Contributing factors to repeat breeding and post-partum anestrus and pregnancy rate subsequent to hormonal intervention in crossbred dairy cows

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Abstract

A cross-sectional study was conducted from January to July 2021 to determine the prevalence and contributing factors to repeat breeding (RB) and postpartum anestrus (PPA) in crossbred dairy cattle in the central highland of Ethiopia. Furthermore; a retrospective study was used to collect data on the occurrence of RB and PPA and the associated risk factors. The pregnancy rate was determined by rectal palpation on day 60 after hormonal therapy using double $PGF_{2\alpha}$ and GnRH in combination with $PGF_{2\alpha.}$ (Ovsynch). The prevalence of RB and PPA was 33.85% and 30.73%, respectively. Body condition score, parity, milk yield, herd size, abortion, and mastitis all revealed a statistically significant association ($p < 0.05$) with the prevalence of RB and PPA. Cows with a previous history of abortion were 2.58 times at risk of repeat breeding than those without. Similarly, cows with a previous history of mastitis were 2.63 times more at risk of RB than cows that didn't encounter the disease. Previously aborted and older cows with greater parity numbers were 2.89 and 1.23 times more affected by PPA, respectively. Moreover, endometritis and retained fetal membrane showed significant association ($p<0.05$) with postpartum anestrus. The pregnancy rates in cows treated with double $PGF_{2\alpha}$ and GnRH + $PGF_{2\alpha}$ (Ovsynch) were 41.46% and 29.17%, respectively. It is concluded that the occurrence of RB and PPA in the research area was influenced by parity, body condition, and daily milk yield of the cow. Reproductive health problems including abortion retained fetal membranes, endometritis, and mastitis affected the prevalence of RB and PPA. Although the use of double $PGF_{2\alpha}$ or in combination with GnRH has resumed cyclicity in RB and PPA dairy cows, the pregnancy rates are yet low. Hence, a study that utilizes detailed hormonal assay profiles and reproductive-related blood metabolites should be done.

Keywords: Anestrus; dairy cow; GnRH; $PGF_{2\alpha}$; postpartum; repeat breeding.

Introduction

Reproductive inefficiency is one of the most costly and production-limiting factors facing the dairy industry. Dairy cattle reproductive and production efficiency in Ethiopia is lower than the average African levels (CSA, 2021). Limited genetic potential of dairy cows, inadequate nutrition, and prevailing animal diseases (Belay *et al.*, 2012) as well as reproductive problems (Hadush *et al.*, 2013) that are related to an ovarian malfunction (Cenariu and Jospe, 2017), fertilization failure, and embryonic mortality (Levine, 1999), are among the major limitations resulting in poor reproductive performance (Bartlett *et al.*, 1986; Ibrahim *et al.*, 2011). Repeat breeding (RB) and postpartum anestrus (PPA) are among the major causes of infertility in dairy cows (Dinka, 2013) resulting in economic losses, replacement costs as well as loss of genomic improvement.

Repeat breeding is a breeding problem that results in substantial financial losses for dairy producers due to lower conception rates and inadequate milk yield (Lafi *et al.,* 1992). The causes of RB are multifactorial (Sarder *et al.*, 2010; Nath *et al.*, 2014) that include managerial Asaduzzaman *et al.*(2016) and Malik *et al.*(2019), dietary and reproductive diseases and disorders including endometritis, ovarian cysts, and anovulation (Wodaje and Mekuria, 2016; Cenariu and Jospe, 2017). Poor estrus detection and untimely insemination also contribute to repeat breeding (Keskin *et al.*, 2010). Similarly, PPA is considered a reflection of faulty management, poor nutrition (Rhodes *et al.*, 2003; Montiel and Ahuja, 2005), chronic illnesses, or uterine and ovarian disorders (Opsomer *et al.*, 1996; Gundling *et al.*, 2012). In addition, the incidence of PPA is thought to be influenced by season, breed, age (McDougall, 1994; Mwaanga and Janowski, 2000), parity number, dystocia, and by extended effects from the previous pregnancy (Opsomer *et al.*, 2000).

Various hormonal interventions have been suggested to treat repeat breeding and postpartum anestrus, the potential causes of infertility (reduced or absent capacity to produce viable offspring) in female cattle (Tiwari *et al.,* 2019; Ambrose, 2021). The most commonly applied treatment option is to stimulate or induce estrus and ovulations using GnRH (Fricke *et al.*, 2003) or prostaglandins ($PGF_{2\alpha}$) or in combination with both hormones (Karki *et al*., 2018; Tiwari *et al*., 2019).

In Ethiopia, studies that have been conducted on reproductive problems in dairy cattle are fragmented and focused on identifying the major reproductive disorders (Mitku *et al*., 2012; Haile *et al*., 2014). However, the extent of occurrences of RB and PPA in crossed dairy cows is not yet well documented. Moreover, there were no reports related to the potential risk factors and hormonal intervention on PPA and RB in the study area. Though the study area belongs to the central highland of Ethiopia with a relatively large number of crossbred dairy cows and is one of the main milk sheds of the country that supply milk and milk products to Addis Ababa, the reproductive and productive efficiency of dairy cows remained low. Therefore, the current study was conducted to assess the prevalence, contributing risk factors, and pregnancy rate subsequent to hormonal intervention in RB and PPA dairy cows.

Material and methods

Study area

The research was conducted in Debre Berhan town and its surrounding from January to July 2021. The town is found in Amhara Regional State of North Shoa Zone, some 120 kilometers north of Addis Ababa, at 9^0 30 and 9^0 50 latitudes and 39^0 20 and 39^0 44 longitudes with an Altitude of 2,840 meters above sea level. The average annual temperature is 20 °C, 7°C, and 8.2°C during the day and at night, respectively with 964 milliliters of precipitation. The mean monthly temperature of the study area ranges from 2.8 to 21.9°C with a mean of 13.18°C, and the mean annual rainfall ranges from 698.5 to 1083.5mm with a mean annual of 920 mm (EMA, 2017).

According to the classification by Nish *et al.* (2018), extensive, semi-intensive, and intensive farms are prevalent in the study area. Cows in extensive management conditions were grazing and given straw as a supplement feeding and they were housed on a conventional floor without any additional facilities. Cows that were held in a semi-intensive system were provided with hay, straw, and some concentrate feeding; they were kept in farms with concrete floors. Cows in intensive farms were housed in a standard barn with a concrete floor and were provided with feed consisting of hay, and concentrate with vitamin and mineral mixture before and after calving, they were also fed brewery by-product (spent grain) and straw. Water in most cases was given ad libitum.

Study population

Dairy cows belonging to small-scale (extensive and semi-intensive) farmers and commercial (intensive) dairy farms keeping Zebu*HF crosses located in and around Debre Berhan were considered as the study population. The farms were selected based on the owners' willingness and accessibility to records. Cows that were in estrus for at least three cycles and have been inseminated but failed to conceive were classified as repeat breeders. Cows that have not experienced estrus for more than 60 days succeeding parturition were designated as postpartum anestrus.

Study design and method of data collection

In dairy farms with organized records, retrospective data were collected. In farms without a recorded history of cows, data was collected with a structured questionnaire. The collected information includes herd management-related (herd size, housing, and animal-related (previous calving history, age, parity, milk yield, retained fetal membrane, endometritis, mastitis, and abortion). To determine the nutritional status of the dairy cows, body condition was scored on a one-to-five scale according to Wildman *et al.* (1982). Accordingly, cows were categorized into body condition scores (BCS) of < 3 and ≥ 3.

The sample size required for this study was calculated using Thrusfield's (2005) method. The confidence interval was calculated to be 95 percent, the absolute precision was 5%, and the projected prevalence was estimated to be 50% because there were no comparable reports in the study area. The sample size was calculated as follows:

$\mathbf{n = 1.96^2 Pexp\ (1-Pexp)/\ d^2}$, where Pexp = expected prevalence; d= absolute precision; n =sample size.

Accordingly, the estimated sample size was 384 dairy cows. Animals were obtained by inquiring about 3 commercial farms and 164 smallholder farmers to collect data using a questionnaire survey.

Selection of repeat breeding and postpartum anestrous cows for hormone treatment

Cows identified as repeat breeders failed to conceive after three or more inseminations but were regularly cycling and were subjected to hormonal therapy. Similarly, cows that were identified as having postpartum anestrous were also subjected to hormonal therapy. Additionally, all cows in the treatment were properly evaluated to be free from any detectable malfunctions of reproductive organs, and free of reproductive diseases or disorders that are associated with dystocia, retained fetal membrane, or endometritis.

After selecting repeat breeder cows and those with postpartum anestrus, cows were categorized into two groups based on the presence or absence of CL. Further cows were evaluated for BCS (<3 or ≥3) and parity (≤3, 4-6 or ≥7). All dairy cows were also subjected to rectal palpation and trans-rectal ultrasonography to detect apparent reproductive abnormalities and the presence of the corpus luteum.

Treatment group I (double $PGF_{2\alpha}$**)**: The first group consists of dairy cows with CL (n=50) on either of the ovaries that received 2 ml of $PGF_{2\alpha}$ (Synchromate ®, Bremer, GMBH, Warburg, Germany) intramuscular on day zero (Figure 1). Estrus detection was started 24 h after the first $PGF_{2\alpha}$ administration and cows in estrus were inseminated 12-24 hrs after standing heat. If the cows failed to show estrus signs within five days of the first $PGF_{2\alpha}$ injection, a second $PGF_{2\alpha}$ was administered on day eleven, and insemination followed after 18 hrs of standing heat.

Figure1. Diagrammatic representation of double $PGF_{2\alpha}$ **protocol**

Treatment Group II (Ovsynch): All cows without detectable CL (n=24) at rectal palpation and ultrasonography were injected with 100 µg Gonadotropin Releasing Hormone (GnRH) (Vetoquinol, Canada) on day 0 (Figure 2) followed by $PGF_{2α}$ (Synchromate ®, Bremer, GMBH, Warburg, Germany) administration on day 7. The cows were then monitored for signs of estrus three times per day for two days. When cows were seen in estrus, two inseminations were performed; the second insemination was conducted 12 hrs later. This was done to improve pregnancy rates by minimizing mistakes in estrus detection and the disparity between insemination time and ovulation.

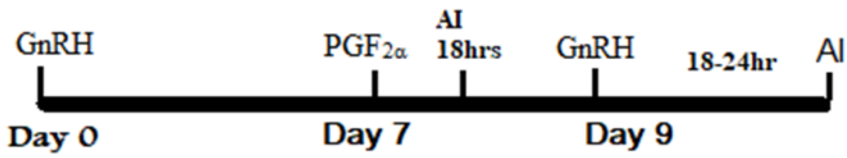

Figure 2. Diagrammatic representation of Ovsynch protocol

Dairy cows that did not come to heat/estrus within 48 hours of administration of $PGF_{2α}$ were given the second shot of 100 µg GnRH on day nine and were inseminated 19 hrs later without estrus detection (fixed time AI). In both groups, when cows were seen in estrus, two inseminations were performed; the second insemination was conducted 12 hrs later. This was done to improve pregnancy rates by minimizing mistakes in estrus detection and the disparity between insemination time and ovulation. To minimize individual variance due to inseminator skill, every cattle was inseminated by the same inseminator and with semen from the same batch of production from the Kality Livestock Development Institute (LDI).

Pregnancy diagnosis

To eliminate individual variance, pregnancy diagnosis was performed by the same professional after 60 days of insemination using rectal palpation, where the asymmetry of the uterine horns and CL on the ovary ipsilateral to the pregnancy and palpation of the allantoin-chorion (membrane slip) are very good indicators of pregnancy (ultrasonography was not used due to organizational limitations).

Data analysis

Statistical Package for Social Science (SPSS) software version 25 was used to sort, code, compute, and analyze the data that had been collected. Descriptive statistical analysis was used to calculate the prevalence of repeat breading and postpartum anestrous in percentages. The chi-square (X^2) test was used to determine the associations between risk factors with the prevalence of repeat breading and postpartum anestrous. Logistic regression was also employed to check the influence of the risk factors on the occurrences of RB and PPA, $p < 0.05$ was considered to declare a significant effect.

Results

Repeat Breeding (RB)

Out of 384 dairy cows that were included in the study, 130 (33.85%) were repeat breeders (RB). In cows with a previous history of repeat beading, the associated potential risk factors such as body condition score, parity number, and milk yield were statistically significant (p<0.05) as shown in Table 1.

Table 1. Association of cow-related risk factors with repeat breeding

Risk Factors	Categories	N (% Prevalence of RB)	X^2	P-value
BCS	≥3 <3	61 (22.93) 69 (25.94)	14.9336	0.001
Parity (number)	≤3 4-6 ≥7	105 (39.47) 22 (8.27) 3 (1.14)	20.4143	0.016
Daily milk yield (litters)	<10 L ≥10 L	33 (12.41) 97 (36.47)	49.2897	0.000

Among management-related variables; herd size revealed a significant association (p<0.05) with the prevalence of repeat breading (Table 2), whereas the production system has no statistically significant relationship (p>0.05) with repeat breading.

Table 2. Association of management-related factors with repeat breeding

Risk Factors	Categories	N (% Prevalence of RB)	X^2	P-value
Production system	Extensive Intensive Semi-intensive	40 (15.04) 24 (9.02) 66 (24.81)	3.6968	0.157
Herd Size	≤10 >10	106 (39.85) 24 (9.02)	17.2584	0.000

Among reproductive disorders and diseases; abortion and mastitis showed a strong association ($p<0.05$) with repeat breeding. Cows with an abortion history had 2.58 times more chance of becoming repeat breeder cattle than those that were not affected cows. Similarly, cows with mastitis exhibited 2.63 times more risk of becoming repeat breeder cattle (Table 3) than those that were not affected by mastitis. Dairy cows with lower BCS (<3), higher milk production, and smaller herd size had a statistically significant ($p<0.05$) association with the prevalence of repeat breeding (Table 3).

Table 3. Strength of association (logistic regression) between risk factors and repeat breeding

Risk Factors	Odds Ratio	p-value	[95% Conf. Interval]
BCS	0.36	0.000	0.22 - 0.61
Milk yield	0.16	0.000	0.09 - 0.27
Herd Size	0.85	0.000	0.80 - 0.91
Abortion	2.59	0.044	1.03 - 6.51
Mastitis	2.63	0.005	1.35 - 5.14

Postpartum anestrus (PPA)

Of 384 dairy cows, 118 (30.73%) were post-partum anestrous (PPA), cows that didn't return to estrus within 60 days of parturition. Body condition score and milk yield were among the risk factors that had statistically significant ($p<0.05$) associations with postpartum anestrus. The management-related factors such as production system and herd size had no eminent relationship ($p>0.05$) with postpartum anestrus (Table 4).

Table 4. Association of management-related factors with postpartum anestrus

Risk Factors	Categories	N (% Prevalence of PPA)	X^2	P-value
Production system	Extensive	35 (13.78)	2.9683	0.227
	Intensive	31 (12.2)		
	Semi-intensive	52 (20.47)		
Herd size	≤10	77 (30.33)	27.9611	0.112
	>10	41 (16.14)		

From the reproductive health problems (risk factors), when an equal number of cows without reproductive health problems were compared with those

with reproductive problems, abortion, endometritis, retained fetal membrane (RFM), and mastitis all showed significant association ($p<0.05$) with postpartum anestrus.
Among the risk factors, RFM has shown a strong association with PPA, and cows that had previous RFM were 9.904 times more at risk of facing postpartum anestrus than those cows free of RFM. Moreover, cows with older age and thus with greater parity were 1.23 times more at risk of suffering from postpartum anestrus than those cows that were younger.

Table 5. Strength of association (Logistic regression) between risk factors and PPA

Risk Factors	Odds Ratio	p-value	[95% Conf. Interval]
Parity	1.24	0.002	1.08-1.42
BCS	0.24	0.000	0.14-0.42
Daily Milk Yield	0.29	0.000	0.17-0.48
RFM	9.90	0.006	1.95-7.29
Endometritis	3.58	0.013	1.31-9.75
Mastitis	2.56	0.041	2.32-5.89
Abortion	2.89	0.025	1.15-7.29

Response to hormonal treatment and associated pregnancy rate

The overall pregnancy rate of repeat breeder and postpartum anestrus female cattle that were treated with double $PGF_{2\alpha}$ and Ovsynch protocols is given in Table 7.

Table 6. Pregnancy rate of repeat breeding and postpartum anestrus cows by hormonal therapy type

Treatment protocol	No. of treated animal	No. of animals in estrus	No. of pregnant animals (%)
Double $PGF_{2\alpha}$	50	41	17 (41.46)
GnRH+$PGF_{2\alpha}$ (Ovsynch)	24	24	7 (29.17)
Total	74	65	24 (36.92)

Among the total of 50 cows that were diagnosed as having corpus luteum (CL) on either of the ovaries, 33 cows exhibited overt signs of estrus after the first injection of $PGF_{2\alpha}$. Consequently, 12 (36.36%) cows were conceived after artificial inseminations. A second shot of $PGF_{2\alpha}$ was given to the rest of the cows (n=17) of which 8 cows exhibited estrus signs and were inseminated, from which 5 (62.5%) were diagnosed pregnant on rectal palpation 60 days after insemination.

Twenty-four (24) cows that did not possess CL upon ultrasonography examination received GnRH injection on the day of start, among which six cows showed estrus signs and inseminated, where only 1 (16.67%) cow confirmed pregnant. Consequently, from 18 cows that failed to show estrus and further received $PGF_{2\alpha}$ injection after 7 days of GnRH administration, three cows showed estrus and inseminated, from which 1 (33.3%) cow was able to conceive. The remaining 15 cows were given the second GnRH injection on day 9 of the first GnRH injection and inseminated 19 hrs later (fixed time AI) regardless of manifestation of estrus signs, where 5 (33.3%) cows were found pregnant on rectal palpation 60 days of insemination.

Discussion

The prevalence of repeat breeding (33.85%) in the cross-sectional study is consistent with the findings of Bartlett *et al.* (1986), who reported the prevalence of repeat breeding (RB) ranging from 14.6 and 36.8 %. In disparity to studies that indicated a prevalence of 7.03%, 13.08%, and 8.72% (Mitku *et al.,* 2012), (Haile *et al.*, 2014) and (Haftu and Gashaw, 2009) in various regions of Ethiopia, respectively and 10.1%, 11.3%, 27.6% and 20% (Gustafsson and Emanuelson, 2002), (Nath *et al.*, 2014) (Malik *et al.*, 2019) and Sarder *et al.*, 2010), respectively from abroad, the prevalence of RB in the present study was quite high. The high rate of occurrence of RB may be the consequence of insufficient estrus detection and errors in timing insemination (Keskin *et al.*, 2010). Moreover, the variances across these studies may be a result of study design, individual variation, topographical variances, and discrepancies in the interpretation of repeat breeding.

The findings of the present study revealed a significantly higher prevalence of RB ($p < 0.05$) in dairy cows with low BCS (<3) than in cows with BCS of ≥3. This result was consistent with previous works of Asaduzzaman *et al.* (2016) and Malik *et al.* (2019), who also found a substantial link between decreasing body condition consequent to inadequate supply of nutrition and reproductive problems including RB. Dairy cows that have insufficient nutrition tend to have low BCS, thus putting them at a higher chance of repeat breeding. More specifically, poorly fed dairy cows are more likely to develop a negative energy balance, which in turn affects the release of reproductive hormones and hence, increases the risk of fertilization failure and/or early embryonic mortality that leads to a higher prevalence of RB (Malik *et al*., 2019).

The current investigation also revealed that milk yield had a statistically significant ($p < 0.05$) impact on the prevalence of RB. The findings show that the higher a dairy cow's milk output is, the greater the chances of being a repeat breeder. In agreement with the current study, Gustafsson and Emanuelson (2002), Nath *et al.* (2014), and Malik *et al.* (2019) observed that the high-producing cattle were more susceptible to RB. The reason could be changes in the reproductive physiology of higher milk-supplying cows experiencing RB and hence subjected to lower fertility (Gustafsson and Emanuelson, 2002). In addition, Yusuf *et al.* (2010) reported that shorter days in milk at first AI were among the risk factors for repeat breeding.

The present study indicated that dairy cows in smaller herds ($n \leq 10$) were more likely to be repeat breeders. The result was consistent with Gustafsson and Emanuelson (2002), who also reported a direct relationship between the decrease in herd size and the rise in the number of repeat breeder cows. However, Asaduzzaman *et al.* (2016) found that RB was considerably less common on farms with fewer animals. The differences may be explained by management differences in those herds; with the assumption that herds with fewer animals were of part-time livestock production and larger herds tend to be commercial farms and hence more attention was given to fertility/reproductive and productive performance of the cows (Asaduzzaman *et al*., 2016). In the present study, dairy cows with a history of reproductive problems of abortion and mastitis during the previous calving had a considerably greater prevalence of RB ($p < 0.05$): dairy cattle that had abortion and mastitis were 2.58 and 2.63 times more likely to turn out to be a repeat breeder, respectively. This finding agreed with the reports of Levine (1999) and Gustafsson and Emanuelson (2002).

The 30.73% prevalence of postpartum anestrus in the present study fairly agrees with the findings of Ducrot *et al.* (1994) who reported a 32.5% prevalence of PPA. In contrast, Hadush *et al.* (2013) and Haile *et al.* (2014) reported considerably lower prevalence of 12.9% and 10.26%, respectively in various regions of Ethiopia; and McDougall (1994), Walsh *et al.* (2007) and Kamal *et al.*

(2012) of 19.5%, 17.2% and 18.5%, respectively from abroad. The difference in prevalence between these studies might be topographical variations, differences in breed studied, and individual variations (Mwaanga and Janowski, 2000). Cows with a body condition score (BCS) of less than 3 and poorly fed animals had a substantial association with the occurrence of postpartum anestrus in the present investigation. The findings were in agreement with reports of Ducrot *et al*. (1994), Rhodes *et al*. (2003), and Montiel and Ahuja (2005), who found a strong interrelation between PPA, body condition scoring, and poor nutrition. The findings of the present study also revealed a substantial association between the higher milk yield of dairy cows and postpartum anestrus. Comparable results were also reported in previous studies of Mwaanga and Janowski (2000) who found that higher-yielding cattle were more at risk of PPA.

Among the reproductive problem-related risk factors considered in this study, endometritis, RFM, mastitis, and abortion was found to have a strong association ($p < 0.05$) with PPA. These results correlate with the findings of Opsomer *et al*. (2000) who were able to show that the risk of postpartum anestrus increases with multiple reproductive disorders and diseases.

The pregnancy rate obtained in the present study when using double $PGF_{2\alpha}$ protocol was 41.46%, which is lower than the findings of Mansour *et al*. (1999) (65.2%) and El-Shahat and Badr (2011) (71.42%) who also used the same treatment protocol. The variations may lay on differences in the breed of cattle used for the study or inseminator skill. In contrast, the hormonal intervention using the Ovsynch protocol resulted in a pregnancy rate of 29.17%, which is fairly close to the findings of Caraba and Velicevici (2013) who reported a rate of 25% for the same hormonal therapy. On the other hand, Zobel *et al*. (2011) found a higher pregnancy rate of 79.88%, in 131 out of 164 treated cows. The difference can be justified by the breed of cattle studied and or the skill of the inseminator as well as the late pregnancy diagnosis time of 60 days post insemination in the present study.

Conclusions

This study confirmed that repeat breeding and postpartum anestrus are among the frequent causes of infertility in dairy cows in the study area. The occurrence of RB and PPA was influenced by parity, reproductive health problems including abortion, retained fetal membranes, endometritis and mastitis, body condition, and daily milk yield of the dairy cows. Treatment of cows with RB and PPA with double $PGF_{2\alpha}$ and/or Ovsynch protocol brought them back to cyclicity thereby increasing the chance of becoming pregnant up on insemination. It is thus, recommended that smallholder and commercial dairy farms need to improve the dairy management and reproductive health monitoring strategy since most risk factors of RB and PPA arise from poor care for dairy cattle. Moreover, hormonal therapy can be used to reduce the wastage of dairy cows that are prone to culling due to RB and PPA.

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Conflict of interest

The authors declare that they have no competing interests.

References

Ambrose, Divakar J., 2021. Postpartum Anestrus and Its Management in Dairy Cattle, Book Editor(s): Richard M. Hopper DVM, Diplomat ACT. https://doi.org/10.1002/9781119602484.ch34

Angesom, H., Abdella, A. and Regassa, F., 2013. Major prepartum and postpartum reproductive problems of dairy cattle in Central Ethiopia. *JVMAH*, 5(4), 118-123. DOI 10.5897/JVMAH2013.0205

Asaduzzaman, K.M., Bhuiyan, M.M. U., Rahman M.M. and Bhattacharjee, J., 2016. Prevalence of repeat breeding and its effective treatment in cows in selected areas of Bangladesh. *Bangladesh J. Vet. Med,* 14(2), 183-190.

Bartlett, P.C., Kirk, J.H. and Mather, E.C., 1986. Repeated insemination in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology, and estimated economic impact. *Theriogenology,* 26(3), 309-322.

Belay, D., Yisehak K. and Janssens, G.P.J., 2012. Survey of major diseases affecting dairy cattle in Jimma town, Oromia, Ethiopia. *Glob*. Vet.*,* 8, 62-66.

Caraba, I. V. and Velicevici, S., 2013. Using Ovsynch protocol versus Cosynch protocol in dairy cows. *Anim. Sci. Biotechnol.*, 56, 63-65.

Cenariu, M.and Jospe, E., 2017. Management of repeat breeding syndrome in cows. *Bull. UASVM Vet. Med.,* 74(2)/2017 doi: 10.15835/buasvmcn-vm:0045

CSA (Central Statistical Authority) 2021. Agricultural Sample Survey 2020/21 (2013 E.C). Report on Livestock and Livestock Characteristics. Volume II, Statistical Bulletin 589, Central Statistical Authority, Addis Ababa, p13.

Dinka, H., 2013. Reproductive performance of crossbred dairy cows under smallholder condition in Ethiopia. *AJDFMP,* 1(5), 101-103.

Ducrot, C., Gröhn, Y.T., Humblot, P., Bugnard, F., Sulpice, P., *et al*., 1994. Postpartum anestrus in French beef cattle: an epidemiological study. *Theriogenology,* 42(5), 753-764.

El-Shahat, K.H. and Badr, A., 2011. Comparative study on the efficacy of different Medicaments on postpartum anestrus Dairy cows. *J. Appl. Biol. Sci.,* 5(3), 59-63.

EMA (Ethiopia Meteorology Agency) 2017. Weather Data of North Shoa Zone from Debre Berhan and Debre Berhan Station from 1986 to 2017, Ethiopia Meteorology Agency, Ethiopia.

Fricke, P.M., Caraviello, D.Z., Weigel, K.A. and Welle, M.L., 2003. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. *J. Dairy Sci.,* 86(12), 3941-3950.

Gundling, N., Feldmann, M. and Hoedemaker, M., 2012. Hormonal treatments for fertility disorders in cattle. *Tierarztl. Prax. Ausg. G: Grosstiere, Nutztiere*, 40(4), 255-263.

Gustafsson, H. and Emanuelson, U., 2002. Characterization of the repeat breeding syndrome in Swedish dairy cattle. *Acta Vet. Scand.,* 43(2), 1-11.

Haftu, B. and Gashaw, A., 2009. Major reproductive health problems of dairy cows in and around Bako, west Ethiopia. *Ethiop. J. Anim. Prod.,* 9(1), 89.

Haile, A., Tsegaye Y. and Tesfaye, N., 2014. Assessment of major reproductive disorders of dairy cattle in urban and per urban area of Hosanna, Southern Ethiopia. *Anim. Vet. Sci.,* 8, 22.

Ibrahim, N, Abraha A. and Mulugeta, S., 2011. Assessment of reproductive performances of crossbreed dairy cattle (Holstein Friesian x Zebu) in Gondar town. *Glob. Vet.,* 6, 561-566.

Kamal, M.M., Rahman, M.M., Momont H.W. and Shamsuddin, M., 2012. Underlying disorders of postpartum anoestrus and effectiveness of their treatments in crossbred dairy cows. *Asian J. Anim. Sci.,* 6(3), 132-139.

Karki, B., Raut, R., Sankhi, K. P., Mandal, U., and Gautam, G., 2018. Fertility improvement by Ovsynch Protocol in repeat breeder cattle of Kathmandu valley. *Int. J. Appl. Sci. Biotechnol.* 6(3), 261-264. doi 10.3126/ijasbt.v6i3.21183

Keskin, A.A., Gumen, G., Yilmazbaş-Mecitoglu, E., Karakaya, U., Tasdemir, *et al.*, 2010. The effect of Progesterone Based Ovsynch Protocol and GnRH treatment after artificial insemination on conception rate in repeat breeder cows. Uludag Univ. *J. Fac. Vet. Med.* 29, 65-70.

Lafi, S.Q., Kaneene, J.B., Black J.R. and Lloyd, J., 1992. Epidemiological and economic study of the repeat breeder syndrome in Michigan dairy cattle. II. Economic modeling. *Prev. Vet. Med.*, 14(1-2), 99-114.

Levine, H.D., 1999. The repeat breeder cow. *The Bovine Practitioner*, 97-105.

Lucy, M.C., 2007. Fertility in high-producing dairy cows: reasons for the decline and corrective strategies for sustainable improvement. *Soc. Reprod. Fertil. Suppl.*, 64, 237–254.

Madhuri, G., Rajashri M. and Kesharwani, S., 2017. Post-partum anoestrus in dairy cows: a review. *Int. J. Sci. Environ. Technol.*, 6(2), 1447-1452.

Malik, M.H., Verma H.K. and. Sharma, R.K., 2019. Prevalence, risk factors and constraints in control of repeat breeding in dairy cows of Punjab. *Indian J. Anim. Sci.,* 89(8), 837-842.

Mansour, M.M. and Abdo, G.A., 1999. Effect of different routes of PGF2α administration and progesterone on the treatment of infertility in buffaloes. *VMJ-G*, 48(2), 267-276.

McDougall, S., 1994. Postpartum anestrus in the pasture grazed New Zealand dairy cow: Massey University (Doctoral dissertation).

Mitiku, T., Ramaswamy, V., Chanie, M. and Fentahun, T., 2012. Commonness of reiterate breeder in dairy cattle in Gondar, Ethiopia. *Adv. Biol. Res.*, 6(6), 226-230.

Montiel, F. and Ahuja, C., 2005. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. *Anim. Reprod. Sci.*, 85(1-2), 1-26.

Mwaanga, E.S. and Janowski, T., 2000. Anoestrus in dairy cows: causes, prevalence and clinical forms. *Reprod. Domest. Anim.*, 35(5), 193-200.

Nath, B.K, Das, B.C., Bari M.S. and Rahman, M.A., 2014. Prevalence and risk factors of repeat breeding in commercial dairy farms of Chittagong district of Bangladesh. *Int. J. Nat. Sci.*, 21-27.

Nishi, S. A., Sarder, M.J., Islam, M.H., Uddin, J. and Sharmin, S., 2018. Management factors influencing anoestrus problems in dairy cows, *J. Vaccines Vaccin..*, 9, doi 10.4172/2157-7560.1000393

Opsomer, G., Mijten, P., Coryn, M. and de Kruif, A., 1996. Post-partum anoestrus in dairy cows: A review. *Vet. Q.*, 18(2), 68-75.

Opsomer, G., Gröhn, Y.T., Hertl, J., Coryn, M., Deluyker, H., *et al.*, 2000. Risk factors for post-partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology*, 53(4), 841-857.

Rhodes, F.M., McDougall, S., Burke, C.R., Verkerk G.A. and Macmillan, K.L., 2003. Invited review: treatment of cows with an extended postpartum anestrous interval. *J. Dairy Sci.*, 86(6), 1876-1894.

Sarder, M.J.U, Moni, M.J.Z. and Aktar, S., 2010. Prevalence of reproductive disorders of crossbred cows in the Rajshahi district of Bangladesh. *SAARC J. Agric.*, 8(2), 65-75.

Short, R.E., Bellows, R.A., Staigmiller, R.B., Berardinelli, J.G. and Custer, E.E., 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J. Anim. Sci.*, 68(3), 799-816.

Thrusfield, M., 2005. Veterinary epidemiology, 3rd ed., Black well Science Ltd. London.

Ishwari, T., Rubina, S., Krishna, K., and Milan, G., 2019. Treatment Approach of Different Hormonal Therapy for Repeat Breeding Dairy Animals in Nepal. *Arch Vet. Sci. Med.* 2 (3), 028-040. doi: 10.26502/avsm.007.

Walsh, R.B., Kelton, D.F., Duffield, T.F., Leslie, K.E., Walton, J.S., *et al.*, 2007. Prevalence and risk factors for postpartum anovulatory condition in dairy cows. *J. Dairy Sci.,* 90(1), 315-324.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, Jr.H.F., *et al.*, 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.,* 65(3), 495-501.

Wodaje, H.B. and Mekuria, T.A., 2016. Risk factors of repeat breeding in dairy cattle. *Adv. Biol. Res.,* 10 (4), 213-221.

Yusuf, M., Nakao, T., Ranasinghe, R.B.K., Gautam, G., Long, S.T., *et al.*, 2010. Reproductive performance of repeat breeders in dairy herds. *Theriogenology*, 73(9), 1220-1229.

Zobel, R., Tkalcic, S., Buic, V., Pipal, I., Geres, D., *et al.*, 2011. Repeat breeder syndrome in dairy cows: influence of breed and age on its prevalence and the success of a hormone therapy. *Turk. J. Vet. Anim. Sci.*, 35(6), 405-411.