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Efficacy of Prostaglandin F2 Alfa in Diagnosis of Early Pregnancy in Bovines

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Abstract: The objective of this study was to determine the efficacy and accuracy of a non- luteolytic dose of PGF2α injection on the diagnosis of early pregnancy in Holstein-Friesian cross breed cows. The diagnosis of early pregnancy depended on the increased intra-mammary pressure response due to release of luteal oxytocin induced by intravenous administration of non-luteolytic dose of PGF2α. The study was performed on 32 lactating cows from 18-22 days post insemination, 3 hours after morning milking. The cows were divided into 3 groups, group I (N=11) and II (N=11) were administered with 125µg and 250µg of PGF2α intra venous, respectively, while group III (N=10) was administered with 2ml of normal saline intra jugular and served as control. The pregnancy status was confirmed by rectal examination on 45-60 days post AI. The positive responses of the test were 54.54% (6/11) for group I, 72.72% (8/11) for group II and 0.00% (0/10) for group III. While the negative responses were 45.45% (5/11), 27.27% (3/11) and 100% (10/10) in group I, II and III, respectively. The time interval for test response were 153.91 ± 44.45 and 85.00 ± 16.73 seconds in groups I and II, respectively, which did not differ significantly (P>0.05). Overall test sensitivity and specificity were 75% and 66.67%, respectively. Accuracy of predicting pregnancy and non-pregnancy were 85.71% (12/14) and 50% (4/8), respectively; with an overall diagnostic accuracy of 72.72% (16/22). It was found that the accuracy of the test varied between groups. The test being inexpensive, rapid, easy to interpret, field friendly and does not require highly qualified personnel to perform, can be used for early pregnancy diagnosis with reasonable accuracy.

Key words: Accuracy · Cow · Luteal Oxytocin · Pregnancy Diagnosis · Prostaglandin F2α · Sensitivity · Specificity

INTRODUCTION

The diagnosis of pregnancy (cyesiognosis) has been sought since long by farmers for curiosity, however; it is essential for profitable animal husbandry especially in the productive animal species. To achieve maximum milk and meat production results and reproductive performance, early pregnancy diagnosis is essential [1].

For an economical dairy farm, cows must calve every year and to maintain this sequence, identifying pregnant animals at an early date is imperative. In the current systems of planned breeding, diagnosis of pregnancy would help to evaluate the therapies at an early date and devise alternative manipulations. In some situations in the pet practice pregnancy may not be desirable by the owners and an early diagnosis would help to terminate these unwanted pregnancies. An early pregnancy diagnosis is essential in mares to tease them if non pregnant and try to get them pregnant in the same season. It therefore, appears that early diagnosis of pregnancy is essential in animal management for economic reasons. In many developing countries, farmers often present their animals for pregnancy diagnosis very late when much of their time is lost in maintaining non pregnant cows [2].

High reproductive efficiency is a prerequisite to realization of high life time production from dairy animals. Early pregnancy diagnosis is crucial to shortening the calving interval through enabling the farmer to identify open animals so as to treat and/or rebreed them at the earliest opportunity. Ideally a 60-days post parturient barren interval in dairy animals is recommended for breeding. Dairy farmers need to recognize non pregnancy at the earliest opportunity so as to rebreed the dam at the very next opportunity [3]. The methods of early diagnosis of pregnancy are of particular importance due to timely detection of non-gravid animals which are subjected to re insemination or therapy in due time [4]. Various methods of early laboratory diagnosis based on blood or milk progesterone level determination by Radio Immune Assay (RIA) or Enzyme Linked Immune Sorbent Assay(ELISA) methods are available and often used [5].

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Some of the rapid methods, based on milk progesterone level determination, are available for field conditions. Unlike RIA and ELISA which are quantitative methods, rapid field tests are qualitative. The determination of progesterone concentration is the most frequently used method for early laboratory diagnostics of gravidity [6]. High progesterone level is not necessarily a reliable proof of vital embryo existence and pregnancy, but only of preserved luteal function. On the other hand a low concentration is a positive indicator of lack of pregnancy [7].

Field measurements and interpretation of results on the farm is the advantage of immune enzyme milk sample testing in relation to RIA tests of blood or milk sampleswhichrequire sample transport to laboratories and thus disable prompt re insemination. RIA, as well as immune enzyme progesterone tests, reaches satisfactory accuracy of 70-75% for the pregnant and almost 100% for non pregnant, 21 day post insemination [8]. Although easy to handle, immune enzyme kits are expensive [9] and need 20 to 40 minutes to interpret the results [10] which impede their wider use in field conditions.

An early and precise pregnancy diagnosis is an important criterion for profitable animal husbandry practice by shortening the calving interval. Though various methods of early pregnancy diagnosis based on blood and milk are available, most of them are time consuming, not cost effective and requires highly qualified personnel. Hence to find out an early, rapid, inexpensive, easy to interpret, field friendly pregnancy diagnosis methods is a paramount importance in the field condition. All these are reasons that have led us to try to establish the existence of early pregnancy corpus luteum, not by their secretion of progesterone, but by oxytocin. Corpus luteum can synthesize this peptide [11] whose tissue concentrations decline earlier than those of progesterone in the case of failed fertilization [12].

Prostaglandin F2α by positive feedback stimulates the luteal tissue to synthesis and secrete oxytocin [13]. Intravenous administration of up to 600 µg of PGF2α doesn't bring up a risk of luteolysis, but leads to a release of luteal oxytocin and milk ejection that can be recorded by measuring the intra-mammary pressure or volume of milk ejected out through the teat cannula [14].

Administration of non-luteolytic PGF2α stimulates the luteal tissue in the corpus luteum to synthesis and secret oxytocin leads to alveolar smooth muscle contraction, release of alveolar milk and engorgement of teat. This engorgement of teats indicates the positive sign of the presence of luteal cells and detected pregnancy in bovines 18-22 days after insemination [15].

Therefore the objective this study was to study the efficacy of non-luteolytic dose of PGF2α in detecting early pregnancy and to study the reliability of the test by measuring the accuracy, sensitivity and specificity on early pregnancy diagnosis

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in dairy farm of College of Veterinary Medicine, Mekelle University, Mekelle. Mekelle is a city in the Northern part of Ethiopia, located in Tigray regional state. It is the capital of Tigray regional state located in 783km North of the capital city, Addis Ababa at a latitude of 30° 29'N and longitude of 39°28'E with elevation of 2000-2200 meters above sea level (m.a.s. and the average rain fall and temperature are 600 mm and 19 °C, respectively. The city covers an area of about 53 square kilometers, with an estimated population of about 310,000 people [16].

Study Population: This study was carried out at livestock farm belonging to College of Veterinary Medicine, Mekelle University, Mekelle, Ethiopia from November 2016 to May 2017. Healthy parous (parity between 2 and 5) Holstein Frisians lactating cross breed cows which completed 60 days of post partum were included in this study. All the animals were subjected to through gynaco-clinical examination before included into the experiment.

Sample Size: A total of 32 healthy parous Holstein Frisian lactating cross breed cows which were completed 60 days post partum from Mekelle University dairy farm were selected.

Study Design: The clinical trial experimental study design was applied. Healthy parous Hostein Frisians lactating cross breed cows which completed 60 days post partum were subjected in this study. The selected sample animals were synchronized. After synchronization animals were observed for heat signs twice a day. Once the animals exhibit the estrus signs, it was inseminated artificially by a trained inseminator. After insemination, the animals were closely monitored for returning into estrus. Those animals which showed heat signs were excluded from the study and the test was performed only in non-return cows, on 18-22 days post insemination.

Preparation of Non-Luteolytic Dose of PGF2α and Protocols: One ml of PGF2α (Lutalyse, Pfizer, Belzium) which contains 5000 micro grams of Dinoprost tromethamine was reconstituted in 39 ml of distilled water to arrive at a final concentrations of 125 micro gram/ml.

Table 1: Study design

S.No	Group	No. of animals	Non-luteolytic dose of PGF2α (µg)	Rout of administration
1	I	11	125	I/V
2	II	11	250	I/V
3	III (control)	10	2 ml normal saline	I/V

The animals under the study were randomly distributed into 3 groups. Then the udder and one or four teats??? Please explain four of the teats was washed with 1 % potassium permanganate solution. A sterile cannula was placed in the left fore teat to empty the cistern milk. Subsequently a non-luteolytic dose of PGF2α Dinoprost tromethamine (Lutalyse, Pfizer, Belzium) Group I: The dose of 125µg/cow (n= 11); Group II: The dose of PGF2α 250µg/cow (n = 11); was administered intra venous, 3 hours after morning milking and Group III(Control) received 2ml normal saline (n = 10). After the injection the time duration of increased intra-mammary pressure (disappearance of the wrinkles on the teat and engorgement of the teat) or alveolar milk ejection was observed and recorded. Increased intra-mammary pressure or alveolar milk ejection was the option which was used in diagnosis of pregnancy. Lack of increased intra-mammary pressure or new milk release after 5 minutes of administration of non-luteolytic dose of PGF2α was assumed as a sign of absence of functional corpus luteum. This was considered as a negative pregnancy test result. All animals which showed increased intra-mammary pressure or let down of free flow of alveolar milk within a few minutes after injection were subjected for rectal examination to confirm the pregnancy on 45- 60 days of gestation. All the collected data were analyzed statically.

Accuracy: The accuracy of the diagnosis was expressed by sensitivity, specificity, positive and negative predictive values. They were calculated as described by Wayne Martin *et al*. [17].

Sensitivity: The sensitivity of the test is its ability to detect pregnant animals and is defined as the proportion of the pregnant animals that test positive.

Specificity: The specificity of the test is its ability to detect non-pregnant animals and is defined as the proportion of the non-pregnant animals that test negative.

Positive Predictive Value: It is defined as the percentage of actual pregnant animals out of the total number of animals diagnosed pregnant through any diagnostic test.

Negative Predictive Value: It is defined as the percentage of actual non-pregnant animals out of the total number of animals diagnosed non-pregnant through any diagnostic test.

Diagnostic Accuracy: It is defined as the percentage of the correct diagnosis out of total number of examinations done by the test. Based on the test results, the animals under study were classified into the following categories.

True Positives (A): Animals diagnosed pregnant with the test and subsequently confirmed pregnant on palpation per rectum on 45-60 days.

False Positives (B): Animals diagnosed pregnant with the test but subsequently confirmed non-pregnant on palpation per rectum on 45-60 days.

False Negatives (C): Animals diagnosed non-pregnant with the test but subsequently confirmed pregnant on palpation per rectum on 45-60 days.

True Negatives (D): Animals diagnosed non-pregnant with the test and subsequently confirmed non pregnant on palpation per rectum on 45-60 days.

Sensitivity, specificity, positive and negative predictive values for PGF2α injection test was calculated as per the formulas given by Wayne Martin *et al*. [17] and depicted as follows.

Number of pregnant animals = A+C.
Number of non-pregnant animals = B+D.
Sensitivity = A/(A+C) ×100.
Specificity = D/(D+B) ×100.
Positive predictive value = A/(A+B) ×100.
Negative predictive value = D/(D+C) ×100.

Overall diagnostic accuracy = A+D/(A+B+C+D) ×100. Sensitivity, specificity, positive and negative predictive values and overall diagnostic accuracy were calculated and presented.

Data Management and Analysis: Statistical analyses of the collected data of test results with descriptive analysis to compute the mean and Independent-Samples T Test to determine the association between dose and time initiation of increased intra-mammary pressure with confidence interval of 95% were performed by using

statistical software (SPSS, version 20.). The correlation between the obtained results was determined as well. Statistically significant results were considered those with P=0.05.

RESULTS

Response of NonLuteolytic PGF2α on Intra Mammary Pressure or Milk Ejection: The positive and negative response of non-Luteolytic PGF2α injection on teats engorgement were 54.54% (6/11), 72.72% (8/11) and 0.00% (0/10) and 45.45% (5/11), 27.27% (3/11) and 100.00% (10/10) in group I, II and III respectively.

Table 2: The response of non-luteolytic PGF2α on engorgement of teats.

	Non-Luteolytic PGF2α response in(%)	
Group	Positive	Negative
I	54.54 (6/11)	45.45 (5/11)
II	72.72 (8/11)	27.27 (3/11)
III	0.00(0/10)	100.00(10/10)

The percentage of respondent and non-respondent animals by administration of non- luteolytic dose of PGF2α on intra-mammary pressure.

The time interval for non-luteolytic dose of PGF2α injection response on teats engorgement were 153.91 ± 44.45 and 85.00 ± 16.73 seconds in groups I and II, respectively and did not differ significantly (P>0.05).

Table 3: Time interval in seconds between administrations of non-luteolytic dose of PGF2αand teat engorgement response (Mean ± S.E)

Group	Time response in(seconds)
I	153.91 ± 44.45
II	85.00 ± 16.73
t- value	0.16

NS-Non-Significant (P=0.05)

PGF2α Injection Intra-Mammary Pressure Test vs. Pregnancy Rate: In group I, out of 6 animals which showed positive response for PGF2α, 5 (83.33%) were found pregnant upon rectal examination on 45-60 days post AI and one animal (16.67%) was observed non-pregnant. Of the remaining 5 cows which did not reveal positive response for PGF2α, two were found pregnant (40%) and three were observed non-pregnant (60%) upon rectal examination on 45-60 days post AI. In group II, out of 8 animals which showed positive response for PGF2α, 7 (87.50%) animals were found pregnant upon rectal examination on 45-60 days post AI and one animal (8.33%) was found non-pregnant. Of the remaining 3 cows which did not reveal positive response for PGF2α, two were found pregnant (66.67.0%) and one was observed non-pregnant (33.33.0%) upon rectal examination on 45-60 days post AI. In group III, all the animals (n=10) showed negative response for normal saline. Among them, 8 (80.0%) were found pregnant and only two animals (20.0%) were observed non-pregnant upon rectal examination on 45-60 days post AI.

Table 4: Non-luteolytic dose of PGF2α injection intra-mammary pressure response vs. Pregnancy rate.

Attribute		Pregnant	Non pregnant
Group I	PGF2α positive	5/6 (83.33%)	1/6 (16.67%)
	PGF2α negative	2/5 (40%)	3/5(60%)
Group II	PGF2α positive	7/8 (87.50%)	1/8 (12.50%)
	PGF2α negative	2/3(66.67%)	1/3 (33.33%)
Group III	Normal saline positive	-	-
(control)	Normal saline negative	8/10 (80%)	2/10 (20%)

Figures in parenthesis indicate the number of observations.

Accuracy of PGF2α Injection Intra-Mammary Pressure Test The values of sensitivity and specificity calculated for non-luteolytic PGF2α Injection on intra-mammary pressure test for group I and group II were 71.43% and 75% and 77.78% and 50.0%, respectively. Irrespective of the groups, the overall PGF2α injection test sensitivity was 75% and specificity 66.67%. The accuracy of predicting pregnancy (PPV, %) based on PGF2α injection test positive response were 83.33% and 87.5% in groups I and II, respectively, whereas the accuracy of predicting non-pregnancy (NPV, %) was 60% in group I and 33.33% in group II. Irrespective of the groups, the accuracy of predicting pregnancy and non-pregnancy were 85.71% (12/14) and 50% (4/8) with an overall diagnostic accuracy of 72.72% (16/22).

Table 5: Accuracy of non-luteolytic dose of PGF2α injection intra-mammary pressure test in the diagnosis of pregnancy on day 18-22 post insemination.

S. No	Particulars	Group I (n=11)	Group II (n=11)	Total tested animals (n=22)
1	True positive	5	7	12
2	False positive	1	1	2
3	False negative	2	2	4
4	True negative	3	1	4
5	Sensitivity (%)	71.43	77.78	75.00
6	Specificity (%)	75.00	50.00	66.67
7	Positive predictive value (%)	83.33	87.50	85.71
8	Negative predictive value (%)	60.00	33.33	50.00
9	Overall diagnostic accuracy (%)	72.72	72.72	72.72

DISCUSSIONS

Response of Non-LuteolyticPGF2α on Intra-Mammary Pressure or Milk Ejection: Non-luteolytic dose of PGF2α on intra-mammary pressure test was conducted in Holstein Fresians cows by administering a non-luteolyticdose of PGF2α at 125µg (Group I, n=11), 250µg (Group II, n=11) and normal saline (Group III – control, n=10) on days 18-22 post AI. Cows showing clear engorgement of teats within 5 minutes of injection were considered positive for the test.

It was observed that 54.54%, 72.72% and 0.00% cows in groups I, II and III, respectively showed positive response for the test (Table 2) which implies that 45.45%, 27.27% and 100% cows in these three groups, respectively did not respond (negative response) as revealed by absence of teat engorgement. This result is comparable with the finding of Ashok *et al*. [18] who stated that the response of PGF2α administration test conducted on days 18-22 post insemination in cross bred cows were 68% (17/25), 80% (20/25) and 0.00% (0/10) for group I, II and III, respectively.

In the present investigation, the mean time interval between administration of PGF2α and milk ejection as revealed by clear engorgement of teats was 153±44.45 and 85.00±16.73 seconds for group I and II, respectively without significant difference (P>0.05) between them (Table 3). Elsewhere several authors reported this time lag in seconds as 86±35, [19] 189.5±18 [20] and 166.47±19.07 and 135.50±16.29 seconds in group I and II, respectively [18].

The observed difference in the duration of increased intra-mammary pressure response might be attributed to the dose of PGF2α, stage of lactation, production capacity and breed. The test implies that the positively reacted cows possess a functional CL and the oxytocin released from CL following administration of non-luteolytic dose of PGF2α caused the engorgement of the teats.

The values of sensitivity and specificity calculated for PGF2α injection test carried out on 18-22 days post insemination for group I were 71.43% and 75% and for group II were 77.78%and 50.0%, respectively. Irrespective of the groups, the overall PGF2α injection test response sensitivity was 75% and specificity was 66.67%. The accuracy of predicting pregnancy based on test positive response were 83.33% and 87.5% in groups I and II, respectively, whereas the accuracy of predicting non-pregnancy were 60% in group I and 33.33% in group II. This result is consistent with the values of sensitivity and specificity calculated for PGF2α injection increased intra-mammary pressure test carried out on 18-22 days post insemination which were 73.68% and 50% in group ^{2}; and 85.71% and 50% in group ^{22}, respectively [15].

PGF2α Injection on Intra-Mammary Pressure Test Vs. Pregnancy Rate: Based on the intra-mammary pressure response (positive or negative) and subsequent pregnancy rate, the accuracy of PGF2α injection test in predicting pregnant and non-pregnant animals were estimated (Table 4 and 5). In this study, the accuracy of predicting pregnancy was as high as 83.33% Group I and 87.5 % in Group II. However, the accuracy of predicting non-pregnancy (60% in group I and 33.33% in group II) was low.

The results of this study were comparable with those of Narasimha and Sudhakar [15] who reported that PGF2α administration test was 100% accurate in predicting pregnancy as against only 42.0% in predicting non-pregnancy. On the other hand [19] reported that the accuracy of predicting pregnancy based on increased intra- mammary pressure response was only 72.3% as against 100% predicting of non-pregnancy with almost the same result of [15] findings, the accuracy estimates of pregnancy was 83.3% whereas that of non-pregnancy 100%.

It was also observed in this study that 16.67% (1/6) and 12.5% (1/8) cows in group I and II, respectively showed positive response to PGF2α injection test but turned out non-pregnant upon rectal examination on 45-60 days post AI (Table 4). This might be attributed to factors like early embryonic mortality, presence of persistent luteal tissue. Animals that fall in these categories would have luteal oxytocin released following administration of non-luteolytic dose of PGF2α and hence showed response to PGF2α administration test [15]. Conversely 2 out of 5 cows in group I (40%) and 2 out of 3 cows in group II (66.67%) which showed negative response to PGF2α administration test were found to be pregnant upon rectal examination on 45-60 days post AI. Unreliability of test in these four cows might be explained by factors such as unexpectedly improper administration of drug, disturbed milk ejection due to unfamiliar surroundings and elevated cortisol levels [23]. Therefore repeating the test by controlling of other risk factors like early embryonic death, proper examination of the ovarian status before administering of the hormone, correct sampling with increased amount of samples, appropriate administration of the hormone and avoiding unfamiliar environment would improve the efficacy, accuracy, sensitivity and specificity of the test.

CONCLUSION AND RECOMMENDATIONS

The values of sensitivity for non luteolytic PGF2α on intra-mammary pressure test were 71.43% and 77.78% in group I and II respectively. It implied that the percentage of animals responded to this test appears to be dose dependent, since in group II which received high dose of non-luteolytic PGF2α had high sensitivity. The present observation suggests that the response to non-luteolytic PGF2α was closely associated with the functional status of the corpus luteum. The overall diagnostic accuracy and sensitivity of this test were 72.27% and 75.00% respectively was satisfactory. However, the specificity of the test 66.67% waslower than that of other laboratory diagnostic methods and need further study. This method of diagnosis of pregnancy has reliability and lower price so it is simple alternative in early pregnancy diagnosis. The non luteolytic PGF2α being inexpensive, field friendly and does not require highly qualified personal to perform, could be used as alternative method for early pregnancy diagnosis in field condition with reasonable accuracy.

Based on the above conclusion the following points are recommended:

- Further studies encompassing ultrasound guided analysis of luteal status at the time of prostaglandin F2α injection and nullifying other variable factors like parity, lactational stage and milk yield, breed have to be conducted.
- Generation of enormous data on the efficacy and accuracy of prostaglandin F2α in diagnosing early pregnancy as well as non-pregnancy should be carried out.
- Training should be given to the field veterinarians on recent diagnosis of pregnancy at an early date.
- Educate the dairy owners on the importance of early pregnancy diagnosis to run the farm in profitable manner by training and mass media.

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