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Epidemiology of Repeat Breeding of Dairy Cows in Bangladesh

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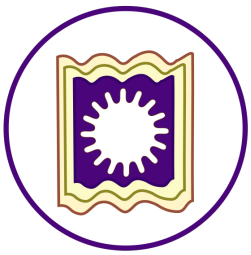
EPIDEMIOLOGY OF REPEAT BREEDING OF DAIRY COWS IN BANGLADESH



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By

MAHABUR RAHMAN

September, 2013

INSTITUTE OF BIOLOGICAL SCIENCES
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Dedicated

To

My Beloved Parents

and Wife

DECLARATION

*I do hereby declare that the whole work submitted as a thesis entitled "**Epidemiology of Repeat Breeding of Dairy Cows in Bangladesh**" is the results of my own and original investigation in the Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh under the supervision of Professor Dr. Md. Jalal Uddin Sarder & Co-supervisor Dr. Shah Md. Abdur Rauf, Associate Professor & Chairman, Department of Animal Husbandry and Veterinary Science, Rajshahi University, Rajshahi, Bangladesh in fulfillment of the requirement for the degree of Master of Philosophy (M. Phil).*

The thesis or any part of it has not been submitted to any other University or elsewhere for any degree or for other similar purpose.

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CERTIFICATE

*We have the pleasure in certifying the thesis of Mahabur Rahman and his thesis title "**Epidemiology of Repeat Breeding of Dairy Cows in Bangladesh**" to the Institute of Biological Sciences, Rajshahi University for the Degree of Master of Philosophy (M. Phil).*

We hereby certify that-

i) the candidate has fulfilled the residential requirement.

ii) the research work embodied in the thesis were carried out by the candidate and

iii) the data to the best of our knowledge, is genuine and original. No part of the research work has been submitted in substance for any degree else.

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ABSTRACTS

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The present study was to investigate the repeat breeding of dairy cows in relation to breed, age, parity, body weight, body condition score, farm type, farm size, housing system, floor type, feed quality, breeding method, preventive measure and treatment provider in Bangladesh. A total 1207 dairy cows in Rajshahi district during the period from August, 2011 to January, 2013 to study the overall incidence of repeat breeding as well as isolation of organism and antibiotic sensitivity test of repeat breeder cows at Rajshahi in Bangladesh and 100 cows for therapeutic approach to evaluate the efficacy of drugs against repeat breeding. Extensive survey and data were collected from private dairy farm from Rajshahi district of Bangladesh and a government farm namely Rajshahi Dairy and Cattle Improvement Farm, Rajabarihat, Rajshahi. The data collected by using structured quiestionnaire and direct interview of the farmer. Sorted data and complied with the help of computer software SPSS and analyzed and finally commented by the incidence & chi-square test. The overall incidence of repeat breeding of dairy cows was 20.4%. The highest incidence of repeat breeding observed in cross breed cows (19.7%), L×F genotype (8.5%), 6-<8 years of age (8.7%), 3rd parity (6.4%) 250-<350 kg body weight (10.3%), private farm (17.4%), large farm size (5.8%), AI breeding method (15.4%), Occasionally preventive measure (7.3%) and traditionally treatment provider (10.7%) on repeat breeder cows in Rajshahi. The lower incidence of repeat breeding were observed in local (0.7%) L×SL×SL genotype (3.0%), <4 years (3.3%), 6th parity (1.0%), >350kg body weight (4.1%), good body condition score (2.7%), government farm (3.0%), medium farm size (5.5%), good housing system (4.4%), natural breeding method (5.0%), no preventive measure (6.1%) and treatment by veterinarian (2.0%) had been taken.

In total 100 (n=100) cows were selected for collection of uterine sample from repeat breed cows at Rajshahi district. A significant number of bacteria were identified from diseased samples and they were *Fusobacterium* spp 14 (45.16%) (p=0.000), *Actinomyces* spp 10 (32.25%) (p=0.003), Escherichia coli 12 (38.70%), *Pseudomonas* spp 11 (35.48%) (p=0.002) and *Haemophilus* spp 9(29.0%) (p=0.012). Identified Staphylococcus spp was 11 (35.48%) and *Streptococcus* spp 12 (38.78%). In average, Azithromycin was highly sensitive (99.38%), Penicillin (51.85%) and Oxytetracyclien (25.30) were highly resistance. Streptomycin was moderately sensitive (48.76%). Ciprofloxacin (93.20%), Kanamycin (73.45%), Gentamycin (55.55%) and Cephalexin (53.70%) were also sensitive.

Therapeutic approach had significant effect (P<0.05) on treatment of repeat breeding of cows. Among therapeutic drugs Acriflavin + Metronedazole + Gentamycin Sulphate was the best group of all. The present results suggests that L×SL×SL, <4 years of age, 6th and above parity, <350 kg body weight, good body condition, Natural service and veterinarian treatment support significantly reduce the incidence of repeat breeding in dairy cows. Additionally, a lot of microorganism were identify which are very harmful effect on fertility status of dairy cows in this study area as well as Azithromycin and Ciprofloxacin were highly sensitive 99.38% and 93.20% respectively.

LIST OF ABBREVIATIONS

%	=	Percentage
<	=	Less than
>	=	Greater than
±	=	Plus minus
×	=	Cross/breeding
°	=	Degree
µ	=	Microns (s)
AI	=	Artificial Insemination
BCS	=	Body Condition Score
BW	=	Body Weight
BW	=	Body weight
C	=	Celsius
CR	=	Conception Rate
CS	=	Culture sensitivity
d	=	Day (s)
DF	=	Degree of Freedom
DLS	=	Directorate of Livestock Services
DNA	=	Deoxy Ribonucleic Acid
EMB	=	Eosin Methylene Blue
et al	=	Et alia
F	=	Friesian
FA(AI)	=	Field Assistant (Artificial Inseminator)
FAO	=	Food and Agricultural Organization
FDA	=	Food and Drug Administration
Fig.	=	Figure
FSH	=	Follicle Stimulating Hormone
G″	=	Girth in inch
GDP	=	Gross domestic product
gm	=	Gram (s)
GnRH	=	Gonadotropin Releasing Hormone
HF	=	Holstein Friesian
i.u	=	International Unit
Kg	=	Kilogram (s)
L	=	Local

l	=	Litre (s)
L″	=	Length in inch
LH	=	Luteinizing Hormone
LPS	=	Lipopolysaccharides
LSD	=	Least Significant Difference
m	=	Month (s)
mg	=	Milligram (s)
mill.	=	Million
min	=	Minutes(s)
ml	=	Millilitre
n or N	=	Number of Observation
NGO	=	Non Government Organization
no.	=	Number
NS	=	Natural Service
O.R.	=	Odd Ratio
P	=	Probability
PBS	=	Phosphate Buffer Saline
PPM	=	Potassium per manganate
RB	=	Repeat Breeding
RBC	=	Red Blood Cell
RDCIF	=	Rajshahi Dairy and Cattle Improvement Farm
S/C	=	Service per Conception
S/N	=	Serial Number
SAARC	=	South Asian Association for Regional Cooperation
SD	=	Standard Deviation (s)
SE	=	Standard Error
SL	=	Sahiwal
SPSS	=	Statistical Package for the Social Sciences
v/v	=	Volume by Volume
WHO	=	World Health Organization
yrs	=	Years

CHAPTER I

Introduction

CHAPTER II

Review of Literature

CHAPTER III

Materials and Methods

CHAPTER IV

Results

CHAPTER V

Discussion

CHAPTER VI

Conclusions and Recommendations

CHAPTER VII

References

CONTENTS

Page no.

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER I
INTRODUCTION

Cattle is an integral part of the mixed crop-livestock smallholder farming systems in the developing countries of the Asia-Pacific region. Apart from being a crucial source of high quality food (meat and milk), dairy farming provides employment, sustainable income and social security to millions of smallholder farmers within the region. Also, attaining food security and self-sufficiency in livestock products is a high priority development goal of most countries in this region.

The profitability of milk and meat production from cattle depends to a large extent on the efficiency of reproduction. Maximizing reproductive efficiency requires the matching of genotypes to the production environment, together with appropriate husbandry practices, in order to ensure that the intervals from calving to conception are short and the rates of conception to natural or artificial breeding are high. This will result in short calving intervals, yielding more lactations and calves per lifetime of each breeding cow. The outcome will be greater economic benefits to the farmers.

Bangladesh is an agricultural country. Livestock is an integral part of agriculture in our country. Cattle are the important species of livestock in Bangladesh. Cattle is an important factor in agricultural operation which provides valuable food of animal origin like milk, meat, milk products; industrial raw materials like skin and manures. Moreover it also direct impact on socio-economic relevance a security income generation and human nutrition and stability to farming system. They obtain meat for

human consumption as well as to provide same additional cash. The sale of skin and utilization of dung as organic fertilizer and fuel are the other utilities of cows (Husain, 1988). To date, a large number of mini-dairy farm with cross-bred cows through artificial insemination (AI) program established at private level in urban and rural areas in Bangladesh as a mean of milk producer. The current production of milk is grossly inadequate to meet the present requirement and total deficit is about 80 to 85.9%. To meet the deficit the country has to import milk and milk products from abroad every years spending huge amount of foreign currency amounting to Tk. 1310 million (DLS, 1998). So attempt was made to reduce these deficits by establishing dairy farm privately. Animal diseases of dairy cattle substantially limit production performances. About 20% large animals are estimated to die annually due to various infectious diseases causing less milk production at 15% (Haque, 2002). Reproductive diseases are of great concern to dairy producers worldwide. The estimation of loss and effects of diseases on milk production, fertility and survival are of great importance to assess cost-benefits of diagnosis, treatments and prevention efforts (Bar and Ezra, 2005). Most reproductive diseases viz. incidence of repeat breeding, retained placenta, metritis, ovarian cysts, milk fever, ketosis and mastitis have adverse effects on future fertility. Recently, most of the infectious disease of cattle in Bangladesh is controlled by routine vaccination programme and farmers awareness. But the dairy industries are threatened due to several reproductive diseases viz. repeat breeding, abortion, stillbirth, anoestrus, retained placenta, metritis, endo-metiritis, pyometra, uterine prolapse, vaginal prolapse, cystic ovaries, silent heat as

well as metabolic diseases such as milk fever, ketosis and mastitis and lameness which are major problems to improve the dairy cattle.

Repeat breeding can be a major factor involved in infertility. A “repeat breeder” is generally definedas any cow that has not conceived after three or more services associated with true estrus (heat). In herds of normal fertility, where conception rates are commonly at 50-55%, about 9-12% of the cows are expected to be repeat breeders. As the conception rate decreases, the number of cows requiring additional services increases. As a result, repeat breeding rapidly becomes a significant Problem. As with other reproductive problems the key to identifying or confirming a repeat breeding problem lies in a good set of records. By keeping and analyzing good estrus and breeding records one can calculate the percent of repeat breeders in a herd. It is important however, when evaluating the significance of repeat breeding in an infertility problem, to keep the definition of a repeat breeder in mind, so only cows requiring more than three services are considered. If natural service is used on the farm, frequent pregnancy exams will be especially helpful in identifying repeat breeders. In general, if more than 15% of the cows require more than three services, repeat breeding should be considered a significant problem warranting further investigation.

When a group of repeat breeders that lack anatomical defects are bred, approximately 25% will become pregnant to a single service. In approximately 15% of the cows, ova are either missing or ruptured. Ova are not fertilized in 25 to 35% of the cows and early embryonic mortality occurs in the other 25 to 35% of the cows. Thus most repeat breeders are not sterile, rather they suffer from lowered fertility. A very comprehensive

analysis of the entire reproductive program is necessary to effectively diagnose the complete cause of a repeat breeding problem. To accomplish this most successfully all parties involved in the reproductive management program, namely, the producer, inseminator and veterinarian, need to evaluate the problem and review the herd records together. This approach is the most rewarding since it frequently results in the identification of a number of factors, which alone may be minor, but collectively result in a herd problem. Inadequate estrous detection is the most frequent cause of repeat breeding. Therefore, when diagnosing the cause of a repeat breeding problem, the estrous detection program should always be thoroughly evaluated. When estrous detection is faulty, standing estrus is less likely to be observed and more cows are inseminated on the basis of signs other than standing estrus. This results in inaccurate timing of insemination, which in turn results in a failure to conceive. If a herd problem exists and natural service is used, then a thorough breeding soundness examination of the bull is indicated. This should include a complete diagnostic workup to identify any contributing physical factors or infectious agents. Sometimes the contributing factors are not readily apparent and only become evident after all members of the management team have had ample opportunity to work together for an extended time. Thus, it is important when attempting to diagnose the causes of repeat breeding that the producer, inseminator and veterinarian continue to work together in an ongoing comprehensive herd health program.

This causes a direct loss to the farmer due to delayed calving leading to a lengthy period between births (calving intervals) and hence low milk production. It is unhygienic to milk a cow with decomposing afterbirth hanging on it.

It causes considerable economic losses in the herd due to decreased milk production, illness and treatment cost, beside a decreased market value of the animal. An economic calculation made by adding the losses due to increased calving interval, increased culling rate, loss of milk production and the costs of veterinary treatment and drugs. A computer farm simulation model, based on a stochastic determination of events, was used to make calculations for circumstances closely resembling those on farms. The economic effects of repeat breeding were similar in magnitude in herds of high or low productivity and high or low fertility. Sensitivity analysis showed that the greatest financial losses were caused by loss of milk production, followed by the number of animals suffering from complications.

The incidence of repeat breeding appears to be varying from area to area and from year to year. Incidence rate was observed in various age. The number of calving was related with the incidence of repeat breeding. Nutritional status (body condition score) might also be associated with repeat breeding.

Bangladesh suffers from acute shortage of livestock products like milk, meat and eggs. Bangladesh government has given the priority on the development of dairying at farmers level to increase the supply of milk from small dairy farms. Dairy farmers target a maximum period of 60 to 90 days open and to get one calf per cow per year.

Repeat breeding (RB) is a substantial problem in cattle breeding leading to large economic loss for the dairy producer due to more inseminations, increased calving interval and increased culling rates. Repeat breeding

has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities.

The need for RB i.e. a return to oestrus after a mating or artificial insemination (AI) could be caused by either fertilization failure or embryonic death. Numerous studies have led to the conclusion that in female cattle with normal fertility the incidence of fertilization failure is approximately 10% and early embryonic death within 3 weeks following fertilization accounts for approx. 30% leading to a total early pregnancy loss of close to 40% during the first 21 days post AI. This means that on average 40% females will return to oestrus after each AI or mating. Several environmental factors e.g. nutrition, climate, as well as intrinsic animal factors have been suggested to be the cause behind this early embryonic loss in cattle. It has also been proposed that early embryonic loss should be regarded as "normal" due to an early elimination of unfit genotypes.

During the last 50 years RB has been the object of several investigations. There are different opinions among scientists about the cause of RB. A number of experiments have reported a higher proportion of embryos with deviated morphology collected from RB animals compared to control animals suggesting an increased embryonic death rate as the cause of RB. Recently, higher progesterone levels during oestrous in RB cows compared to control animals have been reported, indicating hormonal deviations as one possible cause of RB. These findings suggest physiological alterations linked to individual animals as a possible cause of repeat breeding. On the other hand, other investigators have reported normal pregnancy rates in repeat breeders when an additional

insemination was performed under controlled conditions suggesting management and environmental imperfects as the most important factors behind the RB syndrome. A third theory has been proposed by claiming the RB phenomenon as solely the result of a probability distribution since the same proportion of animals will be pregnant after each AI and there will always be a number of not pregnant individuals after a number of AIs.

There are only a few studies analyzing risk factors using field data. These studies have found factors such as season, herd size, age and nutrition influencing the incidence of RB. Some of the earlier studies, however, have the weaknesses that the material is restricted to a limited number of herds and animals and that the RB animals are not strictly selected according to the definition: "absence of detectable abnormalities".

Repeat Breeding prolongs days open which results in decrease milk production fertility, market value of the animals and increase calving interval, culling rate and treatment and labor cost. There are many factors influencing the incidence of repeat breeding like abortion, dystocia, multiple birth, poor body condition score, age, nutritional deficiencies, hormonal imbalance (Grunert, 1986; Alam and Dobson, 1987) season of the year and premature birth. It was evident that *E. coli* LPS (Lipo polysacharide) plus oxytocin effectively reduced the uterine inflammation and infection, thus increasing the overall reproductive efficiency in chronically sub fertile mares. LPS is a potent secretagogue for a variety of inflammatory mediators and immune regulatory cytokines from endometrial cells and leukocytes. However, from clinical and economical point of view prevention rather than therapy of this disorder is important with regard to the reproductive and general heath status of the herds.

Uterine infection is a major problem in reproductive management and to inefficient estrus detection. Azizunnesa (2000) stated that to develop sustainable dairy farm in Bangladesh good management, introducing crossbred dairy cows and reducing the cost for milk production should be introduced. The bovine uterus is sterile prior to calving and the cervix provides a barrier against intra-uterine invasion by pathogens during gestation (Sheldon and Dobson, 2004). Cows with uterine infection in the early postpartum period generally have lower conception rates at subsequent breeding. A study was conducted in Washington demonstrates that cows with server uterine infection experienced an 8 percent reduction in first service conception rate compared with herd –mates that were not infected. This effect would likely have been more severe if the herds had not been participating in a routine heard health programme in which uterine infections and other postpartum reproductive problems were detected and treated early. Mild urine infections also adversely affected conception rates. A high percentage of cows have bacteria present in the uterus during the first two weeks after calving and may be considered infected. However, by two months post-calving the prevalence of uterine infection decline to less than 10 percent. Even thought, pathogenic bacteria persist in some cows causing uterine disease by preventing physiological uterine involution resulting in sub fertility in individual cows or even in the whole herds.

Among that reasons repeat breeding of cows is one of the major problem which causes huge loss of cattle production and finally obstructs to reach our milk and meat production and ultimately protein requirement. So I select my research topic “Epidemiology of Repeat Breeding of Dairy Cows in Bangladesh” that can help our teachers, students, NGOs, and

researchers to know about accurate data and improve the consciousness of our farmers about repeat breeding that ultimately help us to contribute in our national income (GDP).

It is important to reduce the incidence of repeat breeding of dairy cow in Bangladesh. Therefore, the present study was undertaken to investigate the repeat breeding of dairy cows in relation to breed, age, parity, body weight, body condition score, farm type, farm size, housing system, floor type, feed quality, breeding method, preventive measure and treatment provider isolation of microorganism and antibiotic sensitivity test of repeat breeder cows as well as the therapeutic drugs were used to repeat breeder cows to determine the efficacy of drugs in reducing the risk of repeat breeding in Bangladesh with the following objectives.

The objectives of this study are as follows:

- To study the over all incidence of repeat breeding of dairy cows in Bangladesh.
- To evaluate the effects of breed, age, parity, body weight and body condition score of dairy cows on incidence repeat breeding
- To determine the effect of farm type, farm size, housing system, floor type and feed quality of dairy cows on prevalence of repeat breeding.
- To observe the effect of breeding method of dairy cows on risk of repeat breeding.
- To identify the common organisms of repeat breeder cows.
- To chose the best antibiotic for the treatment of repeat breeder cows by using antibiotic sensitivity test.
- To investigate efficacy of therapeutic drugs on recovery of repeat breeding of dairy cows.
- Identification of the most suitable drugs for the treatment of repeat breeding of dairy cows.

CHAPTER II
REVIEW OF LITERATURES

Failure of cattle to become pregnant after repeated AI is a source of frustration and economic loss to dairy producers. In a recent review, incident of repeat breeders in various countries ranged from 10 t0 18% (Aylon, 1984). Repeat breeding is a substantial problem in cattle breeding leading to large economic loss for the dairy producer due to more inseminations, increased calving interval and increased culling rates (Barlett *et al*., 1986; Lafi *et al*., 1992). Repeat breeder is the cow that has returned to estrus after a third service, has no evidence of abnormalities of the genital organs detected by rectal palpation and has no abnormal genital discharges. Repeat breeding has also been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Zemjanis, 1980). The present review focuses on the factors related to occurrence of repeat breeding and its remedial measures in cows under different section.

2.1 Repeat Breeding of Cows

When repeat breeding is a real problem the first step in correcting it is to diagnose its cause or causes. Unfortunately this can be a difficult task since many factors can, and frequently do, contribute to a failure to conceive or maintain pregnancy. Furthermore, the cause may be a herd problem or a variety of individual cow problems.

Herd problems are by far the most common, and those most often causing repeat breeding include:

1. Inadequate estrous detection, resulting in:

I. Improper timing of insemination in relation to the onset of standing estrus

II. Cows being inseminated that have not actually been in estrus

2. Semen and insemination techniques:

I. Inadequate semen quality

II. Insufficient numbers of sperm

III. Improper insemination techniques

IV. Infertile bull

3. Cow factors:

I. Metritis and/or endometritis (uterine infections)

II. Cervicitis and/or vaginitis (cervical/vaginal infections)

Individual cow problems also can cause repeat breeding. Although they are less common and usually not a major factor, they are a part of the problem and cannot be overlooked. Metritis, endometritis, cervicitis and vaginitis can be individual cow problems as well as herd problems.

Other common cow problems include:

l. Endocrine (hormonal) disorders

I. Cystic ovaries (may also cause irregular or short cycles)

II. Delayed ovulation

2. Ovulation disorders (these may also be hormonal)

3. Obstructed oviducts

4. Defective ova
5. Anatomical defects of the reproductive tract
6. Early embryonic death (may also cause abnormally long cycles)

2.2 Factors Related to Repeat Breeding in Cows

Sometimes it is dismissed that the term "Repeat breeder" is meaningless because it includes some healthy cows. The important factors related to repeat breeding in cows are discussed here under oestrus detection errors and incorrect time of insemination, body condition score (BCS) of cows, uterine infections, fertilization failure and embryonic losses.

Repeat Breeder Cows are a heterogeneous group of sub fertile cows with no anatomical abnormalities or infections that exhibit a variety of reproductive disturbances in a consistent pattern over three or more consecutive heat cycles of normal duration (17-25 days). One of the major constraints of profitable dairy farming is low conception rate (Alam and Ghosh, 1994; Shamsuddin *et al*., 2001). Early embryonic death (<42 days) is a major factor in reproduction failure, which in turn causes economic loss to the dairy industries (Rahman *et al*., 1996). Shamsuddin (1995) reported 5% repeat breeding cases in Bangladesh. Gani *et al*. (2008) found positive correlation (r = 0.94) between repeat breeders and bacterial infection of uterus. They detected bacteria in 62% repeat breeding cases in contrast to only 28% bacterial infections from normal fertile cows where *Staphylococcus* was predominant 37%, followed by *Bacillus* 35%, *E. coli* 29%, *Pseudomonas* 18% while Gram negative minute rod shaped bacteria was 24%. The isolates of *Pseudomonas* and Gram negative minute rod shaped bacteria were obtained only from repeat breeder cows with mucopurulent uterine

discharges. Antibiotic sensitivity of their study showed moderate to high sensitivity to amoxicillin, oxytetracycline and ciprofloxacin. Kamal *et al*. (2010) found that double insemination with intrauterine antibiotic improve conception in repeat breeding dairy cattle. Kamal *et al*. (1999) was claimed fungal infection to be cause of repeat breeding in cows and fungi belonging to *Candida*, *Aspergillus* were predominantly isolated (26%) in varying proportions. Repeat breeding cows from which *Aspergillus* were isolated had been suffering from endometritis with mucopurulent utero-vaginal discharge. Alam *et al*. (2007) also found bacteria and fungal infections in varying proportions in repeat breeder cows of Bangladesh.

2.2.1 Estrus detection errors and incorrect time of AI

Poor accuracy in estrus detection leads cows to be repeat breeder. In such errors, a cow is bred, when it is not actually in heat or rebred when it is already conceived. The later cow may subsequently losses its conception and increases the risk of becoming a repeat breeder. Heat detection accuracy was 30% in Bangladesh (Shamsuddin *et al*., 2001). A number of environmental conditions either stimulate or restrict interactions between cows and thus influence the behavioral manifestation of estrus. For instance, cows show less mounting activity that are eating or are crowded in holding pens or alleys on housed on slippery alleys, frozen ground, or any surface that makes footing tenous (Britt *et al*., 1986). If the other cows are tied rather than the loose and cows that have foot problems irrespective of the structural, subclinical or clinical problem, the estrus activity will be reduced (Leonard *et al*., 1994). As herd size increases and more cows are confined to concrete floor (Vailes and Britt, 1990). In lactating dairy cows, heat stress decreased AI submission rates resulting

from decreased expression or detection of estrus or both (Imtiaz-Hussain *et al*., 1992).

2.2.2 Body condition score (BCS) of cows

A negative energy balance during early lactation results in an increased risk of a variety of metabolic diseases in addition, a majority of infectious diseases, especially mastitis, become clinically apparent during the first two weeks of lactation (Goff and Horst, 1997). Loss of BCS between calving and AI may negatively influence conception (Suriyasathaporn *et al*., 1998; Shamsuddin *et al*., 2001). Domecq *et al*. (1997) found negative balance during the first 30 DIM contributed to the failure of conception in the first service in multiparous cows, which was more than any other variable. Ferguson *et al*. (1996) reported first service conception rates were 55.9%, 49.5%, 46.3%, 43.2%, 37.0%, 28.6% for +0.75, +0.25, 0.0, -0.25, -0.75, -0.15change in BCS from calving to breeding respectively. Milk production and dry matter intake of dairy cows are stimulated by increased dietary protein, but unfortunately, early embryonic death increases when cows are fed on excess ruminal degradable protein (Butler, 1998). Concentrations of milk urea nitrogen exceeding 19 mg/dl are associated with altered uterine p^H and reduced fertility when the negative energy balance is exacerbated by excess ruminal degradable protein intake (Butler, 1998; Butler *et al*., 1996). Excess loss in BCS after calving also reduced fertility in cows (Broster and Broster, 1998). Severe deficiency of minerals rch as Se, I, Co, Cu and possibly P, Mg and Mn can cause conception failure (Rogers,2001).

2.2.3 Fertilization failure

Fertilization failure is one of the main causes of conception failure in cows resulting repeat breeding. Two main causes are of conception failure are-

2.2.3a Semen and insemination factors

Saacke *et al*. (1991) reported that abnormalities of bovine spermatozoa may be either compensable, those that are not able to participate in fertilization, or uncompensable, those that are able to initiate but not complete fertilization or sustain embryo development. Chromatin aberrations in morphologically normal or near normal spermatozoa appear to be the best candidates for the uncompensable deficiency (Saacke *et al*., 2000). Irrespective of causes, morphologically abnormal spermatozoa fail to fertilize the oocutes (Shamsuddin and Rodriguez-Martinez, 1994). Normal acrosome contains enzymes which assist in penetration of sperm into the cumulus-oocyte complex (Roldan and Gomendio, 1992). Therefore, increased frequency of acrosomal defect is likely to atociate with reduced fertilizing capacity of spermatozoa (Shamsuddin and Rodriguez-Martinez, 1994).

Not following recommended procedures for retrieving, thawing and protecting straws until placing semen safely inside the cows results in damaged sperm membranes, cold- and heat- shocked sperm, or impaired sperm motility (Foote and Parks, 1993; Shamsuddin *et al*., 2001). It appears that AI beyond the optimum time (4 to 18 hours after detected estrus) will reduce conception rate (Shamsuddin *et al*., 2001). Improper placement of the semen in the reproductive tract can be a limiting factor for fertilization (Hawk, 1983). Semen placement errors are common

among professional technicians and below-average technicians only placed semen in the body of uterus about one-third of the time compared to 85.7 accuracy by above average technicians (Graham, 1966).

2.2.3b Ova and ovulation factors

Defective ovum is one of the causes of conception failure. Jainuddin and Hafez (2000) reported that several types of morphologic and functional abnormalities of ovum have been observed in unfertilized eggs e.g. giant eggs, oval-shaped eggs, lentil-shaped eggs and eggs with ruptured zona pellucida. Milk fever, Ketosis and other metabolic diseases may affect the oocyte by altering normal follicular development contributing to defective follicles that are recruited for ovulation during the optimum breeding interval (Farin and Slenning, 2001). Failure to undergo fertilization and embryonic development may be due to inherent abnormalities of the eggs or to elevated ambient temperature prior to breeding (Jainudden and Hafez, 2000).

Higher incidence of low conception rate, ovulation failure, delayed ovulation and multiple ovulations were observed in lactating cows. These were attributed to lower circulating steroid concentrations in spite of larger ovulatory follicles and luteal structures (Sartori *et al*.,2004). Anovulation may results from lack of preovulatory LH surge. Wiltbank *et al*.(2002) classified, a) anovulation with follicle growth not progressing beyond the 'emergency' stage of development; b) anovulation with follicle growth to less than ovulatory follicle size and c) anovulation with follicular growth to larger than ovulatory follicle size).Sometimes, ovulation may delay or docs not occur at the predictable time probably due to delayed onset of LH surge and that ovum may reach to the

fertilization site after passing the viable fertile lilfespan of the inseminated spermatozoa and hence, fertilization failure results.

The fertilizable lifespan of the bovine ovum has been estimated to be up to 24 hours after ovulation (Thibault1967), whereas the functional lifespan, during which normal fertilization results in a viable embryo, has been suggested to be less than 8 to 12 hours after ovulation (Hunter, 1988). Delayed fertilization has been shown to lead to an increase incidence of degenerated embryos (Schiewe *et al*., 1987), possibly due to decreased normal ova activation competence (Ducibella,1998).Aged ova can not successfully prevent the polyspermy because of losing its ability to sustain important biophysical and biochemical reactions to prevent additional sperm entry. If even a normal fertilization occurs in an aged ovum with pronuclei formation and without polyspermy, the embryo can not Sustain its subsequent development and resorted alter a period of time (Jainudeen and Hafez, 2000).

2.2.4. Uterine infections

Uterine infection has great effect on subsequent conception in cows. One of the common uterine infections is retained placenta. Retained placenta or metritis can cause reduced conception rate and nutrition can have an inliucnce on these problems. The incidence of retained placenta was 7.7% in the USA (Muller and Owens. 1974) and 42.26% in Bangladesh (Shamsuddin *et al*., 1988). Cows fed 8.5% instead of l5% crude protein diet and feeding Selenium deficient diets during the airy period resulted in a high incidence of retained placenta (Julllien *et al*., 1976). Cows with retained placenta have an increased risk for metritis (Correa *et al*., 1993)

and metritis is thought to be the main factor by which retained placenta affects fertility (Laven and Peters. 1996).

Chronic metritis can impair facility (Francos and Mayer, 1988). The incidence of metritis was lower in normal (14%) than in over-conditioned (31%) cows during dry period (Butler and Smith, 1989). Early luteal activity may negatively impact uterine involution. After ovulation, progesterone concentration increase, which supress the uterine immune system and thereby incidence of uterine infections (Lewis, 1997). This leads to reduced fertility (Smith md Wallace, 1998). Cows become sick in the first 2-weeks postcalving when actinomyces pyogenes, Fusobacterium species, Bacteroides species and Clostridium species overwhelm uterine defenses, hence acute metritis ensues (Giffin *et al*.,1974). Endometritis due to gram negative anaerobes, Pseudomonus species and Haemophyllus species were responsible for most of the unsuccessful services (Rahman, l996). Failure to clear Actinomyces pyogens from the uterus beyond 3-weeks post calving results in chronic endometritis and reduced conception rate if the infection is severe enough (Whitacre,1992), however, mild to moderate endometritis has little effect on fertility (Roberts,1986).

2.2.5 Early embryonic loss

Fertilization failure and early embryonic mortality are major contributors to reproductive failure in cattle (Zavy, 1994). In dairy cattle only 48% o1` embryos were classified as normal on day 7 after Al (Weibold, 1988). 7% to 12% of embryos die after the second week of pregnancy (Britt, 1992). Additional embryonic losses can occur during the period of 25 to 40 days after breeding (Perry, 1981). Losses between day 27 and 98 vary from 7

to 56% across many herds and are affected by seasons and stage of pregnancy (Cartmill *et al*., 2001a, 2001b).

Specific physiologic mechanisms responsible for pregnancy loss in lactating any cows are unknown. Although pregnancy loss may occur due to lactation stress, negative energy balance, toxic effects of urea and nitrogen are often identified as causes (Butler and Smith, 1989; Nebel and McGilliard, 1993; Butler *et al*., 1995). Reduced ability to respond to increased environmental temperature, lack of maternal recognition and pregnancy diagnosis by rectal palpation are also the causes of embryonic loss (Geisert *et al*., 1918; Thurmond *et al*., 1990; Hansen *et al*., 1992). The male inherent genotype may lead to incompatibility between spermatozoa and mother, between spermatozoa and egg, or zygote, as a result early embryonic loss or conception failure may occur (Jainudeen and Hafez, 2000). Twinning, feeding toxic plants and excess protein intake also result in pregnancy loss (James *et al*., 1992; Elrod and Butler, 1993; Jainudeen and Hafez, 2000). Cows that had clinical mastitis during the first 45 day of gestation were at 2.7 times greater risk for abortion during the next 90 days than cows without mastitls (Risco *et al*., 1999). Cows conceived in early lactation pose greatest risk of abortion due to the incidence of mastitis (Cullor, 1990; Risco *et al*., 1999). The mechanism of abortion due to mastitis seems related to the secretion of PGF2α (Risen *et al*., 1999). Uterine environment was described interior in repeat breeders and inferior uterine environment failed to support normal embryo development (Tanabe *et al*., 1985). Decrease in uterine pH was associated with higher intake of rumen degradable protein and possible, cause of conception failure (Elrod *et al*., 1993).

Heat stress has been shown to alter the duration of estrus (Gangwar *et al*., 1965). Conception rate (Howell *et al*., I976), uterine function (Wolfenson *et al*., 2000), endocrine status (Howell *et al*., 1994), follicular dynamics (Wolfenson *et al*., 2000), follicular growth and development (Wilson *et al*., 1998), oocyte quality (Wolfenson *et al*., 2000), luteolytic mechanisms (Wilson *et al*., 1998), uterine blood flow during breeding (Roman-Ponce *et al*., 1978) and early embryonic development (Biggers *et al*., 1987). Heat stress during oocyte maturation, near ovulation. from 8 to 40 days of pregnancy has deleterious effects on embryonic development or conception rate (Biggers *et al*., 1987; Cartmill *et al*.. 2001b; Al-Katanani *et al*., 2002). Heat stress antagonizes the inhibitory effects of the embryo on the uterine secretion of PGF2α (Wolfwstone *et al*., 1993). Heat stress between Day 8 to 17 of pregnancy can alter uterine environment and thereby hampers the development of conceptus (Geisert *et al*., 1988). Cartmill *et al*. (2001b) reported that for every 10-unit increase in average temperature and humidity index (THI, <72 unit means no heat stress and ≥72 means heat stress) on the day of calving, the subsequent fertility would decrease by 4.1 to 4.7%. Further, embryo survival tended to decrease by 7.1 ±3.8% as a carryover effect of heat stress. Every 10-unit increase in average temperature and humidity index at. Al decreased conception rate and pregnancy rate by 2.2 to 2.5% and for every 10-unit increase on the day of GnRH administration embryo survival decreased by 4.9±2.5% (Cartmill *et al*., 2001b).

CHAPTER III
MATERIALS AND METHODS

The study was conducted a total 1207 dairy cows in Rajshahi district during the period from August, 2011 to January, 2013 to study the incidence of repeat breeding as well as identification of the common organism of repeat breeder cow, antibiotic sensitivity test and 100 cows for therapeutic trial to evaluate the efficacy of drugs against repeat breeding of dairy cows at Rajshahi in Bangladesh.

3.1 Study Area

The animals were selected from 9 upazila and 4 Metro Thana of Rajshahi. The name of 9 upazila viz. Poba, Godagari, Tanor, Mohonpur, Bagmara, Puthia, Durgapur, Bagha and Charghat and 4 Metro Thana were Boalia, Rajpara, Motihar and Shamukhdum at Rajshahi district in Bangladesh. The said area has been selected for region of available cattle population, well communication, crossbred and government dairy farm.

3.2 Selection of Cows

Different breeds of 1207 dairy cows from 1st parity to 8th parities an absolutely for dairy purpose was considered as experimental materials for the present study. Extensive survey and data were collected using structured questionnaire (see appendix 1) from private dairy farm of Rajshahi district, Bangladesh and a government farm namely Rajshahi Dairy and Cattle Improvement Farm, Rajabarihat, Rajshahi and 100 repeat breeder cows from selected dairy farms for therapeutic treatment.

- Red dot indicate the study area

Fig. 3.1. A map of study area (Rajshahi district)

3.3 Data Collection Procedure

Firstly a questionnaire was prepared **(Appendix-1)** including information were name and address of farmer, breed, age, body weight, parity, body condition, farm type, farm size, housing system, floor type, feed quality, breeding methods, preventive measure, treatment provider, isolation and identification of bacteria, antibiotic sensitivity test and therapeutic approach for achievement of the study. The data collected directly from owner of dairy farms and register books of government and private farms.

3.4 Management of Dairy Cows

In study area the individual cow owners were housed their cows were traditional housing and most of them feeding straw and green grass, few concentrate feeding and little feeding balanced diet; very few were deworming and vaccinating their cows and most of the farmer. But mini and large sized dairy farmers had improved practice on rearing, feeding, deworming and vaccination.

3.5. Grouping of Experimental Cows

To achieve the goal animals were group according to following considering factors:

3.5.1 Breed

The cows was classified broadly in Indigenous or Local (n = 27) and Crossbred (n = 1180), Selected cows were further group into their genetic composition. These were -

Group-I **:** Local (n = 27),
Group-II **:** Local × Sahiwal × Sahiwal (n = 180),
Group-III **:** Local × Holstein Friesian (n = 663),
Group IV **:** Local ×Holstein Friesian × Sahiwal (n = 134) and
Group V **:** Local × Sahiwal (n = 203).

3.5.2 Age

Age of these repeat breeder cows were determined from birth register and examined by teeth and cornual ring reading. After confirmation of age of these cows and then classified as follows-

Group I **:** < 4 years (n=187),
Group II **:** 4 to <6 years (n=420),
Group III **:** 6 to <8 years (n=451) and
Group IV **:** >8 years (n=149).

3.5.3 Parity

The cows those gave 1st calf considered as parity 1 (P_1), those gave 2nd calf consider as parity 2 (P_2), 3rd calf as parity 3 (P_3) and so on. Cows were different parities and up to 6 parities cows were considered for the study. The cows were divided in the following groups considering parity: These were

P_1 **:** 1st calving; (n=300),
P_2 **:** 2nd calving; (n=262),
P_3 **:** 3rd calving; (n=352),
P_4 **:** 4th calving; (n=163),
P_5 **:** 5th calving; (n=53) and
P_6 **:** 6th calving and above (n=77).

3.5.4 Body weight

The body weights of dairy cows will be calculated according to the Shaeffer's formula adopted by McNitt (1983).

$$\text{Body weight} = \frac{L''\times(G'')^2}{300\times2.2}\ \text{kg}$$

Where,

L = Length from point of elbow to pin bone in inch

G = Length of heart girth in inch

The research cows were again classified according to their body weight such as:

Group 1 **:** <250 kg (n = 249),

Group 2 **:** 250 to <350 kg (n = 716) and

Group 3 **:** >350 kg (n = 202).

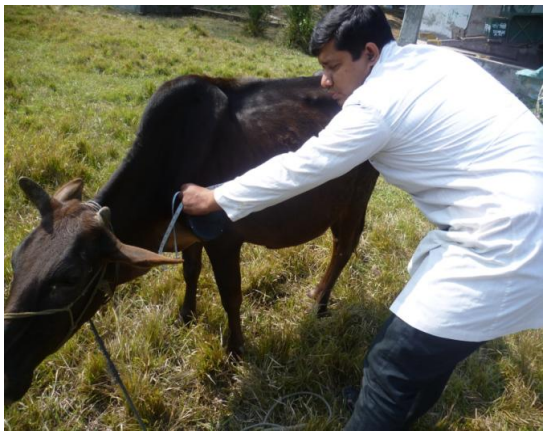



Plate 1: Researcher measured body weight with the help of measuring tape.

3.5.5 Body Condition Score (BCS)

In order to record the health condition the lumber vertebral processes of the cows were used as landmark. The body condition of the cows were divided into three classes such as-

Group 1 **:** Poor (n=270),

Group 2 **:** Fair (n=787) and

Group 3 **:** Good (n=150).

Where,

Poor = Marked emaciated.

Fair = Ribs are usually visible, little fat covered dorsal spines are rarely visible.

Good = Animals are smooth and well covered but fat deposits are not marked.

Plate 2: Observation of body condition score of cow.

3.5.6 Farm type

The farm types were separated according to the ownership viz.

Private farm (n =1027) and

Government farm (n = 180)

3.5.7 Farm size

The cows were also classify into 3 types to their farm sizes such as-

Single cow **:** Owner having 1 cows (n = 505),

Medium farm **:** Owner having 2-5 cows (n = 310) and

Large size farm **:** Those farm having > 5 cows (n = 392).

3.5.8 Housing system

Cows were different housing system. The cows were divided into following groups considering housing pattern: These were-

Poor housing: Very low ventilation, insufficient sunlight, low space, uncomfortable condition. (n=375),

Medium housing: Low ventilation, medium sunlight, moderate space, less comfortable condition (n=558) and

Good housing: Good ventilation, sufficient sunlight, enough space, comfortable condition (n=274)

3.5.9 Floor type

The cows were divided into 3 categories according to their floor pattern.

Poor condition : The cows were kept in traditional floor; mud, very dirty, no drainage system (n = 274)

Medium condition : The cows were kept with little facilities of semi concrete floor, no drainage (n = 558) and

Good condition : The cows were kept in scientific concrete floor, clean and good drainage system (n = 375).

3.5.10 Feed quality

Feed quality of these studied cows were divided into 3 groups such as-

Poor : Traditional feed supply (only grazing and little straw feeding) (n = 162),

Average : The cows were supplied some concentrate and straw (n = 698) and

Better : The cows were supplied balanced feed (concentrate, vitamin and mineral mixture before calving) diet including green grass and straw (n = 347).

3.5.11 Breeding method

The cows were group on the basis of servicing. The services were-

NS **:** Natural service (n = 548) and

AI **:** Artificial insemination for their breeding condition (n = 659).

3.5.12 Preventive measure

The cows were group on the basis of preventive measure like vaccination, deworming, mineral supply, water supply etc. The preventive measure are-

Nothing **:** No vaccination, deworming, mineral supply (n = 364)

Occasional **:** Irregularly provide vaccination, deworming, mineral supply (n = 356)

Regular **:** Regularly provide vaccination, deworming, mineral supply (n = 487)

3.5.13 Treatment provider:

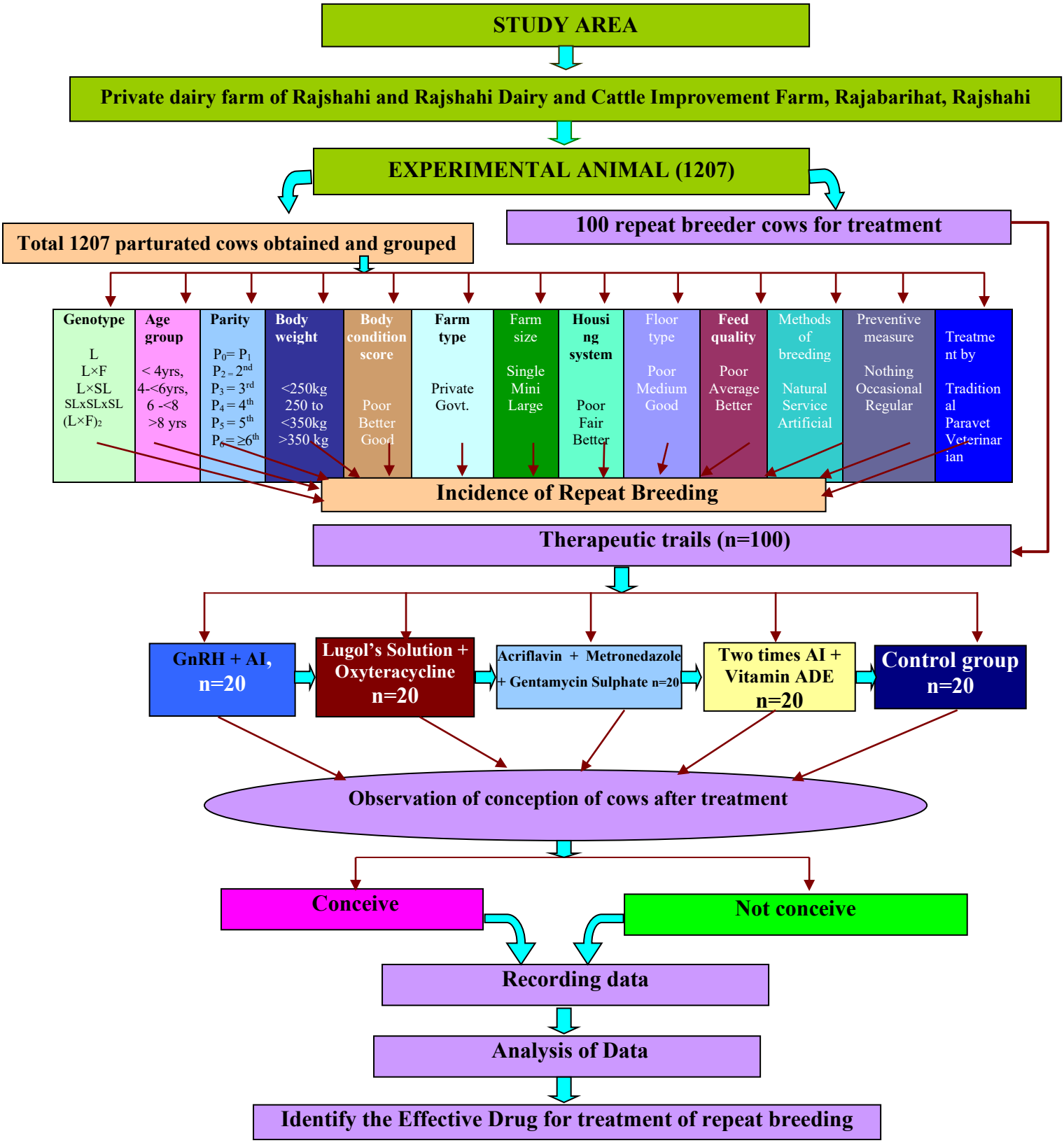
The cows were group on the basis of treatment taken from like traditional, paravet, veterinarian etc. The treatment status are-

Traditional **:** Treatment of animal by traditional way like Kobiraj, village people (n = 711)

Paravet **:** Treatment of animal by paravet who take short training on livestock (n = 397)

Veterinarian : Treatment of animal by veterinarian (n = 99)

3.6 Experimental design



Flowchart: At a glance of partial experimental design

3.7 Clinical examination

Prior to collection of samples all cows were examined clinically. Both physical and clinical examinations were done and reproductive histories of cows were taken and then data were recorded on a prescribed questionnaire. The findings of observation and examination including body temperature, respiration rate, pulse rate, nature of mucus through vagina and other abnormalities if present were recorded for each and every individual cows.

3.7.1 Rectal examination

Before examination the perineal region including tail was washed with water and then potassium per manganate. After taking the necessary precaution and safety measures, the gloved hand was introduced into the rectum, fecal material were evacuated, the cervix was located and grasped properly to follow the orientation of the genital tract. The caudal end of the cervix was palpated with thumb and the external os was examined to establish whether it was open or closed. Both horns were then examined from base to the tip for their size and symmetry between them, tonacity /consistency and thickness of the wall and for possible contents. In case of uterine asymmetry, the bigger horn was palpated to confirm whether it was pregnant or affected by pyometra / hydrometra. The ovaries were palpated to the stages of oestrous cycle upon confirmation of the presence and consistency of corpus luteum together with follicular structures.

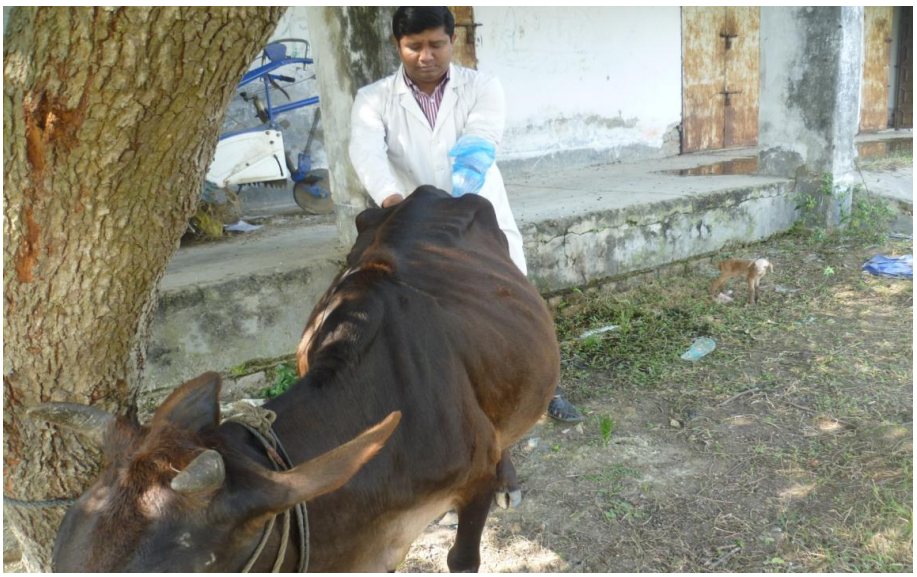


Plate 3: Rectal palpation

3.7.2 Equipments were needed to collected samples

1. Glass made AI tube
2. 10 ml syringe and rubber tube
3. Both aerobic and anaerobic transport media to provide the environment for survival of broad spectrum organisms, e.g. PBS for aerobic and Thio-glycolate broth for anaerobic organism.
4. Vaccuotainer vial to collect sample.
5. Refrigerator for preservation of sample.
6. Anaerobic incubator.
7. Sterilized paraffin oil.
8. Tissue paper.
9. Potassium per manganate (PPM).
10. Vaccuotainer vial container rack.

3.7.3 Uterine sample collection

Before collection of sample a good communication was built up with FA (AI). Most of the samples were collected from dairy farm and others were collected from cows came at AI centre with the history of estrus and unsuccessful insemination. The animal were divided into groups; Group-I and Group-II. Group-I consisting the cows with reproductive problems, and Group-II consisting the cows which were diseases free. Trans cervical uterine sample was collected in this study. Before collection of uterine sample the perineal region of animal were washed and cleaned properly with fresh water and PPM solution. Left handed rectal palpation was performed to collect uterine sample by using AI tube. A total 10 ml (air emptied) syringe adjusted behind the glass made tube with rubber pipe was introduced into the uterus through cervix guided by rectal palpation. Uterine sample was collected by giving a negative pressure to the uterus with applying a back pressure to the syringe by turning out of piston from syringe. Tip of the AI tube was moved into the uterus several times to extract the targeted amount of sample. The sample was transferred to the vaccuotainer tube containing PBS for aerobic culture and Thio-glycolate broth for anaerobic bacterial culture. Paraffin oil was poured to the surface of anaerobic sample to create an anaerobic condition. All these samples were preserved in refrigerator up to culture.

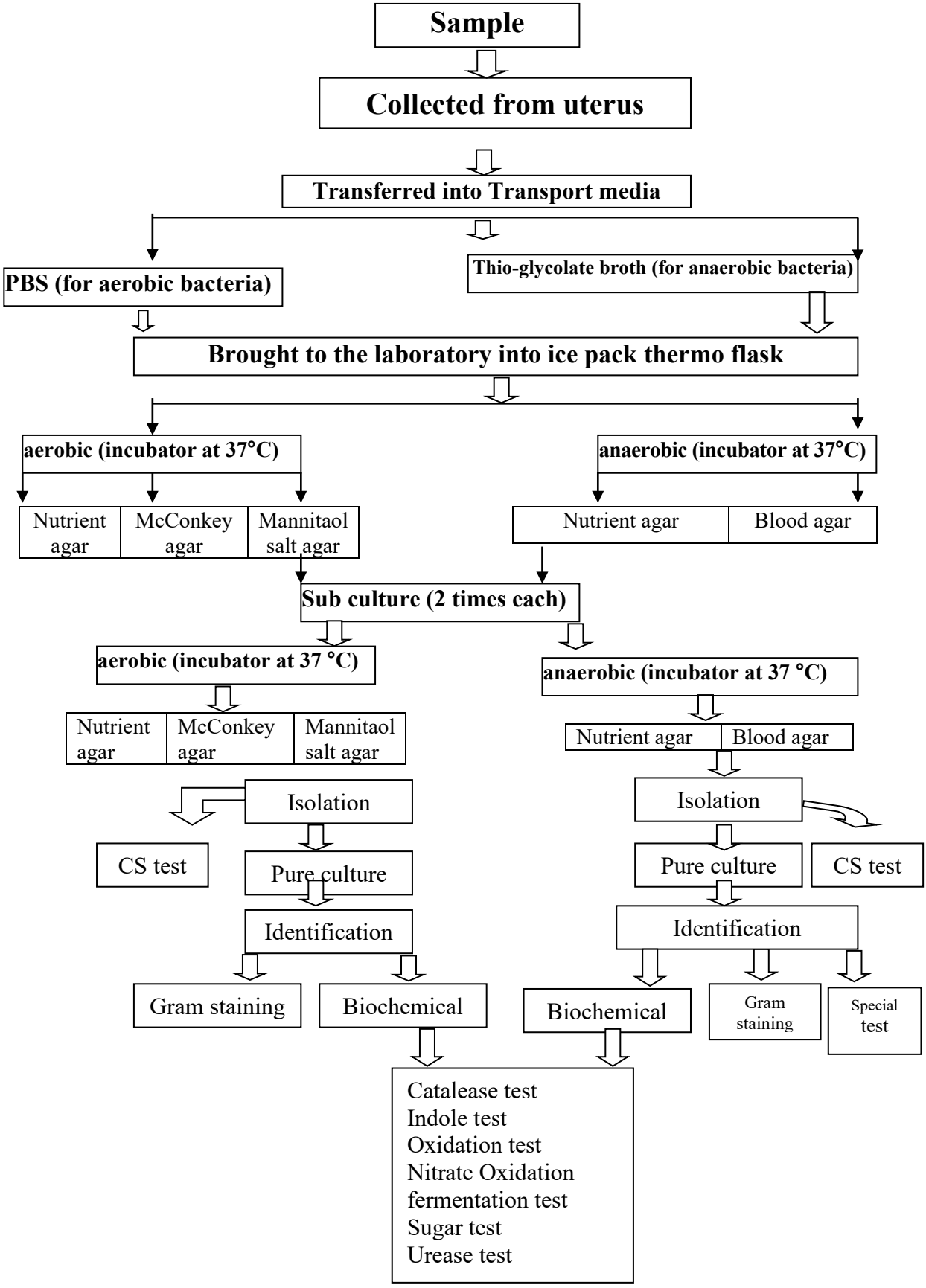


Fig. 3.2. Flowchart for the isolation and identification of organism and antibiotic sensitivity test in repeat breeder cows.

3.8 Preparation of Bacteriological Media

3.8.1 Blood Agar (BA) medium

An amount of 40 g blood base (Hi-Media, India) powder was suspended to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 121° C maintaining a pressure of 15 pound/ square inch for 15 minutes. The autoclaved materials were recommended allowed to cool to a temperature of 45° C in a water bath. Defribinated 5% blood was then added to the medium aseptically and distributed to sterile Petri dishes and allowed to solidify. After solidification of the medium, the plates were allowed to incubation at 37 ° C for overnight to check their servility and stored in a refrigerator for future use.

3.8.2 Streptococcus Agar N° 110

Selective medium for the isolation of pathogenic staphylococci

Composition:

Sodium Chloride 75.00 Dipotassium Phosphate 5.00

Gelatin 30.00 Yesast Estract 2.50

Casein Peptone 10.00 Lactose 2.00

D-Mannitol 10.00 Bacteriological Agar 15.00

Final Ph 7.0±0.2 at 25 ° C.

Preparation:

Suspend 149.5 grams of the medium in one litre of distilled water. Mixed well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121° C for 15 minutes Cool to 45-50° C, Mix well and dispense into plates. The prepared medium should be stored at 8-15 ° C. The color is amber slightly opalescent. The dehydrated medium should be homogenous, free –flowing and clear in color.

3.8.3 MacConkey's Medium:

Exactly 6gm MacConkey's agar (Difco) was suspended in 1 litre distilled water in a flask. The suspension was then heated to boil for few minutes to dissolve the medium completely. The medium was then autoclaved for 30 minute to make it sterile. After autoclaving the medium was put into water bath of 45 °C to cool down its treatment at 45° C. From water bath 10-20 ml of medium was poured into small and medium sized sterile Petridis to make MacConkey's medium plates. After solidifying the medium, they were kept in incubator at 37% for overnight to check their sterility.

3.8.4 Eosin Methylene Blue (EMB) Agar:

Exactly 36 gm of Bacto Metheylene blue agar (EMB, Oxid, UK) was suspended in a litter of distilled water. The suspension was then heated to boil for few minutes to dissolve the power completely. The medium was then distributed in flask to contain 100 ml quantities in each and sterilized in autoclave for 30 minutes at 15Ib pressure per inch (121° C) to make it sterile. After autoclaving the medium was put water bath of 45° C to cool down its temperature at 40° C. From water bath 10-20ml of medium was poured into small and medium size sterile petridish to make agar plate. After solidifying the medium in the plate, the plates were allowed for incubation at 37° C for overnight to check their sterility.

3.9 Isolation of Bacteria in Pure Culture:

For isolation of bacteria in pure culture, the mixed culture was inoculated into Nutrient agar media by streak plate technique to obtain isolated colonies as per suggested by Poindexter (1971).

Step-I:

An inoculums up with a sterile loop and spread on an area of the medium in the Petridish.

Step-II:

The loop was sterilized by being heated as red hot in a flame.

Step-III:

The inoculum was spread over the reminder of the plate by drawing the cooled parallel line. This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

3.10 Morphological Characterization of Organism by Gram's Staining Method

- A loopful of sterile distilled water was placed in the centre of a clean sterile slide.
- A small colony was picked up with a bacterilogical loop and was mixed with distilled water of a slide.
- The colony was made to thin smear on a slide.
- The smear was fixed by air drying.
- 0.5 crystal violet solution was then applied on the smear for one minute.
- Lugols iodine was then added to act as mordant for one minute.
- Acetone alcohol was then added to decolorize for 1-2 second.
- Then the slide wash was with water.
- Safradine was added as counter stain and allowed for one minute.
- Then the slide was then washed with water.

- Then the slide was blotted with blot paper and was allowed to dry.
- The slide was examined under microscope with high power objective (100x) using immersion oil.

3.11 Culture of Organisms

The test for sample to culture, isolation and identification of microorganisms and culture sensitivity (CS) were done at Dept. of Animal Husbandry & Veterinary Science, RU and Institute of Biological Sciences, University of Rajshahi, Bangladesh. For the growth of aerobic bacteria, the samples were cultured in Nutrient agar media, McConkey agar and in Mannitol Salt agar at 37°C temperature for 48 hours and then from each of the culture colonies were taken for subculture (2 times sub culture) to get pure colonies. On the other hand for anaerobic bacterial growth, anaerobic incubator was used. In case of anaerobic bacteria, Nutrient agar media and Blood agar were used and these samples were also sub culture 2 times from the 1st culture to get pure culture. All the procedure was conduced at 37°C temperature for 48 hours.

3.12 Isolation and Identification of Bacteria

After two times of subculture individual colonies of bacteria were identified on the basis of their own characteristics of colony, gram staining, morphology, zone of hemolysis in blood agar and growth on selective media. After that aerobic and anaerobic bacterial colonies were stained with Gram's stain and then Gram's positive aerobic and anaerobic bacteria and gram negative aerobic and anaerobic were separated and marked. Then after presumptive diagnosis the culture plates were sent for specific and biochemical tests. The identification of anaerobes is highly complex and in laboratories different deification systems were used. These include Sugar fermentation, Bile solubility, Esculin, Starch, Gelatin hydrolysis, Casein and

Gelatin digestion, H_2S Production, Coagulase Oxidation fermentation, Urease, catalase, Lipase, Indole production, Nitrate reduction, VPMR, etc.

3.13 Culture Sensitivity Test

Resistance to antibiotics can either be naturally occurring for a particular organism / drug combination or acquired resistance, where misuse of antimicrobials results in a population being exposed to an environment in which organisms that have genes conferring resistance (either spontaneously mutated or through DNA transfer from other resistant cells) have been able to flourish and spread. To see the sensitivity of antibiotics 8 different antibiotics including Azithromycin® 10 µg, Cephalexin ® 25 µg, Ciprofloxacin ® 30 µg, Gentamycin ® 10 µg, Kanamycin ® 30 µg, Penicillin G ® 20 µg, Streptomycin ® 10 µg and Oxytetracycline ® 30 µg; were used in this research.

For CS test Mueler Hinton agar were used. Different pure colonies at primary isolation were picked up from specific Petridis of each samples aseptically with inoculating loop and diluted into the sterilized nutrient broth and then incubate for 8-10 hours (for aerobic bacteria, nutrient broth in aerobic incubator at 37°C and for anaerobic bacteria, nutrient broth in anaerobic incubator at 37°C temperature. For aerobic CS test Mueler Hinton agar was used and for anaerobic (for fastidious organisms like Haemophilus and Actinomyces), Mueler Hinton agar was added with 5% sheep RBC and 1% Yeast extract. These individual diluted samples were therafter thickly poured on agar plates and then Eight (8) different culture sensitivity antibiotic discs (Azithromycin, Cephalexin, Ciprofloxacin, Gentamycin, Kanamycin, Penicillin G, Streptomycin and Oxytetracycline) were placed circularly at

each plate. The result was checked after 24 hours of incubation. This method was followed by WHO, 1979.

3.14 Therapeutic Approach to Repeat Breeder Cows

Out of 246 repeat breeder 100 cows at 5 Metro Thana were applied 4 therapeutic drugs for the treatment of Repeat Breeding to observe the efficacy of studied drugs.

Treatment:

The cows were divided into 4 groups with 20 cows each group. Every group was treated with different therapeutic drugs for the treatment of repeat breeding.

Group-I: GnRH + AI

Group-II: Lugol's Solution + Oxyteracycline

Group-III: Acriflavin + Metronedazole + Gentamycin Sulphate

Group-IV: Two times AI + Vitamin ADE.

Group-V: No treatment

The repeat breeding of cows divided into seven groups and each group of cows were given treatment with separate drugs as follows:

Table 3.1: Therapeutic Treatment to Repeat Breeder Cows

GD	Groups	No. of cows	Treatment protocol
Repeat breeding	Group-I	n = 20	Inj. GnRH like Inj. Fertazyl 2ml (Intervet) + AI
	Group-II	n = 20	Intrauterine wash with Lugol's Iodine 0.2% 10ml help of AI tube and syringe + Inj. Renamycin 10ml vial at rate of 1ml / 10kg body weight parentally + Intrauterine
	Group-III	n = 20	Intrauterine wash with Acraflavin solution Dush can + Inj. Gentamycin Sulphate parentally + Pessaries of Metronedazole 500mg mixed with water and introduce into the uterus with help of AI catheter of both of one time for 3-5 days.
	Group-IV	n = 20	Inj. Vitamin ADE + Two times AI/ service (If heat in morning than service is in afternoon and morning of day after tomorrow).
	Group-V	n = 20	No treatment

3.15 Selection of drugs used for repeat breeding treatment

3.15.1 Gonadotropin releasing hormone (GnRH)

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone, a neuro-hormone consisting of 10 amino acids that is produced in the arcuate nuclei of the hypothalamus. GnRH stimulates the synthesis and secretion of the two gonadotropins—luteinizing hormone (LH) and follicle-stimulating hormone (FSH)—by

the anterior pituitary gland. The effects of GnRH on the secretion of LH and FSH are not exactly parallel, and the variations are probably due to other modulating factors such as the serum concentrations of steroid hormones (substances secreted by the adrenal cortex, testes, and ovaries).

The neurons that secrete gonadotropin-releasing hormone have connections to an area of the brain known as the limbic system, which is heavily involved in the control of emotions and sexual activity.

3.15.2 Lugol's Solution

Lugol's iodine, also known as Lugol's solution, first made in 1829, is a solution of elemental iodine and potassium iodide in water, named after the French physician J.G.A. Lugol. Lugol's iodine solution is often used as an antiseptic and disinfectant, for emergency disinfection of drinking water, and as a reagent for starch detection in routine laboratory and medical tests. These uses are possible since the solution is a source of effectively free elemental iodine, which is readily generated from the equilibration between elemental iodine molecules and triiodide ion in the solution.

It has been used more rarely to replenish iodine deficiency. However, pure potassium iodide, containing the relatively benign iodide ion without the more toxic elemental iodine, is strongly preferred for this purpose. Likewise, in the Chernobyl disaster some Lugol's solution was used as an emergency source of iodide to block radioactive iodine uptake, simply because it was widely available as a drinking water decontaminant, and pure potassium iodide without iodine (the preferred agent) was not available.

Formula and manufacture

Lugol's solution is available in different potencies of 1%, 2%, or 5% iodine. The 5% solution consists of 5% (wt/v) iodine (I_2) and 10% (wt/v) potassium iodide (KI) mixed in distilled water and has a total iodine content of 126.5 mg/mL. Potassium iodide renders the elementary iodine soluble in water through the formation of the triiodide (I_3) ion. It is not to be confused with tincture of iodine solutions, which consist of elemental iodine, and iodide salts dissolved in water and alcohol. Lugol's solution contains no alcohol.

Other names for Lugol's solution are I_2KI (iodine-potassium iodide); Markodine, Strong solution (Systemic); and Aqueous Iodine Solution BCP.

3.15.3 Oxytetracycline

Oxytetracycline was the second of the broad-spectrum tetracycline group of antibiotics to be discovered.

Oxytetracycline works by interfering with the ability of bacteria to produce essential proteins. Without these proteins, the bacteria cannot grow, multiply and increase in numbers. Oxytetracycline therefore stops the spread of the infection and the remaining bacteria are killed by the immune system or eventually die.

Oxytetracycline is a broad-spectrum antibiotic, active against a wide variety of bacteria. However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections

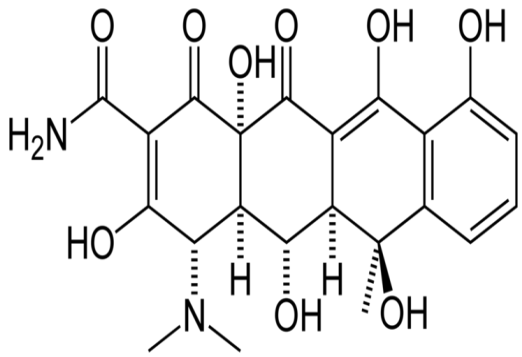


Plate 4. Chemical Structure of Oxytetracycline

Plate 5. Different drugs used for treatment protocol.

3.15.4 Acriflavin

Acriflavine is a topical antiseptic. It has the form of an orange or brown powder. It may be harmful in the eyes or if inhaled. It is a dye and it stains the skin and may irritate. Commercial preparations are often mixtures with proflavine. It is known by a variety of commercial names.

Acriflavine was developed in 1912 by Paul Ehrlich, a German medical researcher and was used during the First World War against sleeping sickness. It is derived from acridine. The hydrochloride form is more irritating than the neutral form.

Acriflavine is also used as treatment for external fungal infections of aquarium fish.

Acriflavine has been shown to have anti-cancer activity by inhibition of HIF-1 which prevents blood vessels growing to supply tumors with blood and interferes with glucose uptake and use.

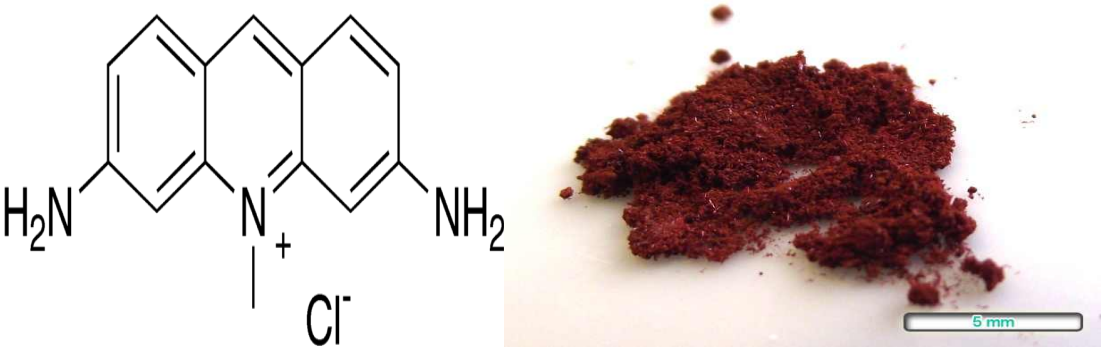


Plate 6. Chemical Structure and colour of Acriflavin used for treatment protocol.

3.15.5 Metronedazole

Metronidazole is one of the mainstay drugs for the treatment of anaerobic infections and the treatment of choice for most patients with mild to moderate Clostridium difficile-associated diarrhea. It is approved by the US Food and Drug Administration (FDA) for the treatment of anaerobic and protozoal infections. Metronidazole exerts its antimicrobial effects through the production of free radicals that are toxic to the microbe.

Mechanism of Action

Metronidazole is cytotoxic to facultative anaerobic bacteria such as Helicobacter pylori and Gardnerella vaginalis, but the mechanism of this action is not well understood.

However, its activity against obligate anaerobes occurs through a four-step process:

- Entry into the microorganism — Metronidazole is a low molecular weight compound that diffuses across the cell membranes of anaerobic and aerobic microorganisms. However, antimicrobial activity is limited to anaerobes.

- Reductive activation by intracellular transport proteins — Metronidazole is reduced by the pyruvate:ferredoxin oxidoreductase system in the mitochondria of obligate anaerobes, which alters its chemical structure. Pyruvate:ferredoxin oxidoreductase normally generates ATP via oxidative decarboxylation of pyruvate. With metronidazole in the cellular environment, its nitro group acts as an electron sink, capturing electrons that would usually be transferred to hydrogen ions in this cycle. Reduction of metronidazole creates a concentration gradient that drives uptake of more drug, and promotes formation of intermediate compounds and free radicals that are toxic to the cell.

- Reduced intermediate particle interacts with intracellular targets — Cytotoxic intermediate particles interact with host cell DNA, resulting in DNA strand breakage and fatal destabilization of the DNA helix.

- Breakdown of cytotoxic intermediate products — The toxic intermediate particles decay into inactive end products.

3.15.6 Gentamycin

Gentamycin is an amino glycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms.

It is synthesized by *Micromonospora*, a genus of Gram-positive bacteria widely present in the environment (water and soil). To highlight their specific biological origins, gentamycin and other related antibiotics produced by this genus (verdamicin, mutamicin, sisomicin, netilmicin,

retymicin) generally have their spellings ending in *~micin* and not in *~mycin*. Gentamycin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis.

Like all aminoglycosides, when gentamycin is given orally, it is not systemically active. This is because it is not absorbed to any appreciable extent from the small intestine. It is administered intravenously, intramuscularly or topically to treat infections. It appears to be completely eliminated unchanged in the urine. Urine must be collected for many days to recover all of a given dose because the drug binds avidly to certain tissues.

Gentamycin is one of the few heat-stable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of some microbiological growth media. It is used during orthopaedic surgery when high temperatures are required for the setting of cements (e.g. hip replacements)

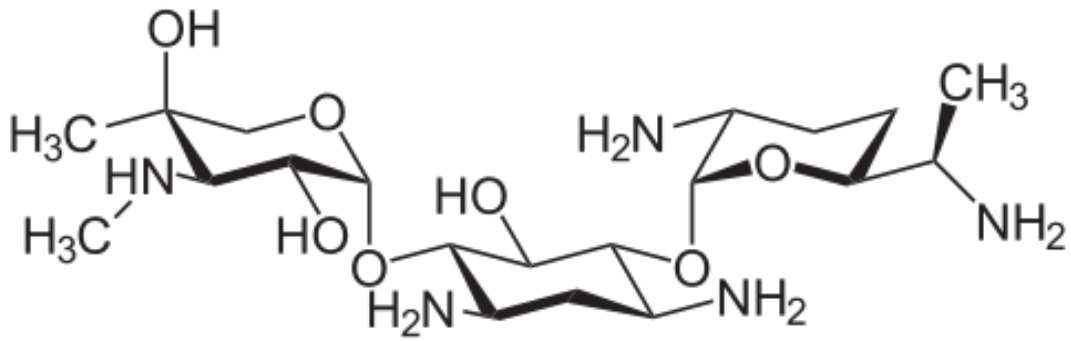


Plate 7. Chemical structure of Gentamycin

3.15.7 Vitamin ADE

Vitamin-A

Vitamin A is found in actively growing green plants. When forages are cut for hay or green feed, vitamin A precursors are oxidized, and levels decline with time. Approximately 90 days after cutting forages, all vitamin precursors are considered to be oxidized and not available to animals, which is why most deficiencies are observed in late fall and winter. Supplementation should be provided from this time forward.

Vitamin A is involved in the maintenance of body tissue, so requirements of the pregnant cow are higher in the last third of pregnancy and immediately after calving. Cows that have a vitamin A deficiency may have night blindness, excessive tear production and problems walking or moving around. Pregnant animals may abort, retain their placenta or develop uterine infections after calving.

Calves may be born weak or blind, or dead. Cows with a vitamin A deficiency conceive normally but return to heat due to the early death of the embryo. Bulls affected by a vitamin A deficiency produce fewer and abnormal sperm that contribute to infertility problems.

Vitamin-D

Cattle usually form adequate amounts of vitamin D through exposure to the sun and the consumption of fresh forages. This vitamin interacts with calcium and phosphorus in bone development and maintenance.

Deficiencies of vitamin D are diagnosed more frequently in animals that are either housed indoors or in areas where sunlight hours are minimal in the winter.

Animals with vitamin D deficiency symptoms have a stiff gait, laboured breathing, weakness and possibly convulsions. Swollen knees and hocks can also occur. Bones may be soft (rickets) or be re-absorbed in older animals. Calves may be born dead, weak or deformed. Cows may not show heat when exposed. Recent research has implicated Vitamin D with heart health, cancer and infectious diseases.

In areas where sunlight is limited or on operations where animals are housed indoors, supplemental vitamin D is required. If an animal is losing weight or has a poor body condition score, vitamin D can be deficient.

Vitamin-E

Vitamin E interacts with selenium, and the two nutrients work together to prevent damage to body tissue. A deficiency of selenium or vitamin E or both can cause white muscle disease in calves and reduce growth rates. In mature animals, reproductive performance can be impaired. A vitamin E deficient ration causes the incidence of retained placentas and mastitis to increase while colostrum and milk quality are reduced.

Plate 8. Preparation of Vit-ADE used for treatment of Repeat Breeding

3.16 Methods of drugs administration

The animals were controlled during administration of drug properly. Intramuscular injections were administrated with the help of 10 ml disposable syringe and 19 G × 1.5 inch needle and the site of intramuscular injection was gluteal muscle, thigh, neck muscle.

3.17 Recording of Repeat Breeder Cows after Therapeutic Approach

After treatment the cows were classified according to their state of recovery such as **"Yes"** means conceive and **"No"** means again show heat. The selected dairy farms at different location and given 4 treatment regimen and control one is presented in table 3.2-3.6.

Table 3.2: Selected dairy farms with therapeutic drugs (GnRH + AI) for effective treatment of Repeat Breeding at 4 Metro Thana.

Name of Farm	Location (Thana)	Treatment By GnRH + AI	Conception Status	
			Conceive	Not Conceive
Johurul Dairy Farm	Motihar	1	1	-
Salam Dairy Farm	Motihar	1	1	-
Babu Dairy Farm	Rajpara	1	-	1
Biren Dairy Farm	Rajpara	1	1	-
Deben Dairy Farm	Rajpara	1	-	1
Emaz Dairy Farm	Rajpara	1	1	-
Ghatu Dairy Farm	Rajpara	1	1	-
Jalal Dairy Farm	Boalia	1	-	1
Johir Dairy Farm	Motihar	1	1	-
Josim Dairy Farm	Boalia	1	-	1
Kader Dairy Farm	Boalia	1	-	1
Khoka Dairy Farm	Boalia	1	1	-
Khokon Dairy Farm	Boalia	1	1	-
M. Ali Dairy Farm	Shamukhdum	1	-	1
Mamun Dairy Farm	Motihar	1	1	-
Mobarak Dairy Farm	Shamukhdum	1	1	-
Moohsin Dairy Farm	Shamukhdum	1	-	1
Mozamel Dairy Farm	Shamukhdum	1	1	-
Nahar Dairy Farm	Boalia	1	-	1
Nannu Dairy Farm	Boalia	1	-	1
	Total	**20**	**11**	**9**

Table 3.3: Selected dairy farms with therapeutic drugs (Lugol's Solution + Oxytetracycline) for effective treatment of Repeat Breeding at 4 Metro Thana.

Name of Farm	Location (Thana)	Treatment By Lugol's Solution + Oxytetracycline	Conception Status	
			Conceive	Not Conceive
Anwar Dairy Farm	Motihar	1	1	-
Robiul Dairy Farm	Motihar	1	1	-
Moslem Dairy Farm	Rajpara	1	1	-
Shofik Dairy Farm	Rajpara	1	-	1
Erfan Dairy Farm	Rajpara	1	-	1
Zia Dairy Farm	Rajpara	1	1	-
KarimDairy Farm	Rajpara	1	1	-
Arif Dairy Farm	Boalia	1	-	1
Ripon Dairy Farm	Motihar	1	-	1
Sarwar Dairy Farm	Boalia	1	1	-
Kader Dairy Farm	Boalia	1	1	-
Rofik Dairy Farm	Boalia	1	-	1
Sohel Dairy Farm	Boalia	1	1	-
Mazed Dairy Farm	Shamukhdum	1	-	1
Bikash Dairy Farm	Motihar	1	1	-
Islam Dairy Farm	Shamukhdum	1	-	1
Taposh Dairy Farm	Shamukhdum	1	1	-
Kabir Dairy Farm	Shamukhdum	1	1	-
Momin Dairy Farm	Boalia	1	1	-
Shohid Dairy Farm	Boalia	1	1	-
	Total	**20**	**13**	**7**

Table 3.4: Selected dairy farms with therapeutic drugs (Acriflavin + Metronedazole + Gentamycin Sulphate) for effective treatment of Repeat Breeding at 4 Metro Thana.

Name of Farm	Location (Thana)	Treatment By Acriflavin + Metronedazole + Gentamycin Sulphate	Conception Status	
			Conceive	Not Conceive
Samim Dairy Farm	Motihar	1	1	-
Mithu Dairy Farm	Motihar	1	1	-
Shuzon Dairy Farm	Rajpara	1	1	-
Sunny Dairy Farm	Rajpara	1	-	1
Sumon Dairy Farm	Rajpara	1	-	1
Sattar Dairy Farm	Rajpara	1	1	-
Murad Dairy Farm	Rajpara	1	1	-
Mamun Dairy Farm	Boalia	1	-	1
Shaju Dairy Farm	Motihar	1	1	-
Azad Dairy Farm	Boalia	1	1	-
Mithu Dairy Farm	Boalia	1	1	-
Nahid Dairy Farm	Boalia	1	-	1
Razib Dairy Farm	Boalia	1	1	-
Milon Dairy Farm	Shamukhdum	1	-	1
Rasel Dairy Farm	Motihar	1	1	-
Touhid Dairy Farm	Shamukhdum	1	-	1
Mahafuz Dairy Farm	Shamukhdum	1	1	-
Robiul Dairy Farm	Shamukhdum	1	1	-
AminDairy Farm	Boalia	1	1	-
Suruj Dairy Farm	Boalia	1	1	-
	Total	**20**	**14**	**6**

Table 3.5: Selected dairy farms with therapeutic drugs (Two times AI + Vitamin-ADE) for effective treatment of Repeat Breeding at 4 Metro Thana.

Name of Farm	Location (Thana)	Treatment By Two times AI + Vitamin-ADE	Conception Status	
			Conceive	Not Conceive
Johurul Dairy Farm	Motihar	1	1	-
Salam Dairy Farm	Motihar	1	-	1
Sajedul Dairy Farm	Rajpara	1	1	-
Bikash Dairy Farm	Rajpara	1	-	1
Arju Dairy Farm	Rajpara	1	-	1
Mojnu Dairy Farm	Rajpara	1	1	-
Kazol Dairy Farm	Rajpara	1	1	-
Rouf Dairy Farm	Boalia	1	-	1
Rana Dairy Farm	Motihar	1	1	-
Uzzal Dairy Farm	Boalia	1	-	1
Tutul Dairy Farm	Boalia	1	1	-
Mannan Dairy Farm	Boalia	1	-	1
Miza Dairy Farm	Boalia	1	1	-
Alom Dairy Farm	Shamukhdum	1	-	1
Torikul Dairy Farm	Motihar	1	1	-
Habib Dairy Farm	Shamukhdum	1	-	1
Hafez Dairy Farm	Shamukhdum	1	-	1
Mozamel Dairy Farm	Shamukhdum	1	1	-
Ibrahim Dairy Farm	Boalia	1	-	1
Sohorab Dairy Farm	Boalia	1	1	-
	Total	**20**	**10**	**10**

Table 3.6: Selected dairy farms as control (no treatment).

Name of Farm	Location (Thana)	No treatment	Conception Status	
			Conceive	Not Conceive
Anowar Dairy Farm	Motihar	1	1	-
Delwar Dairy Farm	Motihar	1	-	1
Bashed Dairy Farm	Rajpara	1	1	-
Robiul Dairy Farm	Rajpara	1	-	1
Kashed Dairy Farm	Rajpara	1	-	1
Eaqub Dairy Farm	Rajpara	1	1	-
Zabbar Dairy Farm	Rajpara	1	1	-
Halim Dairy Farm	Boalia	1	-	1
Nasir Dairy Farm	Motihar	1	1	-
Pintu Dairy Farm	Boalia	1	-	1
Harun Dairy Farm	Boalia	1	-	1
Jahid Dairy Farm	Boalia	1	-	1
Mosihur Dairy Farm	Boalia	1	1	-
Kalam Dairy Farm	Shamukhdum	1	-	1
Chunnu Dairy Farm	Motihar	1	1	-
Belal Dairy Farm	Shamukhdum	1	-	1
Pikul Dairy Farm	Shamukhdum	1	-	1
Rana Dairy Farm	Shamukhdum	1	-	1
Hasem Dairy Farm	Boalia	1	-	1
Liton Dairy Farm	Boalia	1	-	1
	Total	**20**	**7**	**13**

3.18 Estrus (Heat) Detection in Cattle

Estrus in cattle is commonly referred to as heat. It occurs every 18 to 24 days in sexually mature, open (non-pregnant) female cattle when they are receptive to mounting activity by bulls or other cows or heifers.

Heat detection is critical to heat synchronization and breeding programs, particularly artificial insemination and embryo transfer programs. Effective heat detection is often the most limiting factor in an artificial insemination program. Heat detection can also be used to monitor onset of puberty in heifers, regularity of estrous cycles in breeding age females, and breeding effectiveness of natural service sires via returns to heat in the cow herd.

Heat detection efficiency (rate) is the percentage of eligible cows seen or detected in heat. Eligible cows are cows eligible for insemination. Heifers have reached puberty if they have resumed normal estrus function (cycling) after calving (typically 40 days or more post calving), are free of reproductive disorders or reproductive tract infections, and are open. A heat detection rate of 80 to 85 percent should be attainable.

3.18.1 Heat signs and detection method

Several methods of heat detection can be implemented. Some involve using heat detection aids. Several different methods can be combined to improve heat detection rates and accuracy. These include visual observation, heat mount detectors, tail head markers (paint, chalk, crayon, paste), chin ball markers, detector animals, and electronic heat detection devices.

3.18.2 Visual Observation

Visual observation is a commonly used method of heat detection. It involves a trained observer's recognizing and recording signs of heat. Observable signs of heat include mounting or attempting to mount other cattle, standing to be mounted by other cattle, smelling other females, trailing other females, bellowing, depressed appetite, nervous and excitable behavior, mud on hindquarters and sides of cattle, roughed up tail hair, vulva swelling and reddening, clear vaginal mucous discharge, and mucous smeared on rump etc.

Table 3.7: Sign of heat

	Coming into Heat (8 hours)	**Standing Heat (18 hours)**	**Going out of Heat (14+ hours)**
Heat Signs	• Stands and bellows • Smells other cows • Head butts other cows • Attempts to ride other cows but will not stand to be mounted • Red, moist, slightly swollen vulva • Clear mucous discharge from vulva	• Stands to be mounted • Rides other cows • Bellows frequently • Nervous and excitable	• Attempts ro ride other cows but will not stand to be mounted • Smells other cows • Clear mucous discharge from vulva

3.19 Artificial Insemination

Artificial insemination is the technique in which semen with living sperms is collected from the male and introduced into female reproductive tract at proper time with the help of instruments. This has been found to result in a normal offspring. In this process, the semen is

inseminated into the female by placing a portion of it either in a collected or diluted form into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions. Artificial insemination is not merely a novel method of bringing about impregnation in females. Instead, it is a powerful tool mostly employed for livestock improvement. In artificial insemination the germplasm of the bulls of superior quality can be effectively utilized with the least regard for their location in faraway places. By adoption of artificial insemination, there would be considerable reduction in both genital and non-genital diseases in the farm stock.

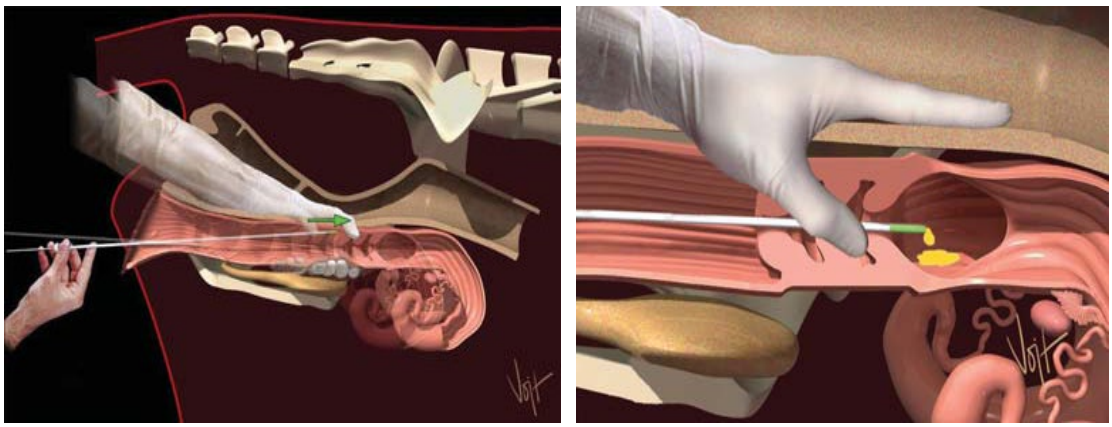


Plate 9: Artificial insemination of cows

Artificial insemination techniques

The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception. Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates. Early methods of AI involved deposition of the semen in the vagina, as would occur in natural mating. Those methods are not satisfactory. Fertility is low and greater numbers of sperm are

required. Another method which gained popularity was the "speculum" method. This method is easily learned, but proper cleaning and sterilizing of the equipment is necessary, making it more impractical to inseminate than with the rectovaginal technique which is the most widely used AI method today.

In the recto-vaginal technique a sterile, disposable catheter containing the thawed semen is inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter is passed through the spiral folds of the cow's cervix into the uterus. Part of the semen is deposited just inside the uterus and the remainder in the cervix as the catheter is withdrawn. Expulsion of the semen should be accomplished slowly and deliberately to avoid excessive sperm losses in the catheter. The body of the uterus is short; therefore, care should be taken not to penetrate too deeply which might cause physical injury. In animals previously inseminated, the catheter should not be forced through the cervix since pregnancy is a possibility. Since research data show little variation in conception rates when semen is placed in the cervix, uterine body or uterine horns, some people recommend incomplete penetration of the cervical canal and deposition of semen in the cervix.

The recto-vaginal technique is more difficult to learn and practice is essential for acceptable proficiency but the advantages make this method of insemination more desirable than other known methods. With practice, the skillful technician soon learns to thread the cervix over the catheter with ease. If disposable catheters are used and proper sanitation measures are followed, there is little chance of infection being carried from one cow to another.

3.20 Statistical Analysis

The researcher was also conduct the experiment with the help of farm owner to trail their therapeutic drugs and recording the conception status. Data which was collected from the study area as per requirement sorted and complied with the help of computer software SPSS and analyzed in accordance with the objectives of the study. Various statistical tools such as percentages, mean, standard deviation, incidence & chi-square test, logistic regression and the significant difference among therapeutic treatment means were identified by least significant difference (LSD) test and finally thesis writing.

The difference between therapeutic treatment and control group between each therapeutic treatment group, and, effects of other variables on repeat breeding were determined by χ^2 test.

$$\text{Incidence of repeat breeding} = \frac{\text{Number of repeat breeding}}{\text{Total number of observed cow}} \times 100$$

Model for χ^2 test:

H_0: There is no relationship between repeat breeding and breed, age, parity, body weight, body condition score, farm type, farm size, floor type, housing system, feed quality, breeding method, preventive measure and treatment provider.

H_1: There is relationship between repeat breeding and breed, age, parity, body weight, body condition score, farm type, farm size, floor type, housing system, feed quality, breeding method, preventive measure and treatment provider. If the calculated value is greater than the tabulated value than the hypothesis is reject and vice versa.

3.21 Model Building

Simple logistic regression model has been undertaken to estimate the impact of different therapeutic treatment groups of GnRH + AI, Lugol's Solution + Oxyteracycline , Acriflavin + Metronedazole + Gentamycin Sulphate, Two times AI + Vitamin ADE with Control group (no treatment) and pregnancy come out of dairy cows at Rajshahi in Bangladesh. The rate of repeat breeding of dairy cows at Rajshahi in Bangladesh is to be estimated by the created index from the logistic model analysis. When it takes the value 1 the probability will be *P* (say) if the respondent contains "Normally conceive" and 0 with probability (1-*P*) if it contains "Repeat Breeding". The independent variable of the analysis are categorical as well as indicator variables as to handle in simple logistic regression analysis the individual category of a variable is converted into the present and absence of a characteristic, usually denoted by 1 and 0, often called dummy variables. Explanations of the variables are shown in Table 3.8.

Table 3.8: Description of Variables Appearing in the Simple Logistic Regression Analysis.

Variables	**Value Levels**	**N**
Dependent: ***Index of Repeat Breeding***	$\Rightarrow$ Y= 0 (Repeat Breeding) $\Rightarrow$ Y = 1 (Conceive)	100
Independent: ***Treatment (therapeutic trials) Group*** GnRH + AI, Lugol's Solution + Oxyteracycline, Acriflavin + Metronedazole + Gentamycin Sulphate, Two times AI + Vitamin ADE Control$^{(r)}$	GnRH + AI = 1 $\Rightarrow X_{11} = x_{11}$ Lugol's Solution + Oxyteracycline, = 2 $\Rightarrow X_{12} = x_{12}$ Acriflavin + Metronedazole + Gentamycin Sulphate, t = 3 $\Rightarrow X_{13} = x_{13}$ Two times AI + Vitamin ADE = 4 $\Rightarrow X_{14} = x_{14}$ Control$^{(r)}$ = 5 $\Rightarrow X_{15}^{\{r\}} = 0$	100

Logistic Regression Model for Variables of Table-3.8

The explanations of the model corresponding to treatment *(therapeutic trials)* groups of GnRH + AI, Lugol's Solution + Oxyteracycline, Acriflavin + Metronedazole + Gentamycin Sulphate, Two times AI + Vitamin ADE, Control and Repeat breeding come out of dairy cows at Rajshahi in Bangladesh variables in Table 1 are as follows:

Expression of P_i is given by

$$P_i = E\left[Y_i = 1 \mid X_{11} = x_{11}, X_{12} = x_{12}, X_{13} = x_{13}, X_{14} = x_{14}, X_{15} = 0\right]$$

(Here the values of the variables corresponding to the reference category are considered as '0')

That is,

$$E\left(Y_i = 1 \middle| X_i\right) = P_i = \frac{e^{(a+bx)}}{1+e^{(a+bx)}} = \frac{1}{1+e^{-(a+bx)}}$$

and

$$1 - P_i = \frac{e^{-(a+bx)}}{1+e^{-(a+bx)}}$$

Therefore,

$$\frac{P_i}{1-P_i} = e^{(a+bX)}.$$

Hence the simple binary logistic regression model is given by

$$\log_e \frac{P_i}{1-P_i} = a + b_{11}X_{11} + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14} \qquad (1)$$

CHAPTER IV

RESULTS

A total of 1207 cows were recorded where 246 cows were failed to become pregnant when bred three cycles continuously, to a fertile insemination. The overall incidence of repeat breeding was 20.4% (Fig. 4.1). The incidence of repeat breeding of dairy cows among the breed, age, parity, body weight, body condition score, farm type, farm size, housing system, floor type, feed quality, breeding method, preventive measure, animal treatment provider as well as to identify the causal agent, antibiotic sensitivity test and to investigate some therapeutic trials to recovery the repeat breeding of dairy cows at Rajshahi in Bangladesh. All the results were presented in Tables 4.1-4.25 and Fig. 4.1-4.16.

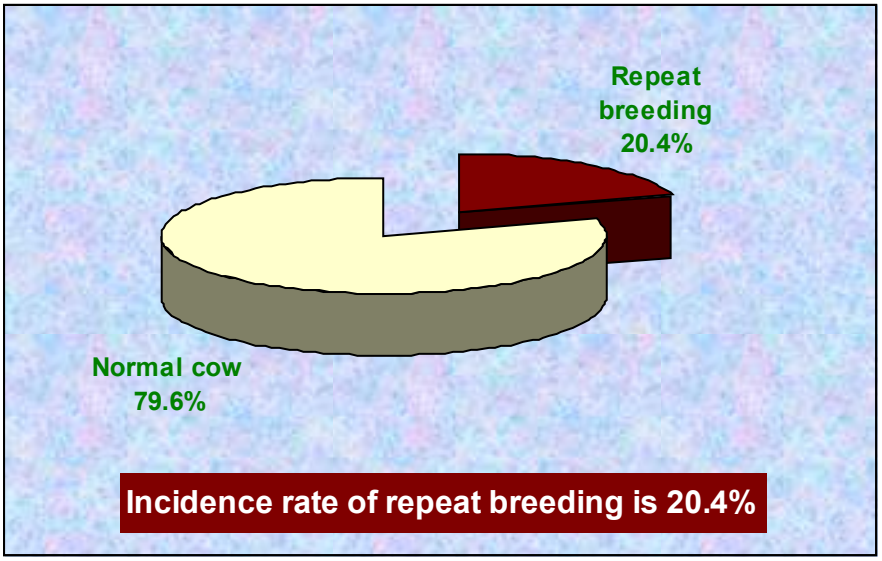


Figure 4.1: Graphical representation of incidence of repeat breeding.

4.1 Factors affecting the incidence of repeat breeding of dairy cows

Various influencing factors on incidence of repeat breeding of dairy cows were studied. These were detailed below:

4.1.1 Effect of breeds

The effect of breed or genotype on repeat breeding are shown in tables-4.1 and 4.2. It was observed that the incidence of repeat breeding is highest number 237 and the percentage of 19.7 were recorded in crossbred cows. And other Local breed was 09 number and 0.7 percentage, respectively. When compared with genetic composition the highest number was 103 and percentage was 8.5 recorded in Local x Friesian crossbred cows and other number and percentage were 61(5.1%), 38(3.1%) and 36(3.0%) in Local × Sahiwal, Local × Holstein Friesian x Sahiwal, and Local × Sahiwal × Sahiwal type, respectively. Figure 4.2 shows the incidence of repeat breeding about 19.7% share in crossbred and 0.7% in Local breed cows. Figure 4.3 observed greater share of repeat breeding was 5.1%, 3.1% and 3.0% in Local × Sahiwal, Local × Holstein Friesian x Sahiwal and Local × Sahiwal × Sahiwal type, respectively. Researcher has found from table 4.1 and 4.2 that the calculated value of chi-square were 27.315, 27.98 on the other hand the tabulated value of chi-square at ($P<0.05$) level of significance and 1& 4 degrees of freedom was 3.841 & 9.49. Since the calculated value of tests were greater than the tabulated value, so researcher may reject the null hypothesis that means there was significant effect ($P<0.05$) of breed and genotype on incidence of repeat breeding of dairy cows.

Table 4.1: Incidence of repeat breeding among cross and indigenous dairy cows.

Repeat Breeding Status	Breed Type		Total	Chi-Square (χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	Local	Cross-bred			
Repeat Breeder	9 (0.7%)	237 (19.7%)	246 (20.4%)	27.315	3.841
Normal Cow	18 (1.5%)	943 (78.1%)	961 (79.6%)		
Total	**27 (2.2%)**	**1180 (97.8%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis

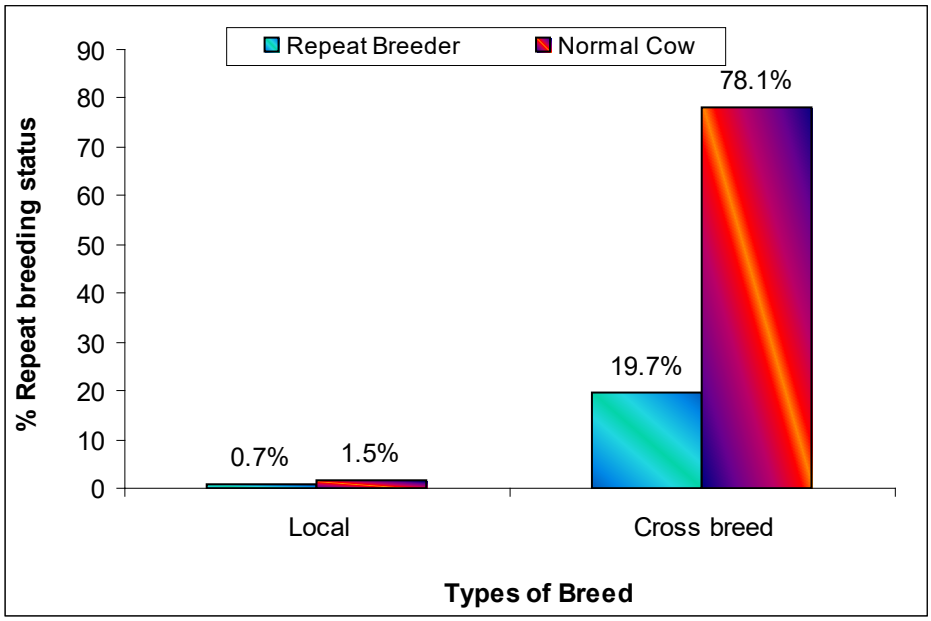


Figure 4.2: Graphical representation of percentages of repeat breeding in relation with breed of cows.

Table 4.2: Genotype wise effect on repeat breeding of dairy cows

Repeat Breeding condition	Genotype					Total	Chi-Square (χ^2) Calculated value	Chi-Square (χ^2) tabulated value
	L	L ×SL	L × F	L×F×SL	L×SL×SL			
Repeat Breeder	8 (0.7%)	61 (5.1%)	103 (8.5%)	38 (3.1%)	36 (3.0%)	246 (20.4%)	27.98	9.49
Normal Cow	19 (1.6%)	142 (11.8%)	560 (46.4%)	96 (8.0%)	144 (11.9%)	961 (79.6%)		
Total	**27 (2.3%)**	**203 (16.8%)**	**663 (54.9%)**	**134 (11.1%)**	**180 (14.9%)**	**1207 (100.0%)**		

L=Local, SL=Sahiwal, F=Friesian;

The percentage of repeat breeding is indicated in parenthesis.

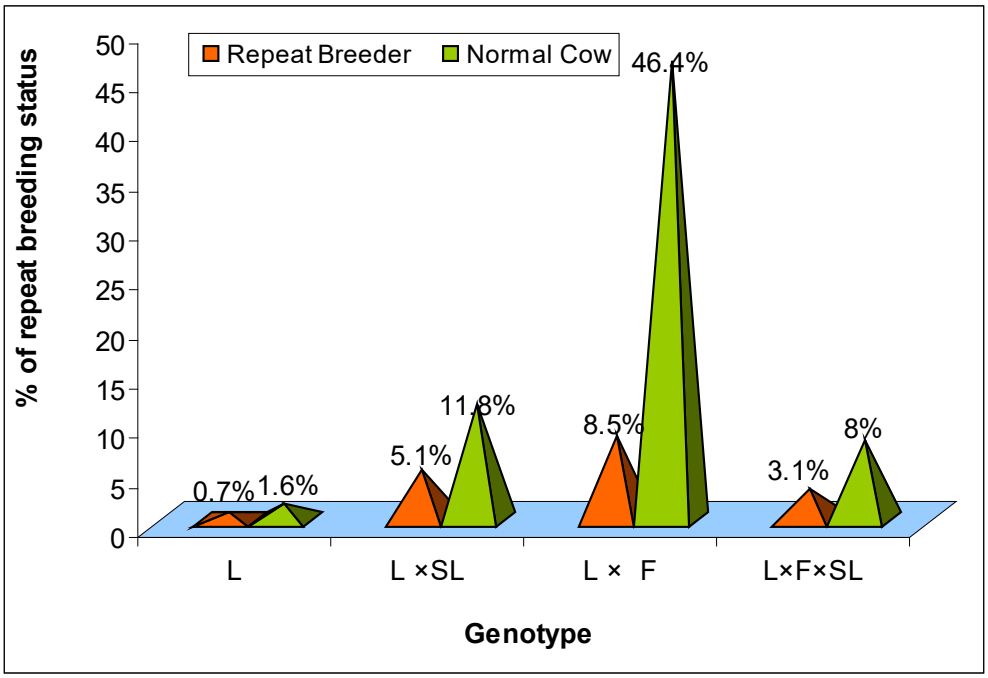


Figure 4.3: Pyramid bar diagram shows the percentages repeat breeding relation with genotype of cows.

4.1.2 Effect of age

The age influences on repeat breeding are exposed in table 4.3. It was found that the highest number repeat breeding was 105 recorded in 6 to <8 years group cows. And others <4 years, 4 to <6 years and >8 years were 3.3%, 4.8%, and 3.6%, respectively. Figure 4.4 observed that greater share of incidence on repeat breeding was 8.7% in 6 to <8 years group cows; thereafter 3.3%, 4.8% and 3.6% share of incidence <4 years, 4 to <6 years & >8 years group of cows, respectively. Researcher had found from table 4.3 that the calculated value of chi-square was 20.23, on the other hand the tabulated value of chi-square at (P<0.05) level of significance and 3 degree of freedom was 7.815. Since the calculated value of the test was greater than the tabulated value, so researcher may reject the null hypothesis that means there was significant effect (P<0.05) of age groups on repeat breeding of dairy cows.

Table 4.3: Effect of age on incidence of repeat breeding of dairy cows

Repeat Breeding Status	Age group				Total	Chi- Square (χ^2) Calculated value	Chi-Square (χ^2) tabulated value
	<4 years	4 years to < 6 years	6 to < 8 years	> 8 years			
Repeat Breeder	40 (3.3%)	58 (4.8%)	105 (8.7%)	43 (3.6%)	246 (20.4%)	20.23	7.815
Normal Cow	147 (12.2%)	362 (30.0%)	346 (28.7%)	106 (8.8%)	961 (79.6%)		
Total	**187 (15.5%)**	**420 (34.8%)**	**451 (37.4%)**	**149 (12.3%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

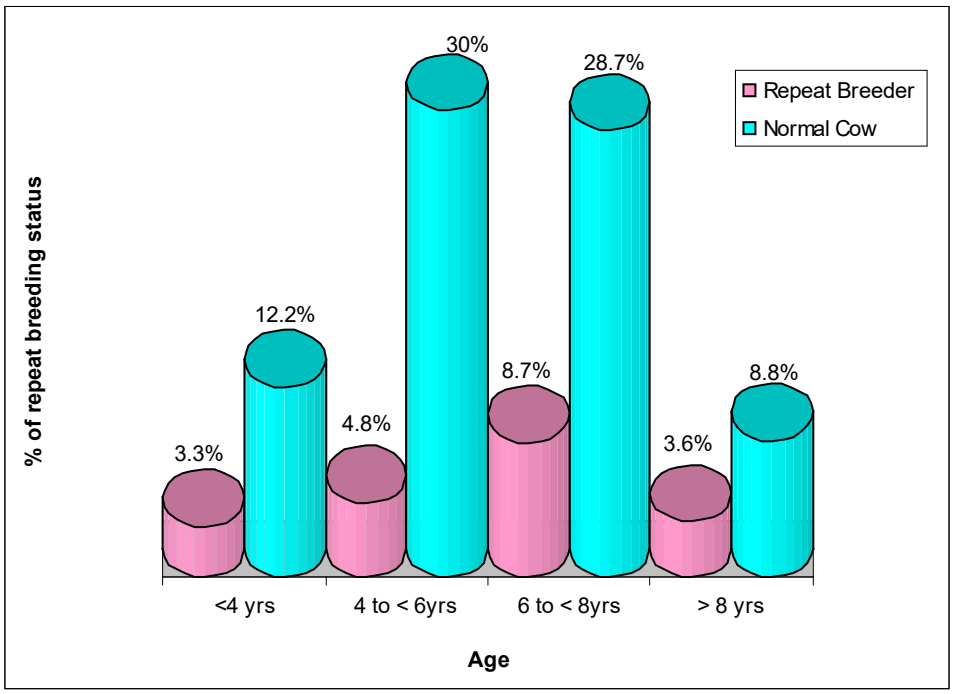


Figure 4.4: Cylinder bar diagram indicates the proportion of repeat breeding and age group of cows.

4.1.3 Effect of parity

The parity of cow's influences on repeat breeding is presented in table 4.4. It was observed that the highest number 77 cases of repeat breeding was recorded in third parity of cows and others were 58, 43, 39,17 & 12 in 1st, 2nd, 4th,5th and 6th parity of cows, respectively. From figure 4.5 the highest percentage of repeat breeding was 6.4% in parity 3 and lowest 1.0% in parity 6 and others values of repeat breeding were 4.8%, 3.6%, 3.2% and 1.4% in parity 1, parity 2, parity 4 and parity 5, respectively. From the table 4.4, the author has got, the calculated value of chi-square was 10.05 and tabulated value of chi-square at ($P<0.05$) level of significance and 5 degrees of freedom was 11.07. Hence the calculated value of the test had lower than the tabulated value, so the author may

accept the null hypothesis that means there was no significance effect of parity (P<0.05) on repeat breeding of dairy cows.

Table 4.4: Effect of parity on the incidence of repeat breeding of dairy Cows

Repeat breeding status	Parity						Total	Chi-Square(χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	P_1	P_2	P_3	P_4	P_5	P_6			
Repeat Breeder	58 (4.8%)	43 (3.6%)	77 (6.4%)	39 (3.2%)	17 (1.4%)	12 (1.0%)	162 (20.4%)	10.05	11.07
Normal cow	242 (20.0%)	219 (18.1%)	275 (22.8 %)	124 (10.3%)	36 (3.0%)	65 (5.4%)	1043 (79.6%)		
Total	**300 (24.9%)**	**262 (21.7%)**	**352 (29.2.2%)**	**163 (13.5%)**	**53 (4.4%)**	**77 (6.4%)**	**1207 (100.0%)**		

P_1=1st calving, P_2=2ndcalving, P_3=3rdcalving, P_4=4thcalving, P_5=5thcalving & P_6=> 6th calving;

The percentage of repeat breeding is indicated in parenthesis.

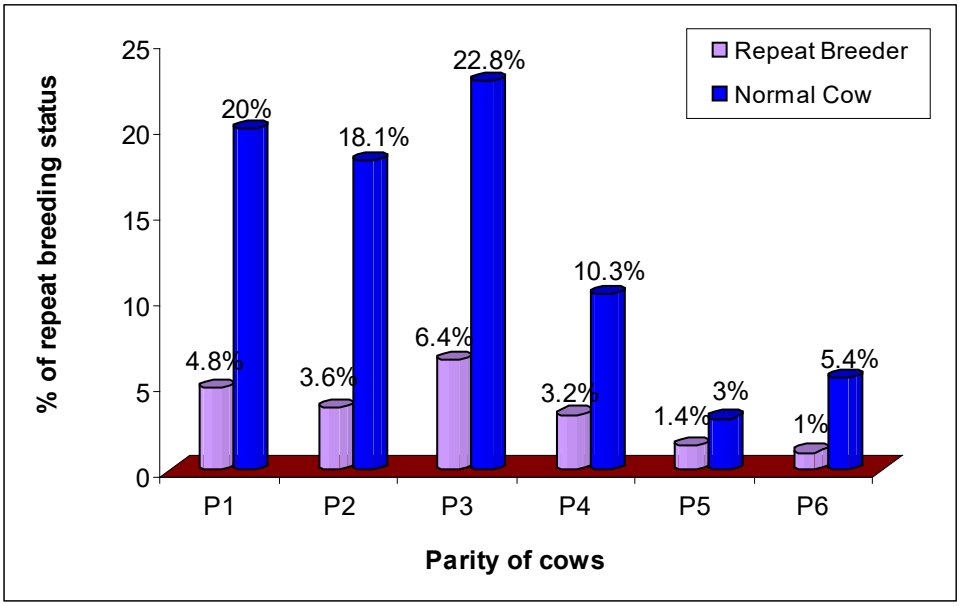


Figure 4.5: Bar diagram demonstrate percentages of repeat breeding with parity of cows.

4.1.4 Effect of body weight

The body weight influences on repeat breeding is summarized in table 4.5 and figure 4.6. It was observed that the highest incidence of repeat breeding was recorded in 124 number in 250 to < 350 kg body weight group of cows and lowest 50 in > 350 kg body weight and others were 72 < 250 kg body weight group of cows, respectively. Researcher had observed from the curve (figure 4.6) on incidence of repeat breeding (pick in 10.3%) 250 to < 350 kg body weight decreasing (6.0%) in <250 kg and descend in (4.1%) in >350 kg body weight accordingly. From table 4.5 that the researcher obtained calculated value of chi-square was 10.17 and the tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.99. Since the calculated value of the test was higher than the tabulated value, so researcher may reject the null hypothesis that means there was significant effect ($P>0.05$) of body weight on repeat breeding of dairy cows**.**

Table 4.5: Effect of body weight on the incidence of repeat breeding of dairy Cows

Repeat breeding status	**Body Weight**			**Total**	**Chi-Square(χ^2) Calculated value**	**Chi-Square(χ^2) tabulated value**
	< 250kg	**250 to < 350kg**	**> 350 kg**			
Repeat Breeder	72 (6.0%)	124 (10.3%)	50 (4.1%)	246 (20.4%)	10.17	5.99
Normal cow	217 (18.0%)	592 (49.0%)	152 (12.6%)	961 (79.6%)		
Total	**289 (24.0%)**	**716 (59.3%)**	**202 (16.7%)**	**1207 (100.0%)**		

The percentage of repeat breeding is indicated in parenthesis.

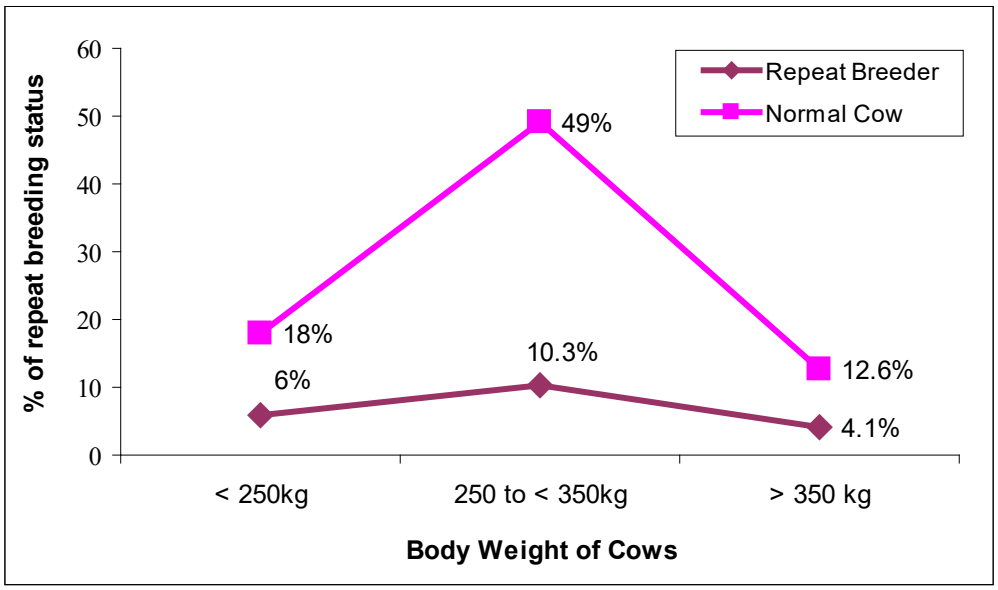


Figure 4.6: Curve represent the percentage of repeat breeding among different body weight of cows.

4.1.5 Effect of body condition score

The body condition influences on repeat breeding are shown in table 4.6. It was observed 45, 186 and 33 number of incidence repeat breeding in poor, fair and good body condition group of cows respectively. Figure 4.7 (tubes) indicated among this group the highest 13.9% of repeat breeding was recorded in fair group body condition cows, lowest was 2.7% in good body condition group and other was 3.8% in poor body condition group cows. Researcher had found from table 4.6 that the calculated value of chi-square was 2.99, on the other hand the tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.991. Since the calculated value of the test was lower than the tabulated value, so researcher might be accept the null hypothesis that means there was no significant effect ($P<0.05$) of body condition score on repeat breeding of dairy cows.

Table 4.6: Effect of body condition score on the incidence of repeat breeding of dairy cows

Repeat breeding status	Body Condition Score			Total	Chi-Square(χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	Poor	Fair	Good			
Repeat Breeder	45 (3.8%)	168 (13.9%)	33 (2.7%)	246 (20.4%)	2.99	5.99
Normal cow	225 (18.6%)	619 (51.3%)	117 (9.7%)	961 (79.6%)		
Total	**270 (22.4%)**	**787 (65.2%)**	**150 (12.4%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

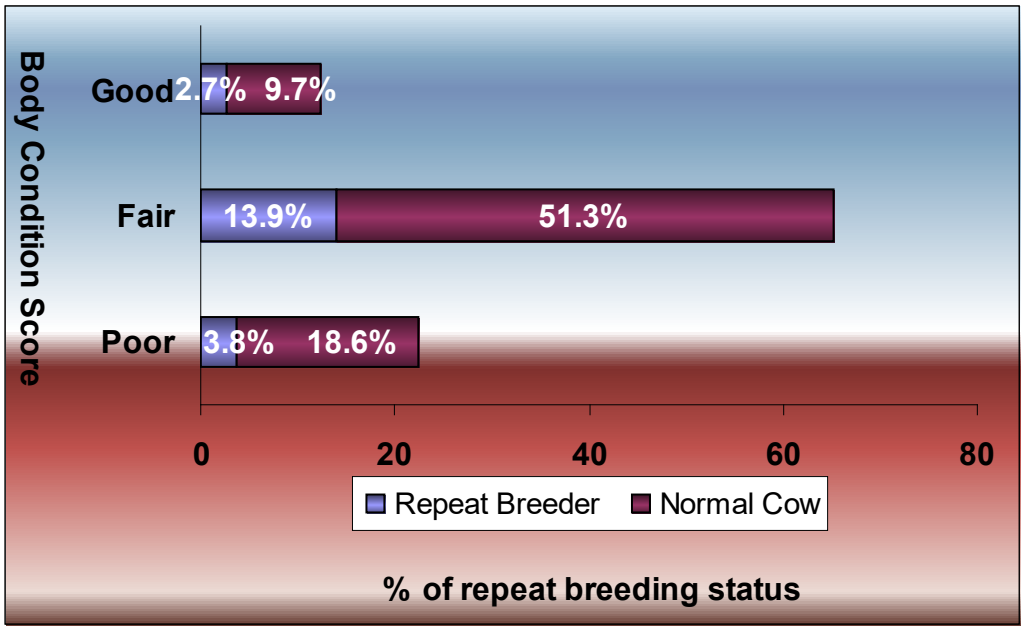


Figure 4.7: Above tubes shows of repeat breeding status relation with various BCS group of cows.

4.1.6 Effect of farm type

The farm type influences on repeat breeding are exposed in table 4.7. It was observed that the highest incidence 210 number of repeat breeding was recorded in private dairy farm and lowest was 36 in government dairy farm and figure 4.8 shows that 17.4% share of repeat breeding in private farm and only 3.0% in government dairy farm. Researcher had found from table 4.7 that the calculated value of chi-square was 1.517 and the tabulated value of chi-square at ($P<0.05$) level of significance and one degree of freedom was 3.841. Hence the tabulated value of the test was greater than the calculated value, so researcher may accept the null hypothesis and comment that there was no significant effect ($P>0.05$) of farm type on repeat breeding of dairy cows.

Table 4.7: Effect of farm type on the incidence of re of dairy cows

Repeat breeding status	**Farm type**		**Total**	**Chi- Square (χ^2) Calculated value**	**Chi-Square(χ^2) tabulated value**
	Government farm	**Private farm**			
Repeat Breeder	36 (3.0%)	210 (17.4%)	246 (20.4%)	1.517	3.841
Normal cow	144 (11.9%)	817 (67.7%)	961 (79.6%)		
Total	**180 (14.9%)**	**1027 (85.1%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

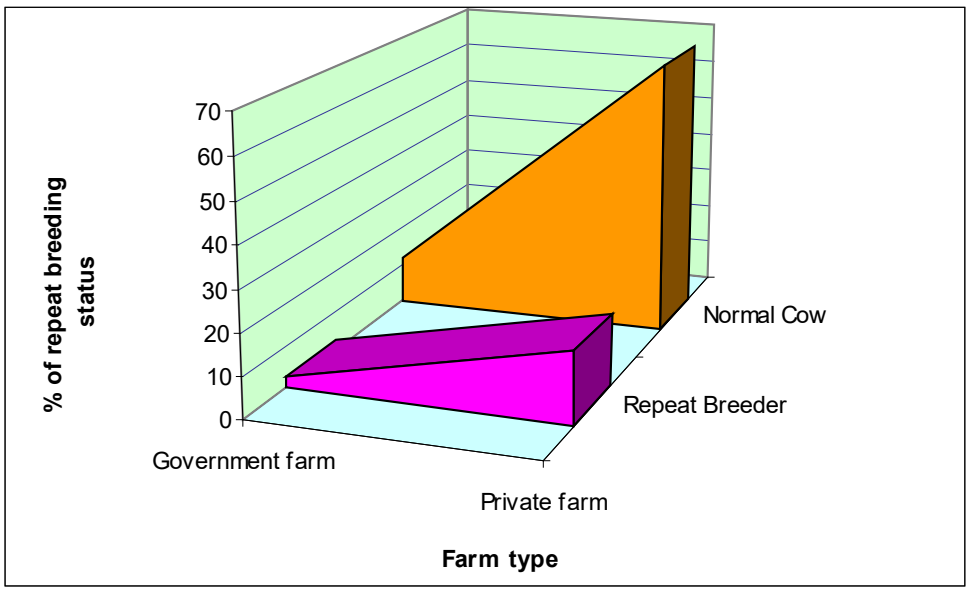


Figure 4.8: Area diagram shows the comparison between government and private dairy farm relation with repeat breeding.

4.1.7 Effect of farm size

The farm size influences on repeat breeding are constructed in table 4.8 and it was observed that the highest number 110 of incidence on repeat breeding was recorded in single cow dairy farms, lowest number (66) in medium and others number (70) in large dairy farms, correspondingly. Figure 4.9 (Cone diagram) shows highest 9.1% share of repeat breeding was in single cow dairy farms and other were 5.5% & 5.8% in medium and large dairy farms, respectively. The author obtained from table 4.8 that the calculated value of chi-square was 2.308 and the tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.991. Since the tabulated value of the test was greater than the calculated value so the researcher might be said that there was no significant effect ($P>0.05$) of farm size on repeat breeding of dairy cows.

Table 4.8: Effect of farm size on the incidence of repeat breeding of dairy cows

Repeat breeding status	Farm Size			Total	Chi-Square(χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	Single cow	Medium	Large			
Repeat Breeder	110 (9.1%)	66 (5.5%)	70 (5.8%)	246 (20.4%)	2.308	5.991
Normal cow	395 (32.7%)	244 (20.2%)	322 (26.7%)	961 (79.6%)		
Total	**505 (41.8%)**	**310 (25.7%)**	**392 (32.5%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

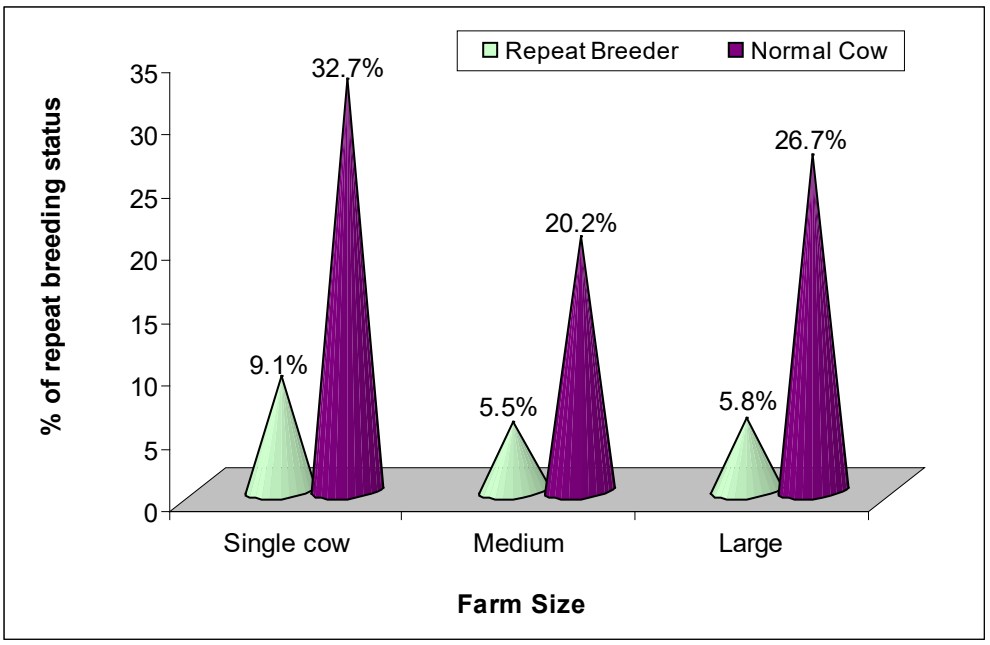


Figure 4.9: Cone diagram revealed the percentages of repeat breeding with farm size of cows.

4.1.8 Effect of housing system

The housing system influences on repeat breeding are given in table 4.9. It was observed that the incidence of repeat breeding was highest numbers (124) in medium house dairy farms than average number (69) in poor condition and thereafter lowest number (53) in good condition house dairy farms. Figure 4.10 shows highest 10.3% percentage of share on repeat breeding was medium house dairy farms and other were 4.4% & 5.7% in good and poor house dairy farms, respectively. Researcher had found (from table 4.9) that the calculated value of chi-square was 2.255, on the other hand the tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.991. Since the tabulated value of the test was greater than the calculated value, so researcher may accept the null hypothesis that means there was no significant effect ($P>0.05$) of breed on repeat breeding of dairy cows.

Table 4.9: Effect of housing system on the incidence of repeat breeding of dairy cows

Repeat breeding status	**Housing System**			**Total**	**Chi-Square(χ^2) Calculated value**	**Chi-Square(χ^2) tabulated value**
	Poor	**Medium**	**Good**			
Repeat Breeder	69 (5.7%)	124 (10.3%)	53 (4.4%)	246 (20.4%)	2.255	5.991
Normal cow	306 (25.4%)	434 (35.9%)	221 (18.3%)	961 (79.6%)		
Total	**375 (31.1%)**	**558 (46.2%)**	**274 (22.7%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

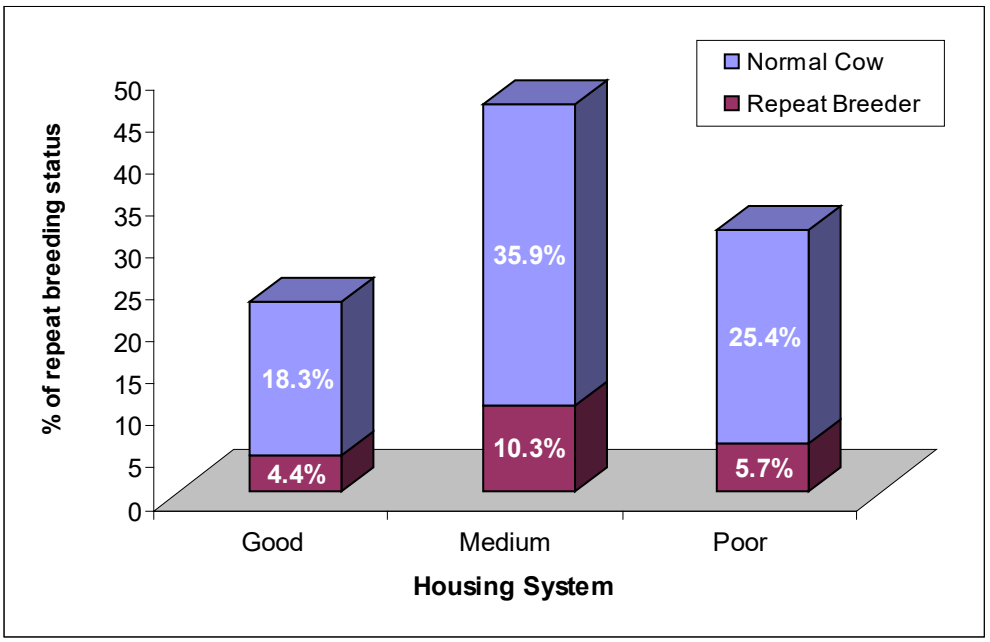


Figure 4.10: Bar diagram indicate percentages repeat breeding relation with housing system of cows.

4.1.9 Effect of floor type

The floor type influences on repeat breeding are furnished in table 4.10. It was observed that the incidence of repeat breeding highest number (124) of incidence of repeat breeding was recorded in medium and others were in 53 & 69 number of incidence of repeat breeding in poor and good condition floor type of farm, respectively. Figure 4.11 (line-column 2 axis) shows the incidence of repeat breeding among different floor type of farm were 4.4%, 10.3% and 5.7% in poor condition, medium condition and good conditions, respectively. From the table 4.10, the author has got, the calculated value of chi-square was 2.255 and tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.991. Since the calculated value of the test was lower than the tabulated value, so researcher might be accept the null hypothesis that means there

was no significant effect (P>0.05) of floor type on repeat breeding of dairy cows.

Table 4.10: Effect of floor type on the incidence of repeat breeding of dairy Cows

Repeat breeding status	Floor Type			Total	Chi-Square(χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	Poor	Medium	Good			
Repeat Breeder	53 (4.4%)	124 (10.3%)	69 (5.7%)	246 (20.4%)	2.255	5.991
Normal cow	221 (18.3%)	434 (35.9%)	306 (25.4%)	961 (79.6%)		
Total	**274 (22.7%)**	**558 (46.2%)**	**375 (31.1%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

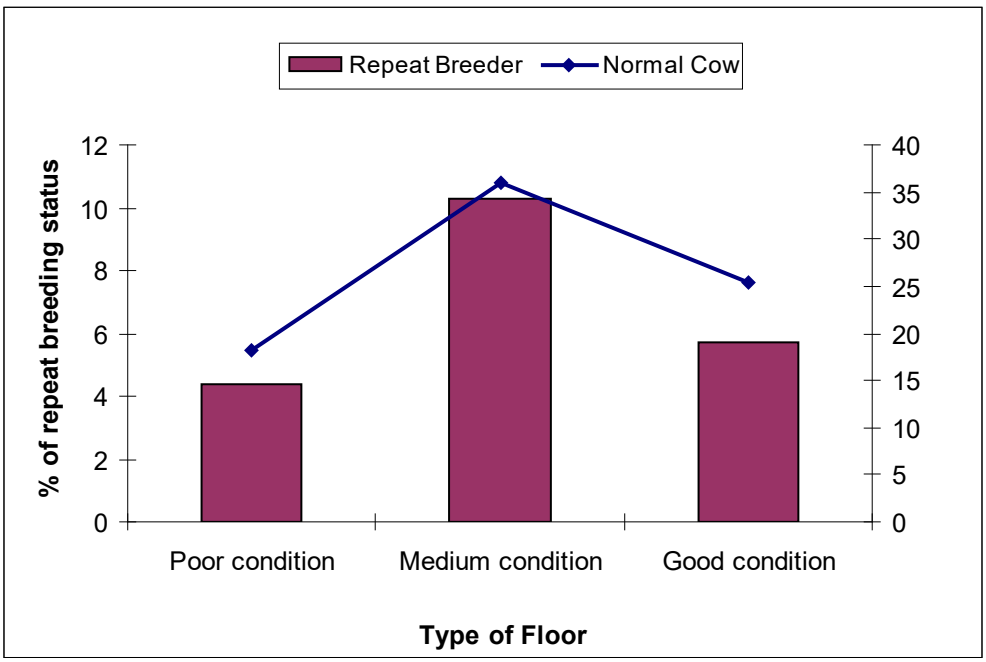


Figure 4.11: Line-column 2 axis diagram shows percentages of repeat breeding relation with various group of floor type of cows.

4.1.10 Effect of feed quality

The feed quality influences on repeat breeding is observed in table 4.11. It was observed that the incidence of repeat breeding highest 152 numbers was recorded in average feeding group of dairy cows, lowest & other were 45 & 49 numbers of repeat breeding recorded in better and average feed quality feeding of dairy cows. Figure 4.12 (pie diagram) shows 3.7%, 12.6% and 4.1% rate of repeat breeding was recorded in poor, average & better feed quality group of dairy farms, consequently. Researcher (from the table 4.11) has found the calculated value of chi-square was 14.679 and the tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.991. While the tabulated value of the test was lower than the calculated value, the author might be reject the null hypothesis and eventually stated that there was significant effect ($P>0.05$) of feed quality on repeat breeding of dairy cows.

Table 4.11: Effect of feed quality on the incidence of repeat breeding of dairy cows

Repeat breeding status	Feed Quality			Total	Chi-Square(χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	Poor	Average	Better			
Repeat Breeder	45 (3.7%))	152 (12.6%)	49 (4.1%	1246 (20.4%)	14.679	5.991
Normal cow	117 (9.7%)	546 (45.2%)	298 (24.7%)	961 (79.6%)		
Total	**162 (13.4%)**	**698 (57.8%)**	**347 (28.8%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

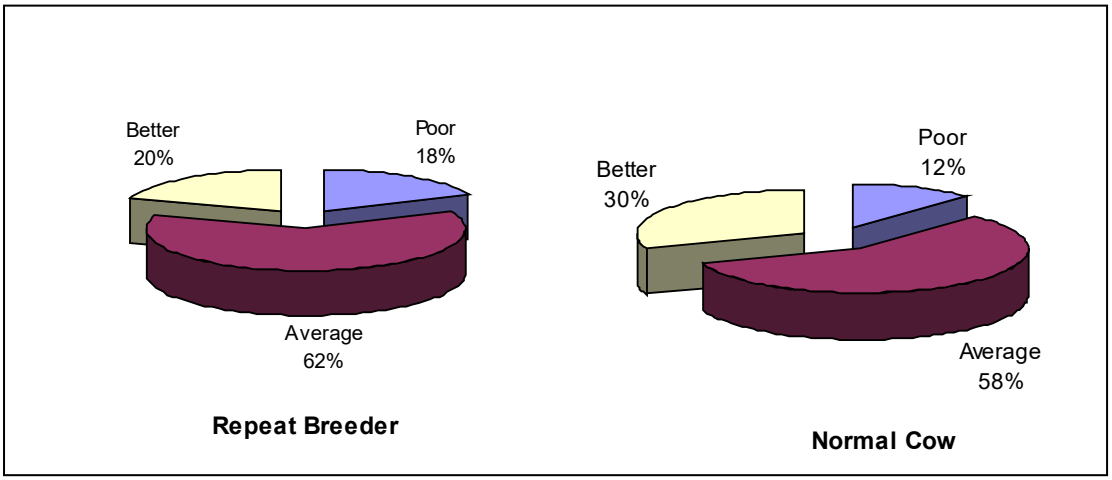


Figure 4.12: Pie diagram shows the proportion of repeat breeding and normal cow relation with feed quality.

4.1.11 Effect of breeding method

The breeding method influences on repeat breeding are available in table 4.12. It was observed that the incidence of repeat breeding highest 186 numbers in artificially inseminated cow and others was 60 numbers in naturally serviced cows and similarly from the figure 4.13 were recorded in 15.4% and 5.0%, respectively. From the table 4.12 researcher has found that the calculated value of chi-square was 55.028 and the tabulated value of chi-square at ($P<0.05$) level of significance and 1 degree of freedom was 3.841. Hence the calculated value of the test was greater than the tabulated value, so researcher might be reject the null hypothesis that means there was significant effect ($P<0.05$) of breeding method on repeat breeding of dairy cows.

Table 4.12: Effect of breeding method on the incidence of repeat breeding of dairy Cows

Repeat breeding status	Breeding Method		Total	Chi-Square(χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	Natural Service	Artificial Insemination			
Repeat Breeder	60 (5.0%)	186 (15.4%)	246 (20.4%)	55.028	3.841
Normal cow	488 (40.4%)	473 (39.2%)	961 (79.6%)		
Total	**548 (45.4)**	**659 (54.6%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

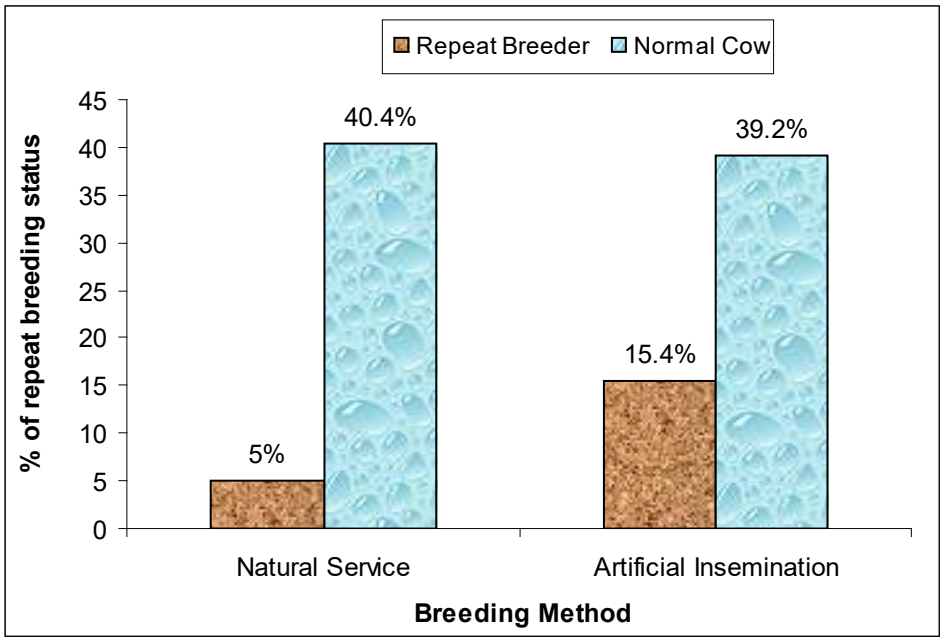


Figure 4.13: Bar diagram represent the percentages of repeat breeding relation with breeding method of cows.

4.1.12 Effect of Preventive measure on repeat Breeding:

The preventive measure (vaccination, deworming, mineral supply etc) influences on repeat breeding is observed in table 4.13. It was observed that the incidence of repeat breeding highest 88 numbers was recorded in occasionally preventive measure group of dairy cows, lowest & other were 73 & 85 numbers of repeat breeding recorded in nothing and regular preventive measure group of dairy cows. Figure 4.14 shows 6.1%, 7.3% and 7.0% rate of repeat breeding was recorded in nothing, occasionally and regular preventive measure group of dairy cows consequently. Researcher (from the table 4.13) has found the calculated value of chi-square was 6.724 and the tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.991. While the tabulated value of the test was lower than the calculated value, the author might be reject the null hypothesis and eventually stated that there was significant effect ($P>0.05$) of preventive on repeat breeding of dairy cows.

Table 4.13: Effect of preventive measure on the incidence of repeat breeding of dairy Cows

Repeat breeding status	**Preventive measure**			**Total**	**Chi-Square(χ^2) Calculated value**	**Chi-Square(χ^2) tabulated value**
	Nothing	**Occasionally**	**Regular**			
Repeat Breeder	73 (6.1%)	88 (7.3%)	85 (7.0%)	246 (20.4%)	6.724	5.991
Normal cow	291 (24.1%)	268 (22.2%)	402 (33.3%)	961 (79.6%)		
Total	**364 (30.2%)**	**356 (29.5%)**	**487 (40.3%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

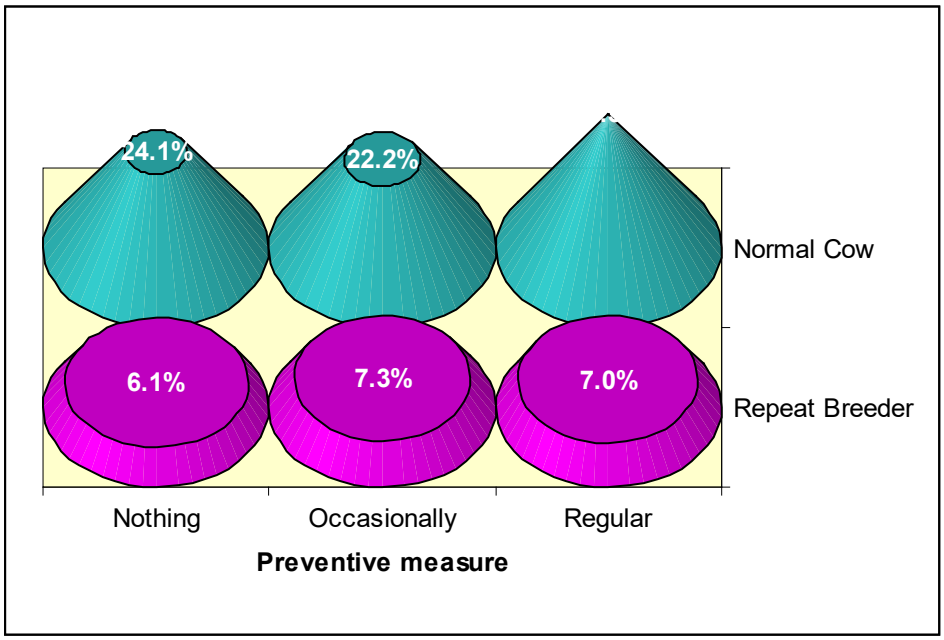


Figure 4.14: Cone diagram represent the percentages of repeat breeding relation with preventive measure of cows.

4.1.13 Effect of Treatment status on repeat breeding

The treatment status influences on repeat breeding is observed in table 4.14. It was observed that the incidence of repeat breeding highest 129 numbers was recorded in treatment by traditional group of dairy cows, lowest and other were 24 and 93 numbers of repeat breeding recorded in treatment by veterinarian and paravet group of dairy cows. Figure 4.15 (pie diagram) shows 2.0%, 7.7% and 10.7% rate of repeat breeding was recorded in treatment by veterinarian, paravet and traditional group of dairy cows consequently. Researcher (from the table 4.14) has found the calculated value of chi-square was 5.371 and the tabulated value of chi-square at ($P<0.05$) level of significance and 2 degrees of freedom was 5.991. Hence, the tabulated value of the test was greater than the calculated value, the author might be accept the null hypothesis and

eventually stated that there was no significant effect (P>0.05) of treatment on repeat breeding of dairy cows.

Table 4.14: Effect of treatment provider on the incidence of repeat breeding of dairy Cows

Repeat breeding status	Treatment by			Total	Chi-Square(χ^2) Calculated value	Chi-Square(χ^2) tabulated value
	Traditional	Paravet	Veterinarian			
Repeat Breeder	129 (10.7%)	93 (7.7%)	24 (2.0%)	246 (20.4%)	5.371	5.991
Normal cow	582 (48.2%)	304 (25.2%)	75 (6.2%)	961 (79.6%)		
Total	**711 (58.9%)**	**397 (32.9%)**	**99 (8.2%)**	**1207 (100%)**		

The percentage of repeat breeding is indicated in parenthesis.

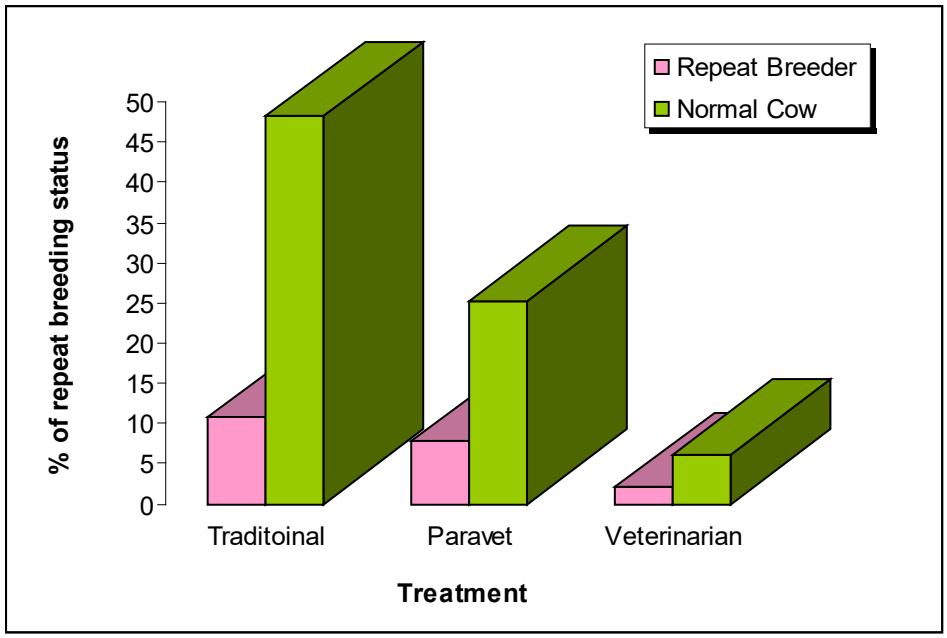


Figure 4.15: Column graph represent the percentages of repeat breeding relation with treatment provider of cows.

4.2. Isolation and Identification of Micro organism

The table 4.15 found that the studied cows were in different parity from 1st to > 5th parity.

Table 4.15. Distribution of animals according to breed, age and parity.

Breed	Total number of animals	Age (years)			Parity				
		<4	4- <8	> 8	1st	2nd	3rd	4th	>5th
Crossbred	70	10	40	20	5	5	25	20	15
Local	30	2	5	3	2	4	12	8	4

For simplifying the study and to make it easy to understand all samples was divided into two groups; Group-I consisting the sample from cows with history of any reproductive diseases. Group-II including the samples from cows with the history of diseases free. Thirty one cows were under group-I of which 22 were crossbred and 9 were local and in group-II out of 69 cows, 48 were crossbred and 21 were local (Table 4.16).

Table 4.16. Distribution of sample from uterus according to diseases and non diseases animals.

Group	Crossbred n=70	Local n=30	Total number n=100
Group-I (Sample from diseases animals)	22	9	31(31.0%)
Group-II (Sample from non diseased animals)	48	21	69 (69.0%)

Here the proportion of reproductive diseases 31.0% which is one

approximately third of total. The prevalence of uterus infection was 16.63% (Gazaw *et al*., 2007) fairly agrees with that of 18.7% reported by Oumermohammed (2003) and 19.6% by Gebremariam (1996) in smallholder dairy cows. Erb and Martin (1980), Kassahun (2003) and Mamo (2004) who reported an incidence rates ranging from 11.5-13.6%.

Table 4.17. Distribution of samples according to reproductive diseases or disorders (n=31)

Diseases	Crossbred	Local	Total (%)	Uterine infection (%)	Total Number n=100 (%)
Pyometra	6	2	8(25.80%)	8 (25.80%)	25.00
Endometritis	8	3	11(35.48%)	11(35.48%)	
Cervisitis	2	1	3 (9.67%)	3 (9.67%)	
Abortion	2	1	3(9.67%)	3(9.67%)	
Vaginitis	1	1	2 (6.45%)		
Bloody discharge	3	1	4(12.90%)		
Total	22	9	31(100)	25(80.64)	

Table 4.18. Reproductive diseases in different age in cows

Breed	Total number of animals	Age (years)			Total n=31
		<4	4- <8	> 8	
Crossbred	22	2 (10.0%)	8 (36.3%)	12 (54.5%)	22 (71.0%)
Local	9	1(11.1%)	3 (33.3%)	5 (55.6%)	9(29.0%)

Table 4.18 showed that positive correlation between age and reproductive disorders.

It also observed that cross breed are most susceptible to reproductive diseases than local cows. It found that 71.0% cows were in diseases condition among the crossbred which was above 8 years old 54.5%, whether 4-<8years were 36.3% and < 4 years were 10.0% only. In case of local cow also observed that 55.6% diseases cows were above 8 years old and only 11.1% cows below 4 years old. Incidence of infection in cows between 6-8 years or above has 50% to 60% more than those of below 6 years Oumermohammed (2003) and Tadesse (1999). Herath *et al* (2006), Shamsuddin *et al*., (2001) and Singh *et al*., proved that increasing age increasing the susceptibility of uterine diseases in cows which may be for decreasing immunity, increasing microbial load day by day, nutritional insufficiency, increasing reproductive disorders etc. age and parity had significant effect on prevalence of reproductive health problems (Gazaw *et al*., 2007). In this study the results shown that increasing age and parity increasing the prevalence of reproductive problems in cows, its may due to more parity give chance to entrance of microorganisms and more scope of contamination during and after parturition.

In this study the prevalence of diseases in crossbred (71.0%) were higher than local cows (29.0%) which is near about 2 times higher is supported by Bretzlaff *et al*., (1982) showed that cross with Holstein Frisian cattle are 2 times higher susceptible to disease than zebu (Bos indicus). Crossbred cattle are more susceptible to diseases than indigenous cattle (Ruder *et al*., 1990). The significantly higher prevalence rate of reproductive problems in crossbred animals than indigenous zebu may be due to the fact that crossbred are less adapted to tropical condition of high temperature and humidity, diseases and low feed quality than zebu cattle (Mukasa-Mugerwa, 1989) making them more susceptible than indigenous zebu.

The organisms including *Fusobacterium* spp, *Streptococcus* spp, *Pseudococcus* spp, *Staphyloccus* spp, *Actinomyces* spp, *Haemophilus* spp, *Micrococcus* spp, *Bacteriodes* spp, *Escherichia coli*, *Pasteurella* spp, *Diplococcus* spp, *Peptocuccus* spp, *Klebsiella* spp, *Enterobactor* spp, *Bacillus* spp, *Nocardia* spp, *Clostredium* spp, *Salmonella* spp and *Proteus* spp were isolated from uterine sample. All bacteria were found in both diseased and non diseased samples except *Nocardium* spp and *Clostredium* spp were not found in diseased and *Salmonella* and *Bacillus* spp were not found in non-diseased samples. A significant number of bacteria were identified from diseased samples and they were *Fusobacterium* spp 14 (45.16%) (p=0.000), *Actinomyces* spp 10 (32.25%) (p=0.003), *Escherichia coli* 12 (38.70%), *Pseudomonas* spp 11 (35.48%) (p=0.002) and *Haemophilus* spp 9(29.0%) (p=0.012) (Table 4.19). The isolates of *Pseudomonas* and Gram negative minute rod shaped bacteria were obtained only from repeat breeder cows with mucopurulent uterine discharges (Gani *et al.,* 2008). *Actinomyces pyogenes* is considered to be a major pathogen of the reproductive tract among others and *Hemophilus* spp is well known to cause uterine infection (www.das. psu.edu/teamdairy/). Bacterial causes are most important behind endometritis in cows. *Arcanobacterium, Staphylococcus, Streptococcus, E. coli* and *Klebsiella* spp are main to cause endometritis in cows (Foldi *et al*., 2006). Identified *Staphylococcus* spp was 11 (35.48%) and *Streptococcus* spp 12 (38.78%). Identification of all other bacteria were between the range of 1 (3.0%) to 6 (18.20%). Gani *et al*., (2008) showed *Staphylococcus* was predominant 14 (37.8%), followed by *E. coli* 11 (29.7%), *Pseudomonas* 7 (18.9%) while Gram negative minute rod shaped bacteria was 9 (24.3%) in repeat breeder cows.

Table 4.19: Isolation and identification of microorganisms from uterine sample in cows.

Name of organisms	Total number n=100	Group-I (Diseased) n=31	Group-II (Non diseased) n=69	P-value
Fusobacterium spp	16 (16%)	14 (45.16%)	2(2.9%)	0.000
Actinomyces spp	12 (12%)	10 (32.25%)	2 (2.9%)	0.003
Escherichia coli	15(15%)	12 (38.70%)	3 (4.34)	0.001
Pseudomonas spp	14(14%)	11(35.48%)	3(4.34)	0.002
Haemophilus spp	12(12%)	9(29.0%)	3 (4.34)	0.012
Staphylococcus spp	16(16%)	11(35.48)	4(5.80%)	0.002
Streptococcus spp	15(15%)	12(38.78%)	3(4.34)	0.002
Micrococci spp	12(12%)	10(32.25)	2(2.90)	0.0134
Pasteurella spp	10(10%)	7(22.58%)	3(4.34)	NS
Enterobacter spp	3(3.0%)	2(6.45%)	1(1.44%)	NS
Klebsiella spp	4(4%)	2(6.45%)	2(2.90%)	NS
Proteus spp	3(3%)	2(6.45%)	1(1.44%)	NS
Bacteroides spp	11(11%)	3(9.67%)	8(11.60%)	NS
Peptococci spp	5(5%)	2(6.45%)	3(4.34%)	NS
Salmonella spp	2(2%)	3(9.67%)	0(00.0%)	NS
Diplococci spp	6(6%)	1(3.22%)	5(7.24)	NS
Bacillus spp	1(1%)	1(3.22%)	0(00.0%)	NS
Nocardia spp	1(1%)	0(00.0%)	1(1.44%)	NS
Clostredium spp	1(1%)	0(00.0%)	1(1.44%)	NS

In non diseased cases the highest number of samples was positive for *Bacteroiedes* spp 8 (11.60%). *Diplococcus* spp 5 (7.24%), *Staphylococcus* spp 4 (5.84%) and *Streptococcus* spp, *Escherischi* coli, *Pseudomonas* spp, *Haemophillus* spp, *Pasteurella* spp and *Peptococci* spp were the same number 3(4.34%). The positive number of *Klebsiella* spp was also same for diseased sample like non diseased sample. Identification number of other bacteria were between the range of 0 (00%) 8 (11.60%) in non-diseased samples.

However, in average, the highest numbers of organisms were encountered from diseased samples and most of those bacteria are pathogenic Gani et.al., (2008) and Singh *et al*. (1999) stated that about 60% of the identified bacteria from uterus commonly found in repeat breeders as well as from diseased animals and the frequency of the isolates were much higher than that of normal cows. Uterine disease is commonly associated with *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Prevotella* species. Indeed, *A. pyogenes*, *F. necrophorum, Prevotell* and *Streptococcus* species have been shown act synergistically to enhance the likelihood of uterus disease Sheldon (2008). In another study Erin *et al*. (2005) showed that most common and economically important bacteria for uterine infection are *Actinomyces, Escherichia coli, Fuosbacterium, Pasteurella, Pseudomonas* and *Staphycoccus*. Williams *et al*. (2005) found muco-purulent discharge was associated with *F. necrophorum* and purulent discharge was associated with greater load of *A. pyogenes, E. coli,* and *Streptococci* (Sheldon *et al*., 2006). From the above, discussion it is easily understood that most of endometrial and pyogenic cases and or uterine infections were caused by *Fusobacterium* spp, *Actonomyces* spp, *E. coli*, *Pseudomonas* spp and

Haemophilus spp, *Staphylococci* spp, *Streptococci* spp, *Pasteurella* spp, *Enterobacter* spp, *Klebsiella* spp and *Proteus* spp were also observed higher in number in diseased sample than non diseased sample though the number of isolates were not significant.

4.2.1 Identification of *Staphylococcus* spp. by different bacteriological methods

Results from cultural examination

Nutrient broth: Nutrient broth was inoculated in the uterine sample and incubated at 37°C for 24 hours. The growth of bacteria was indicated by presence of turbidity.

Nutrient Agar: Nutrient agar plates streaked separately with the organisms revealed the growth of bacterial after 24 hours of incubation at 37°C aerobically and were indicated by the growth of circular, small, convex and gray white or yellowish colonies.

Blood Agar: Blood plates streaked separately with the organism and incubated at 37°C aerobically for 24 hours. β type hemolysis were produced (Plate 19).

Results from Gram's staining: Gram's stained smear from nutrient agar and Blood agar revealed Gram-positive cocci arranged in grape like starcter (Plate 12).

Results from biochemical tests:

Fermentation reaction with five basic sugars: The isolate bacterial fermentated the five sugars (dextrose, maltose, lactose, surcrose and manitol) with the production of acid. The change of color from reddish to yellowish indicated acid production (Plate 14, 15 and 18).

Calalase test: The isolates were catalase positive.

Methyl-red and Voges-proskauer test: The isolates were MR positive and VP negative (Plate 13).

Indole test: The isolates were indole positive (Plate 13).

4.2.2 Identification of *Escherichia coli* by different bacteriological methods

Results from cultural examination

Nutrient broth: Nutrient broth was inoculated in the uterine sample and incubated at 37°C for 24 hours. The growth of bacteria was indicated by presence of turbidity.

MacConkey (MC) agar: MacConkey agar plates streaked separately with the organisms revealed the growth of bacterial after 24 hours of incubation at 37° C aerobically and were indicated by the growth of bright-pink colour smooth colonies.

Eosin Methylene Blue (EMB) Agar: EMB agar plates streaked separately with the organism revealed the growth of bacteria after 24 hours of incubation at 37°C aerobically by the growth smooth, circular, black color colonies with metallic sheen (Plate 27).

Results from Gram's staining: The microscopic examination of Gram's stained smear from MC agar and EMB agar revealed Gram-negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain (Plate 11).

Results from biochemical tests:

Fermentation reaction with five basic sugars: The isolate bacterial

fermentated the five sugars (dextrose, maltose, lactose, surcrose and manitol) with the production of acid. The change of color from reddish to yellowish indicated acid production (Plate 13).

Methyl-red and Voges-proskauer test: The isolates were MR positive and VP negative (Plate 13).

Indole test: The isolates were indole positive (Plate 13).

4.2.3 Identification of *Bacillus* spp. by different bacteriological methods

Results from cultural examination

Nutrient broth: Nutrient broth was inoculated in the uterine sample and incubated at 37° C for 24 hours. The growth of bacteria was indicated by presence of turbidity.

Nutrient Agar: Nutrient agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37 °C aerobically and were indicated by the growth of thick grayish-white or cream coloured colonies.

Blood Agar: Blood agar plates were streaked separately with the organism and incubated at 37°C aerobically for 24 hours. Large, creamy colonies with β-types hemolysis were produced.

Results from Gram's staining: Gram's stained smear from nutrient agar and Blood agar were examined microscopically which revealed Gram-positive, large rod shaped organisms arranged in chain.

Results from biochemical tests:

Fermentation reaction with five basic sugars: The isolate bacterial

fermentated the five sugars (dextrose, maltose, lactose, sucrose and manitol) with the production only acid but partial or incomplete fermentation with small amount of gas production was observed in case of mannitole and lactose. The change of color from reddish to yellowish in the medium.

Methyl-red and Voges-proskauer test: The isolates were MR positive and VP negative (Plate 13).

Indole test: The isolates were indole positive (Plate 13).

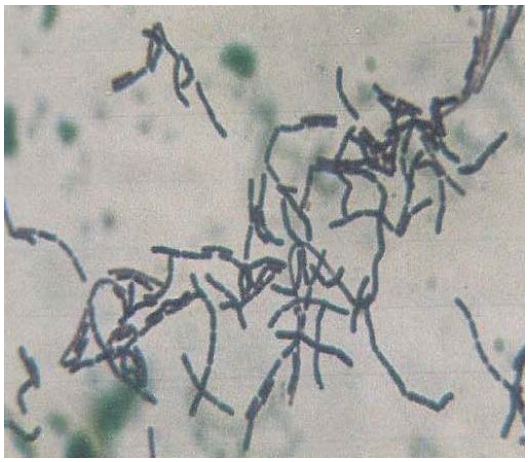


Plate 10: Gram's staining of *Bacillus* spp showing Gram-positive single, paired and also long chain organism.

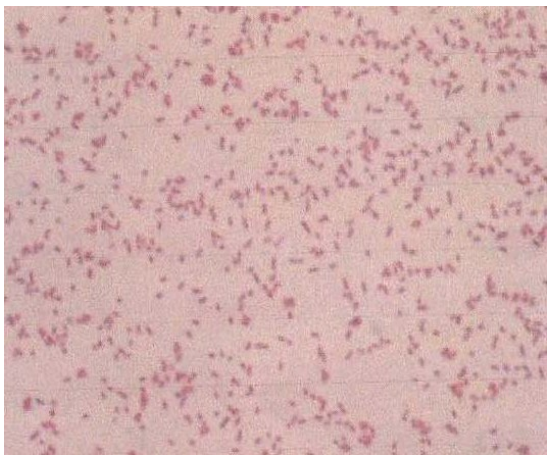


Plate 11: Gram's staining of *E. coli* isolates showing Gram-negative, pink coloured, small rod-shaped, single or paired organism.

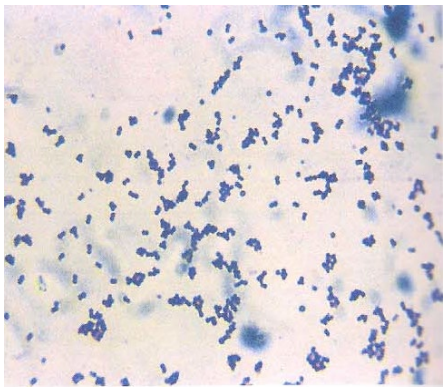


Plate 12: Gram's staining of *Staphylococcus* showing Gram positive cluster form of organism.

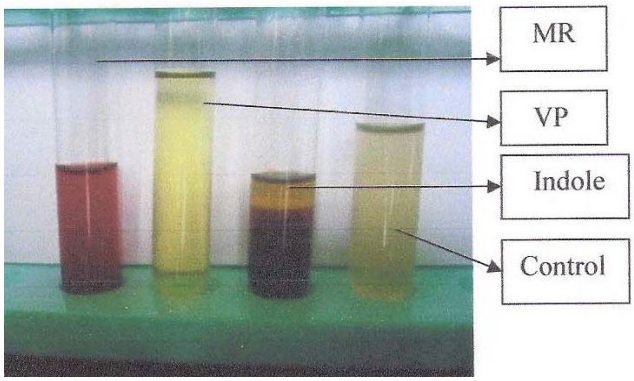


Plate 13: Biochemical properties of *Escherichia coli* (MR, VP, Indole).

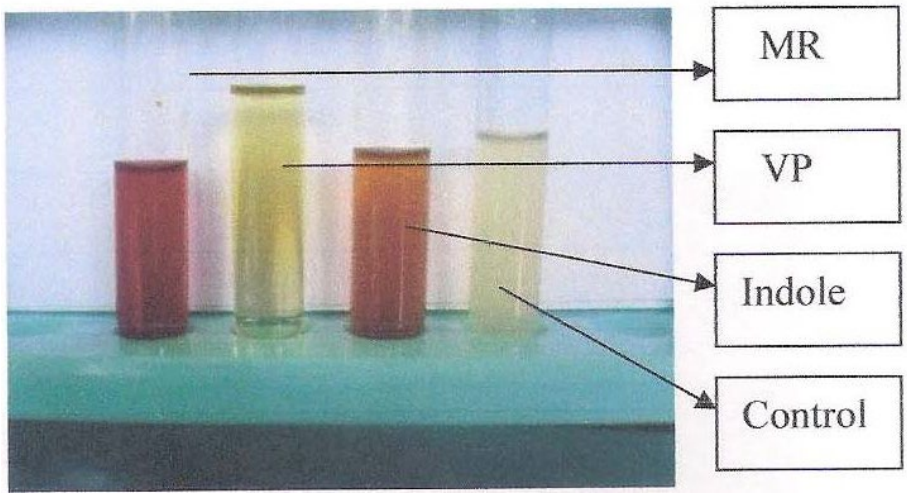


Plate 14: Biochemical properties of *Staphylococcus* spp and *Bacillus* spp (MR, VP and Indole)

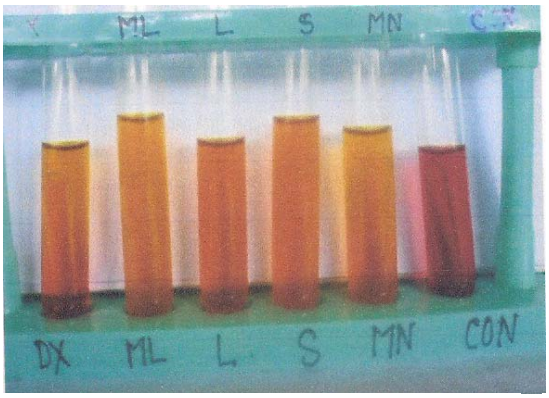


Plate 15: Fermentative activity of *Staphylococcus* with 5 basic sugars (DX=Dextrose, ML=Maltose, L=Lactose, S=Sucrose, M= Mannitol, C=Control) with production of gas. Change of medium color reddish to yellowish.

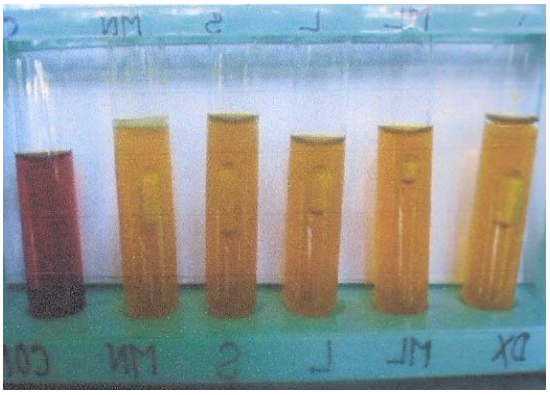


Plate 16: Fermentative activity of *Escherichia coli* with 5 basic sugars (DX=Dextrose, ML=Maltose, L=Lactose, S=Sucrose, M= Mannitol, C=Control) with production of gas. Change of medium color reddish to yellowish and gas bubble formation.

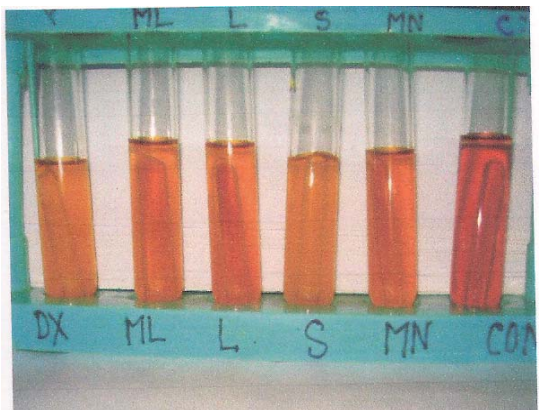


Plate 17: Fermentative activity of *Bacillus* spp. with 5 basic sugars (DX=Dextrose, ML=Maltose, L=Lactose, S=Sucrose, M= Mannitol, C=Control) with production of gas. Change of medium color reddish to yellowish.

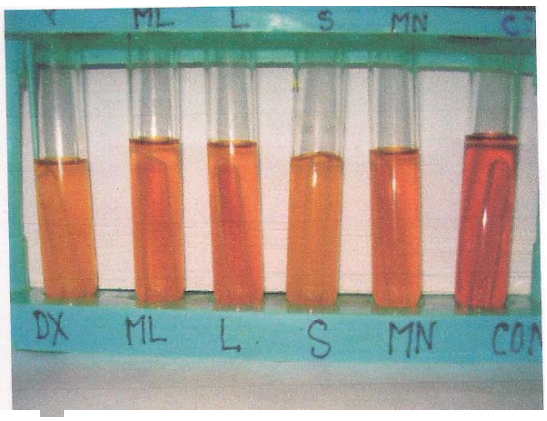


Plate 18: Carbohydrate test (5 basic sugar) of bacteria
DX = Dextrose
ML = Maltose
L = Lactose
S = Sucrose
MN = Mannitol

Plate 19: *E. coli*. (black colony) & *Staphylococcus* sp. (pink colony) In EMB agar

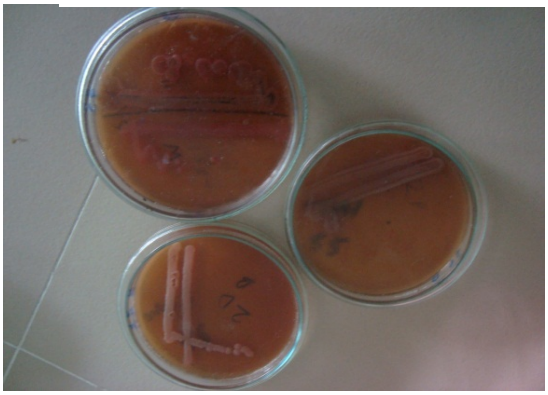


Plate 20: *Staphylococcus* in Mannitol salt agar

Plate 21: *Streptococcus* sp. In nutrient agar

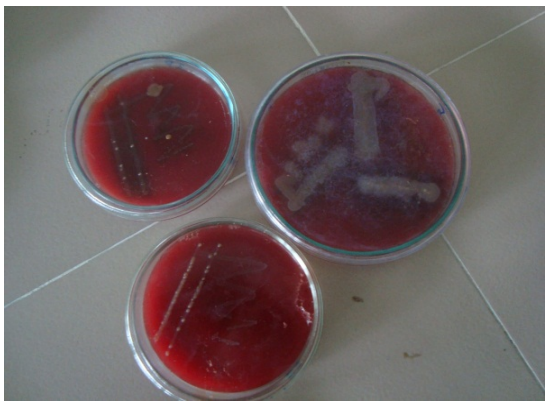


Plate 22: *Staphyloccus* sp. In Blood agar (Haemolytic in right)

Plate 23: *Staphyloccus* sp. in Blood agar (Haemolytic in left)

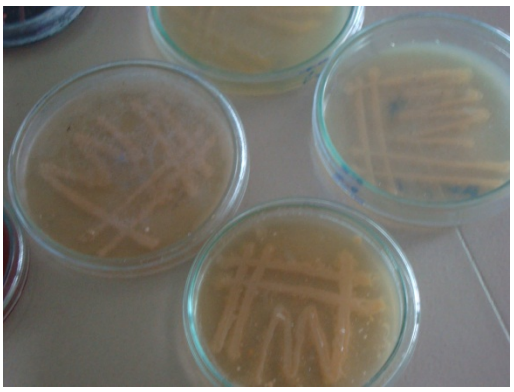
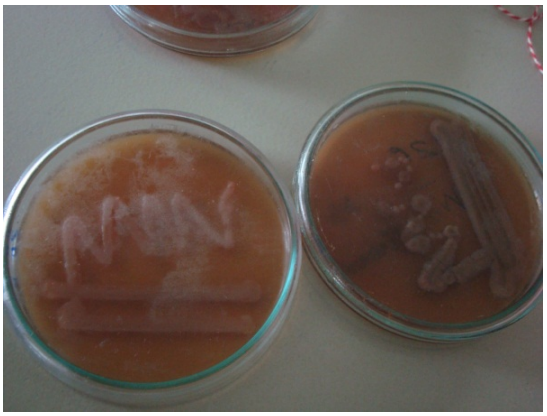


Plate 24: *Streptoccus* sp. in Nutrient agar



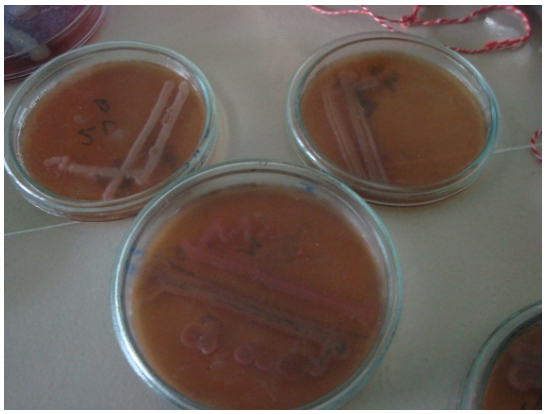


Plate 25: *Staphyloccus* sp. in Mannitol salt agar

Plate 26: *Streptoccus* sp. in nutrient agar

Plate 27: *E. coli* (black colony) & *Staphylococcus* sp. (pink colony) In EMB agar

Plate 28: *E. coli* in nutrient agar

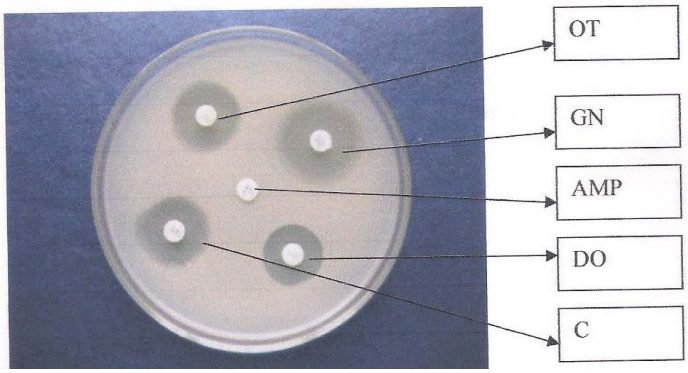


Plate 29:Antibiotic sensitivity test for isolated *Bacillus* spp. on nutrient agar (OT=Oxytetracycline, GN=Gentamycin, AX= Amoxycillin, DO=Doxycycline, C=Cholramphenicol)

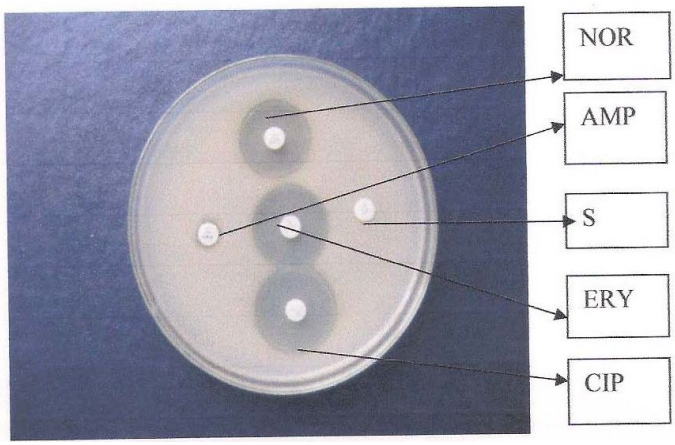


Plate 30: .Antibiotic sensitivity test for isolated *Bacillus* spp. on nutrient agar (ERY=Erythromycin, AMP= Ampicillin, S=Streptomycin, ENR= Enrofloxacin and CIP=Ciprofloxacin.

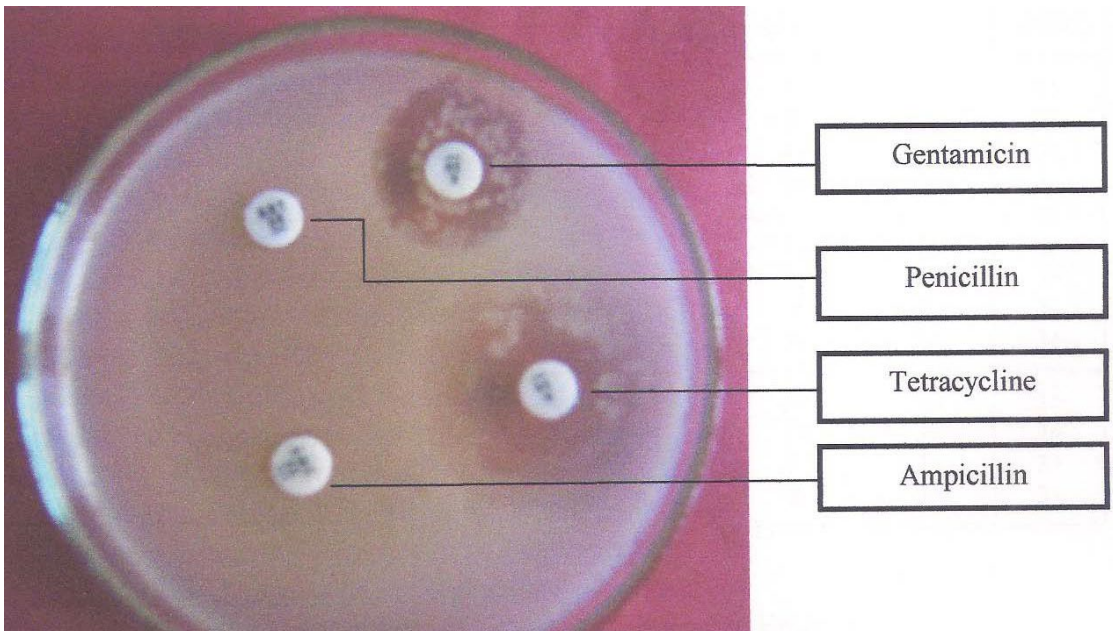


Plate 31: Antibiotic sensitivity test plate of *E. coli* showing sensitive to Tetracycline (3+), moderately sensitive to Gentamycin (2+) and resistant to Penicillin and Ampicillin

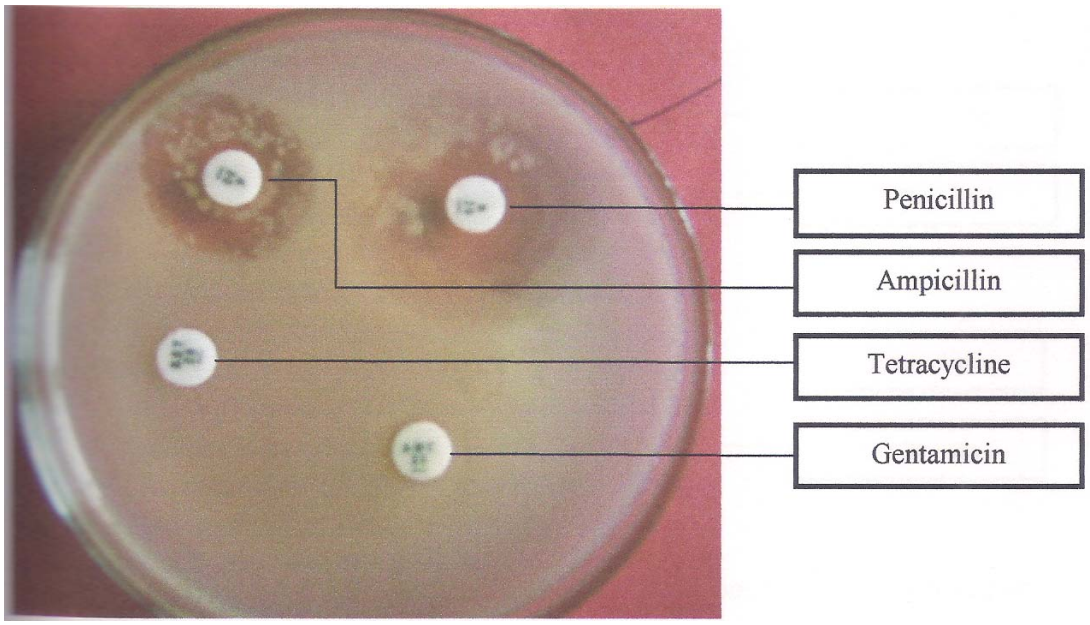


Plate 32: Antibiotic sensitivity test plate of Gram positive *Bacillus* spp. showing highly sensitive to Penicillin (3+), ampicillin (3+) but resistant to Tetracycline and Gentamycin.

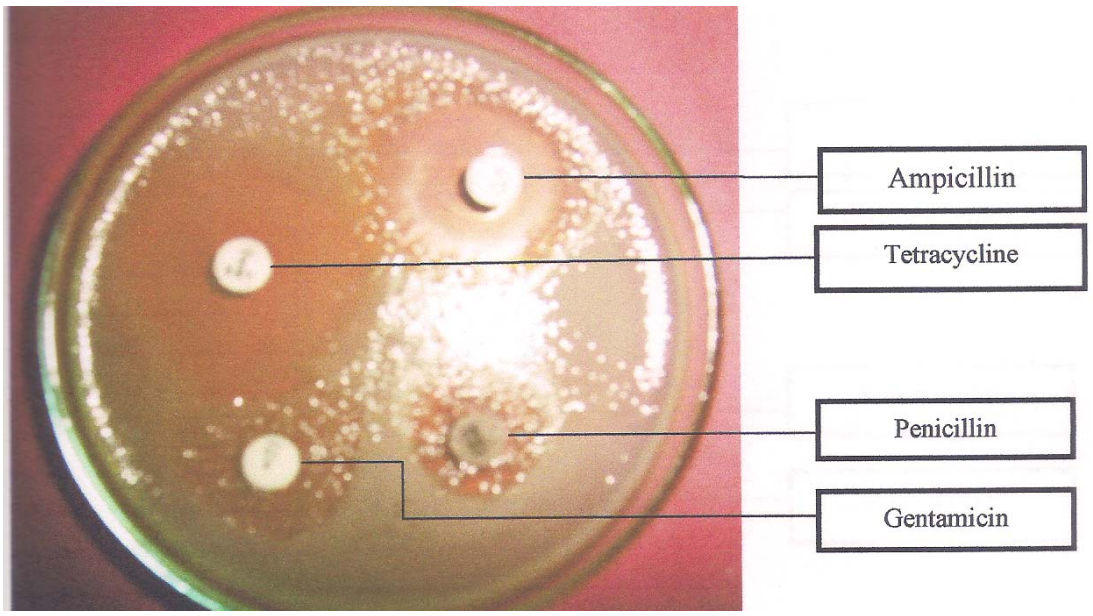


Plate 33: Antibiotic sensitivity test plate of *E. coli* showing highly sensitive to Tetracycline (3+), Moderately sensitive to Ampicillin (2+) and Gentamycin (2+) and sensitive to Penicillin (1+).

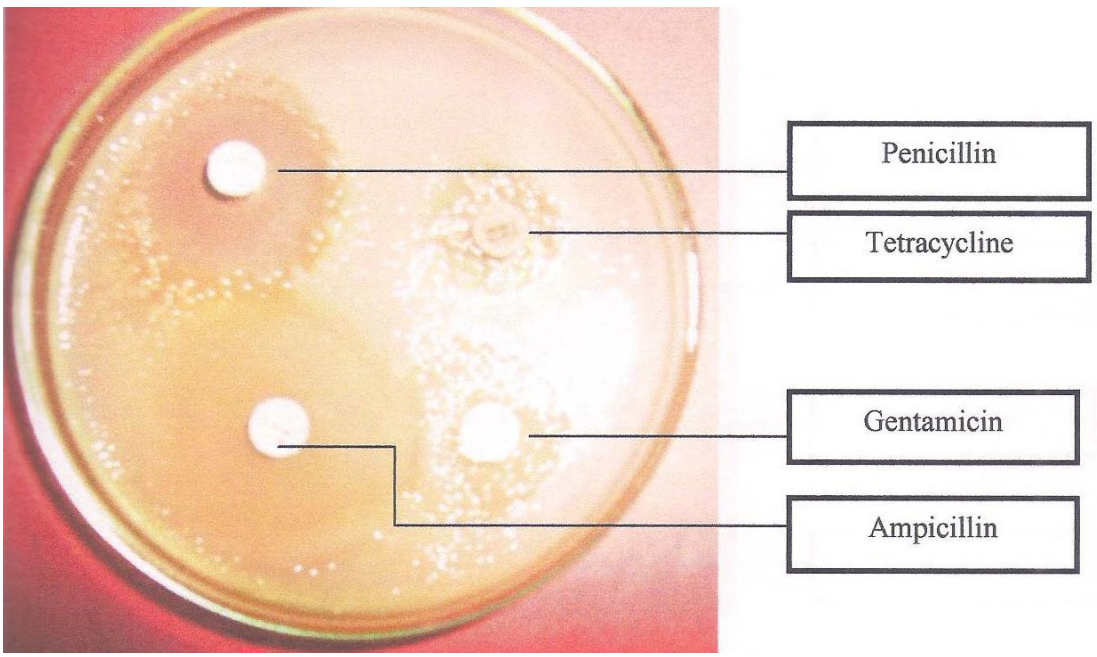


Plate 34: Antibiotic sensitivity test plate of *Staphylococcus* spp. showing highly sensitive to Penicllin (3+) and Ampicillin (3+), Moderately sensitive to Gentamycin (2+) and Tetracycline(2+).

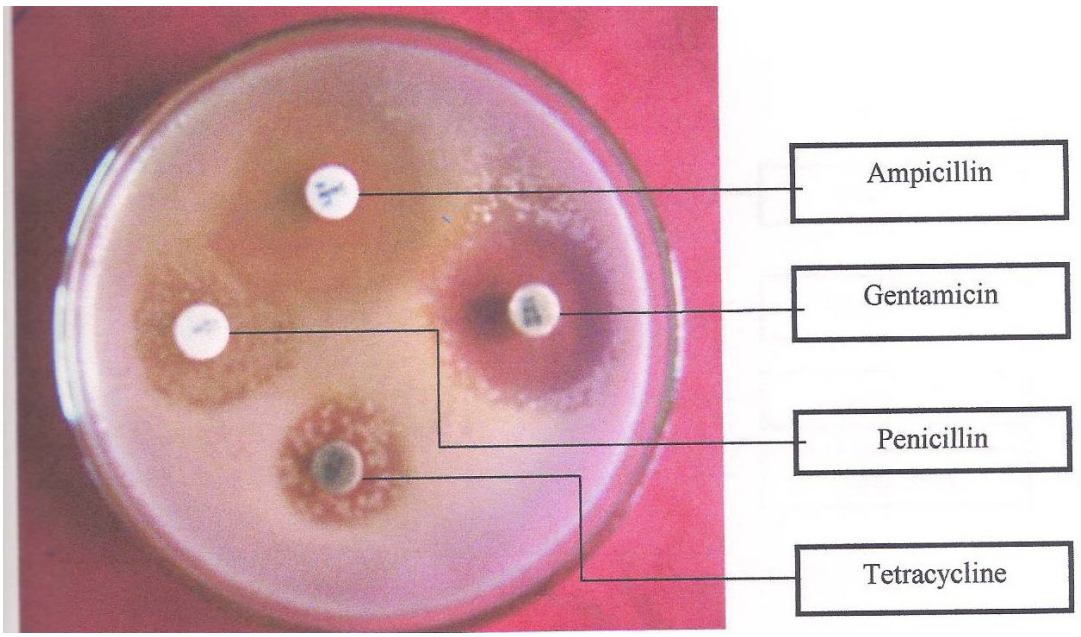


Plate 35:Antibiotic sensitivity test plate of *Staphylococcus* spp. showing highly sensitive to Ampicillin (3+) and Gentamycin (3+), Moderately sensitive to Penicllin (2+) and sensitive to Tetracycline(1+).

Antibiotics including Azithromycin, Ciprofloxacin, Cephalexin, Kanamycin, Gentamycin, Streptomycin, Oxytetracyline and Penicillin were used to sensitivity test. Culture sensitivity test were done both in aerobic and anaerobic condition for samples collected from reproductive diseased and diseases free cows. Table 4.20 showed CT Test in aerobic condition. In aerobic condition Azithromycin was highly sensitive in diseased and non diseased cases. Ciprofloxacin, Kenamycin and Gentamycin also sensitive both diseased and non diseased groups of samples. Cephalexin, Streptomycin and Oxytetracyline were moderately sensitive in disease condition. However, in non diseased condition Cephalexin and Oxytetracycline were highly sensitive but Streptomycin showed moderate sensitivity.

Table 4.20 represented the culture sensitivity test for anaerobic bacteria in diseased and diseased free samples. The highest sensitivity was found Azithromycin in anaerobic condition. Ciprofloxacin, Kanamycin, Cephalexin and Gentamycin were also highly sensitive to organisms. Streptomycin was moderately sensitive both in diseased and non diseased groups of samples of anaerobic condition. Penicillin and Oxytetracyline were resistance in anaerobic condition.

Table 4.20. Culture sensitivity Test for aerobic bacteria

Name of Antibiotics	Group-I (Diseased)			Group-II (Non diseased)		
	Sensitive	Moderate	Resistance	Sensitive	Moderate	Resistance
Azithromycin	31	0	0	50	0	0
Penicillin	2	4	25	18	15	17
Cephalexin	6	24	1	30	14	6
Ciprofloxacin	29	1	1	49	0	1
Gentamycin	15	14	2	30	16	4
Kanamycin	20	6	5	35	12	3
Streptomycin	10	18	3	18	20	12
Oxytetracycline	14	12	5	18	24	8

Table 4.21. Culture sensitivity Test for anaerobic bacteria

Name of Antibiotics	Group-I (Diseased)			Group-II (Non diseased)		
	Sensitive	Moderate	Resistance	Sensitive	Moderate	Resistance
Azithromycin	30	0	1	50	0	0
Penicillin	3	3	24	16	16	18
Cephalexin	19	8	4	32	16	2
Ciprofloxacin	28	1	2	45	1	4
Gentamycin	18	12	1	27	16	7
Kanamycin	28	1	3	36	10	4
Streptomycin	13	16	2	18	25	7
Oxytetracycline	13	10	8	12	18	20

In average, Azithromycin was highly sensitive (99.38%), Penicillin (51.85%) and Oxytetracyclien (25.30) were highly resistance. Streptomycin was moderately sensitive (48.76%). Ciprofloxacin (93.20%), Kanamycin (73.45%), Gentamycin (55.55%) and Cephalexin (53.70%) were also sensitive (Table 4.20). Gain *et al*. (2008) found that *Bacillus* spp and *Esherichia coli* are more or less effective against 6 antibiotics agents (Oxytetracyline, Amoxicillin, Streptomycin, Gentamycin, Ciprofloxacin and Spiromycin). On the other hand, Amoxicillin, Oxytetracycline and Ciprofloxacin are moderate to highly sensitive to the all of isolates. For gentamycin the possible reason of better results in the half life (t ½) and bioavailability have been reported

to be higher in and were 45 hours and 92% (Haddad *et al*., 1987), Gentamycin when administered intrauterine attained maximum plasma concentration within 30 minutes (Al-Guedawy *et al*., 1983).

Table 4.22. Antibiotic sensitivity test

Name of Antibiotics	Average percentage		
	Av. Sensitivity %	Av. Moderate %	AV. Resistance %
Azithromycin	99.38	00	0.61
Penicillin	24.07	23.45	51.85
Cephalexin	53.70	38.27	8.02
Ciprofloxacin	93.20	1.85	4.93
Gentamycin	55.55	35.80	8.64
Kanamycin	73.45	17.90	9.25
Streptomycin	36.41	48.76	14.81
Oxytetracycline	35.18	39.50	25.30

Treatment of Repeat breeding of cows: Repeat breeding of cows divided into seven groups and each group of cows were given treatment with separate drugs. Effect of drugs on repeat breeding of cows is presented in Table 4.23. Repeat breeding cows were recorded such as conception rate and percentage of recovery after 3 months of treatment. Among these, 88% recovery was obtained with antiseptic solution and penicillin and streptomycin injection. The mean duration of recovery from repeat breeding of cows with intrauterine wash with Potassium permanganate solution at rate of 0.01% with help of Dush can + Inj.

Penicillin and Streptomycin parentally injection treatment was shorter (20.5±3.5 days) and higher (43.0±0 days) with control group. Intrauterine wash with Potassium permanganate solution at rate of 0.01% with help of Dush can + Inj. Penicillin and Streptomycin parentally were the most effective in the treatment of Repeat breeding of cows.

Table 4.23. Pregnancy rate of cows with repeat breeding treated using different treatment protocols.

Treatment protocols	**No. of animals**	**No. of cows pregnant**	**Pregnancy rate (%)**
Treatment-I	25	11	44 [b]
Treatment-II	25	13	52[a]
Treatment-III	25	6	24 [c]
Treatment-IV	25	10	40 [b]
Treatment-V	25	7	30 [c]
Control	25	2	8 [d]

The means within the same column with at least one common letter, do not have significant difference (P>0.01).

4.3 Results of Therapeutic Trials

The results of logistic regression analysis of equation (1) have been given in Table 4.24. From table shows that the whole logistic regression model of the test was significant at 4 degrees of freedom at 5% level. That means researcher may reject the null hypothesis and finally commented that there was significance effect (P<0.05) of treatment on repeat breeding of dairy cows. In the logistic regression analysis five therapeutic trials of dairy cows at Rajshahi in Bangladesh has been taken as treatment

groups. The five therapeutic trials were GnRH + AI, Lugol's Solution + Oxyteracycline, Acriflavin + Metronedazole + Gentamycin Sulphate, Two times AI + Vitamin ADE and Control group respectively. From figure 17 (Error bar) shown average conception rate of repeat breeder after administration of 4 types therapeutic drug and a control group to prevent the repeat breeding of experimental dairy cows. To the analysis Control was assumed to be the reference category. All the logistic regression coefficients were found to be positive and statistically insignificant except GnRH ($P<0.05$) and Oxytetracycline ($P<0.10$). The odds ratios of GnRH + AI, Lugol's Solution + Oxyteracycline, Acriflavin + Metronedazole + Gentamycin Sulphate, Two times AI + Vitamin ADE Oxytocin 2.154, 3.051($P<0.10$), 4.846 ($P<0.05$) and 2.154, respectively which means recovery rate from repeat breeding of dairy cows and better than the Control group.

Table 4.24: Effect of treatment protocol on the conception rate of repeat breeder.

Group	Therapeutic Group	No. of cows Inseminated	No. of Cows conception	Conception Rate
I	GnRH + AI	20	11	55%
II	Lugol's Solution+ Oxytetracycline	20	13	65%
III	Acriflavin + Metronedazole + Gentamycin Sulphate	20	14	70%
IV	Two times AI+Vitamin-ADE	20	10	50%
V	Control	20	7	35%

The percentage of repeat breeding is indicated in parenthesis.

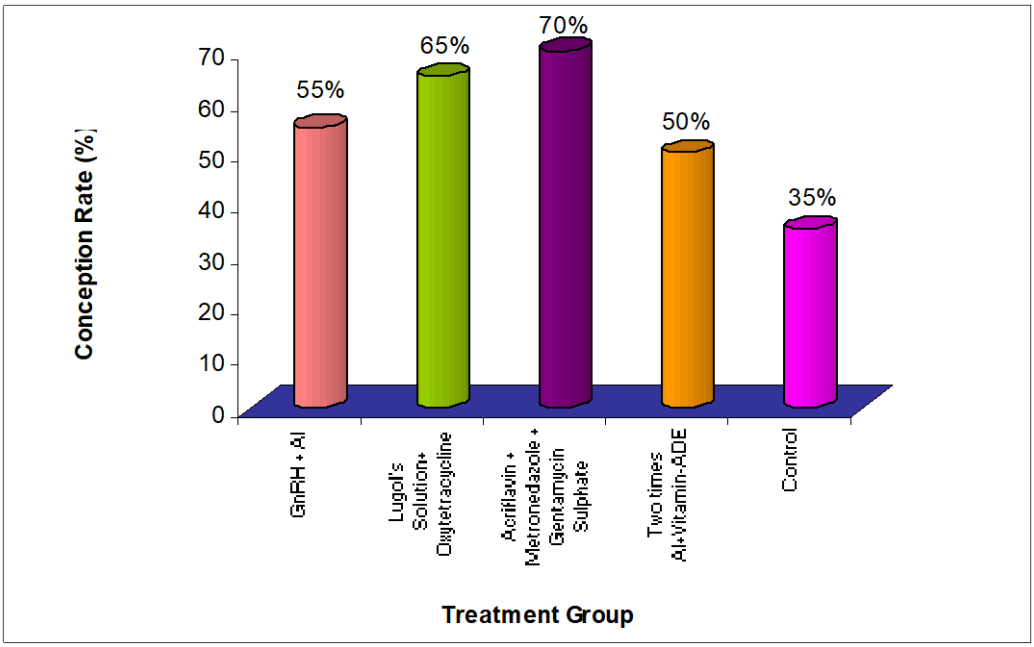


Figure 4.16: Cylinder diagram represent the percentages of conception of repeat breeder relation with treatment protocol.

Table 4.25: Logistic Regression Estimates of the Odds Ratios of Treatment Group (therapeutic trials) and Index of Repeat Breeding of dairy cows at Rajshahi in Bangladesh.

Independent Variable	**B**	**S.E**	**Exp(B)**
Treatment Group			
GnRH+AI	0.767	0.730	2.154
Lugol's solution + Oxytetracycline	1.116	0.782	3.051**
Acriflavin + Metronedazole + Gentamicin Sulphate	1.578	0.881	4.846*
Two times AI + Vit-ADE	0.767	0.730	2.154
Control$^{(r)}$	-	-	1.000
Constant	0.619	0.488	1.857
Chi-square (calculated)	11.647*		
-2 Log likelihood	89.361		
df	4		
Cox & Snell R Square	0.234		
Chi-square (tabulated)	**9.488**		

r = Reference Category; B = Logistic Regression Coefficient; and Exp (B) = Odds Ratio
'*' = Significant at 5% level, '**' = Significant at 10% level

CHAPTER V
DISCUSSION

Most of the influencing factors viz., breed, age, parity, body weight, body condition score, farm type, farm size, housing system, floor type, feed quality, breeding method, preventive measure, treatment by on investigation of repeat breeding of 1207 dairy cows at 9 upazilas and 4 metro thanas of Rajshahi from August 2011 to January 2013 have been studied and discussed below. The over all incidence of repeat breeding was 20.4%.

Repeat breeder cows are a heterogeneous group of sub-fertile cows with no anatomical abnormalities or infections that exhibit a variety of reproductive disturbances in a consistent pattern over three or more consecutive heat cycles of normal duration (17-25days). One of the major constraints of profitable dairy farming is low conception rate (Alam and Ghosh, 1994; Shamsuddin *et al.*, 2001). Early embryonic death (<42 days) is a major factor in reproduction failure, which in turn causes economic loss to the dairy industries (Rahman *et al.,* 1996). Shamsuddin (1995) reported 5% repeat breeding cases in Bangladesh.

Kumar *et al.,* (1986) performed a survey on reproductive disorders in non descript cattle. In this survey of 810 indigenous cattle 52% were normal and 48% had reproductive disorders. Anoestrus was found in 19%, ovarian hypoplasia in 1.7%, atrophy/hypoplasia of uterus in 7% underdeveloped/ infantile genitalia 9.5%, cervicitis in 5% and endometritis in 1.2% of the cases. The frequency of various other disorders was each less than 1%.

Shamsuddin *et al.,* (1988) to detect various reproductive disorders in crossbred cows. The study was undertaken to find out the relative incidence of reproductive disorders after birth in cows of savar Dairy Farm. The relative percentage of retained placenta, metritis, pyometra, endometritis, cervicitis, persistent corpora lutea, cystic ovaries and non functional ovaries were 42.26%, 10.38%, 8.15%, 27.39%, 1.52%, 1.17%, 3.13% and 5.98% respectively. They concluded that the persistent corpora lutea and cystic were in right ovaries, whereas higher number of non functional ovaries were found in both the ovaries.

Rao *et al.,* (1993) comparative studied the incidence of reproductive disorders among 1860 crossbred Hersy and Holstein cows and heifers that were classified as half bred, three quarter bred or higher (seven- eight). The overall incidence of anatomical functional and infectious (non specific) from of infertility were 3.0, 56.4 and 40.6 respectively. The highest incidence of specific conditions was for cystic ovaries 49.7% and endometritis 32.8%.

Chawdhury *et al.* (2000) to assess the reproductive status, pregnancy wastage and incidence of gross genital abnormalities in cows slaughtered at Maiduguri abattoir, 7375 female genitalia were examined over a period of 36 months from July 1997 to June 2000. A total of 55.49% organs were cyclic while 44.51% were non-cyclic. The cyclic organs included 12.64% at pro-estrus, 12.0% at estrus, 13.03% at met estrus and 17.82% at dioestrus stage of the estrous cycle. Out of 3283 non-cyclic organs, 1676 were gravid from which 1676 fetuses were recovered. The fetal crown-rump (CR) lengths ranged from 6 to 85 cm with corresponding age range of 60 to 265 days. Juvenile organs with smooth ovaries were 3.78%. The gross abnormalities of the genitalia recorded included cystic

ovaries (3.35%), ovaro-bursal adhesion (2.9%), ovarian hypoplasia (2.2%) and endometrocervicitis (1.7%). Oviductal occlusion accounted for 0.75%, hydrosalpinx 0.54%, pyometra 0.48%, par ovarian cyst 0.26%, hypo plastic uterus 0.24% and uterine cyst 0.08% cases.

Fathalla *et al.* (2000) conducted a survey in Northern Jordan to determine the incidence of gross reproductive tract abnormalities in cattle. A total of 200 specimens of bovine reproductive tracts were collected from cows slaughtered. at a local abattoir in Irbid, Jordan between 1993 -1994. The results of the investigation showed that a large number of slaughtered cows (n=27; 13.5%) were pregnant. A total of 27 (13.5%) specimens had lesions. The predominant lesion of the ovaries was ovarian inactivity (21 cases; 10.5%), ovaro-bursal adhesions (16 cases; 8%) and cysts (14 cases; 7%). Other, interesting rare pathological lesions of the ovaries were bilateral ovarian haematoma and tuberculosis. Twenty specimens (10%) had uterine lesions, the most common of which were infections, presenting as metritis and pyometra. Seven specimens (3.5%) had oviduct lesions, which included hydrosalpinx, pyosalpinx and haemosalpinx.

Douthwaite (2000) studied the accuracy of different common methods of differentiating between follicular and luteal ovarian cysts, and to monitor the response of the cysts to 12 days treatment with a progesterone-releasing intravaginal device (PRID). On the basis of agreement between the different methods 25 of the 46 cases examined were diagnosed as follicular and 14 as luteal cysts; for the other seven cases the methods disagreed. The use of ultrasound was more accurate in diagnosing follicular cysts than luteal cysts, and combined with plasma progesterone concentrations gave the most accurate assessment of cyst type (92 percent for follicular cysts and 82 percent for luteal cysts). The mean (se) plasma

progesterone concentration was lower in the cows with follicular cysts than in those with luteal cysts (0·29 [0·05] v 3·90 [0·63] mg/ml; P<0-05). Luteal cysts had thicker walls (5·3 [0·04] v 2·5 [0·2] mm; P<0-0001), and the wall thickness of all the cysts was positively correlated with plasma progesterone concentration (r=0·52, P<0·0004). Cows with luteal cysts had more additional follicles greater than 5 mm in diameter (P<0·01). In cows with follicular cysts and other follicles greater than 5 mm in diameter, the mean oestradiol concentration was 7·9 (1·8) mg/ml compared with 24·2 (3·1) mg/ml (P= 0.002) in cows without other follicles greater than 5 mm in diameter on either ovary. At the time of PRID removal, plasma progesterone concentration had increased in the cows with follicular cysts to 1·59 (0·06) mg/ml (P<0·05) and decreased in the cows with luteal cysts to 0·87 (0·01) mg/ml (P<0·05), although there was no change in original cyst structure in 45 percent of the cases. However, new ovarian structures were frequently observed during the treatment. The overall pregnancy rate for cows with both types of cyst after treatment was 50 percent after three inseminations, but the first service pregnancy rate was only 18 percent for cows with follicular cysts and 28 percent for cows with luteal cysts. After treatment, the fertility of cows with follicular cysts was similar to that of paired herd mates, whereas cows with luteal cysts took 40 days longer to calve again than healthy herd mates. However, the culling rate was higher for cows with follicular cysts (41 v 11 percent).

Farooq (2000) recorded 3760 crossbred cows at various locations in Northwest Frontier Province (NWFP), Pakistan, revealed 379 (10.08%) cases of reproductive abnormalities. Cervicitis had the highest incidence at 45.12%, followed by abnormalities of uterus (38.26%), vagina

(10.29%) and ovary (6.33%). The frequencies of endometritis and vaginitis were 28.69 and 4.76%, respectively. Ovarian cysts were found in 6.33% of the cases. The incidence of pyometra, vaginal tumors, pyometritis and metritis were 6.53, 1.83, 5.56 and 1.58%, respectively. Genetic group, season and locality had significant effect ($P<0.01$) on incidence of various reproductive abnormalities. Cows possessing 75% Holstein Friesian (HF) inheritance were the most susceptible to reproductive disorders. Summer was the peak (35.88%) season and spring the rough (15.57%) season of incidence. The highest incidence of reproductive disorders was found in Peshawar (34.31%), followed by cows in Bannu (26.13%), Mardan (13.18%), Risalpur (8.97%), Nowshera (6.58%), Kohat (6.33%) and Dera Ismail Khan (4.23%). It was concluded that reproductive disorders were more prevalent under stressful environment, within or around thickly populated areas, during hot summer months and among crossbred.

Kotowski (2001) determined the health condition of mammary glands and reproductive organs of cows selected for slaughter. The studies were carried out in Poland in 1999 on 84 black and white cows 3 to 16. It was found that most of the cows subjected to slaughter (31 %) were in the 6-8 age bracket and it was at this age that most of the inactive udder quarters were noticed. Mammary gland irritation was diagnosed in 41 cows, i.e. 48.80%. In the post-slaughter examination fetuses in the uterus were discovered in 6 cows (7.16%) whereas various pathological changes in the reproductive organs were recorded in 49 cows, i.e. 58.34%. The pathological changes more often affected the uterus (59.18%) than the ovaries (40.82%). The bacteriological examination of uterine swabs revealed the presence of potentially pathogenic bacterial flora in all cases.

The predominant microorganisms were of the following types: *E. coli* 33.33%, *Staphylococcus epidermidis* 12.5%, *Pseudomonas aeruginosa* 12.5%, *and Enterococcus sp.* 20.83%. Practical conclusions include: advisable physical check-up of cows prior to slaughter and that chronic illness of the reproductive organs most often affects cows at their peak of performance.

Kubar (2002) observed the reproductive organs of 20 Estonian Holstein Breed (EHF) cows and 3 heifers and culled because of infertility that were examined by palpation perrectumand ultrasonography. In addition, pathoanatomical and histopathological studies were carried out after slaughter. The histopathological study revealed that small cysts less than 2.5 cm in diameter, often (12 animals) existed in culled cows. These cysts were frequently accompanied by changes in secondary and Graafian follicles, rete ovarii, ovarian stroma, and the endometrium. Three cows had follicular cysts in the ovaries, which were 25-35 mm in diameter. Two cows revealed luteal cysts in the ovaries and one of them had vaginal prolapse. Four animals (one heifer and three cows) manifested tumors or tumor-like malformations: ovarian endosalpingiosis, germ and stromal cell tumor, oviductal myolipoma, and haemangiosarcoma in the uterine blood vessels. One heifer had been culled because of 2 abscesses in the -vaginal wall close to the cervix and another had chronic endometritis. The research findings indicated that the most common cause of infertility in the culled cows was cystic degeneration in ovaries (85%), accompanied by pathological changes elsewhere in the reproductive organs.

Asseyl *et al.* (2004) examined the reproductive organs from mature Small East African Zebu (SEAZ) heifers and cows slaughtered at the Morogoro

abattoir were collected twice a month and evaluated over a period of 12 months. Out of the 402 animals from which reproductive organs were taken, 54% were pregnant, 24% were actively cycling and 22% were non-cycling. Various gross abnormalities were observed in the reproductive organs of about16% of the cattle, and the major reproductive abnormality in both total and the noncycling animals was various degrees of fibrous adhesion between the ovary and infundibulum and mesosalpinx. It is concluded that, contrary to common belief, a majority of the female SEAZ cattle that are slaughtered are fertile.

Yener (2004) examined the 'surface epithelial' tumors originating from the modified colonic mesothelial cell covering of the ovary, constitute a group of tumors in dogs and humans but are uncommon in mares, cats and cows. Tumours of this group are subdivided into four categories according to their main morphological features: papillary adenoma, papillary adenocarcinoma, cyst adenoma and cystadenocarcinoma. Cyst adenomas, which appear to arise from the epoophoron and/or rete ovarii, are comparatively rare in domestic animals. This article discusses the occurrence, pathology, histopathology and diagnosis of serous papillary cyst adenoma in the ovary of a cow from Turkey

McDougall *et al.* (2005) examined thirty-six cows that had one or more gross lesions which involved the ovary, uterine tube, uterus or vagina. Bacteria were isolated from the uteri of 22 (21%) cows. Isolates included *Archanobacterium pyogenes* (n=1), *Escherichia coli* (n=1), *Fusobacterium* spp. (n=1), *Haemophilus somnus* (n=5), *Streptococcus acidominimus* (n=12), *S. bovis* (n=2), *S. uberis* (n=1) and *S.salivarious* (n=1). In only five cows were both gross pathology and bacteria detected. There was no relationship between the isolation of bacteria and the

diagnosis of gross pathology of the uterus. There were no differences in the of histopathological changes in the uteri from the three groups of cows examined, and lesions present were minor. Gross pathological changes and intrauterine bacteria were found in 34% and 20% of cows, respectively, but the correlation between the two was poor. Histopathological changes were unremarkable, suggesting the bacteriological findings were coincidental, that causative agents of infertility were not present at the time of examination, or that unrelated causes such as nutritional anoestrus may have been responsible failure of some cows to conceive.

Abalti *et al.* (2006) studied the type and prevalence of abnormalities occurring in the female reproductive tracts of 201 Zebu cattle of Fogera type (161 cows and 40 heifers) slaughtered at Bahir-Dar town, north-west Ethiopia. Out of the 201 female genital tracts collected and examined, abnormalities were recorded in 74 (36.8%). The most common abnormalities encountered were ovaro-bursal adhesion (5.5%), endometritis (3.9%) and cystic ovaries (3.5%). Other abnormalities recorded were ovarian hypoplasia, vaginitis, cervicitis, tortuous cervical canal, mucometra, vaginal cyst, par ovarian cyst, hypo plastic cervical rings, cervical cyst, freemartins, closed external cervical os, uterine and oviducts adhesion, cystic uterine tube, remnant of retained fetal membrane and cyst in the uterine wall. The prevalence of the abnormalities was significantly ($p< 0.05$) higher in parous than in nulliparous cows. Moreover, evidence of ovarian cyclicity was found in 51.6% and 30% of non-pregnant parous and nulliparous cows examined, respectively.

Ali *et al.*, (2006) examined the reproductive tracts of 110 non descriptive

cows, collected from Faisalabad abattoir, were studied for biometrical values and pathological changes during disease condition. The average length of right ovary was 2.40 ± 0.06 cm and that of left ovary was 2.31 ± 0.05 cm. The average width of right ovary was 1.15 ± 0.02 cm and that of left ovary 1, 14 ± 0.03 cm. The average thickness of right ovary was 1.61 ± 0.04 cm and that of left ovary was 1.52 ± 0.03 cm. The average weight of right and left ovaries was 4.29 ± 0.29 and 3.97 ± 0.24 g, respectively. The average size of right and left horns was 0.69 ± 0.59 and 19.76 ± 0.58 cm, respectively. The average length of circumference and cervical rings were 6.0 ± 0.22, 8.40 ±0.21 and 4.62 ± 0.09 cm. The incidences of pathological conditions observed were ovaro-bursal adhesions, cystic ovary, cystic corpus luteum, par ovarian cysts, teratomas, pyometra, metritis, mummified fetus, mucometra, cervicitis, fibrosity of cervix, tortuosity of cervix and double cervices. No abnormalities of oviducts were found.

Patel *et al.* (2007) recorded a total of 4188 animals, composed of 2570 buffaloes and 1618 cows, the reproductive disorders were categorized as anatomical, functional pathological origin. The anatomical abnormalities observed in buffaloes and cattle were infantile genitalia (9.38 and 5.99%), kinked cervix (0.59 and 3.39%) and uterine adhesion (0.93 and 1.36%, respectively). The percentages of functional form of infertility were anoestrus (27.32 and 24.73%), sub-estrus (28.99 and 21.38%), cystic ovarian degeneration (1.48 and 6.62%) and repeat breeding (8.68 and 18.79%, respectively). The pathological causes included salpingitis (0.43 and 0.18%), endometritis (5.80 and 2.90%), pyometra (0.78 and 1.86%), metritis (10.38 and 8.90%) and mummification (0.11 and 0.12%) in buffaloes and cattle, respectively. Ovarian tumor was observed only in

buffaloes (0.11%).

Hormone therapies have been widely used in the treatment of retained placenta (Roberts, 1986). Several prophylactic treatments have been employed in the prevention of retained placenta in the present study. The third group $PGF_2\alpha$ (Dinoprost) showed the best results and 89% efficacy for the expulsion of RFM among groups and 4.85 times more likely than control group. The effect of $PGF_2\alpha$ can be explained on the basis of the fact that $PGF_2\alpha$ increases uterine contractility (Watson, 1984) with dilatation of the cervix, both of which enhance placental expulsion. Similar finding have been made by Majeed *et al.* (1991) and Al-Haidari (1991), it could be concluded that using of $PGF_2\alpha$ is more effective for treatment of RFM than other treatment regimen. The second group methyl ergometrine maleate (Metherspan) showed second highest & 84% response among groups and 3.051 times more likely than control group. Several workers agreed this effect like ergonomic or a similar drug developed from ergot may be more beneficial than oxytocin in cases of atonic myometrium because of its prolonged oxytocic effect (Roberts, 1986 and Roberts, 1986). The first group oxytocin (Oxin) and fourth group patent herbal drug (Utoklin) obtained similar result and an efficacy of 79%. It has been suggested that oxytocin plays a role in dropping of placenta; through a stimulation effect on phagocytosis by uterine leucocyte. Similar observations have been reported that exogenous oxytocin was used to prevent the occurrence of retained placenta immediately postpartum (Roberts, 1986; Azad *et al*., 1988 and Miller *et al*., 1984). Uteoklin and Oxitocon was 2.154 times more like than control group. Beside this, the animal must also be compared as to only cows

which calved normally at term with a healthy calf' same as groups; same type of management and free from well known infection.

The prevalence of uterus infection was 16.63% (Gazaw *et al*., 2007) fairly agrees with that of 18.7% reported by Oumermohammed (2003) and 19.6% by Gebremariam (1996) in smallholder dairy cows. Erb and Martin (1980), Kassahun (2003) and Mamo (2004) who reported an incidence rates ranging from 11.5-13.6%.

Different types of reproductive diseases or disorders were recorded during collection of samples and those were pyometra, endometritis, cervisitis, abortion, vaginitis and bloody discharge. The first four cases were considered as uterine infection due to presence of pus. The prevalence of uterine infection was 25 (80.64%) among different reproductive diseases. The overall uterine infection was 25% (Table-15). Durrani (2009) reported that prevalence of uterine infection in cattle was 12.9%, retention of foetal membranes 30.6%, dystocia 11.3% and repeat breeding 1.65. Variation in the occurrence of uterine infection may be due to the fact it is influenced by factors such as age and parity as well as breed of the sire (Morrow and Noakes, 1986). The bloody discharge was not considered as uterine infection because of no any other sing was observed except blood in the sample. It could be due to genital injury during collection of sample. Incidence of endometritis was highest 11(35.48%) among different reproductive disorders. The incidence of pyometra was 8 (25.80%) followed by cervisitis 3(9.67%) and abortion 3 (9.67%). Oumermohammed (2003) observed the incidence of pyometra was 30.52% in cow which is more or less similar to this result 25.80%. Tadesse (1999) also found the incidence of pyometra in cow was 31.44%. On the other hand, pymetra cases reported by Tigre (2004) 20.71% and

Shiferaw (1999) 18.53% respectively. The overall prevalence of emdometritis during postpartum period was 14.4% in dairy cows in Japan (Gautam *et al*., 2009). IllHwa (2003) reported that 36.6% postpartum endometritis which is close related in the present study (35.48%). LeBlanc (2008) stated that clinical endometritis is one of the most common disorder in dairy cows, causing decrease fertility and large economic losses. One of the previous report showed that the prevalence of vaginitis was 5.79% (Mamo, 2004). Tiger (2004) also found that prevalence of vaginitis was 7.5% in cows, 7.8% and 9.65% vaginitis was also reported by Tadesse (1999) and Kassahun (2003), respectively. In this study the incidence of vaginitis was 6.46% which is same mentioned authors. But other author, Zewdu (1992) reported the incidence of vaginitis was 2.2-4.4% and Oumermohammed (2003) found 4.3%. Cervicitis is one of another reproductive disorders causing infertility. In this study 9.67% sample was collected from cows were suffered from cervicitis. Durrani (2009) stated the incidence of cervicitis was 14.1%. The prevalence of abortion 9.67% recorded in this study is near to 5.33% and 6.3% reported by Shiferaw (1999) and Kassahun (2003) respectively. However 3.19% by Oumermohammed (2003) and 3.2% abortion reported by Durrani (2009).

In non diseased cases the highest number of samples was positive for *Bacteroiedes* spp 8 (11.60%). *Diplococcus* spp 5 (7.24%), *Staphylococcus* spp 4 (5.84%) and *Streptococcus* spp, Escherischi coli, *Pseudomonas* spp, *Haemophillus* spp, *Pasteurella* spp and *Peptococci* spp were the same number 3(4.34%). The positive number of *Klebsiella* spp was also same for diseased sample like non diseased sample. Identification number of other bacteria were between the range of 0

(00%) 8 (11.60%) in non-diseased samples.

However, in average, the highest numbers of organisms were encountered from diseased samples and most of those bacteria are pathogenic Gani *et al.*, (2008) and Singh *et al*. (1999) stated that about 60% of the identified bacteria from uterus commonly found in repeat breeders as well as from diseased animals and the frequency of the isolates were much higher than that of normal cows. Uterine disease is commonly associated with *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Prevotella* species. Indeed, *A. pyogenes*, *F. necrophorum, Prevotell* and *Streptococcus* species have been shown act synergistically to enhance the likelihood of uterus disease Sheldon (2008). In another study Erin *et al*. (2005) showed that most common and economically important bacteria for uterine infection are *Actinomyces*, *Escherichia coli*, *Fuosbacterium, Pasteurella*, *Pseudomonas* and *Staphycoccus*. Williams *et al*. (2005) found muco-purulent discharge was associated with *F. necrophorum* and purulent discharge was associated with greater load of *A. pyogenes*, E. coli, and *Streptococci* (Sheldon *et al*., 2006). From the above, discussion it is easily understood that most of endometrial and pyogenic cases and or uterine infections were caused by *Fusobacterium* spp, *Actonomyces* spp, E coli, *Pseudomonas* spp and *Haemophilus* spp, *Staphylococci* spp, *Streptococci* spp, *Pasteurella* spp, *Enterobacter* spp, *Klebsiella* spp and *Proteus* spp were also observed higher in number in diseased sample than non diseased sample though the number of isolates were not significant.

According to the interviewed veterinary surgeons, penicillin, oxytetracycline, amoxicillin and streptomycin are the most commonly used intrauterine antibiotics on dairy cows for therapeutic purposes.

Unqualified prescribers frequently treated the cows with these antibiotics and did not maintain the proper doses and time which may enhance the resistance of antibiotics. Sahoo *et al*. (2010) stated that unqualified people are the important factor to causing resistance of antibiotic by prescribing antibiotics without maintaining doses. In the results Penicillin and Oxytetracyline were resistance to microorganisms both in aerobic and anaerobic conditions. The reasons behind the resistance could be due to frequently use of these antibiotics with unauthorized practioners without maintaining proper does. On the other hand Axithromycin, Ciprofloxacin, Kanamycin, Cephalexin and Gentamycin showed highly sensitive to organisms. According to field history it was observed that veterinarians in our country do not choose the mentioned antibiotics yet for treatment of uterine infection except Gentamycin which is used very rare. It indicates that for getting better result new generation of antibiotics maintaining proper doses and duration should be used.

CHAPTER VI
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

At the end of the study it might be concluded the followings-

- The overall incidence of repeat breeding of dairy cows was 20.4%.
- The lower incidence of repeat breeding observed in L×SL×SL breeds (3.0%).
- The cows which having 6 to < 8 years of age had more chance of repeat breeding among the others.
- Lower (< 250 kg) and higher body weight (> 350 kg) showed less prevalence of repeat breeding than 250 to < 350kg weight of group.
- Incidence of repeat breeding is more after 3rd calving.
- Good body condition of cows less prone to repeat breeding.
- The maximum incidence of repeat breeding was observed in private dairy farms than government dairy farm.
- Single cow dairy farm more chance of repeat breeding.
- Good housing system observed lowest incidence of repeat breeding.
- The cow supplied available concentrate, green grass and straw was less prone to repeat breeding
- The lowest rate of repeat breeding of dairy cows was identified in naturally serviced cow than Artificial Insemination.
- The influencing factors including breed, age, body weight, feed quality, breeding method, preventive measure and had significant

effect (P<0.05) on repeat breeding of dairy cows at Rajshahi in Bangladesh.

- A significant number of bacteria were identified from diseased samples and they were *Fusobacterium* spp 14 (45.16%) (p=0.000), *Actinomyces* spp 10 (32.25%) (p=0.003), Escherichia coli 12 (38.70%), *Pseudomonas* spp 11 (35.48%) (p=0.002) and *Haemophilus* spp 9(29.0%) (p=0.012). Identified *Staphylococcus* spp was 11 (35.48%) and *Streptococcus* spp 12 (38.78%). Identification of all other bacteria were between the range of 1 (3.0%) to 6 (18.20%). In non diseased cases the highest number of samples was positive for *Bacteroiedes* spp 8 (11.60%). *Diplococcus* spp 5 (7.24%), *Staphylococcus* spp 4 (5.84%) and *Streptococcus* spp, *Escherischi coli*, *Pseudomonas* spp, *Haemophillus* spp, *Pasteurella* spp and *Peptococci* spp were the same number 3(4.34%). The positive number of *Klebsiella* spp was also same for diseased sample like non diseased sample. Identification number of other bacteria were between the range of 0 (00%) to 8 (11.60%) in non-diseased samples.
- In average, Azithromycin was highly sensitive (99.38%), Penicillin (51.85%) and Oxytetracyclien (25.30) were highly resistance. Streptomycin was moderately sensitive (48.76%). Ciprofloxacin (93.20%), Kanamycin (73.45%), Gentamycin (55.55%) and Cephalexin (53.70%) were also sensitive.
- Therapeutic trial had significant effect (P<0.05) on recovery of repeat breeding of cows. Among therapeutic drugs Acriflavin + Metronedazole + Gentamicin Sulphate was the best group of all.

6.2 Recommendations

- The concern authority should give special attention to the breeding policy.
- The farmers should give special management support to the cross-bred dairy cows.
- The farmers of cross-bred cows should practices Veterinary care regularly.
- Before treatment the gynaecological disease, diagnosis should be done for identify the specific etiology.
- The farmer should choice the effective drugs for their GD of crossbred cows for recovery as early to improve the fertility status of dairy cows.
- This study has been observed out of risk factors responsible for the occurrence of repeat breeding in cows that will reduce the malady and in turn will increase the productivity of the food animals and reduces the economic loss.
- Finally the findings of present study would be contributing technology for the improvement of the dairy cows in Bangladesh.

CHAPTER 7

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APPENDICES

Appendix-1: Surveyed Questionnaire

Investigations of Repeat Breeding in Dairy Cows at Rajshahi in Bangladesh with Special Reference to some Therapeutic Approach

Farmer Name and Address:-

A. General information of farm owners (√ Marks)

- **Framer occupation:** i) Service holder ii) Agriculture farmer iii) Private jobs iv) Others
- **Types of farm:** i) Government ii) Private
- **Size of farm:**
  a) Single (1 cow) b) Mini farm (2 to 5 cows)
  c) large size farm (> 5 cows)

B. Managemental Information:

- .**Housing system**: a) Poor b) Medium c) Good
- **Type of floor:** i) Poor condition (earthen) ii) Medium (semi concrete) iii) Good (concrete)
- **Feeding System:**
  i) Poor (traditional feed supply)
  ii) Average (concentrate and straw and quantity average)
  iii) Better (concentrate, green grass and straw quantity available)
- **Breeding method :** i) Natural service ii) A.I
- **Preventive measure:** i) Nothing ii) Occasional iii) Regular
- **Treatment By:** i) Traditional ii) Paravet iii) Veterinarian

C. Over all farm condition (parameter):-

- **Breed:** a) Local b) Cross

- **Genotype:** a) Local b) Local × Sahiwal c) Local × Friesian d) Local × Friesian × Sahiwal e) Local × Sahiwal x Sahiwal
- **Age group:** a) < 4 years b) 4 to < 6 years c) 6 to < 8 years d) > 8 years
- **Body weight:** a) <250 kg b)250 to <350 kg c) >350 kg

- **Body condition score:** a) Poor b) Fair c) Good

- **Parity (calving number):** P_1 / P_2 / P_3 / P_4 / P_5 / ≥ P_6

- **Repeat Breeding Status:** i) Repeat Breeder ii) Normal cow
- **Prophylactic treatment after delivery by:**
  i) GnRH + AI
  ii) Lugol's Solution + Oxyteracycline
  iii) Acriflavin + Metronedazole + Gentamycin Sulphate
  iv) Two times AI + Vitamin ADE
  v) Without any treatment (Control group)

- **Recovery after treatment:** Yes / No

(Name and Signature of Data Collector)

Appendix-2: Chi-square Table

Degrees of Freedom	Probability, p				
	0.99	**0.95**	**0.05**	**0.01**	**0.001**
1	0.000	0.004	3.84	6.64	10.83
2	0.020	0.103	5.99	9.21	13.82
3	0.115	0.352	7.82	11.35	16.27
4	0.297	0.711	9.49	13.28	18.47
5	0.554	1.145	11.07	15.09	20.52
6	0.872	1.635	12.59	16.81	22.46
7	1.239	2.167	14.07	18.48	24.32
8	1.646	2.733	15.51	20.09	26.13
9	2.088	3.325	16.92	21.67	27.88