

# Assessment of major reproductive health problems, their effect on reproductive performances and association with brucellosis in dairy cows in Bishoftu town, Ethiopia

## Abstract

A cross sectional study was conducted in Bishoftu town from November 2015 to May 2016 to investigate major reproductive health problems in dairy cows. From a total of 519 crossbred dairy cows, 30.1% (n=156) were found to be affected by at least by one reproductive health problems. Hence, retained fetal membrane, repeat breeder, abortion, anestrus and dystocia were found to be the major reproductive health problems containing 10%, 8%, 8%, 7% and 4%, respectively. Other reproductive health problems observed with lower prevalence include still birth, uterine prolapse, mixed and clinical metritis accounting for 2%, 1%, 5% and 1%, respectively. The effect of body condition on the prevalence of major reproductive disorders increased from good to poor body conditioned animals with prevalence of 4.2% and 13.1%, respectively. On the other hand, the number of parity on the prevalence of major reproductive disorders has a significant ( $P<0.05$ ) influence for the occurrence of reproductive problems and the effect increased progressively from pri-miparous to multi-parous with the prevalence of 4% and 26% respectively. Furthermore, there was significantly higher ( $p<0.05$ ) incidence of retained placenta, repeat breeding and abortion recorded in multiparous than in primiparous cattle. Incidence of overall reproductive problems decreased from heifer to older cows with prevalence of 14.3% and 0.4% respectively. 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. The overall mean±S.D of ages at first service and ages at first calving were 20.3±5.1 and 29.4±5months respectively. This study also indicated that the overall mean±S.D of calving interval, days open, calving to conception interval and number of services per conception were, 13.4±2.4months and 114±7, 130.2±71.8days, and 1.8±0.1 respectively. Two hundred ninety one sera samples were collected and Rose Bengal Plate Test (RBPT) was used as a screening test for brucellosis. RBPT showed that 15 (5.2%) of the tested samples were screened as positive for brucellosis. From 15 RBPT positive sera 12 (4.1%) were also confirmed to be positive by complement fixation test (CFT). From the total study animals 39(8.7%) and 52(18.3) were recorded with abortion and retained fetal membrane, respectively. Abortion was found to be significantly associated with sero-positivity of brucellosis in the present study ( $p<0.05$ ). This study showed that reproductive disorders highly affected the reproductive performance of dairy cows.

**Keywords:** bishoftu, crossbred dairy cattle, reproductive performance, reproductive problems

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**Abbreviations:** AAU, addis ababa university; AFC, age at first calving; AFS, age at first service; CFT, complement fixation test; CI, calving interval; CVMA, college of veterinary medicine and agriculture; NSC, number of services per conception; PGF2 $\alpha$ , prostaglandin f2 alpha; PPA, post-partum anoestrus; RBPT, rose bengal plate test; RP, retained placenta

## Introduction

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.<sup>1</sup> So, high reproductive efficiency is necessary for a successful dairy operation and requires a calving interval that maximizes milk production within the herd.<sup>2</sup> Good estrus

detection, good insemination technique, quality semen, and a healthy uterine environment are critical components of high reproductive efficiency.<sup>3</sup> 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.<sup>4,5</sup> 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.<sup>6</sup> In addition to constraints of low production; it continues to cause heavy economic losses

and public health concern throughout the world.<sup>7</sup> In Ethiopia, the differences in management (production) systems and environmental conditions under which cattle are maintained could greatly affect the occurrence of reproductive disorders.<sup>8</sup> Bishoftu 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.<sup>9</sup>

The livestock population of DebreZeit registered cattle 1,147,173 from this cattle population 513,039 is female cows.<sup>10</sup> Although, there are enough dairy farms in and around Bishoftu, ample studies have not been conducted on the major reproductive health disorders in dairy animals.<sup>11</sup> 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 Bishoftu town.

## Materials and methods

### Description of the study area

This study was conducted in Bishoftu town which is located at the distance of 45km 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.<sup>12</sup>

### 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. The number of farms sampled for survey studies was determined as follow:<sup>13</sup>

- i.  $N=0.25/SE^2$ ,
- ii.  $N=$  sample size,
- iii.  $SE=$ 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.

### 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. 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 prolapsed, anestrus, repeat breeding, management systems and parity. Body condition score was based on the criteria adopted by Matthew MR<sup>14</sup>

- a) In BSC 1 (very poor body condition) individual short ribs have a thin covering of flesh,
- b) In BCS 2 (poor body condition), individual short ribs can be felt but are not prominent,
- c) In BCS 3 (good body condition), ends of short ribs can be felt by applying slight pressure,
- d) In BCS 4 (fat), individual short ribs are distinguishable only by firm palpation,
- e) In BCS 5 (very fat), bony structures of backbone, short ribs, and hook and pin bones are not apparent.

Subcutaneous fat deposit very evident. Animals were grouped in to those having the major reproductive problems and those without these problems. 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 2years 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 2years 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 24h and if any abnormal vaginal discharge occurred without regular frequency of visit. Sample collection and laboratory tests Blood samples (5ml) were collected from the jugular vein of each animal, using sterile needles and plain vacuoner tubes. The blood samples were allowed to stand overnight at room temperature and centrifuged at 1500×g for 10min. to obtain the serum. Sera were decanted into cryovials, and were stored at-20°C in college of veterinary medicine and agriculture, Addis Ababa University (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 Centre (NAHDIC), Sebeta, Ethiopia.

### 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).

## Results

### Major reproductive health problems

From a total of 519 dairy cows included in the study period

30.1%(n=156) were found to be affected at least by one reproductive health problems. Overall prevalence of reproductive health problems cited by farmers/attendants or veterinarians in crossbred dairy cattle on different method of study is presented in Table 1. 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%, 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 2). 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 (Table 3). 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 4.2% and 13.1% respectively, (Table 3). 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 3.7% and 26.2% respectively. Furthermore, there was significantly higher ( $P<0.05$ ) incidence of retained placenta, repeat breeding and abortion was recorded in multiparous than in primi-parous cattle. Incidence of overall reproductive problems decreased from heifer to older cows with prevalence of 14.3% and 0.4% respectively. Age was not significantly affected within the different age groups ( $P$ -value=0.2291,  $\chi^2=1.5$ ).

**Table 1** Reproductive disorder 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	26.50%
Observational study	109	19	3.70%
Total	519	156	30.10%

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. 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 Tables 3 & 4. The data indicated that the overall mean  $\pm$  S.D of ages at first service and ages at first calving were 20.3 $\pm$ 5.1 and 29.4 $\pm$ 5 months respectively. Variables such as body condition score and age has no statistical significant influence on age at first service and age at first calving. 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 $\pm$ 2.4 months

and 113.9 $\pm$ 66, 130.2 $\pm$ 71.8 days, and 1.8 $\pm$ 0.1 respectively. According to the present research finding variables such as age and body condition score has statistical significant influence  $p<0.05$  on days open and number of service preconception. Old age cows had longer calving interval and days open than others. Besides, those who have poor body condition score have longer number of service preconception than from that of medium and good once (Table 4).

**Table 2** 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(3.7%)	52 (10%)
Repeat breeding	33(8.04%)	7(6.4%)	40 (7.7%)
Abortion	34(8.3%)	5(4.6%)	39 (7.5%)
Anoestrus	17(4.1%)	2(1.8%)	19 (3.7%)
Dystocia	17(4.1%)	1(0.9%)	18 (3.5%)
Still birth	10(2.4%)	0(0%)	10 (1.9%)
Uterine prolapse	4(1%)	0(0%)	4 (0.8%)
Clinical metritis	3(0.7%)	0(0%)	3 (0.6%)
Mixed disorder	26(96.3%)	1(3.7%)	27(5.2%)

\*Mixed problems include abortion and retained placenta, anoestrus 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)

Each performance trait measured was adversely affected by reproductive abnormalities of cows. Abnormal cows had longer days open, calving to conception interval and required more service per conception that 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. Among 291 tested samples, 15(5.2%) were found positive by RBPT and 12 (4.1%) were found positive by CFT. Therefore, in the present study, an overall seroprevalence was estimated to be 4.1% by CFT. In the present study, abortion, repeat breeder and retained fetal membrane were observed as some major reproductive health problems with the prevalence of 10%, 7.7% and 7.5% respectively. 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 seropositive of brucellosis. But a history of previous retain fetal membrane had not been significantly related to seropositive of brucellosis. 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 6.8

(Table 5). 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 2.1. 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.

**Table 3** Influence of different risk factors on major reproductive problems in the study area

Variables	AFS(m)			AFC(m)		
	No	Mean±SD	P-value	No	Mean±SD	P-value
<b>BCS</b>						
Poor	122	19.4±0.5	0.544	122	28.8±0.4	0.464
Medium	306	20.5±0.3		304	29.6±0.3	
Good	91	19.9±0.5		91	29.2±0.5	
<b>Age</b>						
2-4years	276	20.4±0.3	0.73	274	29.5±0.3	0.181
5-8years	231	19.8±0.8		231	29.1±0.3	
>8years	12	20.9±1.7		12	31.1±1.9	
<b>Overall</b>	<b>20.27±5</b>			<b>29.38±5</b>		

**Table 4** Calving interval, days open, number of services per conception and calving to conception interval of dairy cows in the study area

Variables	CI(m)		DO(d)			NSC			CCI(d)			
	No	Mean±sd	p-value	No	Mean±sd	p-value	No	Mean±sd	p-value	No	Mean±sd	p-value
<b>Parity</b>												
Primiparous	-	-		9	-		36	-		7	-	
Multiparous	483	13.36±2.41		483	113.61±66.5	-	483	1.78±0.08	-	483	129.83±72.39	-
<b>BCS</b>												
Poor	117	13.84±2.53	0.071	118	116.82±59.15	0.935	122	2.3±1.12	0	117	144.56±75.40	0.055
Medium	281	13.27±2.6		288	115.33±75.88		306	1.56±0.82		287	127.14±77.65	
Good	85	13.02±1.23		86	106.04±30.42		91	1.69±0.86		86	120.94±38.07	
<b>Age</b>												
2-4years	240	13.14±1.95	0.006	249	109.78±57.17	0.002	276	1.64±0.95	0.076	247	123.61±5.71	0.174
5-8years	231	13.55±2.6		231	117.38±70.34		231	1.9±0.18		231	135.80±77.91	
>8years	12	14.33±5.36		12	142.5±128.63		12	1.75±0.48		12	158.25±156.63	
Overall		13.42±2.40			113.90±66.01			1.75±0.07			130.17±71.83	

M, month; D, day; CI, calving interval; DO, days open; NSC, number of service preconception; CCI, calving to conception interval

**Table 5** Association of RFM and abortion with brucellosis positivity

Clinical signs	N	Prevalence(N)	Confidence interval	OR	P-value
<b>Retain fetal membrane</b>					
Yes	52(10%)	3.8%(2)	0.4-8.6	1.828	0.444
No	467(90%)				
<b>Abortion</b>					
Yes	39(7.5%)	10.2%(4)	1.9-23.5	6.74	0.003**
No	480(92.5%)				

Table Continued...

Clinical signs	N	Prevalence(N)	Confidence interval	OR	P-value
<b>Abortion period</b>					
First trimester	8(1.5%)	0(0)			
Second trimester	18(3.5%)	0(0)			
Not applicable	480(92.5%)	0(0)	1.6-4.172.6		0.000**
Third trimester	13(2.5%)	30.7%(4)			

N, number of observations; OR=odds ratio

## Discussion

In the present study around 30% of dairy cattle were affected by either one or more reproductive disorders. This rate is in the range of 18 to 45% previously described for this kind of pathology in Ethiopia.<sup>15-19</sup> Variations in prevalence may be due to sample size, study methodology, environmental factors, breeds of the animals and variation in management system that is applied in different dairy farms. Retained fetal membrane was found to be 10%, fairly agrees with 11.5% reported by Alemselem B et al.<sup>20</sup> but lower than 14.7% reported by Haile A et al.<sup>21</sup> and 19.2% by Gashaw A.<sup>22</sup> Cows which gave more birth were susceptible to dystocia. 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.<sup>12,23</sup> According to Esheti G<sup>24</sup> 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. The prevalence of repeated breeding revealed 7.7% which agree with the finding of<sup>24</sup> who reported 7.3% in crossbred cows/heifers. Moreover, the incidence of repeat breeding is comparatively lower than the 13.1% and 21% reported by Adane H<sup>15</sup> Alemselem B et al.<sup>20</sup> respectively. High incidence of repeat breeding could be due to lack of nutrition, improper insemination and timing of AI and poor semen quality.<sup>25</sup> Abortion was 7.5% which is a bit greater than the findings of<sup>17</sup> 6.7% in Central Ethiopia. But, lower than 9.1%, 13.9% and 14.6% reported by Hunduma D,<sup>18</sup> Molalegne B,<sup>19</sup> Dawite and Ahmed<sup>26</sup> respectively. The variation could be due to metabolic or hormonal abnormalities, nutritional deficiencies, trauma, toxicities, or infectious agents.<sup>27,28</sup>

Stillbirth was occurring at the rate of 1.9% which is bit less than the findings of<sup>21</sup> 2.8% in Addis Ababa milk shed. But, higher than 3% reported by Dawite and Ahmed<sup>26</sup> Still births can occur due to forceful fetal extraction, hypocalcaemia and various pathogens. Multipara cows were more affected by reproductive disorders than the primipara as has also reported earlier by Dawite and Ahmed<sup>26</sup> in Kombolcha and Adane H<sup>15</sup> Hosanna. Uterine involution was significantly delayed as the parity increases and thereby the interval from calving to ovarian resumption was also prolonged.<sup>29</sup> Furthermore, an increase in parity not only brings reproductive problems but can also lead to serious locomotive, mammary and metabolic disorders.<sup>30</sup> BCS significantly influenced the occurrence of reproductive diseases in crossbred dairy cows, lowest in medium BCS and highest in low BCS also reported by Getachew E.<sup>11</sup> The means  $\pm$  SD (10months to 37months) age at first (AFS) serviced were 20.1 $\pm$ 4.7. This was lower than the report of<sup>31</sup> 32.1 $\pm$ 0.6months. A higher AFS also been reported than the current finding 27.5months and 24.3months respectively.<sup>32</sup> This could be because of 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 (19months to 52months)

of AFS were 29.4 $\pm$ 4.8months in Bishoftu town. In the present study, the average AFS was lower than AFC of 41.2 $\pm$ 0.6 months, which is reported by Belay D.<sup>32</sup>

Moreover, the mean result of AFS in the present study was lower than that of<sup>8,33,34</sup> 34.8 $\pm$ 4, 32.4 and 36.4 $\pm$ 17months, respectively. The high AFS observed here may be related to environmental conditions and husbandry practices which may effect on the cattle growth. Days open (DO) was 114.1 $\pm$ 66.1days which is longer than the findings of<sup>18</sup> 85.6days and<sup>35</sup> 93.1days. This 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 120-130days.<sup>4</sup> 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.<sup>36</sup> The overall mean value for the number of services preconception (NSC) was 1.8 which is in agreement with the results of (1.7 $\pm$ 0.1).<sup>37</sup> However, higher than<sup>33</sup> (1.3  $\pm$ 0.6) and<sup>38</sup> (1.5 $\pm$ 0.9). Two and more number service 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 was 130.2 $\pm$ 72.1days. It is lower than<sup>39</sup> 257days. The differences 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.<sup>40</sup> The overall sero-prevalence of bovine brucellosis was 4.1%, which is agreed with 3.1% and 4.3% reported by Ibrahim N<sup>41</sup> and Tibesso G<sup>42</sup> respectively. But lower than<sup>43</sup> 8% and<sup>38</sup> 11.2%. The incidence of brucellosis to abortion was 10.2% which is higher than<sup>44</sup> 8.7%, however, lower than<sup>45</sup> 12%. 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 (<0.05) between abortion period and sero-positivity of brucellosis. 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.<sup>46,47</sup>

## Conclusion

Body condition, parity and high prevalence of abortion and retained

placenta together with high incidence of brucellosis were the possible risk factors for major causes of low reproductive performances of dairy cows in Bishoftu town, Ethiopia.

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## Conflict of interest

Authors declare there is any conflict of interest.

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