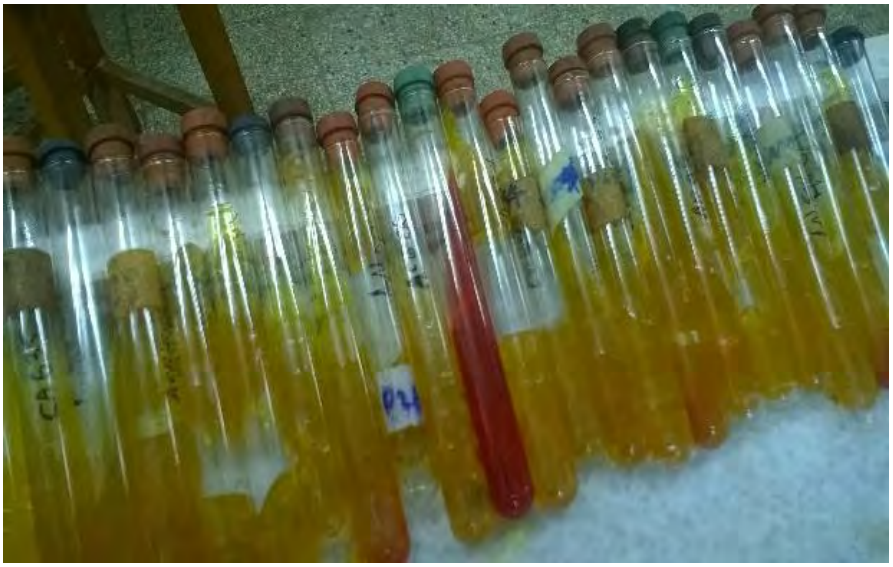
Methyl red and Vogues Proskauer test

*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 ºC 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.

Photograph during sample colection and decontamination of uterine tissue samples for bacteria isolation



Photograph during laboratory processing