Scientific Journal of Engineering & Pharmaceutical Science (https://damaacademia.com/sjeps/)
Volume 1, Issue 5, pp.01-06, May 2019
Published by: Dama Academic Scholarly & Scientific Research Society (www.damaacademia.com)

# Evaluation of the Accuracy of Bovipreg® kit for Early Pregnancy Diagnosis in Indigenous Arsi cattle at Adami Tulu Agricultural research Center, Oromia, Ethiopia

**[1]Alemayehu Arega | [2]Sandip Banerjee | [3]Solomon Gizaw**
*Oromia Agricultural Research Institute, Adami Tulu Agricultural Research Center*
*Hawassa University, College of Agriculture, School of Animal and Range Sciences*
*International Livestock Research Institute, LIVES project*
*Email: fenalex2016@gmail.com*

*Abstract*
*Evaluation of the Accuracy of Bovipreg® kit for early pregnancy diagnosis in indigenous Arsi cattle was done on some selected cattle at AdamiTulu Agricultural Research Center. The result indicates that the overall accuracy of Bovipreg® kit in diagnosing pregnancy were 68.6%. There was a significant difference in the accuracy of Bovipreg test between the milk and blood samples used for diagnosing pregnancy. The overall accuracy of the Bovipreg® were 89.3% and 57.1% by using milk and blood samples respectively. The result indicated that there was no statistically significant difference between the collection dates on the accuracy of Bovipreg® kit. Parity of the animals has no effect on the accuracy of Bovipreg® result. Bovipreg® is one of the technology which allows early detection of pregnancy it has to be evaluated and demonstrated to dairy producers and different stakeholders in the dairy sector.*

***Key words:*** *Bovipreg® kit, pregnancy diagnosis, accuracy, Arsi cattle*

## 1.0 INTRODUCTION

Pregnancy diagnosis is essential for profitable animal husbandry. Early pregnancy diagnosis would help to evaluate the presence of the fetus. Furthermore, early diagnosis of pregnancy is essential for several economic reasons. There is a need to check heifers/cows for pregnancy as early as possible so as to improve the lifetime productivity of the cows, by decreasing the inter calving interval. Pregnancy diagnosis in cattle is a widely practiced procedure and has come to be accepted as a highly recommended management technique in dairy and beef cow herds (Whittier, 2013). Early identification of non-pregnant dairy cows and heifers post breeding can improve the reproductive efficiency and pregnancy rate by decreasing the calving interval and increasing the numbers of lifetime calf produced (Paul, 2010). For successful integration into a reproductive management system, an ideal early pregnancy test for dairy cattle would be sensitive (i.e., correctly identify pregnant animals), specific (i.e., correctly identify non pregnant animals), inexpensive, simple to conduct under field conditions, and able to determine pregnancy status at the time when the test is performed (Paul, 2010). A variety of approaches have been evaluated and developed over the years, for early pregnancy diagnosis in farm animals. Most of the techniques are fairly accurate however like all techniques each of them have some limitations or the other (Natnael *et al.,* 2016). Generally, methods of diagnosing pregnancy are divided into direct and indirect approaches or it can be divided into visual, clinical and laboratory methods (Purohit, 2010).

Amongst all the methods identified for the assessment of pregnancy status of a cow trans rectal palpation of the uterus is quite popular at a global scale and with experienced technicians the method is also fairly accurate. Besides the birth of the cow calves the process has become more popular because of its non invasive nature and hence the method is relatively cheap. However, there are also cases of misdiagnosis of pregnancy especially at early stages (Purohit, 2010). Over the years there have been usages of chemicals and medical equipment's to assess the early pregnancy status of farm animals. One such currently popular equipment is the use of transrectal ultrasonography. However, as indicated before this method too relies on the skill of the technician/s (Paul 2010). Another approach to access the pregnancy status of a cow especially at early stages is the use of hormones which are associated with its pregnancy status (Paul 2010). Presence of specific bio molecules in the maternal body fluids can serve as indirect indicators of the presence of a viable pregnancy (Natnael *et al.,* 2016).Among the popular noninvasive methods to access the pregnancy status of the female livestock to access the levels of endocrine hormones such as progesterone, estronesulphate and pregnancy specific proteins such as pregnancy associated glycoproteins, the early pregnancy factor and interferon-tau (Paul 2010). One such commercially available tool for assessment of the hormone status is the use of BOVIPREG® - One Step Pregnancy Test Cassette. This test evaluates the presence of an early pregnancy and suspected pregnancy following the post conception hormonal changes, as early as 18-22 days (Anonyms, 2013). The earlier the diagnosis the better are the chances of the dam being provided with necessary care associated with its pregnancy

Studies have indicated that with the assistance of Bovipreg® the pregnancy status of the cows can be assessed with an accuracy of 98% and 82.6% among the farm animals however embryonic death and other

pathological conditions may give some false positive cases (Anonyms, 2013 and Solomon *et al*, 2016). Thus the current study was conducted with the objective of evaluating the accuracy of BoviPreg® pregnancy diagnosis kit for early diagnosis of pregnancy among the Arsi cattle and to recommend alternate method pregnancy diagnosis for dairy producers.

## 2.0 MATERIALS AND METHODS

### 2.1 Studied location

The study was conducted at Adami Tulu Agricultural Research center located at 167km south of Addis Ababa and situated at latitude of $7^0$ 9` N and $38^07$`E longitude in semi-arid middle rift valley of Ethiopia. The area is situated at 1500 meters above sea level and the soil type of the area is fine, sandy loam with sand, clay in the proportion of 34:48:18 respectively. The average annual rain fall is 760mm. The minimum and maximum temperature are 12.6 and $27^0$c, respectively (ATARC, 2003).

**Selection criteria of experimental animals and procedures followed:** Arsi cows reared in the center which was used as dam line were selected based on their age, parity and body condition. Progeny tested semen of different sire of Holstein Frisian breed was used as a sire line. The parity of the selected cattle ranged between 2 to 3 and at different stages of lactation with active and functional corpus luteum (CL) were selected.

**Oestrus synchronization:** All cows had been palpated for presence of active CL, and PGF2α analogue (Lutylase®) hormone was administered. Lutylase® was administered to each cow at 2ml/cow after confirmation of receptive corpus luteum through rectal palpation and animal's shows heat signs naturally was inseminated directly.

**Heat detection:** Cows administered with Lutylase® was fitted with Kamar® heat mount detector and also monitored by herdsmen and night guards for signs of heat in order increase the efficiency of heat detection.

**Breeding:** Cows noted in heat in the morning were inseminated that afternoon and those identified in the afternoon were inseminated the next morning based on the "AM- PM guideline" (Peter and Ball, 1995). A standard semen handling and insemination procedure recommended by IAEA (2005) was used to inseminate animals.

**Pregnancy diagnosis:** For this purpose blood and milk samples were collected totally from 77 cows at interval of 18- 22 days post insemination all animals had diagnosed for pregnancy by using BoviPreg® kit. For this purpose 15- 20 inseminated animals was assigned into three groups/ treatments because the pregnancy will be diagnosed from milk, blood and urine samples at day $19^{th}$ , $20^{th}$ and $22^{nd}$ by using BoviPreg® kit. Initially we proposed to look for both blood, milk and urine samples but later on urine samples were ignored based on the latest information we obtain from the company manufacturing Bovipreg® kit.

Samples were collected from animals across each groups but milk samples were collected from only lactating animals whereas blood sample were collected form all animals. Then just add 3 drops of milk/urine or blood serum in the cassette/ kit and get the result in 5 minutes for blood serum and 10-15 minutes for milk/urine. After 90 days animals were rectal palpated for conformation. The accuracy of Bovipreg® test was determined by corresponding with whether the animals repeated or not post the test and result of rectal palpation after 90 days after insemination.

### 2.2 Specimen collection and preparation

**Milk:** Fresh whole milk was used for evaluation. The first couple of squirts had been discarded and then a small amount of milk-about 5 to 10 ml in a cup will be collected in sterile container. The samples would be well mixed before using it for the test. Sometimes milk with high viscosity prevents its spreading to all areas of the kit. So, the specimen could not go up to the whole membrane and apparently stopped in the middle of the test. To solve this problem, it will need to keep the milk still (at rest) for half an hour after collection, and then draw the milk from the bottom layer using the dropper and use that milk on the test kit. Please note that the milk cream usually floats on the surface (Anonyms, 2013).

**Blood serum:** Blood would collected through vein puncture and allowed to clot for some time, the clear straw colored fluid(serum) which was collected on the top of the clot will be used for assessing the pregnancy status of the cows. To facilitate easily separation of the serum from the blood we put the collected blood sample in vacutainer tube and then putted in centrifuge. Then 3-4 drops of the serum dropped on the circular well of the BoviPreg® kit.

**Assay procedures:** The test device and dropper was removed from its foil wrapper by tearing along the slice. Using the dropper, the milk/serum sample collected in a cup was withdrawn and slowly dispensed 3-5 drops into the circular sample well. The sample liquid moves slowly to the other end due to the capillarity. Then finally, we observe one or two red lines in the result window.

### 2.3 Interpretation of results

**Positive:** If both T band and C band are visible, the test result is positive, which means the cow is pregnant.
**Negative:** If only C line is visible, the test result is negative, which indicates the cow is not pregnant.

**Invalid:** When control line does not appear on the membrane, the test is invalid due to improper test procedure or deterioration of reagents. It is recommended that the test be repeated (Anonyms, 2013).

### 2.4 Design of the experiment

For Bovipreg® early pregnancy diagnosis milk and blood samples were collected from all animals across each treatment at different day interval between 18-22 days post insemination.

Treatment 1- Day $19^{th}$
Treatment 2- Day $20^{th}$
Treatment 3- Day $22^{nd}$

## 3.0 STATISTICAL ANALYSIS

Collected data's were coded and processes into Microsoft Excel and imported and analyzed by using Statistical Package for Social Science Version 20.0 (SPSS).The Chi-square ($\chi 2$) test procedure of SPSS Version 24.0 was used to analyze the qualitative data and quantitative data were analyzed using the General Linear Model Procedure (univariate). The variation between groups was considered significant when the $P < 0.05$. Experimental data were analyzed by using factorial analysis statistical methods and GLM procedure of SPSS v24.

### 3.1 Model used for evaluating Bovipreg accuracy

$Y_{ijk}= \mu+ st_i+ cd_j + p_k + e_{ijk}$
$Y_{ijk=}$ Overall accuracy
$\mu$= Over all mean
$st_j$= Random effect of $i^{th}$ sample type ( j=2, blood and milk)
$cd_j$ = Random effect of $j^{th}$ sample collection date (k=3, day 19, day 20 and day 22)
$p_{k=}$ Random effect of $k^{th}$ parity (l=2, second and third)
$stcdp_{ijk}$ = Interaction effect of sample type, collection date and parity
$e_{ijk=}$ Residual error

### 3.2 Results

Overall there is a significant difference between the Bovipreg® and rectal palpation results in diagnosing pregnancy. Totally, seventy- seven cows were checked for pregnancy using Bovipreg® on $19^{th}$, $20^{th}$ and $22^{nd}$ days post insemination. Then cows were checked for repeating after first insemination and also finally rectal palpated after 90 days of insemination. The correspondence between Bovipreg®, attending for repeating and rectal palpation for pregnancy were used to estimate the accuracy of Bovipreg® result.

Overall the accuracy of Bovipreg® kit in diagnosing pregnancy were 68.6%. The probability of reporting false positive and false negative pregnancy results from the Bovipreg® tests considering attending for repeating after test and rectal palpation as a reference were 44.0% and 7.4%, respectively. Early embryonic mortality between day 21 of Bovipreg® test and 90 days of rectal palpation post insemination which was not easily noticed by herdsmen and herd attendants might be the major reason for discrepancies between the results of Bovipreg® and rectal palpation.

Table 1. Overall accuracy of Bovipreg®

| Accuracy | Bovipreg® results | | Chi-square($\chi 2$) |
|---|---|---|---|
| | Positive | Negative | |
| Accurate | | | |
| N | 28 | 22 | |
| % age | 56.0 | 44.0 | 0.001 |
| Not Accurate | | | |
| N | 22 | 2 | |
| % age | 44.0 | 7.4 | |
| Overall | | | |
| N | 53 | 24 | |
| % age | 68.8 | 31.2 | |

### 3.3 Effect of sample types on Bovipreg® test result

The findings as presented in Table 23 indicate that blood milk and urine samples were recommended for Bovipreg® test. However, information received from the manufacturers indicate that urine samples may at times provide conflicting results and hence was avoided in the present study. There was a significant difference in the accuracy of Bovipreg test between the milk and blood samples used for diagnosing pregnancy. The result indicates that the accuracy were higher by using milk samples than blood samples.

Table 2. Sample types used for Bovipreg® test.

| Accuracy | Sample type | | Chi- square ($\chi 2$) |
|---|---|---|---|
| | Blood | Milk | |
| Accurate | | | |
| N | 28 | 25 | 0.003 |
| % age | 57.1 | 89.3 | |
| Not Accurate | | | |
| N | 21 | 3 | |
| % age | 42.9 | 10.7 | |
| Total | | | |
| N | 49 | 28 | |
| % age | 100 | 100 | |

**3.4 Effects of sample collection date**

As per recommendation of the manufacturers of the Bovipreg® kit it was advised to detect pregnancy between 18$^{th}$ to 22$^{nd}$ days post insemination. In current study to see the effects of the sample collection date animals were grouped into three groups 19$^{th}$, 20$^{th}$ and 22$^{nd}$ days post insemination and both blood and milk samples were collected across each groups.

The result pertains to the accuracy of Bovipreg® kit test of pregnancy is indicated in Table 24. The result indicated that there was no significant difference between the collection date on the accuracy of Bovipreg® kit but samples collected on day 22$^{nd}$ has relatively higher accuracy when compared to those collected from the other dates.

Table 3. Sample collection date for Bovipreg® pregnancy diagnosis

| Accuracy | Collection date | | | Chi-square($\chi 2$) |
|---|---|---|---|---|
| | Day 19$^{th}$ | Day 20$^{th}$ | Day 22$^{nd}$ | |
| Accurate | | | | |
| N | 18 | 20 | 15 | 0.231 |
| % age | 58.1 | 74.1 | 78.9 | |
| Not Accurate | | | | |
| N | 13 | 7 | 4 | |
| % age | 41.9 | 25.9 | 21.1 | |
| Total | | | | |
| N | 31 | 27 | 19 | |
| % age | 100 | 100 | 100 | |

**3.5 Effect of parity on Bovipreg® test**

Parity of the animals has no effect on the accuracy of Bovipreg® result. The current result indicated that there is no significant difference in the accuracy of Bovipreg® result between the two parties. The result indicates the accuracy of Bovipreg® test not dependent on the parity of animals and can allow to diagnosis pregnancy both on heifers and cows.

**4.0 CONLUSIONS**

Bovipreg® kit is one Step Pregnancy Test Cassette. This test evaluates early pregnancy and suspected pregnancy it enables to diagnosis pregnancy early from 18-22 days of insemination. Early pregnancy detection shortens the period of non-pregnancy by way of effective treatment or culling and thus increases the profitability of

maintaining a herd (Anonyms, 2013).Overall there is a significant difference between the Bovipreg® and rectal palpation results in diagnosing pregnancy. Totally, seventy- seven cows were checked for pregnancy using Bovipreg® on 19th, 20th and 22nd days post insemination. Then cows were checked for repeating after first insemination and also finally rectal palpated after 90 days of insemination. The correspondence between Bovipreg®, attending for repeating and rectal palpation for pregnancy were used to estimate the accuracy of Bovipreg® result. Overall the accuracy of Bovipreg® kit in diagnosing pregnancy were 68.6%. The probability of reporting false positive and false negative pregnancy results from the Bovipreg® tests considering attending for repeating after test and rectal palpation as a reference were 44.0% and 7.4%, respectively. Early embryonic mortality between day 21 of Bovipreg® test and 90 days of rectal palpation post insemination which was not easily noticed by herdsmen and herd attendants might be the major reason for discrepancies between the results of Bovipreg® and rectal palpation.

The current result were less than the figure reported by (Solomon *et al*, 2016) under smallholder dairy farms in Tigray region, Ethiopia where overall, accuracy were 82.6%. The probability of reporting false positive was higher than their findings with lower false negative reports. Kaçar, C *et al.*, 2017 in their study indicated that the overall accuracy of Bovipreg were 78.04 % from serum sample and 70.73 % from whole blood. The difference in the accuracy and probability of getting false positive and false negative be due to the variation in the samples used for test. One of the major advantage the Bovipreg® kit was the possibility of using both milk and blood sample for pregnancy diagnosis in addition to allow to early diagnosing pregnancy. In current study both blood and milk samples which have variation in accuracy were used whereas, they use milk samples only for Bovipreg® test which has higher accuracy than blood samples. In addition to having better accuracy milk samples were taken by anyone and there is no need for any special equipment and knowledge *i.e.* any livestock keeper can have the kit collect the milk samples and check for pregnancy.

**4.1 Effect of sample types on Bovipreg® test result**

Generally, blood milk and urine samples were recommended for Bovipreg® test. In current study only blood and milk samples were used and the urine samples were ignored based on the latest recommendation we got from the company as urine samples were not advised for better accuracy. There was a significant difference in the accuracy of Bovipreg test between the milk and blood samples used for diagnosing pregnancy. The overall accuracy of the Bovipreg® were 89.3% and 57.1% by using milk and blood samples respectively. The result indicates that the accuracy were higher by using milk samples than blood samples.

The current result were higher than the figure reported by (Solomon *et al*, 2016) under smallholder dairy farms in Tigray region, Ethiopia where the accuracy of the kit were 82.6% by using milk sample. But the current findings from blood sample on the accuracy of Bovipreg® was less than the findings by Kaçar, C *et al.*, (2017) in which the overall accuracy of Bovipreg were 78.04 % from serum sample and 70.73 % from whole blood.

**4.2 Effects of sample collection date**

As per recommendation Bovipreg® kit allows to detect pregnancy 18th to 22nd days post insemination. In current study to see the effects of the sample collection date animals were grouped into three groups 19th, 20th and 22nd days post insemination and both blood and milk samples were collected across each groups. The result indicated that there was no statistically significant difference between the collection date on the accuracy of Bovipreg® kit but samples collected on day 22nd has relatively higher accuracy in percentage than others. So, anyone can able to check for pregnancy of his cow at any day between 18th to 22nd days post service.

**4.3 Effect of parity on Bovipreg® test**

Parity of the animals has no effect on the accuracy of Bovipreg® result. The current result indicated that there is no significant difference in the accuracy of Bovipreg® result between the two parties. The result indicates the accuracy of Bovipreg® test not dependent on the parity of animals and can allow to diagnosis pregnancy both on heifers and cows.

**4.4 Recommendation**

Early pregnancy diagnosis is essential for dairy producers not only to know the status of their animals but also to increase the profitability of the industry by reducing the calving interval the use of modern dairy technologies are important and thus, Bovipreg® is one of the technology which allows early detection of pregnancy it has to be evaluated and demonstrated to dairy producers and different stakeholders in the dairy sector.

**4.5 Acknowledgement**

The authors are grateful to Oromia Agricultural Research Institute for financial support, Adami Tulu Agricultural Research Center management and technical staffs (especially Dairy Research Team staffs), International Livestock Research Institute LIVES project for supplying inputs for the study and Hawassa University, College of Agriculture, School of Animal and Range Sciences.

***References***

*Anonyms, 2013 Bovipreg pregnancy diagnosis Kit (http://www.twilcanada.com/view/TwilCanada%20BOVIPREG.html. Accessed date August 24, 2016.*

*ATARC, 2003. Adami Tullu Agricultural Research Center, strategic planning and management document, P. 67.*

*Balhara, A.K., M. Gupta, S. Singh, A.K. Mohanty and I. Singh, 2013. Early pregnancy diagnosis in bovines: current status and future directions. The Scientific World Journal, pp: 1-10.*

*Cowie TA. Pregnancy diagnosis tests: A review. Commonwealth Agricultual Bureaux Joint Publication No. 13, Great Britain, 1948 pp 11-17.*

*IAEA (2005). Improving artificial breeding of cattle in Africa. Guidelines and recommendations. A manual prepared under the framework of an IAEA Technical Cooperation Regional AFRA Project on Increasing and Improving Milk and Meat Production, with technical support from the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.*

*Kaçar, Cihan & Karakuş, Orhan & Kaya, Semra & Can Demir, Murat & Ari, Umut & Gaffar Zonturlu, Abuzer & Öztürkler, Yavuz. (2017). Investigation of sensitivity of Fassisi ® BoviPreg test kit for early pregnancy diagnosis in cattle.*

*NADIS, 2016. Fertility in Dairy Herds Part 5-Pregnancy Diagnosis-An essential part of fertility management, available at: http://www. qmscotland.co.uk.*

*Natnael Bekele, Mekeonnen Addis, Nejash Abdela and Wahid M. Ahmed, 2016. Pregnancy Diagnosis in Cattle for Fertility Management: A Review Global Veterinaria 16 (4): 355-364.*

*Omid Mavedati, Abdolreza Rastegarnia, Reza Habibian, Yousef Nasiri Bari and Esmail Bandarian, 2013. Early Pregnancy Diagnosis in Water Buffalo by Early Pregnancy Factor Measurement Using Rosette Inhibition Test. Global Veterinaria, 10(4): 391-393.*

*Peter AR, Ball PJH, 1995. Reproduction in Cattle, Second Edition, Blackwell Pres, Oxford, U.K.*

*Purohit G., 2010. Methods of Pregnancy Diagnosis in Domestic Animals: The Current Status. Webmed Central REPRODUCTION 2010; 1 (12):WMC001305*

*Solomon Gizaw, Tadesse Gugssa, Yayneshet Tesfay, Dawit Woldemariam and Azage Tegegne, 2016. Cattle pregnancy Diagnosis technologies tested in smallholder farms. https://lives-ethiopia.org/2016/10/28/cattle-pregnancy-diagnosis-technologies-tested-in-smallholder-farms/ https://lives-ethiopia.org Accessed November 30, 2017.*

*Whittier, D.W., 2013. Pregnancy Determination in Cattle, a review of Available Alternatives. Applied Reproductive Strategies in Beef Cattle. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, pp: 165-176*