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**ASSESSMENT OF MAJOR REPRODUCTIVE HEALTH PROBLEMS, THEIR
EFFECT ON REPRODUCTIVE PERFORMANCE AND ASSOCIATION WITH
BRUCELLOSIS IN DAIRY COWS IN BISHOFTU TOWN, ETHIOPIA**

MSc Thesis



By

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**ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE
AND AGRICULTURE, DEPARTMENT OF ANIMAL PRODUCTON
STUDIES**

June, 2016

Bishoftu, Ethiopia

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**A Thesis Submitted to College of Veterinary Medicine and Agriculture of Addis
Ababa University in Partial Fulfillment of the Requirement for Degree of Masters of
Science in Tropical Animal Production and Health**

By

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June, 2016

Bishoftu, Ethiopia

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DEDICATION

This thesis manuscript is dedicated to my beloved father, Merid Woldeamanuel, my Mother, Amarech Bashu and my Grandfather, Baranbaras Bashu Bushen for their unreserved encouragement, nursing me with affection and love. They are the key of my success in my entire life.

STATEMENT OF THE AUTHOR

First, I declare that this Thesis is my bona fide work and that all sources of materials used for this Thesis have been duly acknowledged. It has been submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University and is deposited at the University Library to be made available to borrowers under rules of the Library. I solemnly declare that this Thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
AFC	Age at First Calving
AFS	Age at First Service
CFT	Complement Fixation Test
CI	Calving Interval
CSA	Central Statistic Agency of Ethiopia
CVMA	College of Veterinary Medicine and Agriculture
NAHDIC	National Animal Health Diagnostic and Investigation Center
NSC	Number of Services Per Conception
OIE	World Animal Health Organization
PGF ₂ α	Prostaglandin F ₂ Alpha
PPA	Post-Partum Anoestrus
RBPT	Rose Bengal Plate Test
RP	Retained Placenta
RFM	Retained Fetal Membrane
SVM	School of Veterinary Medicine

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ABSTRACT

A cross sectional study was conducted in Bishoftu town from November 2015 to May 2016 with the objectives of investigating major reproductive health problems and their impact on reproductive performance and association with brucellosis in dairy cows. Cross sectional questionnaire survey and observational follow-up were used to determine reproductive parameters and abnormalities. The study was carried out on a total of 519 crossbred dairy cows. The mean age at first service, age at first calving, calving interval, days open, calving to conception interval and number of service preconception were 20.27 months, 29.38 months, 13.42 months, 113.9 days, 130.17 days, and 1.75 ± 1.07 respectively. From the total study animals 30.12% (n= 156) were affected by at least with one reproductive health problem. Among the problems retained fetal membrane, repeat breeder, abortion, anestrus and dystocia were mostly found with their respective prevalence of 10.01%, 7.7%, 7.5%, 3.7% and 3.5%. A total of 291 sera were collected from crossbred dairy cows and Rose Bengal Plate Test was used on sera as a screening test for brucellosis. Rose Bengal Plate Test showed that 15 (5.15%) of the tested samples were screened as positive for brucellosis. Rose Bengal Plate Test positive serum samples were further subjected to Complement Fixation Test for confirmation of brucellosis positivity. From 15 Rose Bengal Plate Test positive sera 12 (4.1%) were also confirmed to be positive by Complement Fixation Test. From the total study animals 39(8.7%) and 52(18.3) were recorded with abortion and retained fetal membrane. Abortion was found significantly associated with sero-positivity of brucellosis in the present study ($P < 0.05$). From the different risk factors studied BCS and parity were significantly associated with reproductive health problems. Also, reproductive trait shows highly significant differences between cows with reproductive abnormalities and those with normal once. This study showed that reproductive disorders highly affected the reproductive performance of dairy cows. Further detailed studies on the major reproductive health disorders in the area should be carried out.

Keywords: *Bishoftu, Brucellosis, crossbred dairy cattle, reproductive performance, reproductive problems*

1. INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country and still promising to rally round the economic development of the country. The Ethiopian total cattle population is estimated to be about 56.71 million. Out of this the female cattle constitute about 55.45% and the remaining 44.55% are male cattle and 98.66% of the total cattle in the country are local breeds and remaining are hybrid and exotic breeds that accounted for about 1.19% and 0.14%, respectively (CSA, 2015). The livestock sector plays a vital role as source of food, income, services and foreign exchange to the Ethiopian economy (Ayele *et al.*, 2003).

Moreover, Ethiopia has diverse animal genetic resources and its relatively large livestock population is well adapted to and distributed among diverse ecological conditions and management system (Lobago *et al.*, 2006). Despite the huge number of cattle and their economic importance, the productivity is low due to the constraints of disease, nutrition, poor management and poor genetic potential. These constraints result in poor reproductive performance of dairy cattle (Masama, 2003).

Reproductive performance is one key component of dairy production and the goal of reproductive management in dairy cattle is to have cows become pregnant in an efficient manner and at a profitable interval after calving (Plazier *et al.*, 2002). So, high reproductive efficiency is necessary for a successful dairy operation and requires a calving interval that maximizes milk production within the herd (Ferguson and Galligan, 2000). Good estrus detection, good insemination technique, quality semen, and a healthy uterine environment are critical components of high reproductive efficiency (Nebel, 1999).

Infectious diseases have direct impact on reproductive performance of dairy cows. They do have a potential to cause abortion, dystocia, retained placenta, pyometra, metritis, prolapse (uterine and vaginal), anoestrus and repeat breeder. They can be classified as before gestation, during gestation and after gestation (Shiferaw *et al.*, 2005; Lobago *et al.*, 2006).

Among the infectious diseases, *Brucellosis* is highly characterized primarily by causing abortion in late pregnancy, Retained placenta, endometritis and infertility in subsequent pregnancies in cattle. Mature animals are much more susceptible to infection, regardless of sex. In female animals, pregnancy has positive contribution to the degree of susceptibility than their age (Seifert, 1996). In addition to constraints of low production it continues to cause heavy economic losses and public health concern throughout the world (OIE, 2003).

In Ethiopia, the differences in management (production) systems and environmental conditions under which cattle are maintained could greatly affect the occurrence of reproductive disorders (Shiferaw *et al.*, 2003). Debre zeit is a potential place which comprises national and international research institutes. Cattle, small ruminant, poultry and equines are the major livestock species kept with fast growing smallholder dairy production (IPMS, 2005). Although, there are enough dairy farms in Debre Zeit, ample studies have not been conducted on the major reproductive health disorders in dairy animals (Getachew and Nibret, 2014).

Therefore, the objectives of this study were;

- ✓ To investigate major reproductive health problems and their impact on reproductive performance, and;
- ✓ To quantify the association between some reproductive disorders and brucellosis positivity in Debre Zeit town.

2. LITERATURE REVIEW

2.1. Overview of Dairy Production Systems in Ethiopia

Dairying is practiced almost all over Ethiopia involving a vast number of small, medium or large-sized, subsistence or market-oriented farms. Based on climate, land holdings and integration with crop production as criterion, dairy production systems are recognized in Ethiopia; namely the rural dairy system which is part of the subsistence farming system and includes pastoralists, agro-pastoralists, and mixed crop–livestock producers; the peri-urban; and urban dairy systems (Dereje *et al.*, 2005). The first system (pastoralism, agro pastoralism and highland mixed smallholder production system) contributes to 98%, while the peri-urban and urban dairy farms produce only 2% of the total milk production of the country (Ketema, 2000).

The rural system is non-market oriented and most of the milk produced in this system is retained for home consumption. The level of milk surplus is determined by the demand for milk by the household and its neighbors, the potential to produce milk in terms of herd size and production season, and access to a nearby market (Tsehay, 2001). Pastoralists raise about 30% of the indigenous livestock population which serve as the major milk production system for an estimated 10% of the country's human population living in the lowland areas. Milk production in this system is characterized by low yield and seasonal availability (Zegeye, 2003).

The highland smallholder milk production is found in the central part of Ethiopia where dairying is nearly always part of the subsistence, smallholder mixed crop and livestock farming. Local animals raised in this system generally have low performance with average age at first calving of 53 months, average calving intervals of 25 months and average lactation yield of 524 liters (Zegeye, 2003).

Peri-urban milk production is developed in areas where the population density is high and agricultural land is shrinking due to urbanization around big cities like Addis Ababa. It possesses animal types ranging from 50% crosses to high grade Friesian in small to

medium-sized farms. The peri-urban milk system includes smallholder and commercial dairy farmers in the proximity of Addis Ababa and other regional towns. This sector owns most of the country's improved dairy stock (Tsehay, 2001). The main source of feed is both home produced or purchased hay; and the primary objective is to get additional cash income from milk sale. This production system is now expanding in the highlands among mixed crop–livestock farmers, such as those found in Selale and Holetta, and serves as the major milk supplier to the urban market (GebreWold *et al.*, 2000).

Urban dairy farming is a system involving highly specialized, state or businessmen owned farms, which are mainly concentrated in major cities of the country. They have no access to grazing land. Currently, a number of smallholder and commercial dairy farms are emerging mainly in the urban and peri-urban areas of the capital (Felleke and Geda, 2001; Azage, 2003) and most regional towns and districts (Ike, 2002; Nigussie, 2006). Smallholder rural dairy farms are also increasing in number in areas where there is market access. According to Azage and Alemu, (1998), the urban milk system in Addis Ababa consists of 5167 small, medium and large dairy farms producing 34.65 million liters of milk annually. Of the total urban milk production, 73% is sold, 10% is left for household consumption, 9.4% goes to calves and 7.6% is processed into butter and *ayib* (cheese). In terms of marketing, 71% of the producers sell milk directly to consumers (Tsehay, 2001).

2.2. Reproductive Performance of Dairy Cattle

Dairy herd profitability is highly dependent on reproductive performance. Reproductive Performance is biologically crucial and vital for the profitability in many animal production systems. Reproductive efficiency of a herd is an important component of dairy cattle productivity in the world. Economic losses because of poor fertility can be attributed to the cost of prolonged calving interval, increased insemination costs, reduced returns from calves born and forced replacements in the event of culling. A delay in conception because of poor fertility increases calving interval mostly due to the increase in the number of days from calving to conception (Nishida *et al.*, 2006). The productivity of dairy cattle breeds depends mainly on their reproductive performance and efficiency of service per conception. The most common indices of reproductive performance are age at first service (AFS) and age at first calving (AFC), day's open (DO), calving interval (CI) and number of service preconception (NSPC) are the bases of profitable production for dairy farm (Hammoud *et al.*, 2010).

2.2.1. Age at First Service

Age at first service (AFS) is the age at which heifers attain body condition and sexual maturity for accepting service for the first time (Gidey, 2001). Age at first effective service is one of the most important fertility properties in dairy cattle. It has direct impact on age at first calving since duration of gravidity has physiologically constant value. Age at first effective service includes the period from the birth of heifer to first insemination at the age when animal has reached breeding maturity enabling it normal gravidity. Age when animal reaches sexual and breeding maturity for long time was in the center of attention of expert and scientific public in regard to the possibility for shortening of the generation interval. Age at first effective service is determined within defined time limits. Bottom limit is date of birth, and top limit date of conception (Novaković *et al.*, 2011).

Age at first breeding coupled with reproductive efficiency to first and subsequent breeding determines age at first calving because gestation length is a fixed interval (282 days) once conception occurs. Thus, the major reproductive challenge for breeding age heifers is to achieve conception by 14 to 16 months of age. Rearing heifers to initiate puberty by 11-13 months so that breeding can occur in a timely fashion is critical to the overall success of a heifer rearing program (Head, 1992).

Age at onset of puberty is related to body weight and Holstein heifers normally exhibit their first estrus at a body weight of between 550 to 650 pounds. Nutritional management resulting in poor body weight gain early during development causes reproductive problems because it delays onset of puberty, age at first breeding and ultimately age at first calving. By contrast, research has consistently shown that nutritional management resulting in excessive pre pubertal weight gain reduces milk yield during first and subsequent lactations due to subnormal development of mammary secretory tissue. Thus, nutritional management of pre pubertal heifers profoundly influences age at puberty, age at first breeding and hence age at calving (Head, 1992).

2.2.2. Number of Services per Conception (NSC)

The number of services per conception (NSC) is the number of services (natural or artificial), required for successful conception. It expresses the fertility level of the dairy herds. It is simple and easy to calculate and understand and it is a good measure of reproductive status, but still, it usually does not indicate reasons on heifers and cows that fail to conceive. The number of inseminations required to produce a live calf is one of the most useful parameters of reproductive efficiency which mainly depends on the breeding system used (Young, 2002). It is higher under uncontrolled natural breeding and low where hand-mating or artificial insemination is used.

The optimum recommended NSC for profitable dairy cow's ranges from 1 to 1.7 (Evelyn, 2001). Although several studies estimated NSC in Ethiopia. Number of service per conception is significantly affected by season; that is related to availability of feed, placenta expulsion time, lactation length and milk yield and parity (Gebeyehu *et al.*,

2005; Gebrekidan *et al.*, 2012). The number of service per conception was 1.52 in Asella town Ethiopia (Hunduma, 2012) which is closely to 1.62 reported by (Shiferaw *et al.*, 2003) and 1.67 reported by (Yifat *et al.*, 2009). (Nuraddis *et al.*, 2011) reported mean NSC of 1.29 for crossbred dairy cows in North Gondar town. A comparable value of 1.73, 1.62 and 1.56 NSC were reported by (Demeke *et al.*, 2004, Gebeyehu *et al.*, 2005) and (Belay *et al.*, 2012) in crossbreds in Jimma, Andassa ranch and Holetta Research Center Ethiopia, respectively.

Similar NSC value of 1.8 was reported by (Tadesse *et al.*, 2010) for Holstein Frisian (HF) cows under intensive production system in and around Addis Ababa, Ethiopia. However, (Emebet, 2006) reported higher mean NSC of 2.2 for crossbred cows in commercial farm in Dire Dawa, Ethiopia. Heritability of NSC is low and most of the variation is attributable to environmental factors. (Demeke *et al.*, 2004) and (Gebeyehu *et al.*, 2007) found that year of calving significantly affected NSC. On the contrary, (Nega and Sendrose, 2000) and (Tadesse *et al.*, 2010) did not find significant effect of year of calving on NSC for Holstein Frisian cows in and around Addis Ababa and Holetta state farm, Ethiopia. However, (Gifawosen, 2003) found significantly fewer NSC in the short rainy season than main rainy season for Boran and its crosses at Holetta research farm, Ethiopia. Although the effect was not apparent in Holstein Friesian and crossbred cows under intensive production system in and around Addis Ababa and in Andassa ranch, Ethiopia reported by (Gebeyehu *et al.*, 2005; Tadesse *et al.*, 2010) respectively.

Motlagh *et al* (2013) found that the number of service per conception tends to increase significantly with increase age (47 to 48 month). The possible cause of the low NSC for younger cows was not clear. Razi *et al* (2010) observed that the conception rate of parity 3 and 4 was 77% and 75%, respectively. Cows with parity 3 and 4 have several times increased conception rate than multiparous. Among the age group, the highest conception rate was in between 4 and 5 years, 71% and 74%, respectively and lowest conception rate was in cows of 9 years of age or above. Animals with 1-2 services showed conception rate 61% and animals with 3 and more than three services showed conception rate was 50%. Conception rate was significant Increased in both parity 3 and parity 4 than

However, Habtamu *et al* (2010) working with Jersey cows observed that there is insignificant effect of parity number on number of services per conception. Similar results also were reported by Hammoud *et al* (2010) study on reproductive performance of Friesian cows under semiarid conditions in Egypt, who reported that Parity had no significant effect on NSC. The NSC was highest at the third lactation followed by the first lactation. Estimates of NSC reported by some authors summarized in Table 2.

2.2.3. *Age at First Calving*

Age at first calving is the period between birth and first calving. Influences both the productive and reproductive life of the female, directly through its effect on her lifetime calf crop and milk production and indirectly through its influence on the cost invested for up-bringing (Gebrekidan *et al.*, 2012). Age at first calving is closely related to the rearing intensity, and in a breeding program has impact on generation interval and response to selection. It is affected by nutrition, year and month of birth (Kelay, 2002).

Age at first calving is the earlier measure of reproductive performance in dairy cattle; only after first calving production cycle commences (Dabdoub, 2009). The age at first calving changes the heifer from a non-producing expensive item into an income generating cow. Early AFC reduces unproductive period and higher the AFC will be the additional rearing cost of the animal (Panja and Taraphder, 2012). Under controlled breeding system, heifers are usually mated when they are mature enough to withstand the stress of parturition and lactation. It is recommended that heifers calve between 23 and 25 months of age, which is considered as optimum that increase profitability of the dairy business (Hammoud *et al.*, 2010). AFC influences both the reproductive and productive life of the female directly through its effect on her lifetime, calf crop and milk production and indirectly upon cost invested for her upbringing and the magnitude of genetic progress (Hammoud *et al.*, 2010). Estimates of AFC reported by some authors summarized in Table 1

Table 1: Estimates of age at first calving and calving interval

Breed	Country	AFC (d)	CI (d)	Source
Holstein-Friesian cows	Pakistan	987	505	Sattar <i>et al.</i> (2005)
Friesian cows	Sudan	870	433	Abdel <i>et al.</i> (2007)
Holstein-Friesian cows	Cameron	927	399	Gwaza <i>et al.</i> (2007)
Holstein-Friesian cows	Ethiopia	1272	478	Besufekad (2008)
Friesian cows	Egypt	921	403	Hammoudet <i>et al.</i> (2010)
Jersey cows	Ethiopia	1035	450	Habtamu <i>et al.</i> (2010)
Jersey cows	Pakistan	1010	487	Suhail <i>et al.</i> (2010)
Holstein–Friesian	Ethiopia	1265	561	Amene <i>et al.</i> (2011)
Red Chittagong cattle	Bangladesh	1491	422	Habib (2011)
Friesian X zebu cattle	Ethiopia	1041	419	Nuraddis <i>et al.</i> (2011)
Holstein-Friesian	Pakistan	894	408	Sandhu <i>et al.</i> (2011)
Crossbred dairy cows	Ethiopia	1044	372	Belay <i>et al.</i> (2012)
Zebu X Holstein-Friesian	Ethiopia	1115	640	Belay <i>et al.</i> (2012)
Friesian X zebu cattle	Ethiopia	972	402	Nibret (2012)
Red Chittagong cattle	Bangladesh	1518	454	Amin <i>et al.</i> (2013)
crossbred dairy cattle	Pakistan	1300	543	Hassan and Khan (2013)

d=days, CI=calving interval, AFC=age at first calving

The prolonged AFC of cows could be attributed to factors such as poor nutrition and management practices including poor heat detection at the time of mating the heifers. With good nutrition it is expected that heifers would exhibit fast growth and attain higher weights at relatively younger ages. The optimal AFC for cows reared under tropical conditions is between 24-36 months, as these had the highest first lactation yield, highest estimated life time production and had the highest proportion of total life spent in active milk production (Wathes *et al.*, 2008).

2.2.4. Calving Interval

Calving interval (CI) refers to a time elapsed between two consecutive successive parturitions (Gebrekidan *et al.*, 2012 and Yifat *et al.*, 2012). It is probably the best indicator of a cow's reproductive efficiency and expresses the economic importance of

reproduction. Twelve months calving interval is generally considered the most economically desirable period for dairy cows. However, such a standard lactation length might not work for smallholder dairy cows in which the lactation length is extended considerably in most cases (Msangi *et al.*, 2005). Yifat *et al* (2012) reported that cross breeds have slightly shorter calving intervals than indigenous Cattle. Another study supporting this verdict reported in North Showa zone indicated that indigenous breeds have larger calving interval than crossbreeds (Mulugeta and Belayneh, 2013).

Calving interval can be divided into three periods: postpartum anestrus period (from calving to first estrus), service period (first postpartum estrus to conception) and gestation length. Estimates of calving interval in zebu cattle range from 12.2 to 26.6 months (Gebrekidan *et al.*, 2012). In most modern dairies, the general practice is to breed cows early, with the aim of establishing a calving interval of 12 to 13 months, which is considered optimum; hence, calving interval is considered an important index of reproductive performance (Arbel *et al.*, 2001).

Short calving interval has great advantage to commercial producer, because long calving interval means long dry periods and great loss of feed and carrying of unproductive females (Farin and Slenning, 2001). Attaining short calving interval enable to exploit the early peak milk yield in each lactation. Temperate dairy cattle breeds kept in the warm climate often show longer calving interval than the ideal period of one year (Farin and Slenning, 2001). It has been reported that the mean CI for Holstein Friesian cows under different farm in Ethiopia ranged from 445- 459 days (Gebeyehu *et al.*, 2007; Tadesse *et al.*, 2010). A slightly lower value of 418 days was reported for Holstein Friesian dairy cow at North Gonder (Nuraddis *et al.*, 2011). However, Emebet (2006) and Belay *et al* (2012) reported higher CI of 534.9 and 640.9 days, respectively for crossbred cows in Dire Dawa and Jimma town commercial dairy farms, Ethiopia. Calving interval has a low heritability. (Chagunda *et al.*, 2004) reported a heritability value of 1% for first calving interval in Holstein dairy cattle. This indicates that calving intervals of cows mainly influenced by environmental and management factors. (Tadesse *et al.*, 2010) noted that longer CI might be attributed to the result of longer CFSI and DO obtained which could

be related to environmental factors, mismanagement practices like poor housing, poor nutrition or failure to detect heat by the farmer.

Furthermore, Tadesse *et al* (2010) stated that year, season and parity were significantly affected CI for Holstein Friesian cows in and around Addis Ababa Ethiopia. Similarly, Gebeyehu *et al* (2007) and Habtamu *et al* (2010) reported that year and parity had significant effect on CI for crossbred cows at Stella dairy farm and Jersey dairy cows at Wolaita Sodo, Southern Ethiopia. However, Season has no significant effect in Friesian and Jersey cows, respectively. However, Melendez and Pinedo (2007) reported that season of calving has significant effect on CI in Holstein cattle. But, Haileyesus (2006) indicated a non-significant effect of season on calving interval for crossbred cows at North Gonder town. Length of calving interval depends on reproduction management of the herd (Suhail *et al.*, 2010). Estimates of CI reported by some authors summarized in Table 1.

2.2.5. *Days open*

Days open (also called calving-to-conception interval) is the period between calving and conception in cows (Tewodros, 2008). Days open is influenced by the length of time for the uterus to completely involutes, resumption of normal ovarian cycle, occurrence of silent ovulation, accuracy of heat detection, management, semen quality and skill of inseminator or efficiency of bull (Yosef, 2006; Melaku *et al.*, 2011). Days open affect lifetime production and generation intervals, and hence the annual genetic gain (Yosef, 2006).

According to Gebeyehu *et al* (2007) and Tadesse *et al* (2010) a herd average of less than 85 open days indicates that cows are being breed early, 85 to 115 days considered as optimum for dairy herd, 116 to 130 indicate slight problem, 131 to 145 moderate problems, while more than 145 open days considered as sever reproductive problem in the dairy herd. Although several studies estimated DO in Ethiopia, the results are variable due to various reasons. DO have been reported to be 185, 177, 135, 148 and 85.6 days for dairy cows (Shiferaw *et al.*, 2003; Gebeyehu *et al.*, 2007; Yifat *et al.*, 2009; Tadesse *et*

al., 2010 and Hunduma, 2012), respectively. However, higher value of 305 and 218.47days was also reported for crossbred dairy animals by (Gebeyehu *et al.*, 2005; Emebet, 2006), respectively. Season has no significant effect on day open (Haileyesus, 2006; Kefena *et al.*, 2006; Gebeyehu *et al.*, 2007; Habtamu *et al.*, 2010). However, Melendez and Pinedo (2007) and Hammoud *et al* (2010) detected significant effect of season of calving on DO in HF dairy cows, although, the effect was not significant in crossbreds (Haileyesus, 2006 and Nuraddis *et al.*, 2011). Estimates of DO reported by some authors summarized in Table 2.

Table 2: Estimates of days open and number of service per conception.

Breed	Country	DO(d)	NSC	Source
Holstein–Friesian	Egypt	120	1.95	Oudah <i>et al.</i> (2001)
Holstein cattle	Turk	114	2.01	Turkyilmaz (2005)
Holstein–Friesian	Ethiopia	174	2.01	Yosef (2006)
Friesian cows	Ethiopia	177	1.72	Gebeyehu <i>et al.</i> (2007)
Holstein–Friesian	Ethiopia	206	2.1	Besufekad (2008)
Friesian cows	Egypt	130	2.1	Hammoud <i>et al.</i> (2010)
Holstein–Friesian	Ethiopia	285	1.69	Gizaw <i>et al.</i> (2011)

Friesian X zebu cattle	Ethiopia	171	1.29	Nuraddis <i>et al.</i> (2011)
Horro x Friesian	Ethiopia	123	1.97	Gizaw <i>et al.</i> (2011)
Horro x Jersey	Ethiopia	109	1.92	Gizaw <i>et al.</i> (2011)
Horro (Zebu)	Ethiopia	134	2.00	Gizaw <i>et al.</i> (2011)
Tunisian Holstein cows	Tunisia	150	2.55	M'Hamdi <i>et al.</i> (2011)
Hcrossbred dairy cows	Ethiopia	85	1.52	Hunduma (2012)
Zebu X Holstein Friesian	Ethiopia	155	1.56	Belay <i>et al.</i> (2012)
Friesian X zebu cattle	Ethiopia	87	1.3	Nibret (2012)
Iranian dairy cattle	Iran	111	2.1	Motlagh <i>et al.</i> (2013)

d=Days, DO=Day open, NSC=Numbers of service per conception

2.3. Reproductive Disorders and Their Effect on Reproductive Performance

Reproductive efficiency is a critical component of a successful dairy operation and acts as an important component of a profitable dairy farm, whereas reproductive inefficiency is one of the costliest problems facing the dairy industry today. Reproductive problems occur frequently in lactating dairy cows and can dramatically affect reproductive efficiency in a dairy herd (Fricke, 2001). Low fertility reduces the profit by decreasing the average milk production and the number of calves per cow per year. Poor reproductive performance is a major cause of involuntary culling and therefore reduces the opportunity for voluntary culling and has a negative effect on the future productivity of a dairy herd. Reproductive performance is influenced by the interactive effect of environment, management, health, and genetic factors (Gröhn and Rajala-Schultz, 2000; Roxström *et al.*, 2001).

Among the major problems that have direct impact on reproductive performance of dairy cows are abortion, dystocia, retained placenta (retention of fetal membrane), pyometra, metritis, prolapse (uterine and vaginal), anoestrus and repeat breeder. They are classified as before gestation (anoestrous and repeat breeding), during gestation (abortion, vagina prolapse and dystocia) and after gestation (retained fetal membrane and uterine prolapse) (Shiferaw, 2005; Lobago, 2006). Reproductive health disorders cause considerable economic loss to the dairy industry due to slower uterine involution, reduced

reproductive rate, prolonged inter conception and calving interval, negative effect on fertility, increased cost of medication, drop in milk production, reduced calf crop and early depreciation of potentially useful cows (Lobago *et al.*, 2006).

2.3.1. Abortion

Abortion in dairy cattle is commonly defined as a loss of the fetus between the age of 42 days and approximately 260 days (Peter, 2000). The diagnosis of abortions often presents a challenge to the herd owner and the herd veterinarian. Although a gradual increase in the abortion rate in a herd may be noted over a period of many years, a sudden and dramatic increase is more commonly seen.

Abortions during early pregnancy result in increased days open. Following late term abortions, there is a loss of potential replacement heifers. These late term abortions often result in early culling of productive cows. Moreover, extended calving intervals reduce production. Increasing the calving interval from 12 to 13 months may result in a loss of 2-5% of the herd's potential calf production. Calving intervals over 14 months will produce loss of greater than 10% in average producing dairy herds (Rafati *et al.*, 2010). Despite ongoing research investigations to diagnose and evaluate the epidemiology of infectious factors that cause abortion, breeders are still confronted with abortion continuously. Hovingh (2009) listed numerous causes of abortion: infectious agents (bacteria, viruses, protozoa, and fungi), toxic agents, heat stress, and genetic abnormalities. Twin pregnancies (Bitaraf Sani and Amanloo, 2007) and mastitis (Santos *et al.*, 2003) have also been implicated. Infectious agents are the most commonly diagnosed cause.

2.3.2. Retained placenta

Retained placenta is defined as the failure to pass all or part of the placenta from the uterus after 12 hours of calving. There are several potential causes for placental retention but the effects on the general health of the cow and her subsequent reproductive performance are costly events to the dairyman (Charles, 1999).

Retention of fetal membranes is the most common condition occurring in domestic animals following parturition (Noakes *et al.*, 2009). Its incidence varies from 4.0-16.1%, but can be much higher in problem herds. Established risk factors for placental retention are; abortion, stillbirth, twins, dystocia, induction of parturition with prostaglandin F₂ alpha (PGF₂α) and metabolic disorders, especially milk fever; specific infections such as: brucellosis, leptospirosis, vibriosis, listeriosis and *infectious bovine rhinotrachietis* (IBR). Delayed placental separation predisposes cows to acute puerperium metritis and endometritis post-partum through: increased bacterial multiplication in the uterus, impairment of immune function of endometrial macrophages and neutrophils by bacterial endotoxins (Hatchinson, 1996). The key element in the pathogenesis of retained placenta in cattle is a failure of timely breakdown of the cotyledon-caruncle attachment after delivering the calf (Davies *et al.*, 2004).

According to (Kaneene *et al.*, 1997) metabolic events associated with energy insufficiency are related to increased risk of metritis and RP. Cows in a greater degree of negative energy balance prepartum are 80% more likely to have RP (Le Blank *et al.*, 2004). Milk fever or hypocalcaemia is a severe per parturient disorder characterized by lowered blood Ca concentration in high-yielding dairy cows and such cows are in increased risk of RP. The factors to be avoided are high body condition score at calving, dietary deficiencies of vitamins and minerals, and hypocalcaemia (Han and Kim, 2005).

2.3.3. *Anestrous*

A state of complete sexual inactivity with no manifestation of estrus for more than two months. If estrus has not been observed in a dairy cow by 60 days postpartum the condition is defined as Post-Partum Anoestrus (PPA), whether she is cycling or not. The cow is not observed in estrus either because she has not come into estrous (not cycling) or because estrus was not detected (cycling). Two types of anoestrus are: True anestrous: The cow does not come into estrus because she has inactive ovaries. Sub-estrous: The cow has normal cyclic activity, but is not observed in estrus due to weak or absent estrous behavior, or insufficient observation. Anestrous cows have small, flaccid uteri and small, inactive ovaries with no palpable corpus luteum or follicle (Hafez, 2004).

Anestrus is a major problem in the tropics and subtropics, where inadequate nutrition, high ambient temperature, high parasite burdens and disease aggravate the problem. Low body weight and poor body condition, in addition to lactation stress, can further extend the post-partum anoestrous period. It is indicated that the long anoestrous period in the lactating cow might be due to an elevated level of prolactin, which appeared to depress the secretion and release of gonadotropin hormone, or that the pituitary may be less responsive to gonadotropin releasing hormone during lactating (Hafez, 2004).

2.3.4. Repeat breeder

A cow or a heifer that failed to conceive for three or more consecutive services or animal exhibiting regular oestrous cycle every 18 to 24 days and normal heat sign but failed to conceive after three or more inseminations are considered as repeat breeders (Grooms, 2006). Repeat breeders are those cows that require three or more service to conceive. Repeat breeding can be caused by a number of factors, including sub-fertile bulls, endocrine problems, malnutrition, reproductive tract infections and poor management. Mean number of services per conception of 1.6 have been reported for organic herds in one study (Defra, 2000). On average, two cows out of 100 would need more than 5 services to conceive. If the number of these "normal" repeat breeders is higher, the situation should be investigated to diagnose possible endometritis, cystic ovaries or other pathological reasons, such as herd infection with *bovine viral diarrhoea* (BVD) or leptospirosis as reasons for non-conception (Grooms, 2006).

Repeat breeder cows may appear physically and physiologically normal on examination, but may have a problem with embryo loss after conception or poor embryo recognition, social stress, nutritional imbalance or other management factors may adversely affect her fertility. Embryo loss, failure by the embryo to attach itself onto uterine wall, is considered an important cause to poor conception rates. The main causes of embryo loss are post calving complications that prevent the uterus from recovering and recognising the embryo. Similarly, if a healthy cow is served too soon after calving, the uterus might

not have recovered sufficiently to recognise the embryo. It is suggested that stress in animals leads to poor conception rates (Defra, 2000).

2.3.5. *Dystocia*

Dystocia, more commonly known as difficult calving and defined as prolonged or difficult parturition is a problem most dairy producer's encounter (Mee, 2008a). There are different causes and risk factors associated with dystocia in dairy cattle which can result from both maternal and foetal factors. The most common reason for dystocia results from a physical incompatibility between the pelvic size of the mother and the size of the calf at birth, also called foeto-pelvic incompatibility (or FPI) (Mee, 2004). This is largely influenced by the weight and morphology of the dam and the calf, respectively. The pelvic area available at birth is affected by the size of pelvis but also by fatness of the dam which might partially obstruct the birth canal. The calf's physical factors contributing to a size mismatch between the calf and the dam may include a calf of a big size or malpresentation. These morphological factors are themselves dependent upon different variables including the age, breed and parity of the dam, twinning, the sex and weight of the calf, the sire and breed of the calf as well as the nutrition of the dam during gestation (Hickson *et al.*, 2006; Mee, 2008a; Zaborski *et al.*, 2009; Ghavi Hossein-Zadeh, 2010b).

2.3.6. *Stillbirth*

Stillbirth was defined as a calf loss from day 260 until the end of normal gestation period. Trait definitions vary slightly between countries, with most defining stillbirths as those calves born dead or dying within 24 h of parturition (Philipsson *et al.*, 1979). The incidence of stillbirth parturition in dairy cows seems to have increased in recent years (Hansen *et al.*, 2004; Bicalho *et al.*, 2007; Ghavi Hossein-Zadeh *et al.*, 2008). The great variation in the incidence of stillbirth by farm suggests that management practices (breeding, genetic selection, husbandry, etc.) adopted by individual farms may influence the incidence of stillborn calves (Bicalho *et al.*, 2008). Different studies indicated that male calves had significantly more stillbirths than heifer calves (Heins *et al.*, 2006; Ghavi

Hosseini-Zadeh *et al.*, 2008). This effect is probably due to greater body size of male calves than female ones at birth.

The economic loss is even greater if the detrimental effects of stillbirth on the dam's lactation performance are considered. Stillbirths were associated with increased risk of developing metritis and retained placenta (Emanuelson *et al.*, 1993), and with decreased risk of conception (Maizon *et al.*, 2004). (Stevenson and Call, 1988) reported that cows experiencing stillbirths were at increased risk for a number of postpartum disorders such as prolapsed uterus, retained placenta, metritis, and displaced abomasum. (Correa *et al.*, 1993) also reported increased odds of developing metritis and retained placenta for cows that had stillbirth. It is possible that the higher incidence of postpartum disorders may decrease the survival of cows that had stillbirths. Also, it is likely that the dead calf inside the uterus is accelerating intrauterine bacterial growth which would cause metritis. Furthermore, retained placenta and metritis have been associated with decreased milk yields.

Bicalho *et al* (2007) reported stillbirths significantly increased the risk of death/culling throughout the lactation. Cows that gave birth to stillborn calves had a 40.9% higher hazard rate of death/culling compared with cows that gave birth to live calves. Chassagne *et al* (1999) reported that body condition score ≥ 4 was an important risk factor for stillbirth. Furthermore, heifers are at a greater risk of been over conditioned around parturition than adult cows; hence, heifers are at higher risk for stillbirth parturitions than older cows (Hansen *et al.*, 2004; Bicalho *et al.*, 2007). Obese heifers are potentially more susceptible to dystocia, ketosis, and other metabolic diseases.

2.3.7. Uterine prolapse

The coming out of the uterus through the vulva commonly shorter after parturition and hanged out with inner surface outer most. It occurs most often immediately after parturition and occasionally up to several hours after wards. In rare cases it may occur 48 to 72 hours after parturition. The layman terms for this condition include 'cast her wethers' and 'lost her calf bed'. This true emergency which is an acute condition that is often associated with hypocalcaemia and may also follow use of great force in foetal

extraction. Prolepsis of the uterus is seen most commonly in pluriparous dairy cows but is not infrequent in poorly grown, thin, debilitated dairy heifers. Diagnosis is fairly obvious; caruncles can be observed on the uterus which is prolapsed through the vulva. The prolapsed can involve other organ such as bladder or intestines. In some cases, the uterine artery may rupture with fatal consequences (Hafez, 2004).

2.3.8. Vaginal prolapse

It is defined as the inversion of the vagina only and also some times with the cervix through the vulva. Not infrequently the entire vagina or cervix is prolapsed through the vulva. Vagino-cervical prolapse is seen in all species of domestic animals, but commonly in the cow. The cause of prolapse of vagina and cervix are probably multiple. It is observed in the cow most commonly the last 2 to 3 months of gestation, when a large amount of estrogenic hormone is being secreted by the placenta. This causes a relaxation of a pelvic ligament and adjacent structures and an oedema and the relaxation of vulva and vulvar sphincter muscles. When the cows lie down the intra-abdominal pressure especially in late pregnancy is transmitted to the flaccid pelvic structures tending to force the relaxed and loosely attached vaginal floor and walls through the vulva (Farquherson, 1994; Williams, 1995) rather vaguely attributed the cause to at any of the reproductive tract and general weakness of the patient.

2.3.9. Brucellosis

Brucellosis also known as undulant fever, “Mediterranean fever,” “Malta fever,” Contagious or Infectious abortion, or Bangs disease is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. Expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling partly account for brucellosis remaining a public health hazard (WHO, 2006).

The disease affects cattle, swine, sheep and goats, camels, equines, and dogs. It may also infect other ruminants and marine mammals. The disease in animals is characterized by abortions or reproductive failure. While animals typically recover, and will be able to

have live offspring following the initial abortion, they may continue to shed the bacteria. Brucellosis in cattle (*B. abortus*) in sheep and goats (*B. melitensis*) and in swine (*B. suis*) are diseases listed in the World Organization for Animal Health (OIE) Terrestrial Animal Health Code (OIE, 2010).

Infected cows usually abort during the second trimester to term of first pregnancy after infection. Thereafter the disease usually localizes in the lymph nodes surrounding the reproductive organs and the udder. Bacteria are shed in milk, foetal membranes and discharges from the female reproductive tract after birth or abortion. Male animals develop orchitis, epididymitis, and hygromas (WHO, 2006; Matopel *et al.*, 2010).

Brucellosis is readily transmissible to humans, causing acute febrile illness – undulant fever – which may progress to a more chronic form and can also produce serious complications affecting the muscle–skeletal, cardiovascular, and central nervous systems. Infection is often due to occupational exposure and is essentially acquired by the oral, respiratory, or conjunctiva routes, but ingestion of dairy products constitutes the main risk to the general public where the disease is endemic. There is an occupational risk to veterinarians and farmers who handle infected animals and aborted fetuses or placentas (Quinn *et al.*, 2002 and Ocholi *et al.*, 2004).

Transmission of *B. abortus* mostly occurs via the oral route because cattle tend to lick aborted fetuses and the genital discharge of an aborting cow. Brucella organisms can also be transmitted through the trans mammary route while calves born to healthy dams are infected when fed on colostrum or milk from infected dams (Radostits *et al.*, 2007). Bulls that remain fertile and functionally active after infection with Brucella shed the organisms with the semen during the acute phase of the disease. During the later stage shedding may cease or become intermittent. In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, since semen is not deposited in the uterus (Radostits *et al.*, 2007).

Bovine Brucellosis is widespread throughout the world except for a number of countries where eradication has been successful (PAHO/ WHO, 2001). In Ethiopia, information on

prevalence, incidence and economic importance of brucellosis is available although it is not well representing the different situations in the country except the single report from the work of the veterinary branch of the American Medical Research Unit in 1970. It is of major economic importance in most developing countries, which have no national brucellosis control and eradication program (Radostits *et al.*, 2007). The economic loss from brucellosis in developing countries arises from the actual abortion of calves and resulting decreased milk yield, birth of weak calves that die soon after birth, retention of the placental membrane, impaired fertility and sometimes arthritis or bursitis. It is difficult to estimate the financial loss caused by brucellosis, as it depends on the type of cattle farming, herd size, and whether it is an intensive or extensive cattle farm (Radostits *et al.*, 2007; Roth *et al.*, 2003).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This study was conducted in Bishoftu town which is located at the distance of 45 km South East of Addis Ababa, Ethiopia. The town is located in east Showa zone of Oromia region and it lays 9° North latitude and 40° East longitude at an altitude of 1850 meters above sea level in the central high land of Ethiopia. It has an annual rainfall of 866mm of which 84% is in the long rainy season (June to September) and the remaining in the short rainy season extending from March to May. The dry season extends from October to February. The mean annual maximum minimum temperatures of the area are 26°C and 14°C respectively, with mean relative humidity of 61.3%. Mixed farming system followed in the area, crop and livestock production are an intensive type of production (NMSA, 2010).

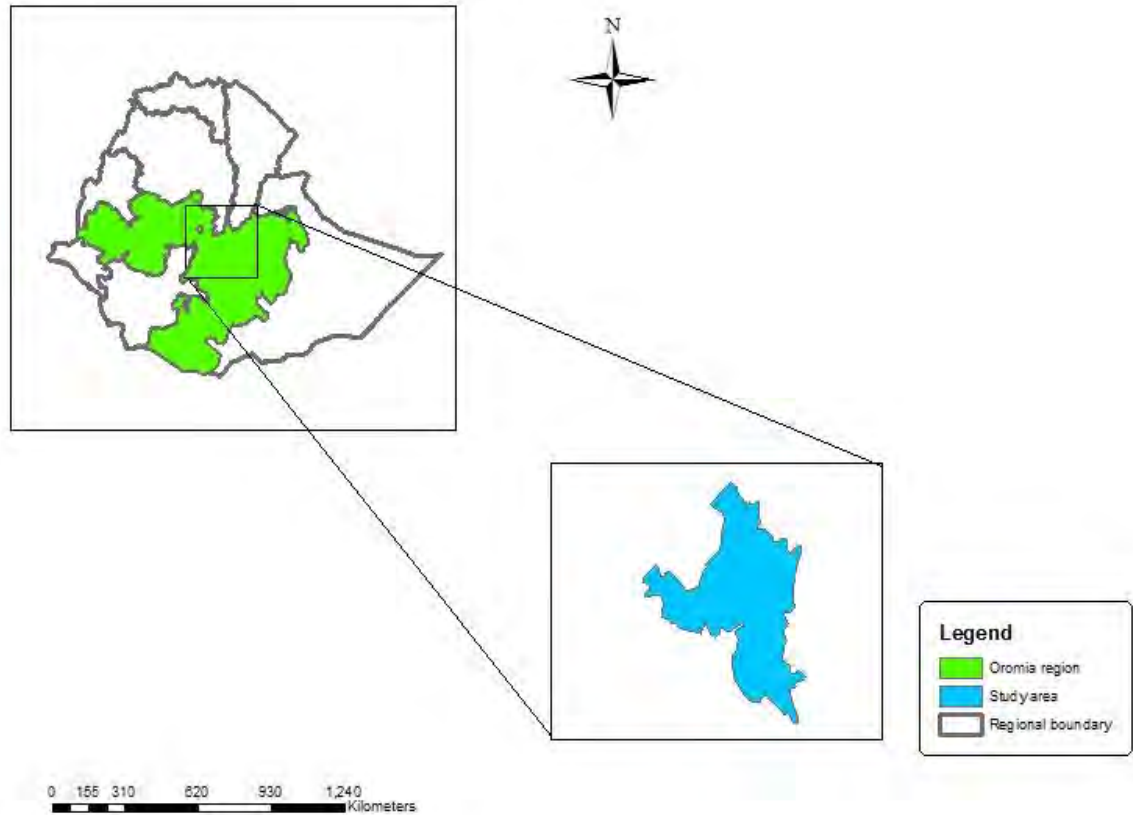


Figure 1: Map of Bishoftu town, East Shoa Zone, Ethiopia

3.2. Study Population and sample size determination

This study was conducted on dairy cows owned by dairy farmers in Bishoftu town. The majority of dairy producers in the town are market oriented smallholder dairy farmers with average herd size of three cows which are organized under, one dairy cooperative called, Ada'a milk and milk products marketing cooperative share company. There is also few government and private owned large (commercial) scale dairy farms with milking heard size of over 50 in the town.

A sampling frame: the list of current members of Adaa Milk and Milk Products Marketing Cooperative was acquired from the administrative office, at the beginning of study. Dairy farms/ herds were purposively selected on basis of herd, ease of access, production system and facility availability. The number of farms sampled in the study was determined by Arsham (2007) for survey studies:

$$N=0.25/SE^2$$

Where, N= sample size

SE=Standard error of the population.

A list of 150 farms housing crossbred dairy animals were considered as the sampling frame. Therefore, a total of 100 farms were selected at 5% standard error with 95% confidence interval. But, because of unwillingness of some farm owners about 9 farms were excluded from the study. Therefore, the study was conducted by the sample size of 91 dairy farms (61 small holders, 19 medium scales, and 11 large commercial scales) were in this manner selected from the cooperation.

Selection of the study animal's: All crossbred dairy cows and heifers aged two years or above were included during the study period. Therefore, a total of 519 crossbred dairy cows with different parity, management and body conditions were used based on willingness to be part of the study. Classification of management systems was done based on the criteria adopted by Richard (1993). Accordingly, by taking the history of animals from owners as: semi-intensive management system for those animals that were kept indoors and fed and watered in their house/shade by cut and carry system while extensive management system for all animals that were kept outdoor during the day time and allowed to graze on a communal or private owned pasture land. The farms were categorized into defined strata based on herd size. Therefore small holders (farms with less than three cows), medium level (farms with 3-10 cows) and large scale (farms with greater than 10 cows) as suggested by ILRI (1996) and cited by Yoseph (1999).

3.3. Study Design

The cross-sectional type of study was carried out on reproductive parameters and abnormalities, which was under taken from November 2015 to May 2016. To manner this study pre-designed structured questioner were developed, pre-tested and administrated through personal interview to farm owners and attendants of all farms considered for this study. Moreover, the study constituted observational study on purposively selected dairy

cows. Retrospective data (Data on reproductive performance was calculated through personal interview and from analysis of farm records) were used to calculate some of reproductive parameters (age at first service, age at first calving, calving interval, number of service per conceptions and calving to conception interval) and reproductive abnormalities respectively.

3.3.1. Questionnaire survey:

In order to get co-operation of the dairy owners and obtain reliable information about their animals, thorough explanation on the objective of the study was given before the start of the interview. Following that, the actual questions were asked about major reproductive problems on individual cattle level like abortion, Dystocia, still birth, retained fatal membrane, metritis, uterine prolapse, anestrus, repeat breeding, management systems and parity. Body condition score was based on the criteria adopted by (Matthew Man, 1993) (Annex 1). Animals were grouped in to those having the major reproductive problems and those without these problems.

3.3.2. Observational study (Longitudinal)

For the observational study, selection of farms was based on the baseline survey information. A regular visit was carried out once per weeks on 18 dairy farms which was purposively selected on the basis of animal size, facility availability, ease of access and production system. Subsequently, about 73 pregnant cows and 36 heifers that have ages of 2 years and above were purposively selected. The study animals were identified by their tag number/ID, parity and pregnant animals that were suspected to give birth within the study period and heifers that has age of 2 years and above were included and follow up from the start to the end of study period. Cows that delivered were observed for the presence of retained fatal membrane left hanging in the vulva in the first 24 h and if any abnormal vaginal discharge occurred without regular frequency of visit.

Body Condition Scoring

For all of the animals under study, body condition was scored in order to assess the nutritional status of the animal and the prevalence of reproductive problems. Therefore, Body condition scoring according to (Matthew Man, 1993) was implemented using 1-5 scale based on palpation of back bone and lumbar process and evaluation of coverage of fat and muscle.

3.4. Sample Collection and Laboratory Tests

3.4.1. Serological blood sample collection

Blood samples (5ml) were collected from the jugular vein of each animal, using sterile needles and plain vacutainer tubes. The blood samples were allowed to stand overnight at room temperature and centrifuged at $1500 \times g$ for 10 min. to obtain the serum. Sera were decanted into cryovials, and were stored at -20°C in CVMA, AAU microbiology laboratory until testing. The Rose Bengal Plate Test (RBPT) was employed as a screening test on the serum samples for the presence of *Brucella* agglutinins. Positive sera were then retested for confirmation by the complement fixation test (CFT) at the National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia.

3.4.2. Serological laboratory techniques

Rose Bengal plate test (RBPT): All sera samples collected were initially screened by RBPT using RBPT antigen (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) according to OIE (2004) and Alton *et al.* (1975) procedures (Annex 2). Sera and antigen were taken from refrigerator and left at room temperature for half an hour before the test to maintain to room temperature and processed following the recommended procedures.

Complement fixation test (CFT): Sera that tested positive to RBPT were further tested using CFT for confirmation using standard *B. abortus* antigen S99 (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom). Preparation of the reagent was evaluated by titration and performed according to

protocols recommended by World Organization for Animal Health (OIE, 2009b) (Annex 3). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative.

3.5. Description and definition of variables

Anestrus: Cows which failed to show clinical heat signs for 90 days or more after parturition (Bekana *et al.*, 1994b).

Repeat Breeders: A cow or a heifer that failed to conceive for three or more consecutive services time (Grooms, 2006).

Abortion: Is the expulsion of a dead fetus of recognizable size at any stage of gestation (Bekana *et al.*, 1994b).

Dystocia: A condition in which prolonged or difficult parturition markedly for more than 6 h and the cow required assistance (Mee, 2008a).

Stillbirth: Calf loss or calves born dead or dying within 24 hours of parturition (Philipsson *et al.*, 1979).

Uterine prolapse: The coming out of uterus through the vulva commonly shortly after parturition and hanged out with the inner surface outer most (Noakes, 1986).

Vaginal prolapse: The protrusion of the vagina and sometimes with the cervix through the vulva (Noakes, 1986).

Retained Fetal Membrane: A lack of expulsion of the placenta with in the first 24 h after calving (Charles, 1999).

Metritis: An inflammation of the uterine wall characterized by reddish brown, white or whitish to yellow mucopurulent, with fetid vaginal discharge along with thickness of uterine wall at transrectal palpation (Robert, 1986; Bekana *et al.*, 1994b).

Age at first service (AFS): The number of days from birth to the date of animals at first service. In the study all cows including heifers served were included (Gidey, 2001).

Age at first calving (AFC): The number of days from birth to the date of animals at first calving. In the study all cows that calved at least once were included (Gebrekidan *et al.*, 2012).

Calving interval (CI): The period between the last two consecutive calving's. Only cows that calved at least two times were included (Gebrekidan *et al.*, 2012).

Calving to conception or Days Open (DO): The number of days between parturition and subsequent conception (Tewodros, 2008).

Number of service per conception (NSC): The number of service be it natural or artificial required for successful conception. The study for this trait was conducted only for cows that conceive and confirmed pregnant (Shiferaw, 2003).

3.6. Data Management and Analysis

Data generated from questionnaire survey, observational study and laboratory investigations were recorded and coded Using Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA 13.0 (Stata Corp LP, College Station, TX, USA). Descriptive statistics such as Mean, percentile and standard deviations were used for both reproductive disorders and reproductive performance. The prevalence of reproductive problems was determined by descriptive statistics as a proportion of affected animals out of the total animal examined. The association of different risk factors with over all prevalence of reproductive problems and compare different variables of reproductive performance and reproductive problems were analyzed by using χ^2 (Chi-square) technique for screening significance as univariate analysis (p- value consideration

as significant was calculated at alpha 0.05 or 95% level of confidence).The point brucellosis prevalence rate was calculated by dividing the number of RBPT and CFT positive animals by the total number of animals tested. Similarly, prevalence of abortion and retained fatal membrane (RFM) was calculated by dividing cows with history of positivity to abortion and RFM cases to the total cows inspected (Thrustfield, 1995). The univariate logistic regression, reporting Odds ratio (OR), was used to test association between abortion, RFM and Sero-positivity.

4. RESULTS

4.1. Characteristics of respondents

Based on the questionnaire study general characteristics associated with household's respondents were distributed by sex, age, marital status, experience in dairy keeping and educational status was presented in Table 3. From the total interviewed respondents (N = 91), the majority (60.4%) of the respondents were male while the remaining (39.6%) were female. The majority age of the respondents in the study area ranges between 30-45 years (49.5%). This result showed that people in the most productive age are actively engaged in dairy activities. Of the total households interviewed, 67% are married. Concerning to level of education, the highest percentage (37.3%) of the respondents had

basic education 6.6% of those respondents had not attended any formal or informal education. A 67% of the respondents had >8 years' experience in dairy keeping.

Table 3: Characteristics of the sample households

Category and variables	Small N (%)	Medium N (%)	Large N (%)	Total N (%)
Sax				
Male	37(60.6)	11(58)	7(63.6)	55(60.4)
Female	24(39.3)	8(42)	4(36.3)	36(39.6)
Age				
<30	11(18)	-	-	11(12)
30-45	31(51)	6(32)	8(72.7)	45(49.5)
>46	19(31)	13(68)	3(27.3)	35(38.5)
Marital status				
Married	39(64)	14(73)	8(73)	61(67)
Single	9(15)	2(11)	2(18)	13(14.3)
Widowed	13(21)	3(16)	1(9)	17(18.7)
Educational level				
None	6(10)			6(6.6)
Basic education	24(39)	10(53)	-	34(37.3)
Primary education	13(21)	5(26)	-	18(20)
Secondary education	10(16)	1(5)	3(27.3)	14(15.4)
Diploma and above	8(13)	3(16)	8(72.7)	19(21)
Experience in dairy keeping				
1-3	8(13)	-	-	8(9)
4-7	20(33)	2(10)	-	22(24)
>8	33(54)	17(90)	11(100)	61(67)

N= Number of Respondents

4.2. Status of dairy farms in the study area

Out of the 91 farms considered in this study, 67% were small scale dairy farms, 20.9% were medium scale dairy farms and 12.1% were large scale dairy farm. The number of animals in each farm size category is given in figure 2. Regardless of the production system or farm size, almost the entire respondent agreed that feeding practice depends on the availability of feed because land space and water that is important factors for cultivation of animal feed is limited in the farm area. Animals in all farm groups were fed natural pasture (cut-and-carry), grass hay, straw, milling byproducts (frushka), dairy concentrate mix, poultry manure, crop residues, some green grasses like alfalfa, elephant grass and beverage by products. Most of the respondents breed their animals by using

artificial insemination 89.0% (n=81) and natural method (bull) 11.0% (n=10).Also 483 (93.1%) cows were managed semi-intensively and 36 (6.9%) cows were managed extensively in the study area.

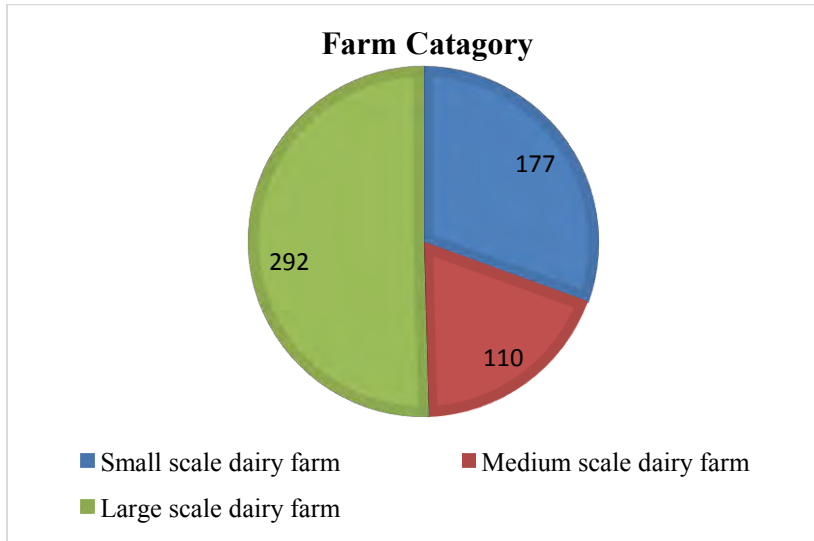


Figure 2: Number of dairy cows in different dairy farm scale in the study area.

4.3. Constraints and Culling reasons of dairy cows in the study area

Each respondent involved in the study was requested to prioritize the challenges of dairy practice. Accordingly, the most important constraints of dairy production in the study areas were feed shortage, high feed cost, and inefficient AI service and heat detection. Figure 3 shows the percentage of constraints of dairy production in the study area.

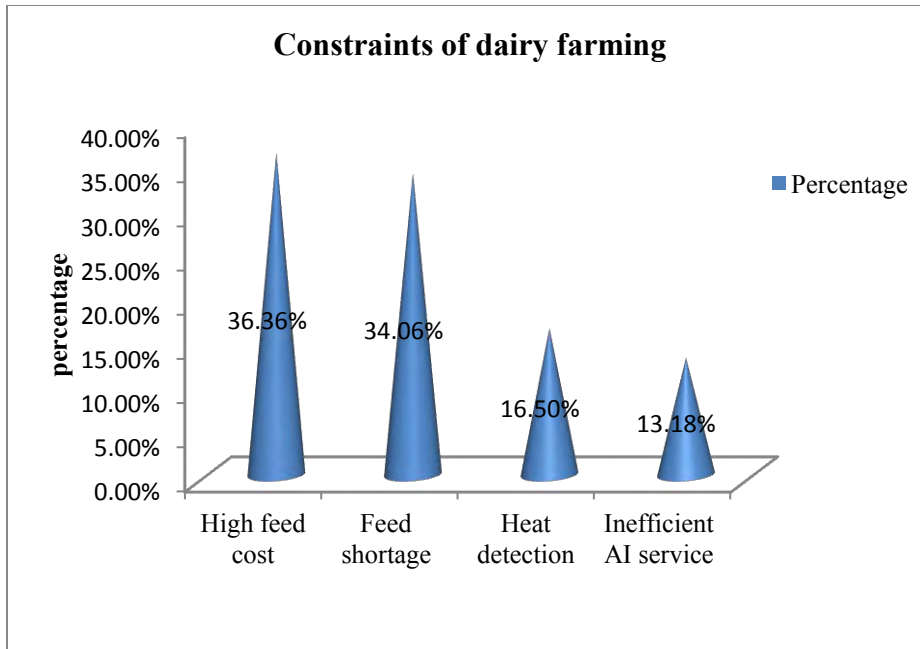


Figure 3: Constraints of dairy farming in the study area

Furthermore, inadequate space, reproductive/ infertility problem, feed shortage, old age, health problems and financial requirement were stated as the major reasons for culling of cows. Figure 4 shows the percentage of culling reasons of dairy cows in the study area.

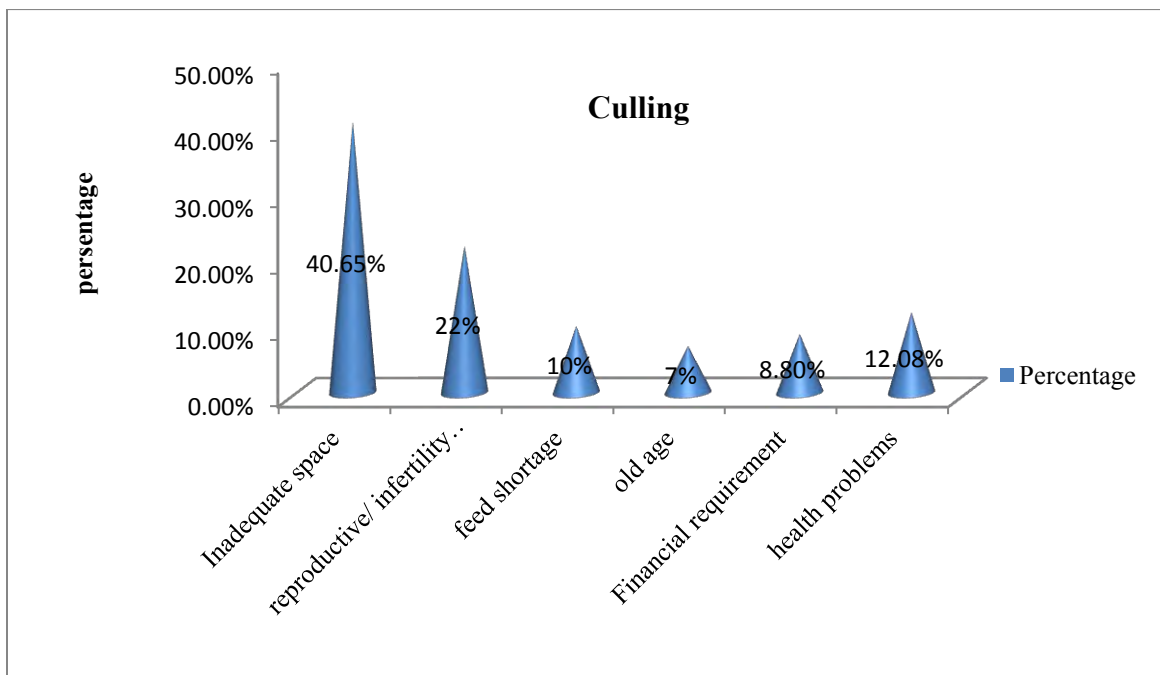


Figure 4: Percentage of culling reasons of dairy cows in the study area.

4.4. Major reproductive health problems

From a total of 519 dairy cows included in the study period 30.12% (n=156) were found to be affected at least by one reproductive health problems. Overall prevalence of reproductive health problem sited by farmers/attendants or veterinarians in crossbred dairy cattle on different method of study is presented in Table 4.

Table 4: Reproductive disorders in dairy cows in Bishoftu town on different method of study.

Method of study	Total No. of observation	No. of cows with reproductive disorders	Percent affected
Questionnaire survey	410	137	26.46%
Observational study	109	19	3.66%
Total	519	156	30.12%

In this study (including questionnaire and observational study) major reproductive health problems were investigated. Hence retained fetal membrane, repeat breeder, abortion, anestrus and Dystocia were found to be the major reproductive health problems containing 10.01%, 7.7%, 7.5%, 3.7% and 3.5%, respectively. Other reproductive health problems observed with lower prevalence include still birth, uterine prolapse, mixed and clinical metritis accounting 1.9%, 0.8%, 5.2% and 0.6% respectively, (Table 5).

Table 5: Relative occurrence of major reproductive disorders in dairy cows in Bishoftu town.

Types of RDs	Questionnaire survey no. (%)	Regular follow up no. (%)	Total (%)
RFM	48(11.7%)	4(3.67%)	52 (10.01%)
Repeat breeding	33(8.04%)	7(6.4%)	40 (7.7%)
Abortion	34(8.29%)	5(4.58%)	39 (7.5%)
Anoestrus	17(4.14%)	2(1.83%)	19 (3.7%)
Dystocia	17(4.14%)	1(0.9%)	18 (3.5%)

Still birth	10(2.44%)	0(0.0%)	10 (1.9%)
Uterine prolapse	4(0.97%)	0(0.0%)	4 (0.8%)
Clinical meterites	3(0.73%)	0(0.0%)	3 (0.6%)
Mixed disorder	26(96.30%)	1(3.70%)	27(5.2%)

RDs = reproductive disorders

*Mixed problems include abortion and retained placenta, anestrus and repeat breeding, and dystocia and retained placenta, repeat breeding, abortion and retained placenta repeat breeding, uterine prolapse, retained placenta, and clinical metritis, (2 and above cases).

4.5. Association of Risk Factors with Reproductive Health Problems of Dairy Cattle

In this study among risk factors; production system, Hygienic condition, Age, heard size, parity, type of insemination and body condition score were considered to assess its association with the occurrence of the reproductive problems as shown on the table 6.

The occurrence of overall reproductive problems was significantly affected within the different body condition and parity. The effect of body condition on the prevalence rate of the major reproductive disorders increased from good to poor animals with prevalence of 24.17% and 64.75% respectively, (Table 6). Generally, body condition had highly significant effect ($P < 0.05$) on the prevalence of major reproductive disorders.

On the other hand, the number of parity on the prevalence rate of major reproductive disorders have a significant ($p < 0.05$) influence for the occurrence of reproductive problems and the effect increased progressively from primiparous to multiparous with prevalence of 52.78% and 28.15% respectively, (Table 6). Furthermore, there was significantly higher ($p < 0.05$) incidence of retained placenta, repeat breeding and abortion was recorded in multiparous than in primiparous cattle (Figure 5).

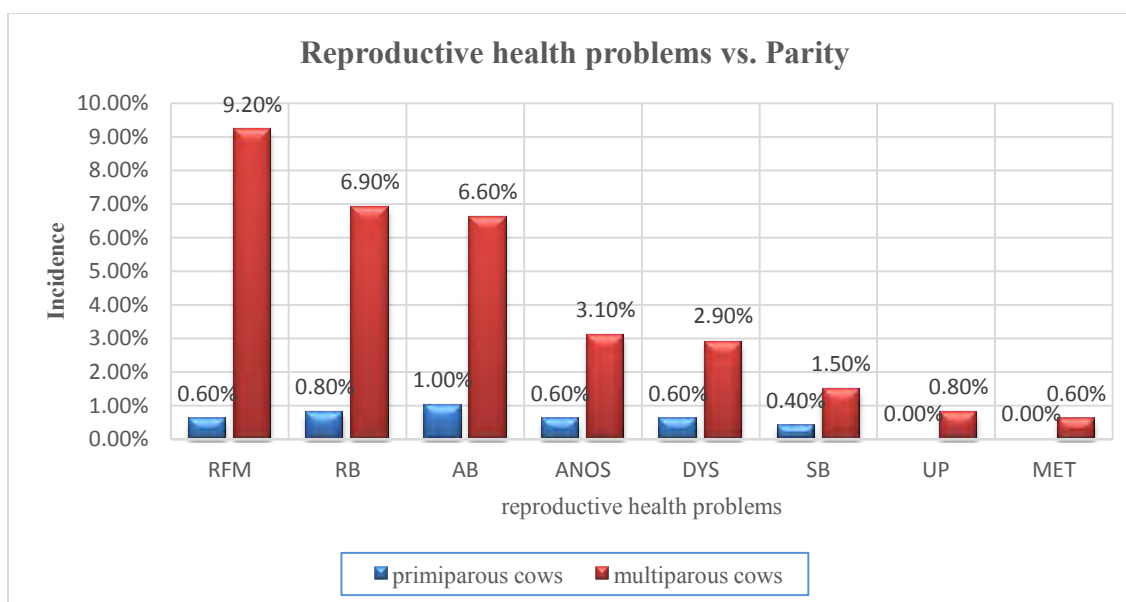


Figure 5: Major reproductive problems vs. parity of crossbred dairy cows.

Incidence of overall reproductive problems decreased from heifer to older cows with prevalence of 26.81% and 33.33% respectively. Age was not significantly affected within the different age groups (p -value= 0.2291, $X^2 = 1.45$). Moreover, the production system, Hygienic condition of the farms as well as type of insemination did not show a significant variation ($p > 0.05$) for the occurrence of reproductive problems. However, reproductive problems were more prevalent in large scale farm (Table 6).

Table 6: Influence of different risk factors on major reproductive problems in the study area.

Factors	Variables	Total no. of		X^2	P-value
		cows examined	cows affected (%)		
BCS	Poor	122	79(64.75%)	31.47	0.0000
	Medium	306	55(18.73%)		
	Good	91	22(24.17%)		
Age	5-8 years	243	81(33.33%)	1.45	0.2291
	2-4 years	276	74(26.81%)		
Parity	Primiparous	36	19(52.78%)		

	Multiparous	483	136(28.15%)	8.87	0.0029
production system	Semi-Intensive	483	147(30.43%)		
	Extensive	36	8(22.22%)	1.14	0.2862
Herd size	small holders	117	32(27.35%)		
	medium level Farm	110	34(31.11%)	0.31	0.5792
	Large scale Farm	292	89(30.48%)		
Type of insemination	Bull	104	33(31.73%)		
	AI	415	122(29.47%)	0.21	0.6434
Hygiene	Good	468	145(31.82%)		
	Poor	51	10(19.61%)	3.05	0.0805

4.6. Reproductive Performance of Dairy Cattle

4.6.1. Age at first service and Age at first calving

The results on the overall means of the reproductive performance of dairy cows in the study area, including ages at first service (AFS), ages at first calving (AFC), calving interval (CI), and days open (DO), number of services per conception (NSPC), and calving to conception interval (CCI) are presented in table 7 and 8.

Table 7: Age at first service and Age at first calving of dairy cows in the study area.

	AFS(m)	AFC(m)	P -- value
Production system	Mean±Sd		
Semi-intensive	19.79±0.83	29.06±0.31	
Extensive	20.37±0.27	29.51±0.28	0.62
Overall	20.27±5.14	29.38±5.01	

m = month

The data indicated that the overall mean \pm S.D of ages at first service and ages at first calving were 20.27 ± 5.14 and 29.38 ± 5.01 months respectively. In the present study production system has no statistical significant influence on age at first service and age at first calving.

4.6.2. Calving interval, days open, number of services per conception and calving to conception interval

The data indicated that the overall mean \pm S.D of calving interval, days open, calving to conception interval and number of services per conception were, 13.42 ± 2.40 months and 113.90 ± 66.01 , 130.17 ± 71.83 days, and 1.75 ± 0.07 respectively. According to the present research finding production system has statistical significant influence $p < 0.05$ on reproductive performance traits. Extensive production system had longer days open and calving to conception interval than semi-intensive production system (Table 8).

Table 8: calving interval, days open, number of service per conception and calving to conception interval of dairy cows in the study area.

	CI(m)	DO(d)	NSC	CCI(d)	P – value
Production system	Mean\pmSd				
Semi-intensive	13.23 ± 0.21	$106\pm 47\pm 4.36$	1.75 ± 0.16	124.06 ± 6.38	
Extensive	13.46 ± 0.11	114.46 ± 3.15	1.75 ± 0.04	130.57 ± 3.42	0.000
Overall	13.42 ± 2.40	113.90 ± 66.01	1.75 ± 0.07	130.17 ± 71.83	

d = days, m = month

4.7. Effect of Major Reproductive Health Problems on Reproductive Performance of Dairy Cow.

Each performance trait measured was adversely affected by reproductive abnormalities of cows (Table 9). Abnormal cows had longer days open, calving to conception interval and required more service per conception than normal cows ($p < 0.05$). Therefore, calving interval were longer in cows with reproductive abnormalities ($p < 0.05$). Overall, the interaction between reproductive health status of cows and performance trait was significant. The present table showed that the difference in reproductive trait with health status of cattle.

Table 8: Mean \pm SD for reproductive traits with reproductive health status

Trait	Reproductive health status		P-Value
	Normal	Abnormal	
CI(m)	12.915 \pm 1.57	14.52 \pm 3.55	0.000
DO(d)	104.69 \pm 44.59	136.49 \pm 97.04	0.000
NSC	1.53 \pm 0.72	2.28 \pm 1.52	0.000
CCI(d)	116.34 \pm 47.36	163.86 \pm 104.02	0.000

NSC=number of service preconception, CCI=calving to conception interval, m=month, d=day, CI= calving interval, DO= days open.

4.8. Sero-Prevalence of Brucellosis with Association of Some Reproductive Problems

Among 291 tested samples, 15(5.15%) were found positive by RBPT and 12 (4.12%) were found positive by CFT respectively. Therefore, in the present study, an overall Sero-prevalence was estimated 4.12% by CFT (Table 10).

Table 9: Results of RBT and CFT of brucellosis.

Serological tests	N	Number Positive	Sero-prevalence
RBPT	291	15	5.15%
CFT	291	12	4.12%

N= number of animal tested; RBPT=Rose Bengal plat test

CFT= complement fixation test

In the present study, abortion, repeat breeder and retained fetal membrane were observed as some major reproductive health problems with the prevalence of 10.01%, 7.7% and 7.5% respectively (Table 4). Out of 39 female animals in the study area with abortion history 4 (10.2%) of them were detected positive for brucellosis. In addition, out of 52 retained fetal membranes 2 (3.8%) of them were positive for brucellosis. However, out of 40 repeat breeder history all of them were negative for brucellosis. A history of previous abortions had been significantly related to sero-positive of brucellosis. But a history of previous retain fetal membrane had not been significantly related to sero-positive of brucellosis (Table 11). Using univariate logistic regression odds ratio was calculated to measure the likely association that could exist between brucellosis and abortion as well as retained fetal membrane. From both cases abortion was strongly associated with brucellosis with values of odds ratio 6.74 (Table 11).

Table 10: Association of RFM and Abortion with Brucellosis positivity.

Clinical signs	N	Prevalence (n)	Confidence Interval	OR	P-Value
Retain Fetal Membrane					
Yes	52(10%)	3.8%(2)	0.38-8.57	1.828	0.444
No	467(90%)				
Abortion					
Yes	39(7.5%)	10.2%(4)	1.93-23.49	6.74	0.003**
No	480(92.5%)				
Abortion period					
1 st trimester	8(1.5%)	0(0)			0.000**
2 nd trimester	18(3.5%)	0(0)	1.62-4.17	2.60	
Not applicable	480(92.5%)	0(0)			
3 rd trimester	13(2.5%)	30.7%(4)			

N= number of observations, OR= odds ratio

On the other hand, there was statistically significant association ($P < 0.05$) between abortion period and sero-positivity of brucellosis with values of odds ratio 2.60. This could be explained by the presence of higher sero-positivity in cows in the last trimester may be due to the preferential localization of *Brucella* in the uterus in which allantoic fluid factors such as erythritol could stimulate the growth of *Brucella* and elevate in the placenta and fetal fluid from about the 5th month of gestation.

5. DISCUSSION

5.1. Constraints of dairy production in the study area

Each respondent involved in the study was requested to prioritize the challenges of dairy practice. As presented in (Figure 3) feed shortage, high feed cost, inefficient AI service and heat detection were the most important constraints of dairy production in the study area. The present study was comparable with the findings of Haftu (2015) who reported that the availability and costs of feeds were noted as major constraints in Hossana town, Ethiopia. Shortage of feed to be the major constraints to milk production, age at first calving and calving interval in crossbred dairy cows in Jimma town, Oromia State, Ethiopia (Belay *et al.*, 2012). Inadequate animal feed resources as one of the important challenges of Ethiopian dairy sector was also reported by Yilma *et al* (2011).

5.2. Major reproductive health problems

In the present study 30.12% (n=156) of dairy cattle in the study area were affected by either one or more reproductive disorders based on questionnaires and follow up study (Table 3). This is in close agreement with 31.76% (n=128) which was reported by Gizaw *et al* (2011) in Central Ethiopia. But it is higher than 18.5% which was reported by Hunduma (2013) in Assela town and 24.8% by Molalegne and Shiv (2011) in Bedelle for major reproductive problems, respectively. This variation in prevalence may be due to environmental factor, breeds of the animals and variation in management system that is applied in different dairy farms. In addition, it is lower than 44.3% which was report by Hadush *et al* (2013) in central Ethiopia and 43.3% by Adane *et al* (2014) in Southern Ethiopia. This difference might be due to sample size, production system, study methodology and breed of animals as well as environmental factors.

The higher prevalence of retained fetal membrane (10.01%) found in the present study is fairly agrees with 11.5% reported by Alemselem *et al* (2015) in and around Mekelle, but lower than 14.7% reported by Haile *et al* (2010) and 19.2% by Gashaw *et al* (2011). The variation in the incidence of RFM may be attributed to variations in predisposing factors

to which the animals are subjected to; among which include nutritional status and management such as lack of exercise. The prevalence rate of RFM in the current study could also be due to dystocia that accounted 3.5% of the problems, which is an important predisposing factor for occurrence of RFM (Table 5). Also might be due to mixed problems that accounted 5.2% of the problems, which is in closed agreement with Gashaw *et al* (2011) who reported 5.6% of mixed problems that might be predisposing factor for the occurrence of retained fetal membrane. These could be due to the interrelationship of reproductive problems as predisposing factors for each other.

Previous report on the prevalence of dystocia by Gashaw *et al* (2011) 3.8% in small holder dairy production system and their reproductive health problems in Jimma town fairly agrees to the prevalence of 3.5% obtained in this study. However, the current finding is lower than the prevalence of 7.75% reported by Dawit and Ahmed (2013) and higher than 2.9% Hadush *et al* (2013). This variation in the occurrence of dystocia may be due to the fact that it is influenced by the factors such as, age and parity of the dam as well as breed of the sire. Inseminating cows with semen collected from large sized bulls without taking into account the size and age of cows is an important factor in precipitating dystocia (Noakes, 1986). According to Anderson *et al* (1996) dystocia primary occurs among first calf heifers as a result of feto pelvic disproportion *i.e.*, because of calf size or pelvic dimension of dam. But, in the present study cows which gave more birth were susceptible to dystocia (Figure 5).

The prevalence of repeated breeding in the present study reveals 7.7% which agree with the finding of Mandefro and Negash (2014) who reported 7.29% in crossbred cow's/heifers in Mekelle city, Ethiopia. But, significantly higher than 3% prevalence rate reported by Bitew and Prasad (2011) from Bedelle, South West Ethiopia. Moreover, the incidence of repeat breeding is comparatively lower than the 13.08% and 21% reported by Adane *et al* (2014) and Alemselem *et al* (2015) respectively. High incidence of repeat breeding could be due to lack of nutrition, improper insemination and timing of AI and poor semen quality Esheti and Moges (2014). Repeat breeding can be caused by a number of factors including sub-fertile bulls, endocrine imbalance, malnutrition, reproductive tract infections and poor management practices such as wrong time of

insemination or faulty heat detection, inappropriate semen handling and insemination techniques (Arthur, 1989). Similarly, the highest record in cross breed heifers which might be due to inseminating at puberty period on the first sign of estrus and low hormonal secretion and due to the susceptibility to environmental influence, inherently lower fertility, more sensitive to housing and other management practices (Mandefro and Negash, 2014).

Also repeat breeding with regard to parity is 0.8% and 6.9% in primiparous and multiparous, respectively (figure 5). This finding agree with Wolfe (1993) who described that reproductive performance declined with increase of parity number of the female cattle. This effect of parity number on the prevalence of the repeat breeding is probably due to repeated exposure of the multipara genital tract to environmental factors, which indeed, caused uterine infections; also older cattle are not as such good in feed intake capacity. Hence the difference between the findings of the current study and previous reports may be attributed to the above-mentioned factors.

The prevalence rate of anestrous observed in this study 3.7% which is lower than (Adane *et al.*, 2014) who reported 10.26% in cross breed dairy cows in urban and per urban area of Hosanna, southern, Ethiopia. This prevalence rate is also lower than the prevalence indicated by Alemselem *et al* (2015) who reported a prevalence of 37.8% in crossbred dairy cattle in and around Mekele, Tigray, Ethiopia. This variation might be due to the age, faulty heat detection, and breed, environment, nutrition, and management system differences.

Abortion is one of the major causes of economic losses in the cattle industry. The prevalence rate of abortion in the present study was 7.5% which is a bit greater than the findings of Hadush *et al* (2013) who reported an abortion rate of 6.7% in Central Ethiopia. But lower than the 9.05%, 13.9% and 14.6% reported by Dawite and Ahmed (2013), Molalegn and Shiv (2011) and Hunduma (2013) respectively. The variation in the occurrence of abortion could be due to metabolic or hormonal abnormalities, nutritional deficiencies, trauma, toxicities, or infectious agents (Ortega-Mora, 2007; Givens, 2006).

Stillbirth in the study was occurring at the rate of 1.9% which is bit less than the findings of Haile *et al* (2010) who reported stillbirth rate of 2.8% in cross breed dairy cow under smallholding in Addis Ababa milk shed. But higher than 3.01% reported by Dawite and Ahmed (2013). Still births can occur due to forceful fetal extraction, hypocalcaemia and various pathogens.

The incidence of metritis in the present study was 0.6% and no inland published report available to compare this finding. It is significantly lower than the prevalence rate (8.7%) reported by Hadush *et al* (2013) in central Ethiopia and 28.5% reported by Amene (2006) in Alage dairy farm. Factors influencing the incidence of metritis may be retention of fetal membrane, negative energy balance, dystocia, and parity of cow had been reported to be associated with metritis (Giuliodori *et al.*, 2013). Other influencing factors for metritis may include unhygienic parturition, unwise handling of parturition, unhygienic AI and injury of uterus by AI guns during insemination.

Uterine prolapse represented a prevalence of 0.8% which agrees with the finding of Adane *et al* (2014) who reported 0.76%. But, higher than 0.43% and 0.56% reported by Dawite and Ahmed (2013) and Bitew and Prasad (2011) respectively. In addition, the prevalence rates of mixed problems in the present study was 5.2% which is similar with the finding of Gashaw *et al* (2011) who reported 5.6% in Jimma, but lower than 1.05% indicated by Simiret (2010). This variation could be due to inter relationship between of reproductive problems as predisposing factors for each other.

5.3. Association of Risk Factors with Reproductive Health Problems of Dairy Cattle

Present study indicated significant effect of parity number on the prevalence of postpartum disorders (Table 6) and (Figure 5). Multipara cows were more affected by reproductive disorders than the primipara as has also reported earlier by Dawit and Ahmed (2013) in Kombolcha, Northeast Ethiopia and Adane *et al* (2014) in urban and per urban area of Hosanna, Southern Ethiopia. Attributed this effect of parity number on the prevalence of the major reproductive disorders is probably due to repeated exposure of the multipara genital tract to environmental factors, which indeed, caused uterine infections. Erb and Martin (1980) found that uterine involution was significantly delayed as the parity increases and thereby the interval from calving to ovarian resumption was also prolonged. Those variations might be due to factors affecting reproduction in cows of greater parity. Aging cows and their reproductive systems probably involves more complex and permanent uterine changes than those cows with lower parities. Furthermore, an increase in parity not only brings reproductive problems but can also lead to serious locomotive, mammary and metabolic disorders (Hebert, 2011).

In the present study, the BCS significantly influenced the occurrence of reproductive diseases in crossbred dairy cows (Table 6). The lowest occurrence of reproductive diseases in cows with medium BCS and the highest occurrence of reproductive diseases in cows with low BCS were also reported by Getachew and Nibret (2014) which agree with the present finding. Since, body condition score is an arbitrary scale for estimating the amount of body fat in cows. BCS has a good reflection on the reproduction as well as reproductive disorders. Cows with good BCS conceived at a higher rate than did thin and over-conditioned ones. BCS at oestrus positively correlate with the conception rate (Jack *et al.*, 2010). In line with the present finding, influence of BCS on occurrence of various reproductive diseases was also reported elsewhere (Shamsuddin *et al.*, 2006a). The authors reported that cows with lower BCS had longer intervals from calving to first ovulation and less detected estrus. Similar reports are available on postpartum cows brought for first service (Siddiqui, 2008). Low body energy reserves of cows greatly increase the probability of suffering from metabolic disorders, reproductive failure, reduction in milk yield and delayed puberty (Montiel and Ahuja, 2005). Moreover, cows

with low BCS may have poor quality oocytes, which do not fertilize normally. Even if fertilized, oocytes of poor BCS cows often do not sustain development to term (Siddiqui *et al.*, 2002).

5.4. Reproductive performance of dairy cattle

5.4.1. Age at First Service and Age at First Calving

In the present study the means \pm SD of age at first serviced were 20.13 \pm 4.73 (Table 7). This was lower than the report of Wassie *et al* (2015) who found that AFS was 32.05 \pm 0.57 months for crossbred dairy cows in Bale zone of Oromia Regional State, Ethiopia. Also Zewdie (2010) and Belay (2012) reported a higher AFS than the current finding which is 27.5 months cross-bred dairy cows in the highlands and central rift valley of Ethiopia and 24.30 months for crossbred dairy cow in Jimma. The largest age recorded in these areas could have resulted from the low level of management and poor feeding of calves and heifers at the earlier stages, which consequently had reduced growth rate and delayed puberty.

The means \pm SD of age at first calving were 29.35 \pm 4.84 months for cross breed cattle in Bishoftu town. In the present study the average age at first calving lower than AFC of 41.16 \pm 0.56 months, which is reported by Wassie *et al* (2015) for crossbreed in Bale zone of Oromia Regional State, Ethiopia. Moreover, the mean result of age at first calving in the present study was lower than that of Hunduma (2012) in Assela, Nibret (2012) in Gonder and Kumar and Tkui (2014) in Mekelle who reported 34.8 \pm 4, 32.4 and 36.4 \pm 17 months, respectively for crossbred cows. The high age at first calving observed here may be related to environmental conditions and husbandry practices which may effect on the cattle growth. These may regard growth rate, delay puberty, reduced fertility and conception thus, the high age at first calving of the imported breed.

5.4.2. *Calving Interval, Days Open, Number of Service per Conception and Calving to conception interval*

In the study presented here in, DO was 114.07 ± 66.13 days which is lower than 176.8 reported by Lemma and Kebede (2011) from Addis Ababa. However, the current finding for days open was longer than the reported by Hunduma (2012) 85.6 days from Asella town and in Gondar town 93.11 days (Niraj *et al.*, 2014). The longer variations might be due to differences in breeding management, health care and attributed to inadequate nutrition particularly energy supply through the concentrates. The major nutritional factor decreasing reproductive efficiency of high yielding dairy cows resulted by negative energy balance that induces a delay in first ovulation after calving (or a low oocyte quality) and increase in embryo mortality incidence with interval from calving to conception that increases over 120-130 days (Rossi *et al.*, 2008). The reproductive performance of cattle, particularly the probability of conception, may be negatively associated with the magnitude and duration of negative energy balance in early lactation (Walsh *et al.*, 2011).

The overall mean value for the number of services preconception (NSC) of cross breed in the current finding was 1.75 which is in agreement with the results of Gebeyehu *et al* (2007) (1.720 ± 0.056) or by Yifat *et al* (2009) (1.67). But it is higher than over all mean value for service per conception reported by Nibret (2012) (1.3 ± 0.6) in crossbred dairy cows in urban and peri-urban areas of Gondar and (Hunduma, 2012) (1.52 ± 0.9) in crossbred dairy cows in Assella. Two and more number of services per conception might be due to lack of semen quality, insufficient nutrition, reproductive disorder, use of improper inseminating techniques, the presence of repeat breeders in the herd and problems of heat detection.

The mean CCI estimated for crossbred dairy cows in the present study was 130.21 ± 72.09 days. It is lower than 219 and 176 days of dairy cow in and around Addis Ababa reported by Lemma and Kebede (2011) and Ali *et al* (2013) who reported 257 days respectively. The difference value of CCI at the present and other reports may be attributed to

management problems such as nutrition and breeding practice. Inadequate nutrition interferes with the resumption of ovarian activity and CCI by impairing the release of luteinizing hormone which consequently delays the occurrence of ovulation (Bolanos *et al.*, 1996).

Calving interval is one of the major components of reproductive performance that influences livestock production system. The means \pm SD for CI was 13.36 \pm 2.41 months for cross breed cattle in Bishoftu town. Results obtained in the current study were comparable with the 13.6 months reported by Yifat *et al* (2009) in Zway for cross breed dairy cows. However, the mean calving interval observed in this study is shorter than 17.8 months reported by Emebet and Zeleke (2007) in Dire-Dawa and 562 days (18.7 months) reported by Amene *et al* (2011). These differences might be due to differences in herds, breeding management, feeding regimes, poor management practices and other environmental stress that could affect the animals return to estrus, heat detection, serving and conception.

5.5. Effect of Major Reproductive Health Problem on Reproductive Performance of Dairy Cow

Approximately 30.12% of the cows showed one or more concurrent reproductive disorders during parturition and *post-partum*, for each reproductive trait measured there were highly significant differences between cows with reproductive abnormalities and those with normal once. This finding agrees with previous results reports by Lema *et al* (2001, Shiferaw *et al* (2005). Reproductive disorders have been found to be a major reason for decreased reproductive efficiency and of lifetime productivity (De-Vecchio *et al.*, 1992). Differences in prevalence in reproductive disorders in different regions may reflect variations in predisposing factors including nutritional status and management (Gashaw *et al.*, 2011).

5.6. Sero-prevalence of Brucellosis and association with risk factors.

The present study revealed that the overall sero-prevalence of bovine brucellosis was 4.12% (Table 11). This finding agrees with the 3.1% and 4.3% reported by Ibrahim *et al* (2010) and Tibesso *et al* (2014) respectively. But lower than the finding of Megersa *et al* (2012) who reported 8.0% and Hunduma and Regassa (2009) who reported 11.2% in pastoral and agro-pastoral areas of East Showa zone, Oromia Regional State, Ethiopia. However, most of these reports were from the extensively managed herds, where cattle from several owners mingle at grazing or watering points.

The present study revealed that the incidence of brucellosis to abortion was 10.2 %. This result higher than (Yayeh., 2003) who reported the prevalence of abortion 6.7% in North Gondar, Ethiopia and (Teferi *et al.*, 2011) who reported the prevalence of abortion was 8.7 % in selected Arsi zone's of Oromia Reginal state, Ethiopia. But lower than 12 % reported by Amin *et al* (2013). This difference in prevalence rate may be due to sample size, study methodology and variation in cattle husbandry management system.

There was statistically significant association ($P < 0.05$) between abortion period and sero-positivity of brucellosis in the present study (Table 11). This could be explained by the presence of higher Sero-positivity in cows in the last trimester may be due to the preferential localization of *Brucella* in the uterus in which allantoic fluid factors such as erythritol could stimulate the growth of *Brucella* and elevate in the placenta and fetal fluid from about the 5th month of gestation (Coetzer and Tustin, 2004; Radostits *et al.*, 2007).

6. CONCLUSION AND RECOMMENDATIONS

The ultimate goal in each herd should be lower calving interval, decrease the number of service per conception thereby increasing heard production. But reproductive health problems such us; retained fetal membrane, repeat breeder, abortion, anestrous, and dystocia were the major causes of low reproductive performance of dairy cows. The Possible risk factors responsible for the occurrence of reproductive health problems identified in this study include body condition and parity. Moreover, the study showed high prevalence of abortion and retained placenta together with high incidence of brucellosis in Bishoftu town. The present study showed that Calving to First Service Interval (CFSI), days open, number of service preconception and Calving Interval (CI) was below the standard. Moreover, the negative effects of preparturiat and post-partum reproductive problems on reproductive performance of cows were marked. Abortion as a potential risk factor that strongly associated with the prevalence rate of brucellosis in Bishoftu dairy farms. However, less association of RFM with the prevalence rate of brucellosis suggest the presence of other causes of reproductive disease in addition to Brucella.

In light of the above conclusion, the following recommendations are forwarded;

- Proper management and feeding is very important to control the reproductive problems as found in this study that those cows having low and high body conditions were affected to some extent by reproductive health problems.
- Other causes of abortion and retained fatal membrane of the cows should be studied in the study area.
- Further detailed studies on the major reproductive health disorders in the area should be carried out.

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8. APPENDICES

Appendix 1: Body condition scoring system

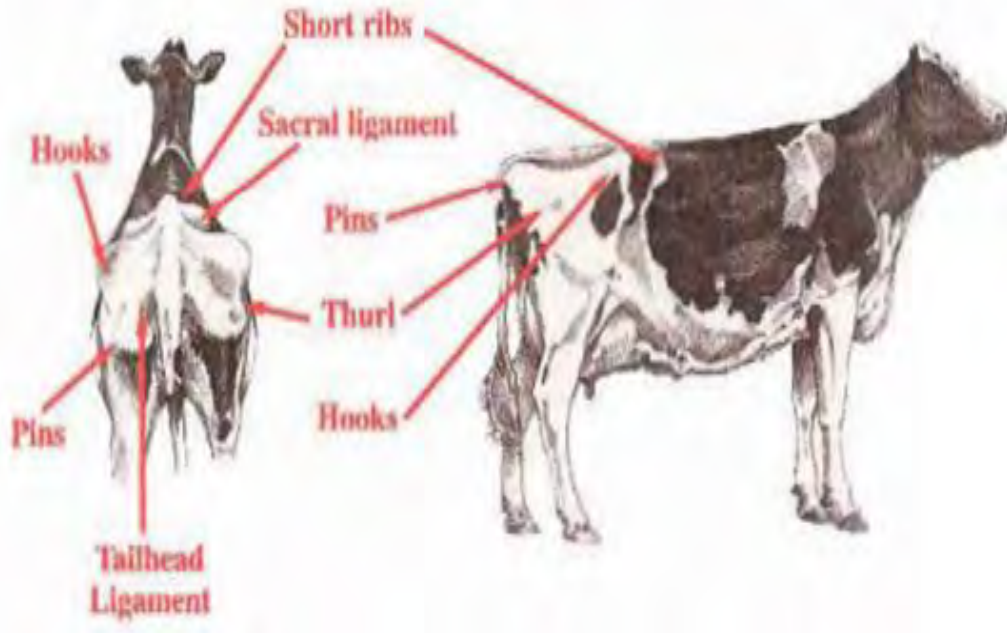


Figure 6: Anatomical regions used for determining BCS

Score of 1 (very poor body condition)

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

Body condition score 2 (poor body condition)

- Individual short ribs can be felt but are not prominent.
- Ends of ribs are sharp to the touch but have a thicker covering of flesh.

- Short ribs do not have as distinct an ‘overhanging shelf’ effect.
- Individual bones in the chine, loin, and rump regions are not visually distinct but are easily distinguished by touch.
- Hook and pin bones are prominent, but the depression between them is less severe.
- Area below tail head and between pin bone is somewhat depressed, but the bony structure has some covering of flesh.

Body condition scoring 3 (good body condition)

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

Body condition score 4 (fat)

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

Score of 5 (very fat)

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

Appendix 2: Rose Bengal Plate Test

Procedure

Sera (control and test sera) and antigen for use were left at room temperature for half an hour before testing, since active materials straight from the refrigerator react poorly

1.30 µl serum was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.

2. The antigen and serum were mixed thoroughly using an applicator stick (a stick being used only once)

3. Rock plate by hand for about 4 minutes

4. Examine for agglutination in a good light

5. Use magnifying glass when micro agglutination suspected

Interpretation

0 = no agglutination

+ = barely perceptible

++ = fine agglutination, some clearing

+++ = coarse clumping, definite clearing

Those samples identified with no agglutination were recorded as negative those with +, ++,

+++ , ++++ were recorded as positive.

Appendix 3: Complement Fixation Test

Procedure

1. Test sera and appropriate working standards are diluted with an equal volume of veronal buffered saline in small tubes and incubated at 58°C for 50 minutes in order to inactivate the native complement.

2. Using standard 96-well U-bottom microtitre plates, 25 µl volumes of diluted test serum are placed in the wells of the first and second rows, and 25 µl volumes of veronal buffered saline are added to all wells except those of the first row.

3. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the second row onwards continuing for at least four dilutions.

4. Repeat steps ii and iii above for each serum to act as anticomplementary serum controls (see below).
5. Volumes (25 μ l) of complement at 1.25 MHD, are added to each well and 25 μ l of antigen, diluted to working strength, are added to all wells excluding those of the anticomplementary controls. These latter wells receive 25 μ l of veronal buffered saline instead.
6. Control wells containing: diluent only, negative serum + complement + diluent, antigen + complement + diluent, and complement + diluent, are set up to contain 75 μ l total volume in each case.
7. The plates are incubated at 37°C for 30 minutes with agitation at least for the initial 10 minutes, or at 4°C for 14- 18 hours.
8. Volumes (25 μ l) of sensitised SRBC suspension are added to each well, and the plates are reincubated at 37°C for 30 minutes with agitation at least for the first 10 minutes.
9. The results are read after the plates have been left to stand at 4°C for up to 1 hour to allow unlysed cells to settle.

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative.

Appendix 4: Questioner survey format

Pre-screening questionnaire to: livestock owners'/farm managers to study major reproductive health problems, their effect on reproductive performance and prevalence of brucellosis in dairy cows in and around Bishoftu town.

Appendix 5: Questioner survey format

Pre-screening questionnaire to: livestock owners'/farm managers to study major reproductive health problems, their effect on reproductive performance and association with brucellosis in dairy cows in Bishoftu town, Ethiopia.

I. GENERAL INFORMATION OF QUESTIONER SURVEY

1. Name of the farm or farm owner _____
2. Address of the farm: _____; phone no. _____
3. Educational qualification:
None secondary education
Basic education Diploma and above
primary education
4. When was the farm established? 1-5 years 6-10 years >10 years
5. Type of farm: intensive semi-intensive extensive
6. Number of cattle in the farm (owned)? _____
 - a. No of cows
 - b. No. of heifers
 - c. No of Bulls
 - d. No. of calves
7. Type of feed cows or heifers are fed:
Hay Straw Concentrate Concentrate and hay or straw
8. Do animals get water regularly? _____; How many times a day? _____

II. ANIMAL INFORMATION

9. Cow /heifer name/ID _____
10. Date of birth _____
11. Age years: >3yrs, 3-6yrs, 6-9
12. Breed: local
Exotic
Cross
13. Parity (the complete #, alive and dead ones) _____
14. Sire's name/ID _____ Breed _____
15. Dam's name/ID _____ Breed _____

16. BCS _____

III. REPRODUCTIVE PERFORMANCE OF INDIVIDUAL DAIRY COW

17. Was she born in your herd or did you get her from somewhere else? _____

18. If born in herd, when was she born? (give date as " year/season ") _____

19. If acquired, when did you get her? (give date as " year/season ") _____

Did you buy her; get her as gift or as dowry?

Was she a calf, heifer or adult at that time?

How old was she at that time?

Why did you get/buy her?

20. At what age did your cow/heifer reach for mating? (age at puberty/AFS)

21. What was the age of your cow when she gave birth to the first calf?

(AFC) _____

a. **Birth date**.....

b. **First calving date**.....

22. Date/ month/ year of **pervious** calving? _____

23. Date/ month/ year of **recent** calving? _____

24. Intervals from calving to first service? _____

25. Intervals from calving to conception? _____

26. Number of service per conception (how many times did you take the cow /heifer for service before got pregnant last time) _____

27. What is the number of pregnant cows/ heifers in the farm?

A) Cows: _____ (trimester: _____ 1st; _____ 2nd; _____; 3rd)

B) Heifers: _____ (trimester: _____ 1st; _____ 2nd; _____; 3rd)

28. Method of insemination? Natural _____ AI _____ Both _____

29. Number of animals in estrous now? A) Cows: _____; B) Heifers: _____

30. How many lactating cows do you have? _____

IV. REPRODUCTIVE PROBLEMS ENCOUNTERED ON INDIVIDUAL DAIRY COW

31. What is the number of anestrus animals in the farm? A) Cows ____;
B) Heifers: ____
32. Were any of your cattle encountered reproductive diseases or problems in the past?
YES NO
33. If yes, what reproductive diseases or problems were observed?

34. Did any of your cows suffer from abortion? YES NO
35. If yes, what month of the pregnancy abortion was occurred?
a. before 3 months c. over 6 months
b. 3 to 6 months d. a, b and c
36. Did any of your cows encounter pregnancy overdue in the past?
YES NO
37. If yes, how many months was the cow pregnant before it was given veterinary assistance?

- Number of cows diagnosed positive? _____
38. Did any of your cows or heifers give birth to a dead calve at a normal gestation length (9 months) in the past?
YES NO
If yes, how many cows? _____
39. Did any of your cows suffer from dystocia in the past? _____ YSE NO
If yes, how many of your cows suffered from dystocia? _____
40. Did any of your cows suffer from Metritis in the past? _____ YSE NO
If yes, how many of your cows suffered from Metritis? _____
41. Did any of your cows or heifers suffer from vaginal, uterine prolapse in the past?
YSE NO

If yes, number of cows or heifers with vaginal prolapse? _____;
uterine prolapse? _____

42. Did any of your cows encounter retained fetal membranes in the past?

How did you solve the problem?

43. Have any of your cows or heifers been repeater (repeat breeder)?

If yes, what do you think the cause/s for the animal to come back in to estrus again?

What measure did/do you take to get the animal pregnant?

44. Did any of your animals come to estrus, but missed insemination or mating in the past?

YES _____ NO _____

If yes, why was the animal not inseminated or mated?

- a. Lack of bull
- b. Lack of AI technician
- c. Lack of means of communication to AI technician
- d. Called AI technician, but did not come to inseminate or did come very late
- e. Reproductive tract problem
- f. Size of the animal was very small to breed
- g. Lack of nutrition
- h. All or some of the above causes

45. Did any of your animals suffer from any health problem other than reproductive health?

If yes, please describe the disease and signs the animals showed.

46. What is your future plan to improve the reproductive performance of your animals?

47. What do you think the government (agriculture, universities, etc.) and private sectors should do to support improve fertility of your animals?

48. Do you have any comment? _____

Appendix 5: Survey and Blood sample collection



Annex 5: Cow with poor good body condition



Appendix 6: Animals managed extensively



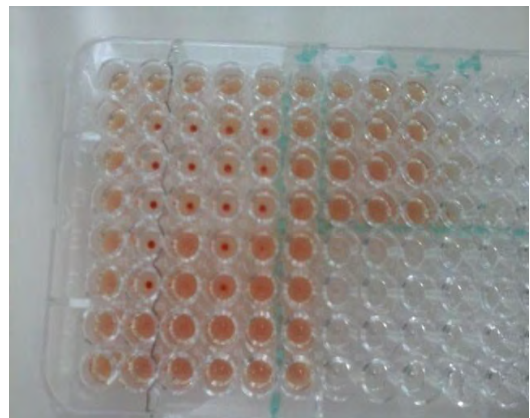
Appendix 7: Pregnancy diagnosis during the study period



Appendix 8: Serological and bacteriological result pictures



Rose Bengal Plate Test



CFT Result of Brucellosis positive