



Early and Late Embryonic Loss of Smallholder Synchronized Dairy Cows Within Ethiopia

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Abstract: This study was carried out on 53 cows selected from smallholder dairy farmers in the Ejerie, Adea Berga and Metarobi western Shoa of Oromia in Ethiopia. All of the cows were investigated in October and November, 2015 for their cyclicity, pregnancy and mainly for early embryonic mortality determination. Milk samples were collected from the 53 of synchronized lactating cows involved in the estrus synchronization programme at the time of AI (day 0). Out of lactating cows, 33 exhibited true heat but due heat detection error, only 26 lactating cows were inseminated in day AI (Artificial Insemination). After it was confirmed that 20 lactating cows were pregnant, then they were continued for further progesterone profiling starting from day 18 to 45 day of post AI at interval of 3 days for determination of early embryonic loss based on progesterone concentration. The milk sample was analyzed by Hormonost Farmer Test. Results of the study indicated that about 60% of lactating cows maintain pregnancy until 45-day post AI whereas 15%, 15% and 10% of early pregnant lactating cow's loss their embryo at 21st to 24th days, 27th to 30th days and 33rd day of post AI, respectively. The average progesterone concentration for pregnant cows was 24.46 ng/ml at day 21 to day 45 post AI. Based on finding of this result monitoring and evaluating reproductive traits with progesterone profiling is recommended to improve the fertility of dairy cattle. Finally, embryonic loss detection may serve as a basis for improvement of fertility of dairy cows mean while it will help for designing appropriate breeding management of dairy cattle.

Keywords: Embryonic Mortality, Milk Progesterone, Progesterone Profiling

1. Introduction

Embryo mortality refers to embryo losses during the period from fertilization to completion of the stage of differentiation, which in the cow occurs at approximately Day 45 of gestation [1]. Embryonic mortality (EM) is regarded as one of the causes of reproductive failure in cattle resulting in reduced pregnancy rates, slower genetic improvement and substantial financial losses to dairy production [2-4]. The timing of the embryonic loss is important and can have different effects on the level of production of a dairy farming enterprise [5]. Embryonic mortality is divided into early embryonic mortality and late embryonic mortality [6]. Early embryonic mortality occurs before day 18 post artificial insemination (AI) whereas late

Embryonic Mortality occurs between 19- and 45-days' post AI.

Identification of non-pregnant animals at the earliest day post-breeding is of prime importance for maintaining an optimum calving interval. Pregnancy diagnosis as early as possible after insemination helps in better management of pregnant animals and early submission of non-pregnant animals for subsequent breeding [7]. Although several methods like non-return to estrus, rectal examination, and ultrasonography are used to diagnose pregnancy in dairy animals, determination of progesterone concentration in milk before 45 days post-breeding/AI continue to be the most common and advanced method.

A lot of investigators have demonstrated the usefulness of measuring milk progesterone for monitoring the cow's reproductive status [8-11].

There is lack of adequate information on embryonic loss in dairy cows associated with various reasons in synchronized dairy cows in western areas of Oromia region. This study will help to fulfill this gap by availing updated information and skill in the areas for further improvement of dairy animals. Therefore, the objective of this study was to investigate and characterize possibility of early and late embryonic loss of hormone synchronized smallholder lactating dairy cows under on farm condition and to determine the progesterone profile of pregnant and non-pregnant lactating cows on day 0, day of AI, day 10-12, day 18 and day 21 up to 45 after breeding.

2. Materials and Method

2.1. The Study Area and Animal Management

This study was carried out on 53 cows selected at on farms small holder dairy farmers in the Ejere, Adea Berga and Metarobi western Shoa of Oromia in Ethiopia. They were selected randomly based on their body condition. All of the cows were investigated in October and November, 2015 for their cyclicity, pregnancy and mainly for early embryonic mortality determination.

The cross-breed cows were kept in roofed structures with open sides, whereas the indigenous cows were kept under free grazing lands. The crossbreed was fed a hay grass with supplementation of wheat and nuge cake. Local cows were mainly fed on grazing land with crop residue supplementation. Estrus detection was carried out by visual observation by the AI technicians. When signs of estrus were noticed, AI was performed by experienced technicians.

2.2. Equipments and Chemicals

Fertility Box Cow is an instrument which measures range of progesterone concentration from 1-30 ng/ml. Microtherme with separate adapter for 220/230V and used for warming up milk diluter and substrate. Tube holder is custom-made for correct handling of special test tubes. 100 μ l-pipettes were used for precise, quantitative sampling of milk and enzyme solutions. Carrying case protects all above Equipments from dust.

Disposable chemicals and materials such as Specific antibody coated Test tubes, Milk Diluter Enzyme, Substrate, two vials of controls, containing predetermined & given concentrations of progesterone and Pipette tips were used. According Hormonost Farmer test the complete test kits were kept under Holleta Biotechnology and molecular scientific refrigerators adjusted at 4°C.

Milk samples were collected from the 53 of total lactating cows involved in the estrus synchronization programme at the time of prostaglandin injection and only 26 out of 33 truly heat exhibited of lactating cows were inseminated at the AI (day 0). After milk progesterone evaluation at day 10, day 18, and day 21 post AI, twenty lactating cows were confirmed as pregnant and then they were continued for further progesterone profiling starting from day 18 to days of

post AI at interval of 3 days for determination of embryonic loss based on progesterone concentration. About 20-30ml whole milk sample was collected from individual cow by cleaned labeled jar. Then the collected milk samples from individual cows were taken to Holleta Biotechnology and molecular laboratory.

The milk sample was analyzed by Hormonost Farmer Test. Hormonost Farmer Test (quantitative rapid assay with test device Fertility Box Cow for determining progesterone content of milk samples of dairy cows) is a standard kit-based test manufactured by biolab GmbH, munchen, Gemen which determines milk progesterone concentration.

Experienced AI technicians were diagnosed the presence of CL through rectal palpation. The principle of milk samples collection at the time of AI (Day 0) were to determine on set of estrus cycle of the cow which is checking presence of CL during injection of prostaglandin based on p4 concentration. A concentration higher than 3ng/ml was considered to indicate the presence of a functional CL and had passed estrus. Corpus Luteum regression was defined by a cow having a milk P4 concentration of greater 3 ng/ml at day AI, which then declining to a level of less or equal to 3 ng/ml at the day the cow coming to estrous. The laboratory procedures were followed as per the standard protocol set by standard kit-based test manufactured by biolab GmbH, munchen, Gemen.

2.3. Data Management and Statistical Analysis

All data collected from laboratory were fed to Ms-Excel (2010). Progesterone profile was summarized and presented by using spread sheet Microsoft excel.

3. Result and Discussion

From the total prostaglandin treated, 53 lactating dairy cows, the milk samples were taken at the day of AI (day 0) for progesterone determination and 33 of them had shown progesterone concentration less than 3ng/ml. But in practical case 26 (24 cows from 1st injection and 2 from second) of 33 were inseminated while the rest seven lactating cows were missed due to heat detection error by technicians.

The milk samples were collected at day of AI, 10-12, day 18 and day 21 of post AI for early pregnancy determination based on progesterone concentration. After day 18 post AI 20 lactating cows (which were pregnant) were continued for milk sample progesterone determination to detect embryonic loss until day 45 post AI at the interval of 3 days for each cow.

The progesterone concentration was continued to raise in 60% of 20 lactating cows on from day 21 to day 45 after post AI. The rest 40% of lactating cows were failed to maintain high progesterone concentration until day 45 of post AI at different days. Therefore, the result 60% of sampled lactating cows were become pregnant while, 15%, 15% and 10% of lactating cows were lost their embryo at 21st to 24th, 27th and 30th to 33rd day of post AI, respectively. The embryonic mortality in this study disagree with result of [12, 13] which

is 26 and 15.2%. This might be due to variation estrus cycle between cows, poor nutrition during postpartum period and poor management [14]. The implication of the above for breeder was progesterone profiling could help to decide fertility management issues quickly. In breeding point of view progesterone concentration had a role to reinstate AI and improve reproductive efficiency by decreasing the interval between AI services [15].

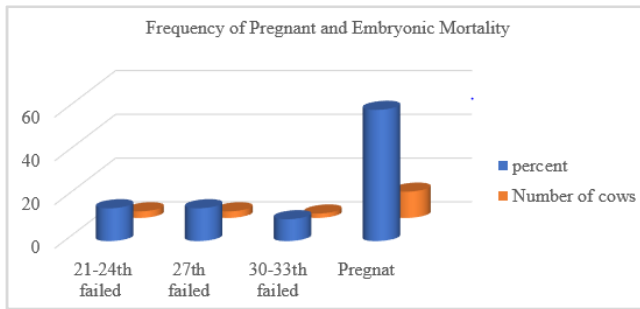


Figure 1. The proportion of pregnant and embryonically lost cows based on progesterone concentrations.

3.1. Characterization of Embryonic Mortality Based on Individual Cow Progesterone

Embryonic mortality, strictly interpreted, refer to fertility losses during the embryonic period, i.e. the period extending from conception to completion of the stage of differentiation which, in the cow, occurs at approximately 45 days [16].

Based on progesterone concentration profile in Figure 2 the embryonic loss of cows (C242, C282 and C618) could be categorized in early embryonic losses while, the other cows (C289, C293, and C288) cows which loss their embryo at 27th day are categorized under late embryonic loss. Similarly, cows fail to maintain pregnancy at 30th to 33rd day after

insemination are classified under late embryonic loss. This result was a little greater with the Studies [17-19] that early pregnancy diagnosis which indicate less than 15% of the viable embryos establish pregnancy by 27th days after ovulation in lactating dairy cows while it was lower than that of beef cattle 69 and 83% of the frozen and fresh semen, respectively, establish pregnancy on day 37 of gestation.

In cow's maternal recognition of pregnancy occurs between day 16 and day 19 of pregnancy facilitated by the secretion of a trophoblast interferon bovine trophoblast protein -1 secreted by the conceptus between day 16 and day 26 of pregnancy identified as antiluteolytic agent in cattle [20]. So embryonic mortality can be detected through progesterone profile at the age of day 21 to 24.

It is widely considered that an increase in the interval between service and return to estrus beyond the usual range of 17-25 days reflects embryonic mortality. Two sources of pregnancy failure exist after breeding, fertilization failure and pregnancy loss. When the interestrus or the interovulatory intervals are extended in bred animals, it usually indicates embryonic loss that occurred around the period of corpus luteum (CL) maintenance [21].

Experiments with continuous measurements of concentrations of progesterone in blood suggest that embryonic death at the time of CL maintenance delayed luteolysis, and extended interestrus interval [22]. Therefore, when embryonic death precedes luteolysis, luteal regression is delayed by at least 3 days after the end of pregnancy [23]. Thus, the result shows that 15% losses of pregnancy prior to day 24 and categorized under early embryonic losses embryonic losses, while 25% losses were grouped under late embryonic losses.

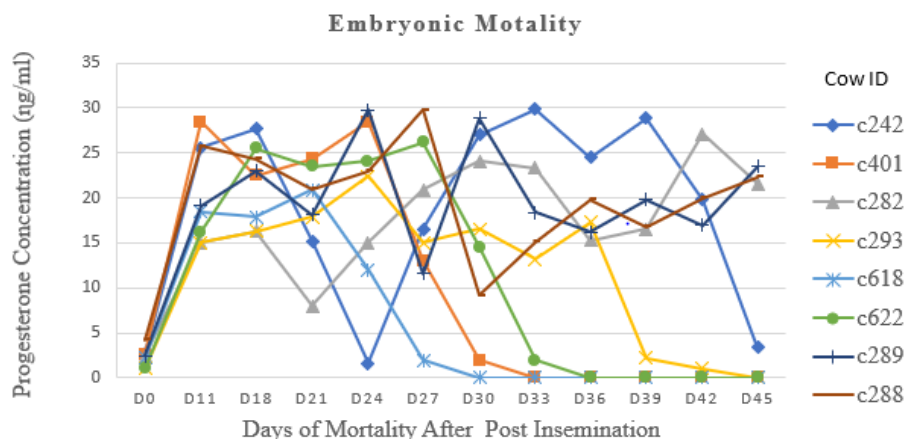


Figure 2. Pattern of milk progesterone concentration for embryonic lost lactating cows.

As it was shown in table 1 from a total of milk sampled local cows 33.33% of them were under incidence of embryonic mortality at different stage of embryo development based on progesterone concentration while 45.45% of crossed lactating cows were loss the embryo at stage of early pregnancy. In studies from California [24], it

would appear that early embryo loss was greater in the modern high producing dairy cow and that a much higher proportion of the embryos after post AI compared with lower producing cows or heifers.

When embryo survival extends beyond the stage of maternal recognition of pregnancy, luteal regression was

delayed, which results in a delayed return to estrus. [25] Suggested that returns to estrus after Day 24 could reflect embryonic losses occurring after Day 16 of gestation. Almost half (47.5%) of the total recorded loss occurred between Days 28 and 42 of gestation. This result agrees with the

report of [26] that more than half (50%) of the total recorded loss occurred between Days 27 and 42 of gestation on late embryo in cattle that were managed on pasture-based systems.

Table 1. Pregnancies and embryonic loss within breed.

Variables	Pregnant		Embryonic loss after post AI ¹					
			Day 21-24		Day 27		Day 30 to 33	
Breed	N	%	N	%	N	%	N	%
Cross	6	54.55	2	18.18	2	18.18	1	9.09
Local	6	66.67	1	11.1	1	11.1	1	11.1
Total	12	60	3	15	3	15	2	10

¹AI = Artificial Insemination.

3.2. Pattern of Progesterone Concentration for Pregnant Lactating

Progesterone (P4) from the corpus luteum (CL) is critical for the establishment and maintenance of pregnancy and plays a major role in regulating endometrial secretions essential for stimulating and mediating changes in conceptus growth and differentiation throughout early pregnancy in ruminants [27]. Numerous studies have demonstrated an association between elevated P4 and acceleration in conceptus elongation [28, 29].

During the follicular phase, progesterone concentration was very low in milk (Figure 3). The actual p4 concentration for cows at estrus from milk samples collected ranged from less than 1 ng/ml to 2.7 ng/ml. This result is in accordance of with the standard of the kit which is less than three for cows in estrus cows. In early embryonic development between day 10 to 12, the progesterone concentration values were found progressively increased. The mean progesterone concentrations at day 10 to 18 were 21.83 ng/ml ranging 16.3ng/ml to 28.6ng/ml. According to Hormonost-Micro lab Farmer Test Standards, the cut-off point milk progesterone level for pregnant cow was 16ng/ml. From the results of milk samples collected lactating cows, the mean progesterone concentrations were 24.46 ng/ml at day 21 of post AI to until day 45. The minimum progesterone concentration was

recorded as 16.3ng/ml and the maximum progesterone concentration was observed as 29.9ng/ml to maintain pregnancy.

Generally based on the milk progesterone test (Hormonost farmer test progesterone determination for fertility) 12 lactating cows were judged as pregnant and the rest 8 were not pregnant. Similarly, those cows were diagnosed by rectal palpation for pregnancy and the result was the same as the test. Finally, it was concluded that 12 lactating cows were pregnant and the pattern of progesterone concentration was sketched in (Figure 3).

3.3. Use of Milk Progesterone Profile to Monitor Pregnancy and Embryonic Mortality

If the cow was bred and becomes pregnant, progesterone in blood and milk remains high until just prior to calving. If the cow does not conceive, the corpus luteum begins to degenerate on approximately day 17 of the cycle, and progesterone declines to minimal concentrations on days 20 as the cow returns to heat. There was strong relationship between progesterone and embryo survival rate. Optimal progesterone concentrations between 7 and 8 ng/mL on 0 to 8 day were associated with maximal pregnancy rates, whereas concentrations that were lower or higher were associated with reduced fertility [30].

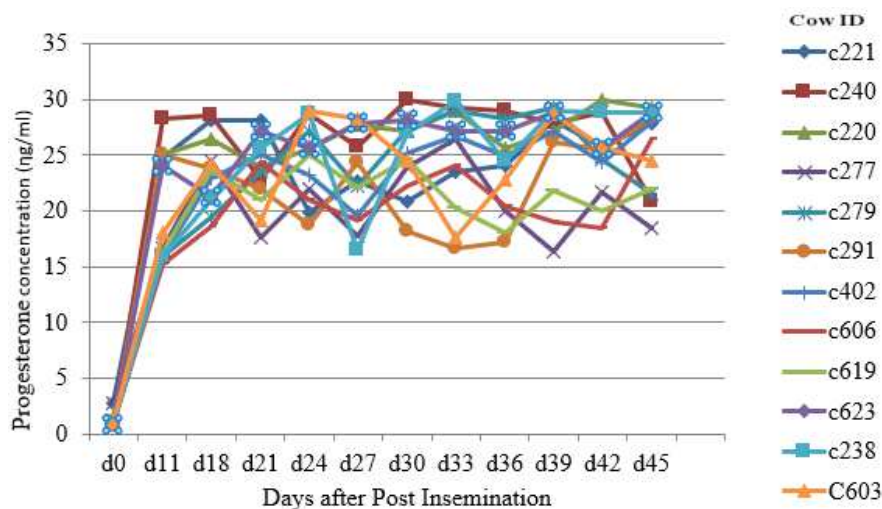


Figure 3. Pattern of milk progesterone individual cows' concentration in pregnant lactating cows at different days.

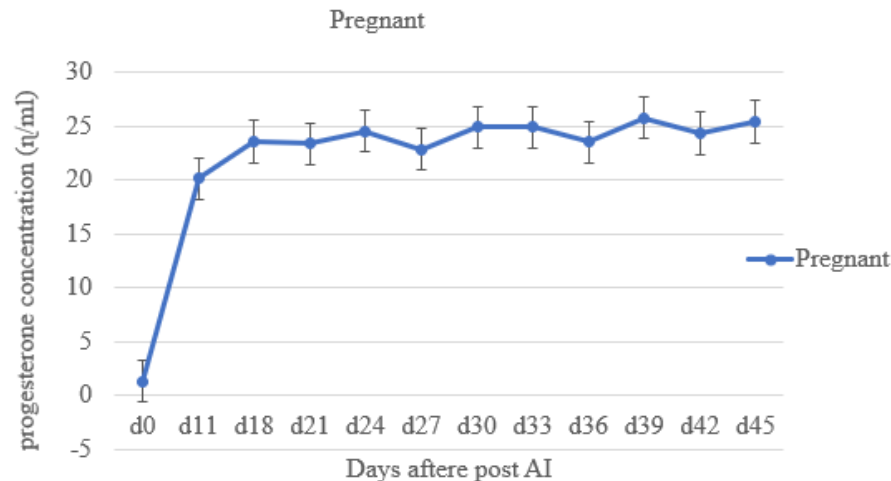


Figure 4. Pattern of average milk progesterone individual cows' concentration in pregnant lactating cows at different days.

From the figure below in pregnant cows mean progesterone concentrations rise from Day 11 to Day 42 to reach 16 to 25.41 ng/mL. However, individual variations exist when measuring progesterone concentrations at different stages of pregnancy. This allows to use this system as a pregnancy test with corresponding positive and negative results of pregnancy results through trans rectal palpation. In the dairy, progesterone concentrations profile can be measured from

Day of AI till the end of pregnancy and can be used as reliable pregnancy test until 45 day. In case of late embryonic mortality associated with luteal maintenance, cows present detectable concentrations that are lower than critical level of the kit concomitant with a larger variation reflecting individual differences in the time at which embryonic death occurs.

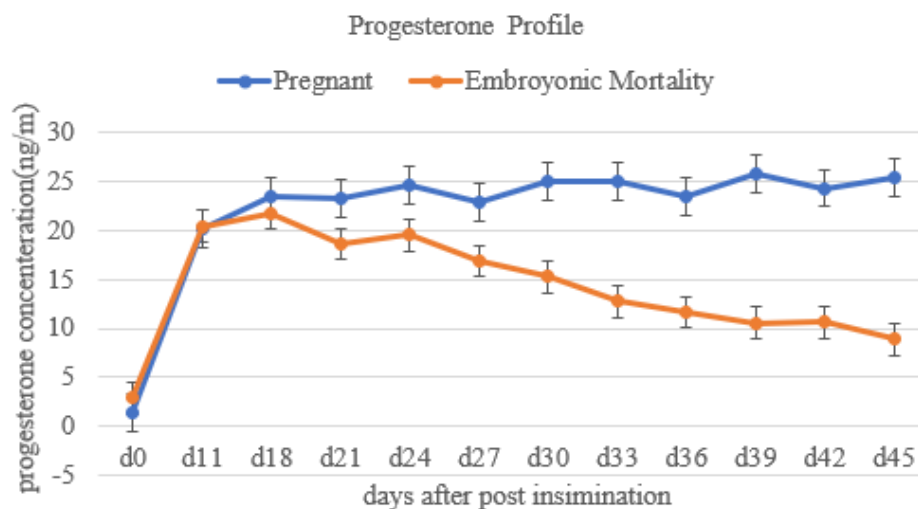


Figure 5. Average pregnant milk progesterone in contrast with Embryonic mortality cows at different days.

4. Conclusion

The result in this study indicates that progesterone concentration in lactating cows at day AI, day 11, day 18 and day 21 can help to judge either the cows pregnant or not pregnant at early stage. By using complete kits of the Hormonost farmer test early pregnancy and late pregnancy embryonic mortality were detected. From milk sampled lactating cows 40% of lactating cows that were confirmed to pregnant embryonic mortality occurred at different stage of pregnancy until 45 days. Therefore, it can be concluded that the use of complete kit Hormonost progesterone

concentration test was helpful for the early and late embryonic mortality detection.

The artificial insemination technician must update recent skills and knowledge. Hormonost® Micro Lab Farmers Test is important tool to improve conception and pregnancy rate in estrus synchronized cattle, by enabling accurate heat detection, conception, embryonic loss and pregnancy status. Hormonost® a solution to invasive rectal palpation to diagnose which farmers disliked. To achieve higher economic return in cattle breeding, monitoring and correcting major causes of embryonic and fatal deaths is necessary. Further on-station research is recommended to address the limitations of this research.

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