

IMPACT OF FOLLICULAR CYST ON UTERINE INFECTIONS WITH LOCAL IMMUNE REGULATION IN COW

A THESIS

BY

MD. SHAHJAHANUR ALAM

Student ID. 1504120

Semester: January- June, 2024

Session: 2022-2023

MASTER OF SCIENCE (MS)

IN

PHARMACOLOGY



**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY

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*DEDICATED TO
MY
BELOVED PARENTS
&
RESPECTED TEACHERS*

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ABSTRACT

The present study was aimed to examine the impact of follicular cyst on local immune system. A total 40 reproductive tract samples were collected from abettors. Ovarian follicular cysts are anovulatory follicular formations found in dairy cows. Cysts were thought to be solid objects that remained for a long duration. Uterine swabs were taken from the uterine endometrium and mixed with 10 millilitres of PBS solution for bacterial cell culture. During uterine samples examination, immune cells, such as neutrophils and lymphocytes were found in follicular cystic conditions. The inflammatory response to the level of bacterial contamination is more important than the presence of a specific type of bacteria. The current investigation provided evidence for the localized impact of uterine bacterial contamination on the ovary after parturition. The microbiological analysis of the uterine sample revealed *E. coli* and *Staphylococcus aureus* bacteria in the uterus of ovarian follicular cystic disease; whereas, the normal uterus did not exhibit any developing bacteria. Additionally, antibacterial antibiotic sensitivity was conducted to determine the antibiotic sensitivity of the colonized bacteria. It was found that there was no antimicrobial resistance at all. Histopathology of the follicular cystic ovary and uterus were carried out, which contained inflammation and fibrous tissue. A well-developed mucosal immune system in the uterus and ovary maintains local immunity by releasing a multitude of substances that change the way underlying resident immune cells function and provide immunological defense against foreign particles. So, in order to prevent metabolic issues and maximize the ration throughout the peri-partum; health measures should be optimized during parturition and postpartum.

Key words: follicular cyst, immunization, ovary, sensitivity and histopathology

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LIST OF ABBREVIATIONS

spp.	= Species
e.g	= Example
i.e	= That is
<i>et al</i>	= Associated with
etc.	= Etcetera
%	= Percentage
HSTU	= Hajee Mohammad Danesh Science and Technology University
+	= Positive
-	= Negative
KOH	= Potassium hydroxide
M.S	= Master of Science
G	= Gram
⁰ C	= Degree of Celsius
µg	= Microgram
Kg	= Kilogram
Lb	= pound
Ltd	= limited
NB	= Nutrient Broth
NA	= Nutrient Agar
MC	= Mac-Conkey agar
EMB	= Eosin Methylene Blue
Mm	= Millimeter
ml	= Milliliter
MR	= Methyl Red
VP	= Voges-Proskauer
TSI	= Triple Sugar Iron
MIU	= Motility Indole Urease
PCR	= Polymerase Chain Reaction
No.	= Number
SL	= Serial number
SC Agar	= Simmons Citrate Agar

PSS	= Physiological Saline Solution
PBS	= Phosphate buffered saline solution
RT	= Room Temperature
AZM	= Azithromycin
TET	= Tetracycline
CIP	= Ciprofloxacin
IMP	= Imipenem
CTR	= Ceftriaxone
NX	= Norfloxacin
CN	= Cefalexin
CX	= Cefoxitin
I	= Intermediate
R	= Resistance
S	= Sensitive
NEB	= Negative Energy Balance
COF	= Cystic Ovarian Follicles
OC	= Ovarian Cyst

CHAPTER I

INTRODUCTION

The ultimate result of the intricate process of fertility is determined by the close and skillful interaction of the brain, pituitary, ovary and uterine. Due to the intricate nature of this process, any interference with the operation of any one of the relevant organs will also have an impact on the overall result of fertility (Samardžija *et al.*, 2006; Kočila *et al.*, 2009; and Kovács *et al.*, 2020).

In dairy cattle, cystic ovarian follicles (COF) are a significant contributor to subfertility because they prolong the time between calvings (Lee *et al.*, 1998; and Fourichon *et al.*, 2000). The dairy farmer experiences financial loss when the COF's treatment costs and calving interval are extended. This nomenclature has to be updated, though, as the focus on cystic follicles has changed over time. In the 1940s, nymphomania and a bull-like appearance in cows were the primary clinical indicators of a state of "disease" that were linked to the existence of cystic follicles on the ovaries (Casida *et al.*, 1944).

The management and economics of dairy herds have changed over the past few decades to the point where postpartum fertility is now crucial. Cystic follicles are rather prevalent during this time and usually don't show any clear clinical symptoms. Nonetheless, normal ovarian cyclicity is delayed, and these cysts should be considered COD even if most individuals do not exhibit the formerly "classical" symptoms of the illness. Furthermore, cysts may cease to be steroidogenic and interfere with cyclicity after a variable amount of time (Dobson *et al.*, 2000; and Noble *et al.*, 2000). As a result, there were no other clinical anomalies at the time the non-steroidogenic cyst was discovered. "Cysts" are frequently diagnosed in dairy herd health programmes, even when there are no obvious clinical symptoms. As a result, it no longer seems suitable to refer to "Cystic Ovarian Disease." Instead, one should use the word "Cystic Ovarian Follicle(s)," which does not always imply a disease condition.

Cattle follicular cysts are large, follicle-like structures that are present on one or both ovaries, clearly interfere with normal ovarian cyclicity, and have a diameter of at least 2.5 cm when no luteal tissue is present. These cysts must last for at least 10 days. (Vanholder *et al.*, 2006). This condition, which is characterised by ovulatory failure, is more prevalent in high-yielding dairy cattle (Oltenacu *et al.*, 1990). In cattle, the incidence varies between 5 and 8%

(Vanholder *et al.*, 2006). In the early postpartum phase (less than 60 days), when cows are under a lot of metabolic stress, follicular cysts are more common.

Its pathogenesis has been connected to a number of conditions, including negative energy balance, ketosis, twinning, prenatal problems, genetic susceptibility, season, and dietary inadequacies (Roberts, 1986). When ovulation fails due to inadequate LH, the ultimate result is an ovarian cyst. A follicular cyst can be diagnosed by a history of nymphomania, a rectal examination, ultrasonography (USG), and a milk or plasma progesterone assay. Ultrasonography is a vital diagnostic tool in the field that helps differentiate between follicular and luteal cysts. When viewed ultrasonographically, follicular cysts show a thin wall (≤ 3 mm) and a consistently anechoic follicular fluid (Jefcoate and Ayliffe, 1995).

A number of therapeutic treatments, including manual rupture, progesterone, prostaglandin F₂ α (PGF₂ α), human chorionic gonadotropin (hCG), gonadotropin releasing hormone (GnRH), and prostaglandin F₂ α (PGF₂ α), have been employed and their post-treatment efficacy assessed (Peter, 2004). Reducing the financial damage to farmers requires early diagnosis and management. Therefore, this study outlines many consecutive methods for treating follicular cysts in the field and enhancing crossbred cattle's ability to reproduce after treatment.

Cystic ovarian follicles are one of the most common and significant ovarian diseases in modern high-yielding dairy cows, and they have been the focus of extensive research in recent decades. However, many features of the condition, including aetiology, remain unknown and inconclusive, as evidenced by the absence of a definite definition. The endocrine and follicular alterations that precede spontaneous cyst formation remain unknown, owing to the disease's variability and unpredictability. Studies have attempted to elucidate the pathophysiology by inducing cysts. However, this may not be the same as normal cyst formation (Kovács *et al.*, 2020).

The current investigation offers fresh perspectives on the abnormal persistence of follicular cysts of immunity development. The disease's hormonal shift features may affect the quantity of proteins involved in the cellular cycle and death processes, which would support the persistence of follicles. Ovarian cysts (OCs) are among the several reproductive diseases that negatively impact dairy cow fertility, but they are one of the most significant reproductive abnormalities. The chronic production of ovarian steroid hormone is caused by the long-term

existence of anovulatory follicular structures in the cyst. Depending on the condition of the estrus cycle, ovarian hormone controls the growth and development of the uterine wall.

There is a lack of information in Bangladesh about follicular cystic condition immunization. This research validates the theory that hormone levels are regulated by ovarian follicular cysts, influencing the mucosal immunological environment in the cow's uterus and oviduct. Thus, more research on ovarian cysts is necessary to ensure that they do not represent a significant threat to the dairy industry.

Objectives

The objectives of this study was,

- i) To observe the presence of immune cells in the uterine fluid of an cystic ovarian sample.
- ii) Investigate the impact of the follicular cystic state on the presence of bacteria in the bovine uterus.
- iii) Analyse the histological alterations in cystic ovarian infections under follicular cystic conditions.

CHAPTER II

REVIEW OF LITERATURE

The goal of this chapter is to give a selective evaluation of the research done in relation to the current topic. This study's literature on bovine ovarian cyst is reviewed.

Definition of ovarian cyst

The term "ovarian cyst" originated in 1831 to describe unusually large ovarian follicles that did not ovulate and caused a shift in cyclic activity, a change in sexual behaviour, and subfertility. McMutt was the first to introduce the word cystic in regard to persistent follicles with a diameter greater than 20 mm (Coleman, 2008). OCs are characterised as anovulatory follicular structures (Silvia *et al.*, 2002), with a diameter of >20 mm (Silva *et al.*, 2012) or $\geq$ 25 mm, surviving on the ovary for at least 10 days in the absence of a functioning corpus luteum (Santos *et al.*, 2009; Polat *et al.*, 2015).

In addition to humans, it can affect a variety of animal species, including ruminants, sows, bitches, and rodents (Francou *et al.*, 2008). Even when the numbers differ, some writers stick to the same definition. According to Calder *et al.* (1999), a cyst is any single or multiple follicular formations that are more than 20 mm in diameter and have a low P4 concentration that last for at least seven days. According to Yotov *et al.* (2014), other writers classified as cystic any ovarian follicle that measures at least 17 mm in diameter, lasts longer than six days, lacks a luteal structure, and can be identified with an ultrasound examination.

According to this assessment, follicles usually ovulate between 13 and 17 mm and remain in this preovulatory stage for five to six days. Therefore, a follicle that remains at 17 mm or higher is classified as cystic (Silvia *et al.*, 2002). Veterinarians typically do not conduct a second examination on the animal within 10 days of the original OC diagnosis to meet *all* the requirements of the criteria, leading one to hypothesise that the OC definition varies across authors and in practice (Vanholder *et al.*, 2006). It is also challenging to determine the relevance of a corpus luteum's existence. Even after another follicle ovulates and a corpus luteum forms, some cysts remain palpable, suggesting that they are not functioning (Zulu *et al.*, 2003).

On the other hand, corpus luteum regression could also be linked to a growing cyst (Yoshioka *et al.*, 1996). This pathology is also known as cystic ovarian degeneration, cystic follicle,

ovarian cyst, or cystic ovarian disease (COD). Some writers contend that the term "COD" is no longer relevant and that "cystic ovarian follicle," which does not always imply a disease condition, should take its place. Because it implies that the ovarian follicle and not other ovarian tissue become cystic, this word is recommended over "ovarian cyst" (Vanholder *et al.*, 2006).

Predisposing factors for Cystic ovarian Follicle (COF)

Milk yield is widely regarded as a risk factor (Hooijer *et al.*, 2001; Lucy, 2001); however, not all authors agree (Nanda *et al.*, 1989). As previously stated, COF is most commonly identified in high-producing dairy cows in the first few months after birth. Furthermore, a genetic relationship between cysts and milk production features has been discovered, implying that selection for production parameters will continue to increase the prevalence of COF in addition to the known genetic predisposition for the condition (Hooijer *et al.*, 2001). It is unclear which genetic factor(s) may lead to cyst development.

However, the absence of cyst development in cows during lactation or ovarian cycles shows that certain stressors, such as high milk output and the resulting negative energy balance (NEB) in the early postpartum period, may promote gene expression or give it functional relevance. Currently, a NEB is caused by greater energy requirements to maintain milk production than calorie consumption. This NEB causes numerous hormonal and metabolic changes that affect ovarian function (Beam & Butler, 1999). To better understand the relationship between COF and production parameters, energy balance may be a more trustworthy statistic than milk output.

Some animals can reduce the effect of milk production on their energy balance by increasing their consumption of dry matter, which balances out the increased milk output (Lucy 2001). This could help explain why some researchers (Bartlett *et al.*, 1986) were unable to discover a connection between ovarian cysts and milk production. However, the results remain uncertain when we focus on energy balance and the presence of COF. Beam [86] observed that although Zulu *et al.* (2002), Refsdal (1982), and Sovani *et al.* (2000) reported a deeper NEB and stronger mobilisation of body reserves in cows producing cysts, the nadir of the NEB came later postpartum in cystic cows than in ovulatory cows.

Furthermore, Beam (1995) discovered that cystic cows used fewer body reserves and that weight loss accounted for a smaller percentage of their milk output. Hooijer *et al.* (2001) were unable to identify a more severe NEB in cows with COF based on the fat/protein ratio in milk, in contrast to ovulatory cows. However, a prior study (Heuer *et al.* 1999) found that a higher fat/protein ratio increased the risk of cyst formation, indicating a more severe non-eye bulge (NEB). According to additional data from sheep, the development of cystic follicles is linked to a higher mobilisation of body reserves, which is an indication of a deeper NEB (Christman *et al.*, 2000).

Although the exact underlying mechanism or mechanisms are still unknown, NEB may affect COF formation at the level of the ovary/follicle and the hypothalamus/pituitary through associated hormonal and metabolic changes (Diskin *et al.*, 2003). NEB causes a decrease in the peripheral plasma concentrations of insulin, glucose, leptin, and IGF-1, while a rise is seen in the quantities of metabolites, including β -hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) (Beam & Butler, 1999; Liebers *et al.*, 2003).

The IGF system is essential for follicular growth and development. IGF-1 and insulin have direct effects on granulosa cells, but they also upregulate the LH receptor, which indirectly stimulates the development of follicles (Davoren *et al.*, 1986). Therefore, anovulation and the subsequent development of cystic follicles may be related to lower systemic IGF-1 concentrations in the early postpartum period, as shown by Zulu *et al.* (2002). However, Beam's (1995) data refute this notion. Moreover, it is widely recognised that insulin plays a major role in inducing follicle cell steroidogenesis and proliferation, both in vitro and in vivo (Armstrong *et al.*, 2002; Silva & Price, 1999).

Recent studies have demonstrated a possible link between reduced circulating insulin concentrations in the early postpartum period and ovarian dysfunction, or cyst development (Vanholder *et al.*, 2005). In addition to having low insulin concentrations, early postpartum high-producing dairy cows also show peripheral insulin resistance (Nestler, 1998). Insulin resistance is considered to be a major contributing factor to the pathophysiology of women's PCOS, or polycystic ovarian syndrome (Yen, 1999) and COF have often been compared to this syndrome, whether or not it is merited. But rather than insulin resistance, it has been observed that COF cows have insulin insufficiency (Opsomer *et al.*, 1999), pointing to a modified pancreatic-level interaction between glucose and insulin.

Furthermore, developing insulin resistance in sheep did not result in cyst development (Christman *et al.*, 2000). In conclusion, low levels of any or both of these hormones may play a role in the development of COF. According to the limited literature on the subject, they are both effective follicle growth stimulants. Additional research should be conducted to confirm the veracity of this notion. Leptin is regarded as the final component linking metabolic circumstances to reproduction. Adipocytes produce a relatively "new" hormone (Barash *et al.*, 1996). The influence on hypothalamic-pituitary function in cattle varies according to their metabolic status (Amstalden *et al.*, 2005). It may have a stimulant effect or none at all.

There is no discernible relationship between leptin profiles and first postpartum ovulation in postpartum dairy cows, despite the fact that a minimally acceptable amount of leptin seems required to trigger the first postpartum LH surge (Liefers *et al.*, 2003). Therefore, leptin may have an early role in the development of postpartum cysts. Zulu *et al.* (2002) and Huszenicza *et al.* (1988) observed that cows forming cysts had higher serum NEFA concentrations during the first week or two postpartum than ovulatory cows; however, Beam & Butler (1999) were unable to observe this. It is noteworthy that in rats, exposure to 48 hours of higher than usual NEFA concentrations can decrease the quantity of insulin released by the β -cells in the pancreatic islets in response to a glucose challenge (Mason *et al.*, 1999).

Moreover, according to Lu *et al.* (2003), NEFA is toxic to a wide range of cell types, including theca and bovine granulosa cells (Vanholder *et al.* 2005). As a result, prolonged exposure to high NEFA concentrations during NEB episodes may impede follicle development and expansion, disrupting the complex endocrine system and increasing the formation of ovarian cysts. Elevated serum ketone concentrations have no negative effects on bovine follicle cells in vitro, but they do increase the risk of delayed cyclicity (Reist *et al.*, 2000) and cyst occurrence postpartum (Andersson *et al.*, 1991). As a result, ketone concentrations in the postpartum dairy cow appear to be a measure of the severity of the NEB rather than a mechanism for mitigating the NEB's effects on ovarian reproduction.

Classification

Follicle cysts (FC) and luteal cysts (LC) are two types of ovarian cysts that can be distinguished by their morphological or functional traits (steroid production) (2000; Silvia *et al.*, 2002; Vanholder *et al.*, 2006). P4 levels in the peripheral circulation are comparatively high in LC patients. Regarding the FC, it secretes more oestradiol (Vanholder *et al.*, 2006;

Silva *et al.*, 2002) in proportions comparable to those of a typical follicle (Odore *et al.*, 1999) and generates less P4. Rectal palpation or ultrasonography can also be used to differentiate FC from LC (Farin *et al.*, 1992). They impact one or both ovaries, have thin walls, and can be single or many (Jeffcoate *et al.*, 1995).

Vanholder *et al.* (2006) describe LCs as echogenic structures with thick walls (> 3 mm) that have been adequately luteinized. These structures can be seen on ultrasonography. As per Odore *et al.* (1999), these cysts are typically exclusive to the ovary. They are FC in the latter stages. This kind spontaneously luteinizes and secretes P4 through thecal cells and granulosa cells (Garverick, 1997). Although Zemjanis (1970) and Carroll *et al.* (1990) indicated that about 30 and 42% of cysts are luteal, respectively, FC are more common than LC; the majority of cysts (70%) are follicular (Garverick, 1997). Another disorder that has been the subject of several investigations is the cavitary corpus luteum (CCL), which is another variation of a normal corpus luteum and is sometimes mistaken for LC.

More specifically, it is a corpus luteum formed following ovulation, with a central cavity of varied size and fluid content (Silvia *et al.*, 2002; Vanholder *et al.*, 2006). The hollow measures 7–10 mm in diameter. Ultrasound imaging can discriminate between LC and CCL (Hanzen *et al.*, 2000). Some researchers found CCL in pregnant cows and concluded that this form could not support gestation. However, others observed that a corpus luteum needed to produce only approximately 100 µg P4 to maintain gestation, indicating that CCL can support a gestational state (Coleman, 2008).

Incidence and consequence

According to Santos *et al.* (2009), the incidence of OC was shown to range from roughly 6 to 30% following diagnosis by rectal palpation, with an average frequency of 10-15% (Calder *et al.*, 1999). In other research, 18–29% of cases were found following an ultrasound scan or hormonal assay (Gümen *et al.*, 2003). Given that over 60% of OC forms prior to the first ovulation after PP heals on its own, this rate might be greater (Peter, 2004). Allrich (2001) reported the following rates based on calving rank, age, and animal production: heifers: 3-6%, pluriparous: 39%, and uniparous: 11%. The prevalence of meat-producing cows is rather low. Osmanu (1979) discovered that OC was the primary cause of infertility in 26% of Ghanaian cows.

An average incidence of 9.3% was found in Canada based on pooled data from various studies, which included 24,356 lactations (Brito and Palmer, 2004). Silvia *et al.* (2002) report that the polycystic ovary is far more common in cystic cows than in normal cows that have multiple ovulations, with 47% of cystic cows having two or more cysts when they are first discovered. It is estimated that 10–14% of dairy cows would experience OC only once in their lives, and of those, 35% would experience this illness on a chronic basis (Peter, 2004).

It can be inferred that there is a significant difference in the frequency of OC among authors, which can be attributed to the definition criteria and diagnostic methods utilised during screening, as well as the number of animals investigated. According to Kaikimi *et al.* (1983), the right ovary is more afflicted (5.1%) than the left ovary (1.2%), with simultaneous involvement of both ovaries estimated at 0.5%. OCs are more common within the first 60 days following calving (Vanholder *et al.*, 2006). In 70% of instances, OC occurred between 16 and 50 days after PP, with an average of 30 to 40 days (Kirk *et al.*, 1982).

Nanda *et al.* (1991) found a high prevalence of OC in the 20–150-dday PP. Cystic cows experience longer or more irregular follicular development cycles than normal cows (Hamilton *et al.*, 1995). Indeed, the negative effects of this condition on fertility are linked to an increase in the calving-calving interval from 22 to 64 days (Silvia *et al.*, 2002), as well as an extension of the calving-1st insemination and calving-conception intervals (by approximately 13 and 33 days, respectively) (Brito and Palmer, 2004). According to Savio *et al.* (1990), the intervals [calving-1st ovulation] between OC and normal cows are 58 days and 12 days, respectively.

Compared to other unaffected cows in the herd, the number of inseminations per conception increases by about 0.8, and the likelihood of culling is increased by 20 to 50%. Research carried out in the United States of America estimated that the cost of OC when it impaired fertility, including labour, veterinary care, treatment, and reform, was \$137 per lactation. These expenses were not offset by the higher milk output of cystic cows, and net losses were calculated to be \$39 (Brito and Palmer, 2004).

Symptoms and diagnosis

Although symptoms of anoestrus are more common, with a prevalence of 62–85%, OC can also be associated with a permanent oestrus state, which supports its link with nymphomania

or, at the latest, with virilism. (Garverick, 1997; Wiltbank *et al.*, 2002). Although the general symptoms associated with this pathology can vary, affected cows typically have an identical physical appearance to normal cows. These symptoms include an absence of tone in the genital tract, relaxation of the pelvic ligaments, elevation of the tail attachment, abrupt changes in milk production, and the development of male characteristics (Allrich, 2001).

Transrectal palpation reveals that the FC has a thin wall that varies in response to pressure. Although it is not as powerful as a corpus luteum, LC has thicker walls and feels stiffer to the touch than FC (Coleman, 2008). CCL is more erratic, softer, and forms after ovulation than a typical corpus luteum (Roberts, 1971). The most common method is transrectal palpation; however, studies have shown that an accurate diagnosis is only achievable in 50% of instances (Farin *et al.*, 1992; Douthwaite and Dobson, 2000), and it does not provide clear discrimination between FC and LC.

About 10% of instances result in a false positive diagnosis because there are huge follicles next to a large CCL, or corpus luteum. Additional information may be obtained by palpating the uterus. In 4% of cases, mucometrium (about 1 litre) or an edematous state of the uterus that causes it to become solid might accompany FC. Using LC, one can palpate a flaccid uterus. When there is hyperoestrus, the cervix is essentially secreting, and the vaginal mucosa may be clogged. According to Al-Dahash and David (1977), it is open enough in 60% of cases to let a finger pass through and in 16% of cases to let a thumb pass through. Because ultrasound allows for the visualisation of ovarian structures, it is a more reliable approach for diagnosing OC. Given that the core CCL cavity's maximum diameter is less than 20 mm, it can discriminate between a CCL and an LC with ease. FC has a wall thickness of 5 to 10 mm and is anechoic (Boyd and Omran, 1991).

Treatment

Non-hormonal treatment:

OC manual puncture and rupture Zschokke first suggested manual cyst rupture in Germany in 1874, and the cure rate was about 45% (Roberts, 1971). Studies have now revealed that, despite being inexpensive, this technique can result in trauma and bleeding, which can cause adhesions and lower fertility (Kahn, 2010). An alternative technique involves transvaginal OC puncturing either with or without ultrasound guidance (ovam-pick-up, OPU). Due to this,

a first oestrus and gestation may be obtained 34 and 55 days after the puncture, respectively (Cruz *et al.*, 2004). Furthermore, it is less costly and riskier than manual rupture than ultrasound-guided puncture (Viana *et al.*, 2003).

Injection of GnRH and PGF2 α seven days later has also been proposed simultaneously (Amiridis, 2009). Out of the five cows who received GnRH treatment concurrently with the OC puncture, 100% of them showed signs of new oestrus. A total pregnancy rate of 68.4% was reported in a trial that combined GnRH at the puncture with PGF2 α 7 days later (Cruz *et al.*, 2004). The outcomes of OC puncture, as reported in several studies, are displayed in Table 1.

Hormonal treatment

hCG (human Chorionic Gonadotropin) protein hormone, with a luteotropic effect, induces luteinization of OC or other follicles present; therefore, endogenous or exogenous PGF2 α causes luteolysis and a new cycle begins. Very expensive and antigenic, this protein can cause anaphylactic shock or a refractory state in cows, as has been demonstrated in rabbits (Roberts, 1971). In response to hCG, 58 to 86% of cows develop luteal tissue. These results are obtained using the different routes of administration, from intravenous to intracystic, and the different combinations of these routes (Nakao *et al.*, 1978).

GnRH Kittock *et al.* (1973) were the first to use GnRH to treat cysts in five cows that seemed to be in heat 20–24 days later. GnRH stimulates LH release and luteinization in the cyst. Ovulation does not occur; however, additional follicles present during treatment may ovulate (Garverick, 1997). Luteinization restores hypothalamic responsiveness to oestradiol, resulting in normal cyclic ovarian activity, endogenous PGF2 α release, and OC regression (Garverick *et al.*, 1976). The percentage of cows that resume cyclicity is between 72 and 85%. The time between therapy and the first oestrus ranges from 19 to 23 days, with a conception rate of 46 to 58%. The reasons for the approximately 20% of cows that did not respond to the therapy are unknown, as the stimulated release of LH was similar in these cows to those that did respond. However, there is no subsequent increase in P4 levels in cows that do not re-establish ovarian activity (Garverick *et al.*, 1976). Nesson *et al.* (1977) conducted a comparison study with hCG and manual rupture, GnRH and manual rupture, and GnRH alone. All three groups had pregnancy rates ranging from 40 to 47%, and they concluded that HCG and manual rupture are unnecessary when GnRH is used. In another comparison

investigation, buserelin (a more potent GnRH analogue) produced effects similar to those seen with GnRH. Probo *et al.* (2011) found that 71% of 133 cystic cows regained ovarian function after receiving 20 µg buserelin. Lecirelin (another equivalent) has been demonstrated to be useful in treating OC in cows, particularly when injected epidurally. GnRH doses of 50–500 µg resulted in a 49–65% first-insemination pregnancy rate, a 70–100 percent total pregnancy rate, and an average gestation duration of 34–87 days. The most successful conventional treatment for OC in cattle is GnRH, which is less allergenic and less expensive than hCG (Peter, 2004).

PGF2α and hormonal interactions Exogenous PGF2α can induce LC regression 7 to 9 days after GnRH or hCG therapy, reducing the time between treatment and oestrus and improving synchronisation. White and Erb (1980) identified PGF2α as the most effective treatment for LC. Leslie and Bosu's (1983) investigation found that cows with low P4 concentrations who did not respond to PGF2α therapy had a higher percentage of cyst turnover than those with high P4 levels.

Furthermore, 75% of cows were in oestrus within 7 days of receiving this therapy, with a 66% conception rate at the first oestrus. Although some authors have not been able to demonstrate the benefit of simultaneous administration of GnRH and PGF2α (Archbald *et al.*, 1991), a recent study reported that concurrent treatment with these two substances resulted in early oestrus re-establishment in cows with LC (50% of cows were in oestrus before a second treatment with PGF2α, 14 days later). López-Gatius and López-Bejar (2002) found that administering a second treatment with PGF2α resulted in more cows responding to FC compared to GnRH alone.

This treatment is effective because distinguishing between FC and LC is challenging. The Ovsynch protocol, which was developed for fixed-time artificial insemination (AI), has been utilised successfully in oestrus synchronisation programmes and ovulation (Hanzen *et al.*, 2003a; 2003b). It consists of:

- 1st injection of GnRH to stimulate follicular growth and induce ovulation of the dominant follicle, which may be present and the subsequent formation of a corpus luteum;
- An injection of PGF2α 7 days later in order to stop the P4 synthesis and to allow the dominant follicle, if present, to continue to grow and to ovulate;

- A second injection of GnRH after 48 hours, to obtain better synchronization of ovulation and the need to prevent the absence of ovulation, and systematic AI carried out 16 to 20 hours later (Hanzen *et al.*, 2008).

In studies with a large dairy herd (3000 lactating cows) in Florida, this technique resulted in a similar gestation rate (about 27%) in cycling and cystic cows (Bartolome *et al.*, 2003). In a study comparing cyst aspiration and hormone treatment, cows who underwent cyst puncture followed by treatment with ovarian cysts in cattle GnRH and PGF_{2α} had higher recovery rates than those who simply received the puncture or hormone treatment (Amiridis, 2009).

Table 1. comparison of the results obtained during the aspiration of the contents of the ovarian cysts

Treatment	Number of animals	Diagnostic method	Rate of oestrus re-establishment	Gestation rate
Aspiration+GnRH	5 FC	Ultrasound Examination	100%	-
Aspiration	18 FC	Ultrasound Examination	94.5%	66.6%
Aspiration+GnRH/ PGF _α	29 FC	Ultrasound Examination	100%	68.4%

Table 2. Results obtained after hormonal

	GnRH	hCG	PRID
Recovery rate (%)	64	66	63
Pregnancy rate (%)	45.2	47.8	46.2
Conception rate (%)	20	22	20
Recovery time (days)	17.9	17.7	19.7

Preventive treatment

Removing PGF_{2α} resulted in effective strategies for reducing OC incidence and improving reproductive success should prioritise disease and stress reduction, as well as optimising food intake during dry-off and PP seasons (Hooijer *et al.*, 2001).

Table 3. Drugs, doses, routes of administration and protocols for the treatment of OC (Brito and Palmer, 2004)

Molecule	Dose Administration route	
Gonadorelline (GnRH)	100 µg	IM
hCG	10 000 U	IM
Dinoprost (PGF _{2α})	25 mg	IM
Cloprosténol (PGF _{2α})	500 µg	IM
Progesterone	1.9 g	Intravaginal device

Treatment protocol

- i) GnRH (or hCG) + PGF_{2α} (day 0); PGF_{2α} (day 9 in the absence of oestrus)
- ii) Ovsynch: GnRH (day 0); PGF_{2α} (day 7) GnRH (day 9); AI at fixed time, 16 hours after the last treatment with GnRH
- iii) Progesterone device over 12 days (not for dairy cows)

In actuality, metabolic abnormalities associated with an increase in free radical production originate at the time of calving, during the involutionary phase, and at the onset of lactation. The best strategy to get rid of or lessen the negative effects of this stress would be to take antioxidants (selenium, β-carotenes) (Ribadu *et al.*, 2000; Aladrović *et al.*, 2018; Folnožić *et al.*, 2019). It is feasible to monitor the development and duration of the negative energy balance using routine body condition score assessments (López-Gatius and López-Bejar, 2002; Folnožić *et al.*, 2015; 2016). The long-term goal ought to be to identify milk-producing cow families that are not genetically susceptible to the illness (Hooijer *et al.*, 2001).

An injection of GnRH 10–15-day PP has been recommended to restore ovarian activity as soon as feasible and limit the incidence of OC (Zaied *et al.*, 1980). To lessen the likelihood of metritis, Richardson (1983) recommends combining this hormone with PGF_{2α} 10–15 days

later. Noseir *et al.* (2013) discovered no significant influence on fertility measures after providing a double dosage of GnRH (receptal and cystorlin) on the 14th and 21st PP days, respectively.

Nutrition is clearly linked to the reproductive function of dairy cows. Excessive or insufficient feeding can undoubtedly have an impact on reproduction. The fundamental issue is that the degree of excess, deficiency, or imbalance that impairs reproduction will not be determined early on, emphasising the importance of early identification.

2.1 Antimicrobial sensitivity test

There are three different test methods: disc diffusion, broth dilution, and agar dilution. When used correctly, antimicrobial susceptibility testing procedures produce predictable and repeatable results.

One of the phenotypic assays that can be used to ascertain an organism's antimicrobial resistance profile, or anti-biogram, is disc diffusion testing. In vitro susceptibility is estimated using disc diffusion tests. Agar plates are infected with standardised bacterial inoculums, and an antimicrobial disc is placed on top of the inoculated agar plate. This is the basic idea behind agar diffusion. An antimicrobial agent in a standardised, known dose is present on the discs used in disc diffusion assays; the agent diffuses into the agar upon contact with its surface. In accordance with guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), the plate is incubated under standardised conditions.

The antimicrobial ingredient diffuses into the agar during incubation, preventing bacterial growth and creating a zone of inhibition around the disc. After incubation, this zone's diameter is measured, and the results are evaluated using conventional guidelines (e.g., CLSI M100) to determine if the sample is resistant, intermediate, or susceptible. The degree of resistance is indicated by the size of the inhibition zone, which may also provide crucial details regarding the resistance mechanism and the resistance genes involved. Furthermore, MIC values can be ascertained via the disc diffusion method if the required reference curves for converting inhibition zones into MIC values are accessible. For all kinds of susceptibility testing, highly standardised procedures are necessary.

These assays are extremely sensitive to alterations in inoculum density, media formulation, pH, and incubation conditions. Furthermore, agar diffusion methods are greatly influenced by agar depth, antimicrobial agent diffusion rate, and specific bacterial growth rate. To ensure the accuracy and reproducibility of antimicrobial susceptibility test results, CLSI recommends that internal quality control testing be undertaken on a regular basis (CLSI M2, 2017).

2.2 Immune cell

Granulocytes consist of basophils, eosinophils, and neutrophils. Basophils and eosinophils play a significant role in host parasite defence. They're also implicated in allergic reactions. Neutrophils, the most prevalent innate immune cells, circulate in the bloodstream to look for abnormalities. They can phagocytose, or consume, germs and degrade them within specific compartments known as vesicles.

Mast cells also are important for defense against parasites. Mast cells are found in tissues and can mediate allergic reactions by releasing inflammatory chemicals like histamine.

Monocytes, which mature into macrophages, patrol and respond to disturbances. They are found in both the circulation and tissues. Macrophages, meaning "big eater" in Greek, are named after their ability to consume and digest microorganisms. When activated, monocytes and macrophages coordinate an immune response by informing other immune cells about the problem. Macrophages also perform vital non-immune jobs, such as recycling dead cells like red blood cells and removing cellular waste. These "housekeeping" functions occur without the initiation of an immune response.

Neutrophils accumulate in minutes at locations of local tissue damage. They subsequently communicate with one another via lipids and other secreted mediators, forming cellular "swarms." Their coordinated mobility and signal exchange direct other innate immune cells, such as macrophages and monocytes, to surround the neutrophil cluster and establish a tight wound closure.

Dendritic cells (DC) are an essential antigen-presenting cell (APC) that can arise from monocytes. Pathogens, host cells, and allergens all produce antigens that adaptive immune cells can recognise. APCs, such as DCs, convert big molecules into "readable" fragments (antigens) that adaptive B or T cells recognise. However, antigens alone cannot activate T lymphocytes. They must be shown the appropriate major histocompatibility complex (MHC)

expression on the APC. MHC functions as a checkpoint, allowing immune cells to discriminate between host and foreign cells.

Natural killer (NK) cells exhibit characteristics of both innate and adaptive immunity. They are essential for identifying and eliminating virus-infected or tumour-infected cells. They contain intracellular compartments known as granules, which are packed with proteins capable of causing holes in the target cell as well as apoptosis, or programmed cell death. It is critical to distinguish between apoptosis and other types of cell death, such as necrosis. Apoptosis, unlike necrosis, does not produce warning signals that can cause increased immune activation and inflammation. Immune cells can use apoptosis to remove infected cells discretely and limit bystander damage. Researchers recently demonstrated in animal models that NK cells, like adaptive cells, may be kept as memory cells and respond to recurrent infections in the same way.

According to Asuncion *et al.* (2020), hyperandrogenism and hormonal imbalance are the primary causes and symptoms of PCOS. Based on mounting data, hormones and immune cells seem to be key players in the development of PCOS. Hyperandrogenism can cause chronic ovarian tissue inflammation, disrupt the maturation of developing follicles in the ovary, and cause cystic follicle formation by stimulating monocyte infiltration in the ovary and increasing the secretion of inflammatory factors like interleukin-6 (IL-6) and tumour necrosis factor- α (TNF- α) (ESHRE & ASRM *et al.*, 2004). Additionally, research has revealed that the peripheral blood of the majority of PCOS women had significantly higher levels of inflammatory elements than that of normal women, including TNF- α , soluble endothelial leukocyte adhesion molecule (SE selectin), soluble intercellular adhesion molecule (sICAM), TNF- α , interleukin-1 β (IL-1 β), interleukin-17 (IL-17), interleukin-18 (IL-18), and monocyte chemoattractant protein-1 (MCP1). PCOS patients' increased visceral fat, in which adipocytes undergo necrosis following hypoxia and assemble a large number of inflammatory cells to release a variety of inflammatory cytokines, is mostly responsible for the low-grade chronic inflammation. Immune cells secrete pro- or inhibitory cytokines that can either trigger or reduce host inflammation. In reaction to antigen activation, immune cells release three types of immunological molecules: complements, antibodies, and lymphokines (Rudnicka *et al.*, 2022). Immune cell dysfunction or an imbalance in variables related to immunity might result from abnormal immune cell activity. Moreover, several immunological molecules have a direct bearing on the immune response and are strongly

associated with the pathological alterations of PCOS. In order to emphasise recent studies on potential roles and regulatory mechanisms in the development of PCOS, this article briefly summarises our current understanding of the many types of innate immune cells and immunological chemicals (Foroozanfard *et al.*, 2017).

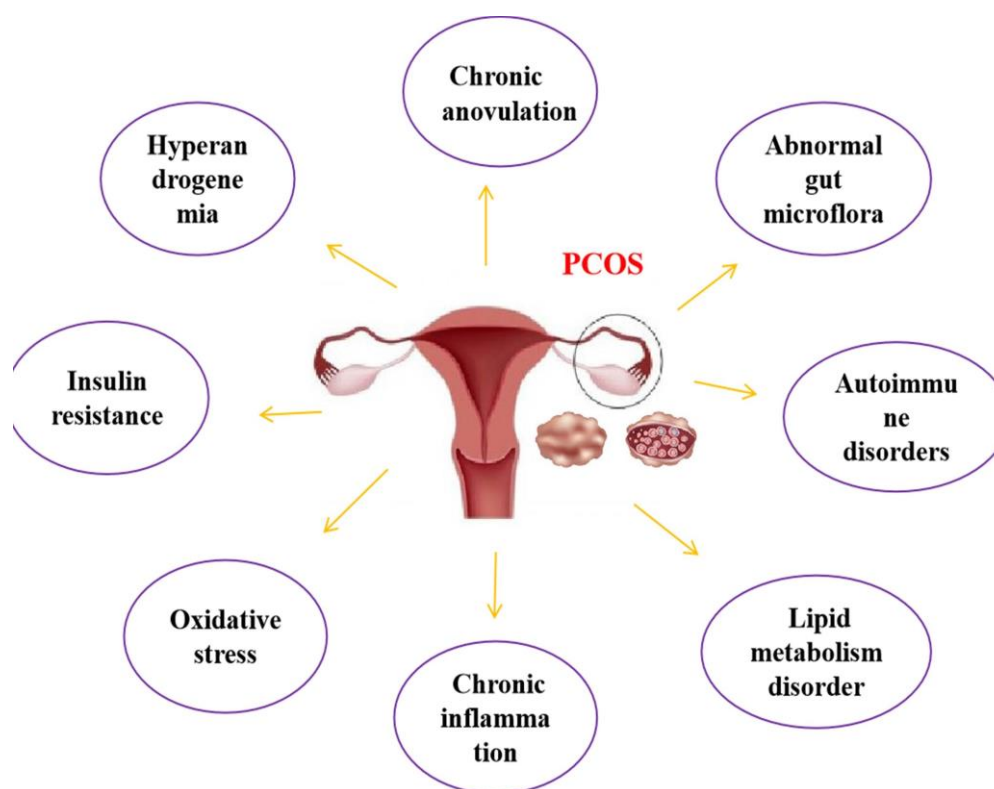


Fig. 1: overview of disorders of PCOS (poly cystic ovarian syndrome) (Luan *et al.*, 2022)

Macrophages are significant in PCOS immune control because they are both anti-infective cells in the body's natural immunological response and antigen-presenting cells (APC) in specific immunity. In slim PCOS patients, there were more macrophages and pro-inflammatory substances in adipose tissue, which led to insulin resistance (IR) (Carron *et al.* 2020). Hyper homocysteinemia, which modulates M2 macrophage polarisation, can enhance IR and adipose tissue inflammation in PCOS mice. One of the most important etiological variables in PCOS is assumed to be insulin resistance. IR affects around 85% of all PCOS patients.

Unusual high insulin levels worsen the pathogenic process of PCOS, increase the synthesis of androgen in ovarian theca cells, reduce insulin receptor autophosphorylation in ovarian granulosa cells, and enhance the ovarian chronic inflammatory response and IR. (Zhu *et al.*,

2020). According to Khashchenko *et al.* (2020), macrophages have the ability to produce cytokines and chemokines into the bloodstream, including IL-6, IL-10, IL-18, MIF, and TNF- α . This can lead to systemic, persistent, low-grade inflammation. Chronic inflammation is assumed to be the primary cause of ovarian dysfunction. Peripheral M1 macrophages increased while M2 macrophages decreased in the DHEA-induced PCOS mouse model (Khashchenko *et al.* 2020). A change in macrophage polarisation from an anti-inflammatory M2 to a proinflammatory M1 state is a characteristic of PCOS (Qi *et al.*, 2017; Khashchenko *et al.*, 2020, Luan *et al.*, 2022).

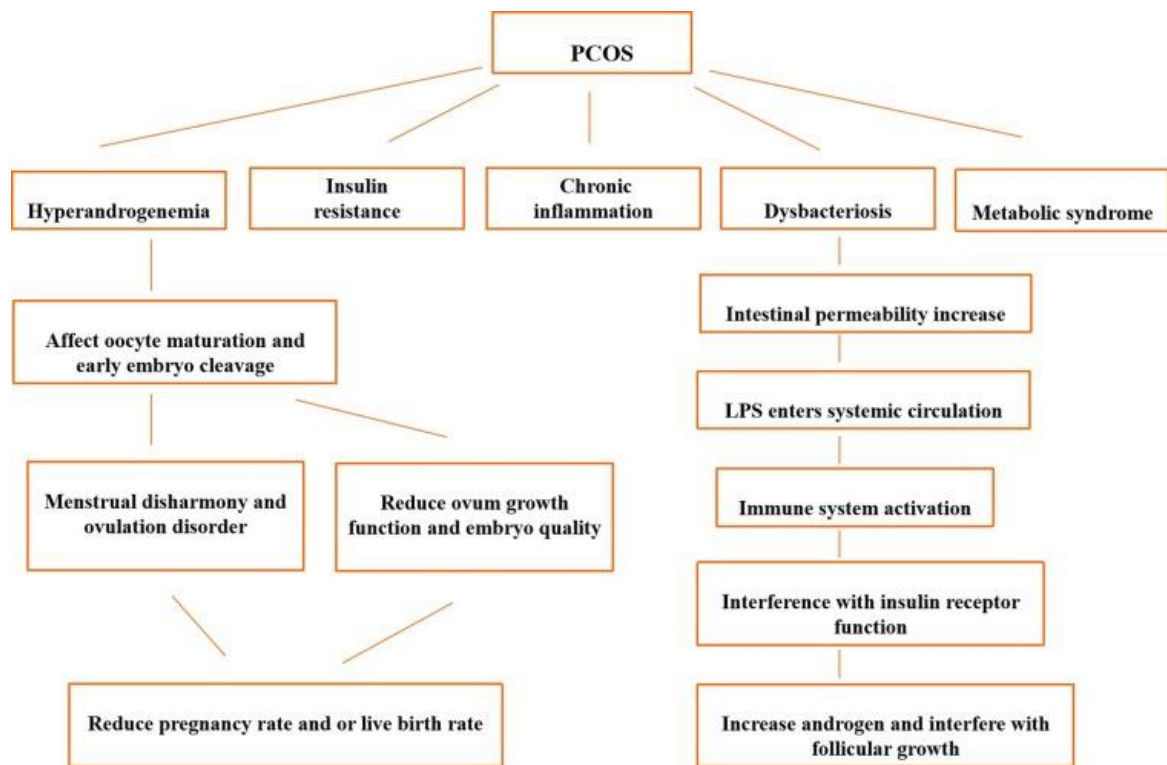


Fig. 2: cystic ovary immunization influence (Luan *et al.*, 2022)

Unusual high insulin levels worsen the pathogenic process of PCOS, increase the synthesis of androgen in ovarian theca cells, reduce insulin receptor autophosphorylation in ovarian granulosa cells, and enhance the ovarian chronic inflammatory response and IR. (Zhu *et al.*, 2020). According to Khashchenko *et al.* (2020), macrophages have the ability to produce cytokines and chemokines into the bloodstream, including IL-6, IL-10, IL-18, MIF, and TNF- α . This can lead to systemic, persistent, low-grade inflammation. Chronic inflammation is assumed to be the primary cause of ovarian dysfunction. Peripheral M1 macrophages increased while M2 macrophages decreased in the DHEA-induced PCOS mouse model

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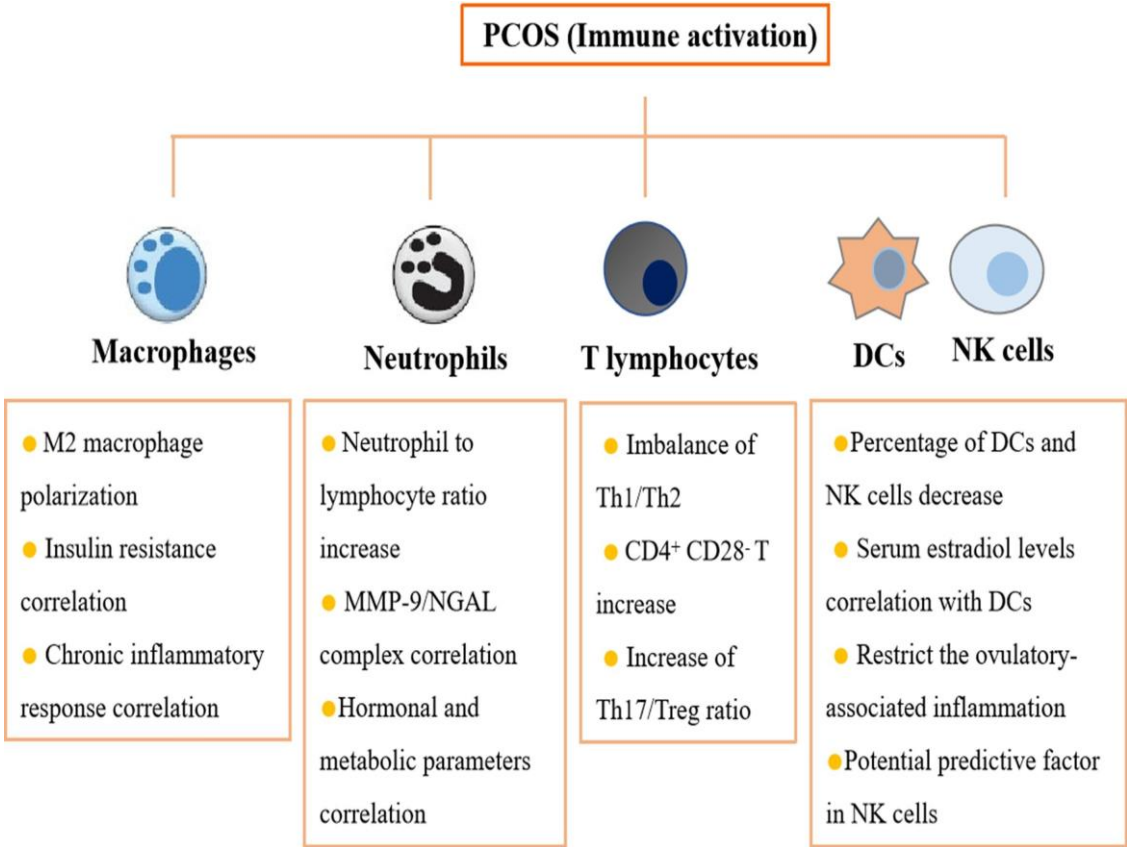


Fig. 3: Cystic ovarian immune cells (Luan *et al.*, 2022)

According to Khashchenko *et al.* (2020), macrophages have the ability to produce cytokines and chemokines into the bloodstream, including IL-6, IL-10, IL-18, MIF, and TNF- α . This can lead to systemic, persistent, low-grade inflammation. Chronic inflammation is assumed to be the primary cause of ovarian dysfunction. Peripheral M1 macrophages increased while M2 macrophages decreased in the DHEA-induced PCOS mouse model (Khashchenko *et al.* 2020). A change in macrophage polarisation from an anti-inflammatory M2 to a proinflammatory M1 state is a characteristic of PCOS (Qi *et al.*, 2017; Khashchenko *et al.*, 2020).

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

To assess the effects of ovarian cysts in dairy cattle, this study was carried out from January to June 2023 at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, in cooperation with the departments of pathology and parasitology, physiology, and pharmacology. Additionally, samples from other abattoirs in the Dinajpur district were taken.

3.2 Experimental layout

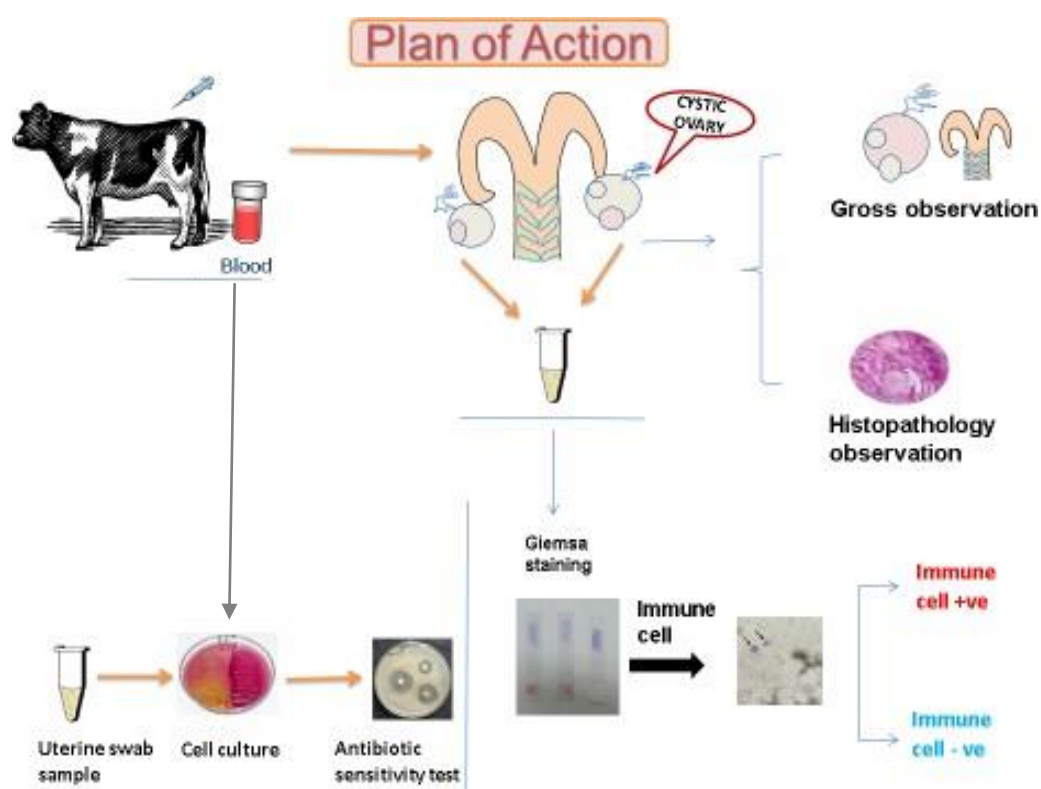


Fig. 4: Experimental layout

3.3 Sample collection

Peripheral blood samples (about 2.5 ml) and uterine samples (about 2.5 ml) were taken from several dairy farms and the abattoir in Dinajpur Sadar upazila, Dinajpur, and placed in an EDTA tube and a saline solution, respectively.



Figure 5: Collection of blood sample and uterus sample

3.4 Transportation and preservation of samples

The samples were transported to the lab in an ice box filled with ice, where they were processed for the subsequent isolation, identification, and characterization of bacteria. Additionally, the remaining samples were kept in storage at 4°C for later use. A formalin solution is used to preserve the histological samples.

3.5 Identification of immune cell

Blood samples were assessed using uterine fluid smear microscopy and stained with Giemsa. The impacts of season and geography were taken into account in this study. Uterine fluid smears that were thick and thin were produced using Giemsa staining. Absolute methanol fixing and air drying were done. The smear was stained with 10% Giemsa's stain following methanol fixation. Magnification under 100x goals follows emulsification, air drying, and washing. Microscopically, the immune cells were identified by applying the techniques described by Benjamin (1978) and Soulsby (1982).

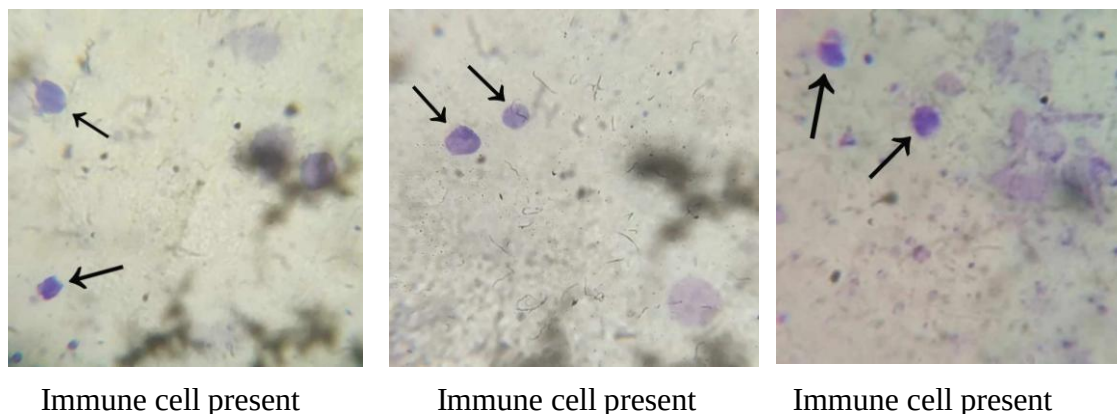


Figure 6: Identification of Immune cell

3.6 Media for culture

Different bacteriological culture media and reagents were employed to isolate, identify, and propagate microorganisms from the obtained materials. The culture media and reagents used in this experiment are listed below.

3.6.1 Liquid media

1. Nutrient broth (Difco)

3.6.2 Solid media for culture

1. Nutrient Agar Medium, (HI-MEDIA, India)
2. Eosin Methylene Blue, (EMB) (HI-MEDIA, India)
3. Mannitol salt agar

3.6.3 Reagents

1. Crystal violet dye
2. Gram's iodine
3. Alcohol
4. Safranin
5. Saline
6. Iodine solution
7. Kovac's reagent
8. Methyl- red solution
9. 3% H₂O₂
10. P – Amino dimethylaniline oxalate
11. Phenol red
12. Phosphate buffered saline (PBS) solution
13. Potassium tellurite (1%)1.5 Reagent

3.6.4 Glassware and appliances

The different types of important equipment used for this work are listed as follow down

1. Distilled water
2. Sterile bent glass or plastic spreader rods.
3. Micropipette (1-5µl; 5-50µl; 10-100µl; 50-500µl; 100-1000µl)

4. Freeze (-20°C)
5. Refrigerator (40C)
6. Spirit lamp
7. Water bath
8. Vortex Mixture
9. Labeling tape
10. Experimental test tube
11. Stopper
12. Petridish
13. Conical flask.
14. Durham's tube
15. Slide
16. Microscope
17. Cotton
18. Immersion Oil
19. Toothpick
20. Autoclave
21. Thermometer
22. Incubator
23. Jar
24. Beaker
25. Cylinder
26. Electric Balance
27. Filter paper
28. Spirit lamp and
29. Bacteriological loop etc.

3.7 Materials used for antibiogram test

- Test tube rack
- Bunsen burner
- Inoculating loop or needle
- Forceps
- Sterile swabs
- Mueller-Hinton or Nutrient agar plates

- Antibiotic disks
- Stock culture of experimental bacteria

3.8 Methods

The following methods were used for the isolation and identification of bacteria.

3.8.1 Laboratory preparations

All essential glassware, including test tubes, pipettes, plates, slides, cylinders, flasks, conical flasks, glass, and vials, was soaked in a home dishwashing detergent solution (Trix Recket and Coleman Bangladesh Ltd.) overnight. Before cleaning, the contaminated glassware was disinfected with a 2% sodium hypochlorite solution. Glassware was cleaned with a brush, washed thoroughly with running tap water, rinsed with distilled water, then sterilised with either dry heat at 160 °C for 2 hours or autoclaving for 15 minutes at 121 °C under 15 lbs. of pressure per square inch. Autoclaved goods were dried in a hot air oven at 50 °C. Autoclaving was used to sterilise disposable plastic products (such as micropipette tips). All glassware was stored in an oven at 50 °C for future use.

3.8.2 Sampling and processing of samples

During the collection operation, proper precautions were taken to avoid sample contamination. The sample tubes were completely tied at the time of sampling to avoid contamination. After arriving at the laboratory, 1g of each sample was mixed with 9 ml of PBS (Phosphate Buffer Solution) in each test tube for serial dilution (10⁻¹ to 10⁻⁷). 50 µl samples were seeded on nutritional agar using the spread plate method. To maintain aseptic conditions, the medium was plated in laminar flow and then incubated at 37 °C for 24 hours.

3.8.3 Plan of the experiment work at a glance

All of the samples were obtained from various surface conditions in Dinajpur district using an ice-containing ice box. The samples were then transported to the microbiological laboratory of the Department of Microbiology at HSTU in Dinajpur, Bangladesh. 50 µl (0.05 ml) of samples were inoculated onto nutrient agar. Nutrient agar, EMB agar, and Mannitol salt agar were then used to identify bacteria. Molecular characterization was also carried out for

genome sequencing. Finally, an antibiotic sensitivity test was done using the pure isolated bacteria.

3.8.4 Preparation of culture media

All the media, broth and reagents used in this experiment were prepared according to instruction of the manufacturer.

3.8.5 Nutrient broth media

A full solution was achieved by suspending 13 grams of dried nutritional broth (HI-MEDIA, India) in 1000 milliliters of distilled water and boiling it. Subsequently, the solution was filled into tubes, sealed with cotton plugs, and autoclaved for 15 minutes at 121°C and 1.2 kg/cm² of pressure. The medium's sterility was verified by overnight incubation at 37°C and storage in an aerator at 4°C for later use. (Carter, 1979).

3.8.6 Nutrient agar media

In a flask, twenty-eight grams of nutritious agar powder (HI-MEDIA) were dissolved in one thousand milliliters of cold distilled water. Next, the medium was autoclaved to achieve sterilization. The medium was put into each sterile Petri dish after it had been autoclaved and allowed to harden. Following the medium's solidification in the petri dishes, these were either refrigerated at 4°C for later use or incubated at 37°C for an entire night to verify their sterility before being utilized for cultural characterization. (Cater, 1979).

3.8.7 Eosin methylene blue agar

To fully dissolve the medium, 36 grams of EMB agar base (HI-MEDIA, India) were added to 1000 milliliters of water in a flask and heated to a boil. To oxidize the methylene blue and regain its blue color, the medium was autoclaved for 15 minutes at 121°C under 1.2 kg/cm² pressure, then cooled to 50°C and shaken. After that, 10 milliliters of the medium were added to each sterile Petri dish and left to solidify. Following the medium's solidification in the petri dishes, these were sterilized by overnight incubation at 37°C. Petri dishes that did not contain any contamination were either employed for cultural characterization or refrigerated at 4°C for later use. (Carter, 1979).

3.8.8 Mannitol salt agar

Boil 1000 milliliters of distilled water with 111 grams of mannitol salt agar suspended in it. Once the medium has completely dissolved, autoclave it at 15 pounds of pressure (121 degrees Celsius) for 15 minutes. Each sterile Petri dish was filled with 10 ml of the medium, which was then left to harden. Petri dishes were utilized for cultural characterization, and sterile petri dishes were employed after the medium solidified in the dishes and they were incubated at 37°C for an entire night. For later use, store the prepared medium at 2-4 degrees Celsius and the dehydrated medium at 24 degrees Celsius.

3.8.9 Preparation of reagents

3.8.10 Methyl- red solution

0.1 g of Bacto methyl-red was dissolved in 300 ml of 95% alcohol to create the indicator MR solution, which was then diluted to 500 ml with the addition of distilled water.

3.8.11 Methyl- red

After dispensing 2 milliliters of the dissolved 17 grams of MR-VP medium in 1000 milliliters of distilled water into each tube, the tubes were autoclaved. The medium-containing tubes were autoclaved, checked for sterility by overnight incubation at 37 degrees, and then refrigerated for later use.

3.8.12 Alpha- naphthol solution

Alpha- naphthol solution was prepared by dissolving 5 gm of Alpha- naphthol in 100 ml of 95% ethyl alcohol

3.8.13 Potassium hydroxide solution

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of Potassium hydroxide crystals in 100 ml of cooled water.

3.8.14 Phosphate buffered saline solution

To prepare phosphate-buffered saline solution, eight grams of sodium chloride (NaCl), 2.89 grams of di-sodium hydrogen phosphate (DSHP), 0.2 grams of potassium chloride (KCl), and

0.2 grams of potassium hydrogen phosphate (KHP) were suspended in 1000 milliliters of distilled water. To completely dissolve the fluid, heat was applied.

After that, the solution was preserved for later use by autoclaving it for 15 minutes at 121°C and 1.2 kg/cm² of pressure.

3.8.15 Indole reagent (Kovac's reagent)

Five grams of para-dimethylaminobenzaldehyde crystals were added to the mixture after 25 milliliters of concentrated hydrochloride acid were dissolved in 75 milliliters of amyl alcohol to create this solution. For later usage, this was then stored in a flask with a rubber stopper.

3.8.16 Crystal violet solution (0.5%)

As the main coloring ingredient, dissolve 500 mg of crystal violet in 75 ml of distilled water, add 25 ml of methanol, and keep at room temperature (Isenberg, 1998).

3.8.17 Gram's iodine

Dissolve 6.7 gram of potassium iodide in 100mL of demonized water, add 3.3 g of iodine; stir to dissolve, then dilute to 1 L. Store in a bottle which acts as a mordant. (Isenberg HD.; 1998)

3.8.18 Safranin

Dissolve 0.1 g safranin in 75 mL of distilled water, then dilute to 100 ml. Filter before use which acts as a counter stain (Isenberg, 1998).

3.9 Isolation of bacteria

3.9.1 Isolation of bacteria in pure culture

As recommended by Poindexter (1986), the mixed culture was injected into nutrient agar media using a streak plate technique to produce isolated colonies in order to isolate the bacteria in pure culture.

3.9.2 Isolation and identification of bacterial pathogens

The obtained samples were directly inoculated onto nutritional agar and incubated at 37°C for 24 hours with the aid of a sterile inoculating loop. After that, the incubated medium was checked for bacterial growth.

Inspection:

The colony features and growth of microorganisms were documented in accordance with the protocols outlined by Carter (1979).

Secondary culture on differential media

The organisms were added to MacConkey agar and cultured at 37°C for 24 hours. The incubated media were then tested for bacterial growth.

Step-1: A sterile inoculating loop was used to pick up an inoculum and disperse it over a specific area of the medium in the petri dish.

Step -2: The loop was sterilized by being heated as red hot in a flame.

Step -3: The inoculums were disseminated over the rest of the plate by pulling the cooled, sterilised loop across a portion of the inoculated region and streaking in a single direction along each parallel line. This process was done as many times as needed to produce a culture with only one type of colony, and usually at least twice more to ensure purity.

3.9.3 Culture on selective media

- EMB agar for *E. coli*
- Mannitol salt agar for *Staphylococcus aureus*

3.10 Isolation of bacteria by colony morphological characteristics

Each of the colonies was seen for size, shape, pigment, diameter, form, margin and elevation.

3.11 Gram's staining

Gramme's staining method was used to investigate the morphological and staining properties of bacteria and to offer information regarding presumptive bacterial identification, as recommended by Merchant and Packer (1967). A pure colony of each isolate was selected, and gramme staining was conducted. The isolates' shapes, arrangements, and gramme reactions were then examined under a microscope.

3.12 Gram's staining method

Clean glass slides were obtained using a sterile technique, and smears of each organism were prepared by placing a drop of normal saline on the slide, then transferring each organism separately on the slide with a sterile, cooled loop and mixing the organisms by circular inoculating loop. The smears were allowed to air-dry before being heat-fixed in the usual manner. Smears were drenched with crystal violet, allowed to stand for one minute, then washed with tap water. Gradually pour Gramme's iodine mordant over the smears and allow them to stand for one minute. Used tap water to gently wash 10 seconds of the 95% alcohol-acetone addition, followed by a gentle tap water wash. For one minute, safranin was applied as a counterstain. Wash gently with tap water, then let the air dry. Then investigated using immersion oil under a microscope with high-power objects (100 X).

Gram's staining observation –

- Gram Negative: pale to dark red
- *E. coli*- Rod shaped, arranged singly or in pairs.
- *Staphylococcus aureus* – blue stained diplococcic
- *Klebsiella pneumonia*- rod shaped

3.13 Antibigram test

The antimicrobial sensitivity test was conducted in accordance with Jan Hudzicki's (2009) Kirby-Bauer disc diffusion susceptibility test technique. Originally created in the 1950s, it underwent refinement under W. Kirby and A. Bauer before being standardized in 1961 by the World Health Organization. Antibiotic resistance and susceptibility testing is most commonly performed using the Kirby-Bauer (K-B) disc diffusion test. Using various commercial antibiotic disc types, sensitivity to antibiotics was investigated on Mueller-Hinton agar plates. Following the placement of the discs on the plates, the plates were turned over and incubated for 16 to 18 hours at 37°C. Following incubation, the diameter of the disc as well as the zones of complete inhibition were measured and recorded in millimeters.

3.13.1 Antibigram discs

To identify the drug resistance pattern of various bacterial isolates, the following antibiotics were tested against the selected organism with their disc concentrations:

Table 4: Antimicrobial agents with their disc's concentration

Antimicrobial class	Name of antimicrobial agents	Disc code	Disc concentration (µg/disc)	Company Name
Aminoglycosides	Amikacin	AK	30	HIMEDIA
Folate pathway Inhibitor	Clotrimazole	COT	1.25/23.75	HIMEDIA
Aminopenicillin	Ampicillin	AMP	25	HIMEDIA
Cephalosporin	Ceftriaxone	CTR	30	HIMEDIA
Phenicol	Chloramphenicol	C	30	HIMEDIA
Fluroquinolone	Ciprofloxacin	CIP	5	HIMEDIA
Aminoglycoside	Streptomycin	ST	10	HIMEDIA
Aminoglycosides	Neomycin	NE	30	HIMEDIA
Tetracycline	Tetracycline	TE	30	HIMEDIA

Note: µg = Microgram

3.14 Histopathological examination

During necropsy, numerous organs with gross lesions were collected and stored in 10% formalin. Formalin-fixed ovary samples from blood protozoa-infected dairy cows were paraffin-embedded, sectioned, and stained with hematoxylin and eosin according to standard protocol (Luna, 1998) for histopathological analysis. Details about tissue processing, sectioning, and staining are provided below.

3.15 Equipment and appliances

- Samples (Ovaries)
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX).
- Microscope
- Microtome

3.15.1 Processing of tissue for histopathology

3.15.2 Collection of tissue and processing

During tissue collection the following point were taken into consideration: The tissues were collected in conditions as fresh as possible.

Normal and diseased tissues were collected side by side.

The thickness of the tissues were as less as possible (5mm approximately).

3.15.3 Fixation

10% formalin was added in the plastic container (10 folds of the tissue size and weight) for fixation of samples.

3.15.4 Washing

The tissues were trimmed into a thin section and washed over night in running tape water to remove formalin.

3.15.5 Dehydration

The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule.

- 50% alcohol — one hour
- 70% alcohol — one hour
- 80% alcohol — one hour
- 95% alcohol — one hour
- Absolute alcohol — three changes (one hour for each change.)

3.15.6 Cleaning

The tissues were cleaned in chloroform for 3 hours to remove ethanol (1 and half hr in each, two changes).

3.15.7 Impregnation

Impregnation was done in melted paraffin (56- 60°C) for 3 hours.

3.15.8 Embedding

Paraffin blocks containing tissue pieces were made using templates and molten paraffin.

3.15.9 Sectioning

The tissues were then sectioned with a microtome to 5-6 micrometre thickness. The portions were stretched out on a lukewarm water bath (40-42°C) before being transferred to a glass slide. A modest amount of gelatin was added to the water bath to improve the section's adherence to the slides. The slides containing sections were air dried and kept in a cold area before staining.

3.16 Routine hematoxylin and eosin staining procedure

Preparation of ehrlich's hematoxylin solution

Hematoxylin crystals 4.0 g Alcohol, 95% 200.0 ml Ammonium or potassium alum 6.0 g Distilled water 200.0 ml Glycerin 200.0 ml Glacial acetic acid 20.0 ml

Hematoxylin was dissolved in alcohol, while alum was dissolved in distilled water and carefully blended. After these are completely dissolved, glycerin and acetic acid are added.

Preparation of eosin solution 1% stock alcoholic eosin

Eosin Y, water soluble 1 g Distilled water 20 ml 95% alcohol 80 ml Eosin was dissolved in water and then 80 ml of 95% alcohol was added.

Working eosin solution Eosin stock solution 1 part Alcohol, 80% 3 parts 0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

Staining protocol

The sectioned tissues were stained as described below:

The sectioned tissues were deparaffinized in three changes of xylene (three minutes in each) and then rehydrated in descending grades of alcohol according to the following schedule:

- Absolute alcohol — three changes (three minutes for each)
- 95% alcohol - two minutes
- 80% alcohol - two minutes
- 70% alcohol - two minutes
- Dipping with distilled water for 10 minutes.
- The tissues were stained with Harris hematoxylin for 10 minutes.
- Washed in running tap water for 10-15 minutes.
- Then the tissues were dipped in ammonia water (few dips).
- Stained with eosin for one minute.
- Differentiated and dehydrated in ascending grade of alcohol.
- 95% alcohol — three changes (2-4 dips for each.)
- Absolute alcohol — three changes (2-3 minutes for each)
- Cleaned in xylene: three changes (five minutes each).

Tissues were mounted with cover slip by using DPX. The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

3.17 Microscopic examination

Samples were fixed, processed, sectioned, stained and observed under microscope using various objectives (X4, X10, and X40).



Fig. 7: Microscopic examination

CHAPTER IV

RESULTS

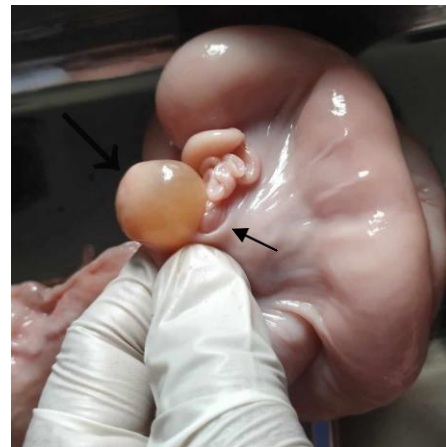
This study investigated immunization, gross lesions, histopathology, identification (staining, selective media), and antibiotic sensitivity (Muller-Hinton agar) of ovarian samples. The study collected 40 reproductive tract samples from abettors in the district of Dinajpur, Bangladesh.

4.1 Gross observation of ovary

40 reproductive tract samples are taken from the Dinajpur district. The levels of progesterone and oestrogen are then measured using blood samples. Below are some images of some unsettling findings, including a cystic ovary (Figure 8).



A. Normal ovary



B. Follicular Cystic ovary



C. Luteal Cystic ovary

Fig. 8: Gross observation of ovary. A: Normal ovary B and C: Here abnormal follicle and anovulatory condition

4.2 Histopathological examination

40 reproductive tract samples were collected from Dinajpur district's abattoir. After that, the histopathology of the normal uterus and ovary (having normal structure) individuals (Figure 9) and follicular cystic ovary and uterus (containing inflammation and fibrous tissue) individuals (Figure 10 and 11) is carried out.

Microscopic analysis of OC was used to define the cystic walls and distinguish between different forms of OC (follicular or luteal cysts). More specifically, changes in OC layers such as granulosa: presence or absence, number of granulosa cell layers, theca: arrangement with the basal lamina, form and type of these cells, and basal lamina: presence or absence, as

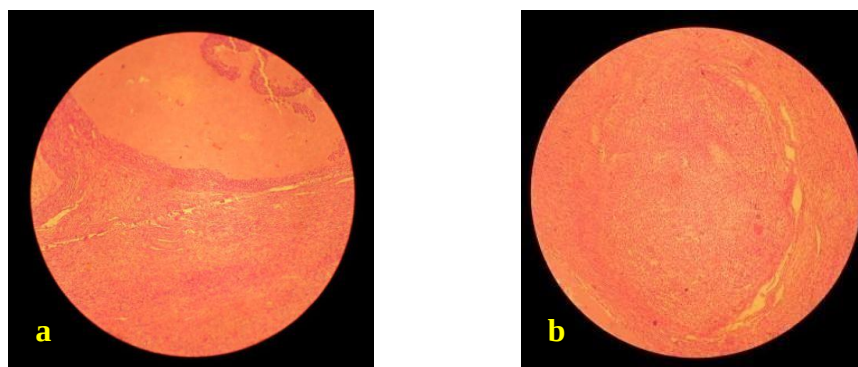


Fig 9: Histopathology of normal control samples (a. uterus; b. ovary)

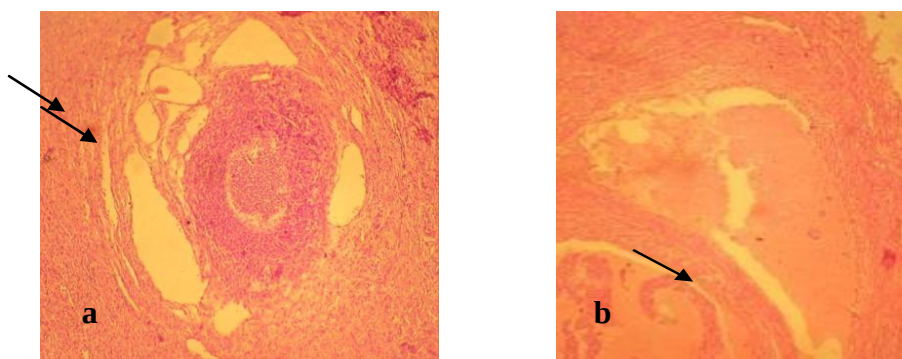


Fig 10: a) Histopathology of follicular cystic ovary (fluid accumulation) & b) no changes of ovary structure

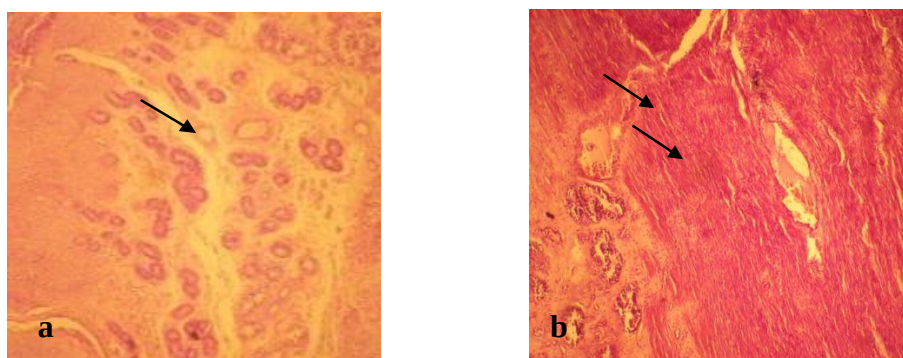


Fig 11. a. Histopathology of uterus having follicular cyst (containing inflammation and fibrous tissue) & b. no changes of uterus structure (control)

4.3 Identification of bacterial load

Important uterine pathogenic microorganisms are linked to more severe clinical disease, greater endometrial inflammation, and lower fertility (Farin *et al.*, 1989; Bonnett *et al.*, 1993). *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, *E. coli*, *Staphylococcus aureus*, and *Prevotella spp.* work together to induce more severe clinical symptoms (Ruder *et al.*, 1981; Olson *et al.*, 1984). Although bacterial load had an influence on folliculo genesis in the current investigation, uterine bacterial pathogens played no meaningful role. This study suggests that the inflammatory response to the level of bacterial contamination is more relevant than the presence of specific bacterial species. The findings of the current investigation support a localised effect of uterine bacterial contamination on the ovary after parturition (Sheldon *et al.*, 2002). Our findings identification of bacterial load in the bellow figure 12 and table 5.

Table 5: The cultural characteristics of *E. coli*, *Staphylococcus aureus*, various media are presented

Sample no.	Name of the uterine cyst	Name of the bacteria
1	control 	No specific bacteria found
2	Folliular cyst 	<i>Escherichia coli</i>
3	Folliular cyst 	<i>Staphylococcus aureus</i>



Fig: No bacteria growth in control sample Fig: Mannitol salt agar(*Staphylococcus aureus*)

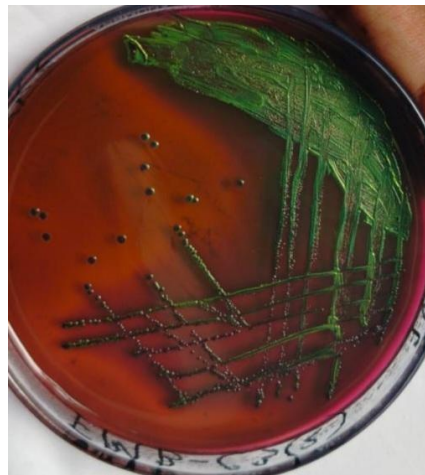


Fig: EMB Agar Media (*E.coli*)

Fig. 12: Cultural characteristics of bacteria

Table 6: Cultural characteristics of *E. coli*, *Staphylococcus aureus* and *klebsiella pneumoniae*

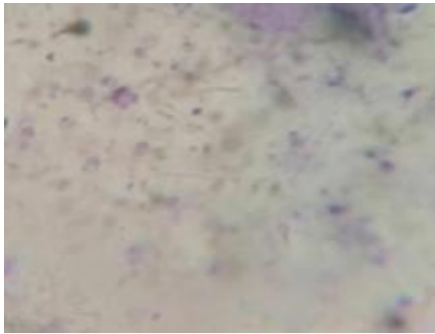
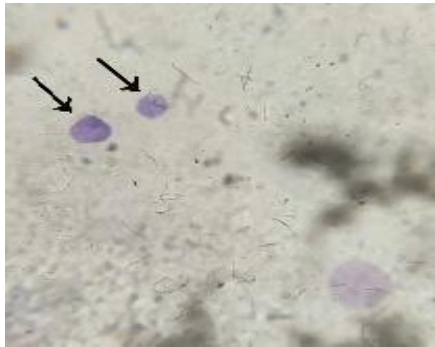
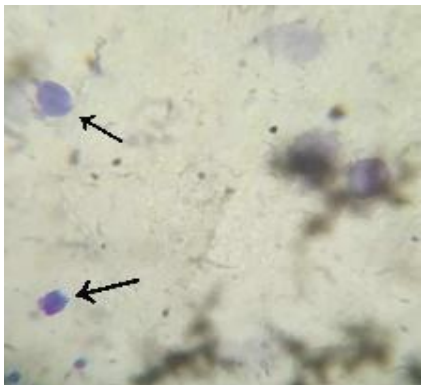
Name of Bacteria	Staining characteristic	Name of Media	Colony characteristics
<i>E. coli</i>	Gram negative large rod shaped, pink color, arranged singly or in pair.	Nutrient Agar	Large, mucoid, white colony.
		Mac-Conkey Agar	Produce large mucoid rose-pink colony.
		EMB agar	Transmitted light blue-black center with a narrow, clear edge. Bluegreen metallic sheen with reflected light.
<i>Staphylococcus aureus</i>	Spherical shape, grape like clusters	Nutrient Agar	Golden yellow and opaque colonies with smooth glistening surface, 1-2mm in diameter
		Mac-Conkey Agar	The colonies are small and pink in color
		Mannitol salt agar	Yellow color colonies
<i>Klebsiella pneumoniae</i>	Nonmotile, encapsulated, Rod-shaped bacillus	Nutrient agar	Colonies are large, thick, greyish white, mucoid, smooth and opaque
		Emb agar	Pink to purple colonies

Here, EMB Agar = Eosin Methylene Blue Agar

4.4 Identification of immune cell

Some variables are immunologically expressed differently in ovarian cystic walls than in the normal group. In this work, the expression of a cytoskeletal protein, an anti-apoptotic factor, an angiogenic molecule, and the inhibin A component was altered in cystic walls. Thus, it is determined that ovarian cysts are an association of multiple illnesses centred on the same pathology in cattle (Mimoune *et al.* 2020). The identification of immune cell such as, t-lymphocytes, neutrophil, monocyte, basophiletc presented in table no 7.

Table 7: The identification of immune cells

Sample no.	Name of the uterine cyst	Immune cell	Name of cells
01	Control	Absent 	
09	Follicular cyst	Present 	lymphocyte
16	Follicular cyst	Present 	lymphocyte

4.5 Antibigram test

For antibiogram test common antibiotics such as amikacin, clotrimazole, ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, colistin, penicillin, mecillinam, rifampicin, streptomycin and levofloxacin were used for *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* 6 antibiotics (Neomycin, clotrimazole, ampicillin, penicillin, streptomycin, ciprofloxacin, penicillin, streptomycin, ciprofloxacin and) were used for antibiogram test.

4.6 Antimicrobial profile

Antibiogram test showed that isolates from every study area resistant to, clotrimazole, any antibiotics where isolates were sensitive to. Neomycin, ampicillin, streptomycin, ciprofloxacin, penicillin percentage of antibiotic resistance pattern for *bacterial samples* was presented in Table no. 8.

Table 8: Antimicrobial profile

Antimicrobial agent	Disc code	Potency (µg/Disc)	Result							
			Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Amikacin	AK	30	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Clotrimazole	COT	1.25/23.75	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(R)
Ampicillin	AMP	25	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Ceftriaxone	CTR	30	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Streptomycin	ST	30	(S)	(S)	(S)	(S)	(S)	(S)	(I)	(I)
Ciprofloxacin	CIP	5	(S)	(R)	(S)	(R)	(S)	(S)	(S)	(S)
Penicillin	PC	10	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Neomycin	NM	30	(S)	(I)	(S)	(S)	(S)	(S)	(S)	(I)
Tetracycline	TE	30	(S)	(S)	(R)	(R)	(S)	(S)	(R)	(S)

Source: CLSI document, 31th edition, 2021 [Note: S=Sensitive, R=Resistant, I=Intermediate]

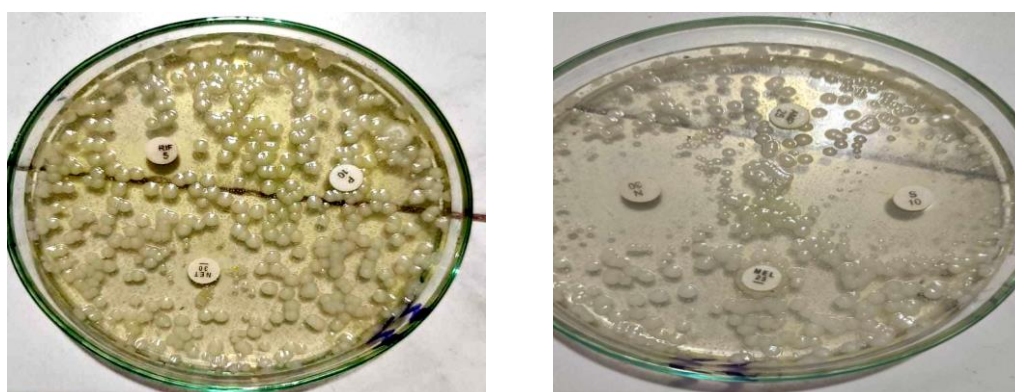


Fig. 13: Antimicrobial resistance test (Red circle denotes sensitive to bacteria)

CHAPTER V

DISCUSSION

Immunization, gross lesions, microscopic characters, biochemical testing, staining, selective media, identification, antibiotic sensitivity (Muller-Hinton agar) of ovarian samples were all examined in this study. In the Dinajpur districts of Bangladesh, 40 reproductive samples were obtained from abettors for these studies.

We found different immune cells in the follicular cyst and similar findings from the different experiments. Certain variables have different immune expression in the ovarian cystic walls compared to the normal group. In this work, the expression of an angiogenic molecule, cytoskeletal protein, anti-apoptotic factor, and inhibin A component was altered in cystic walls. Therefore, it can be said that ovarian cysts in cattle are not characterised as a single condition but rather as a group of disorders centred around the same pathology (Mimoune *et al.*, 2020). The cells of follicular cysts were also immune positive but the staining intensity appeared somewhat more intense compared to large follicles cells (Mimoune *et al* 2020).

The ovary was the first organ in which the most significant angiogenic molecule, vascular endothelial growth factor (VEGF), was initially identified (Shimizu and Miyamoto, 2007). Theca and granulosa cells of cystic follicles were shown to have positive VEGF immunoreactivity in the current investigation. Thus, VEGF is generated in follicular cysts actively. The expression of VEGF in cystic walls can be essential for the transfer of serum components from microvessels to the follicular antrum and for the proliferation of endothelial cells since this factor activates endothelial cells and enhances vascular permeability (Isobe *et al.*, 2005).

A well-developed mucosal immune system in the uterus controls local immunity by releasing a number of chemicals that affect the way underlying resident immune cells function and offer immunological defence against external particles. In the follicular phase of the estrus cycle, PMNs momentarily emerge in the uterine lumen. According to Studer *et al.* (1978), the presence of PMNs in the uterine lumen is considered pathogenic. The subepithelial stroma of the bovine endometrium contains lymphocytes, including T-, B-, and macrophage cells (Leung *et al.*, 2000).

Table 7 illustrates the relationship between immune cells and the follicular cystic ovary. When uterine samples were taken and examined, it was discovered that immune cells, including neutrophils and lymphocytes, were present in follicular cystic conditions, while normal, non-cystic control animals tested negative. This indicates a substantial relationship between immune cells and follicular cystic conditions.

Previous research has shown that macrophages, as a key component of innate immunity, perform a variety of important roles during infection and inflammation, including engulfing and breaking down pathogens during infection and presenting antigens on their surface to helper T cells. Immunohistochemistry research in the bovine ovary used visually staged corpora lutea to describe immune cell populations within the corpus luteum. Spaniel-Borowski *et al.* (1997). Between the corpus luteum's development and regression stages, there was a considerable increase in total leucocyte count. In contrast to our findings, Spaniel-Borowski did not detect T-lymphocytes in luteal tissue. In the walls of the follicular cysts, there was a strong expression of B-cell lymphoma 2 (Bcl-2), a survival protein. Townson and Combelles (2012) state that Bcl2 enhances the antioxidant capacity of cells by mitigating oxidative stress, which is the precursor to early stages of apoptosis. Therefore, this factor's expression may suggest that FC is on the verge of continuing its trend.

Lastly, a number of studies have suggested that oestrogen has a role in the formation of the cystic follicle vascular network (Isobe *et al.*, 2005; Shimizu and Miyamoto, 2007), which is the same as our study findings. The mechanism leading to longer follicular persistence in the current study may be explained by the significant expression of the inhibin alpha subunit in the granulosa of the follicular cyst. A previous study by Stangaferro *et al.* (2014) found a similar result. Inhibin alpha levels and FSH concentrations in cystic follicles have been shown to be inversely connected in the same environment by Li *et al.* (2015). They also mentioned that an imbalance in the production of FSH and LH may be a different factor in the development of ovarian cyst. Indeed, the high LH pulse frequency that causes the dominant follicle to develop and persist excessively is caused by the existence of a suprabasal progesterone concentration. The latter keeps producing estradiol, and inhibin stops FSH release, which leads to the formation of a fresh wave of follicular growth (Todoroki and Kaneko, 2006).

Table 5 and 6 presents the relationship between the presence of bacteria in the uterus and the follicular cystic disease. Significantly connected to the clinical symptoms of dairy cows is the presence of bacteria. The results show that the uterus of normal cyclic animals contains *Klebsiella pneumonia* in addition to dangerous bacteria such as *E. coli* and *Staphylococcus aureus*, which have the same results in the other literature. Significant endometrial inflammation, decreased fertility, and more severe clinical disease are all associated with important uterine pathogenic microorganisms (Farin *et al.*, 1989; Bonnett *et al.*, 1993). To cause more severe clinical symptoms, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, *E. coli*, *Staphylococcus aureus*, and *Prevotella* spp. collaborate (Ruder *et al.*, 1981; Olson *et al.*, 1984). Uterine bacterial infections had no significant effect on folliculogenesis in the current study, despite the fact that bacterial load did. According to this study, the inflammatory response to the degree of bacterial contamination matters more than the existence of a particular species of bacteria. According to Sheldon *et al.* (2002), the results of the present study provide evidence for the localised impact of uterine bacterial contamination on the ovary following parturition.

According to a previous study, certain species of uterine bacteria have been linked to increased endometrial inflammation and more severe cases of clinical uterine illness. Recent research has demonstrated that this association does not exist for other putative uterine infections or opportunistic contaminant bacteria in the uterine lumen. Numerous specific and non-specific genital tract pathogens affect cow infertility. Uterine bacterial infections during the luteal cyst are associated with reduced conception rates, longer intervals between calving and first service or conception, and a higher number of animals culled for infertility. Mammals depend on the functions of the cervix and its secretions to defend against these infections (Blandau and Moghissi, 1973).

Methods for assessing antimicrobial susceptibility that produce consistent and repeatable results. Disc diffusion testing is one of several phenotypic assays available for determining an organism's antimicrobial resistance profile (anti-biogramme). Disc diffusion tests are used to determine in vitro susceptibility. The results revealed that practically all antibiotics were vulnerable. Our findings were similar to those of Frontoso *et al.* (2008), Vlček & Svobodová (1985), Refsdal (2000), and Nasrat (2016).

These investigations provide a unique chance to thoroughly and systematically explore the coordinated activities that determine luteal shape and function. Potential areas of research that

could benefit from recent high-throughput omics methods include determining the underlying initiators and effectors of luteal regression. The corpus luteum's dynamic and transitory character allows for understanding of basic biological processes related to numerous illnesses and disorders.

CHAPTER VI

CONCLUSIONS

In this investigation, ovarian samples were examined for immunization, gross lesions, histopathology, identification (staining, selective media), and antibiotic sensitivity (Muller-Hinton agar). In the Bangladeshi regions of Dinajpur, the study obtained 40 cattle female genital tract samples from abettors. The influence of a follicular cyst on the regulation of the local immune system in the uterus of dairy cows raised the level of progesterone and affected clinical symptoms such as anestrus, recurrent breeding, infertility, and the health of the ovaries and cystic follicles. According to histopathological analysis, follicular cysts with eosinophilic fluid and a fibrous tissue-enclosed corpus hemorrhagicum have formed. *E. coli* and *Streptococcus aureus* were discovered using bacterial cell culture, and evidence of antibiotic resistance was discovered. The current study's findings imply that immunological modulation in the uterus has an impact on dairy cow reproductive success. However, these studies have improved our understanding of the follicular and endocrine alterations that follow cyst development. Strategies focused on risk factor control that aim to prevent or reduce the occurrence of ovarian cysts are prioritized. Therefore, in order to prevent metabolic problems and optimize the peri-partum ration, health interventions should be optimized throughout parturition and postpartum. It is advised to conduct more research on the immune-related gene expression of epithelial cells in order to identify the most effective treatment for mitigating the impact of immune regulation on dairy cow reproductive performance.

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