Pregnancy Detection Techniques in Cattle

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Abstract

Frequently pregnancy in cattle is diagnosed by transrectal ultrasonography of reproductive tract after 30 days or manual palpation of reproductive tract after 35 days to 282 days with 95% sensitivity and specificity which are expensive, invasive and needs an experienced medical practitioner which may lead to abortion in case of mishandling. Non-invasive and non-expert based pregnancy detection assays were developed by analyzing Estrone sulphates or Pregnancy associated glycoproteins or Progesterone levels by Enzyme Linked Immunosorbsent Assay (ELISA), Radio Immuno Assay (RIA) or Latex Agglutination (LA) in which blood or milk or urine or feces is used as sample. Due to the difficulties in sampling or less specificity and sensitivity of the developed assays, they are not succeeded well in the market to produce an efficient commercial kit for pregnancy detection in cattle. As there is no reliable specific molecule found to denote pregnancy status in cattle. Researchers tried to evaluate the pregnancy status with physiological and biochemical markers which have limited applications in the field. So there is a need to increase the sensitivity and specificity of pregnancy detection assays to improve easy, non-invasive, economical and accurate method which will work even with single sample.

Keywords: Cattle Pregnancy, Progesterone, Estrone sulphates, ELISA, RIA, LA.

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Introduction

Milk is a natural and traditional food considered as an important factor in human’s healthy and balanced diet for millennia [1]. India occupied first position in milk production and mostly our dietary milk is produced from cattle. For the last two and half decades per capita availability of milk in India was progressed from 178 to 337 grams/ day [2]. With an occupation of 30 percent planet’s ice free terrestrial surface area, $1.4$ trillion asset value, livestock is emerging as agriculture subsector quicklywith 33 percent occupancy in agriculture GDP [3]. India has blessed with vast dairy resource with an approximate number of 267.6 million cows and 165.3 million buffaloes. According to NDBD (India) data 2015-16, India produced 155.5 million tons of milk in 2016 which will rise to 200 million tons by the end of 2021-22. So it is necessary to maintain annual growth by 4 percent in the next 10 years to reach the demand of milk. But unfortunately the number of total bovines decreased from 304.4 million to 299.6 million from 2007 to 2012. To improve the number of bovines, NDBD had already proposed a breeding policy which included disease free and high quality sperm production, progeny testing and encouraging artificial insemination in cattle. In 1990
government agencies performed 18,718 artificial inseminations whereas it increased to 63,204 in 2015 [2]. It was reported that 2-5% of pregnancies in animals are terminated by spontaneous abortions. As insemination of pregnant animal is also a cause for abortion, it is important to monitor the crossed or inseminated animal to detect pregnancy [4].

Pregnancy Detection

Frequently pregnancy in cattle is diagnosed by transrectal ultrasound where the changes in the ovarian follicular population and reproductive tract will be evaluated by ultrasound imaging technology after 30 days of conception or it will be diagnosed by traditional manual palpation of reproductive tract where the experienced medical practitioner palpate the reproductive tract through rectal wall by his arm after 35 days of conception. These two methods can diagnose pregnancy up to 282 days with 95% sensitivity and specificity, but these are expensive, invasive and needs an experienced medical practitioner [5-7]. In India, small scale dairy farms are maintaining in almost 60000 villages where the veterinary facilities are not available nearby and on payment basis only they will get these services. There was a report that almost 50% of farmers are not using these services due to economic issues, lack of facilities and other reasons [8]. It is advantageous if we detect pregnancy within 35 days of conception as the 33% of abortions occurs from conception to 35 days [9]. Abortions due to manual diagnosis of pregnancy also reported in 5% cases, so some times these two techniques may not be safe for animals [10]. The above two techniques are accurate with high level of sensitivity and specificity and are most common used techniques across the globe but the failure in detection of early pregnancy before 35 days, availability of an experienced medical practitioner, transport of animals to near veterinary centers for veterinarian and risk of mishandling are the main draw backs of the above techniques, so there is a need to follow noninvasive techniques for the detection of pregnancy.

Non-invasive and non-expert based pregnancy detection assays were developed by using Enzyme Linked Immunosorbent Assay (ELISA), Radio Immuno Assay (RIA) or Latex Agglutination (LA) in which blood or milk is used to detect pregnancy specific marker. Estrone sulphate [11-14], pregnancy associated glycoproteins (PAG) [15-16] and steroid hormones [17-24] are some of the markers that are examining as pregnancy indicators.

As a conjugated Estrogen, Estrone sulphate is synthesized in fetus or placenome’s scotyledinory portion. Estrone sulphate is a highly specific pregnancy indicator produced by fats detected in milk [11-12] and blood [13]. Despite of being a highly specific marker for pregnancy detection, Estrone sulphate availability in blood and milk is a draw back for the technique and the collection of blood from animal is also associated with risk factors. Pregnancy detection by Estronesulphate is not reliable before 100 days of gestation period due to the effect of breed, body size, parity status and environment on Estrone sulphate levels which may lead to of false negative or false positive results [11]. So the above technique is also not succeeded for further.

Pregnancy associated glycoproteins are produced by the placenta and trophoblast, which appear in circulation after 15 days of conception. They are direct indicators of pregnancy. Only modern PAG based tests achieved 95% sensitivity and specificity when they used strategically because so many factors such as placental health, infection and metabolic rates influence PAG levels in cattle. After parturition also PAG levels can persist which leads to false positive diagnosis in cows mated within 8 weeks of calving.

In above mentioned techniques, body fluids will be taken as samples in which the PAG and estrone sulphate will present. Antibodies raised against that respective marker molecule will be coated on micro titer well plates or a gel. Samples will be applied on to the respective matrix for interaction of marker molecules and respective antibodies. The reaction will be evaluated either by radioactive molecules (RIA), or by the formation of agglutination (Latex agglutination) or by converting the specific reaction to colored compounds by using Enzyme-Substrate reactions (ELISA). Generally blood and milk are the major sources for those molecules. Due to the difficulties in availability of milk in dairy heifers and
difficulties in collection of blood samples, the above techniques didn't succeed completely in field level.

Gaetano et al. [25] used a new technique in which they detected pregnancy by combining fetus electrocardiogram and phonocardiogram signal processing. In this technique heart beat, depolarization of cardiac muscles, blood pressure and sound waves generated by fetus through maternal tissues will be generated as electrical signals and the same will be captured, filtered, processed and analyzed by various detectors placed on the surface of the animal. Their results showed 87.6% Sensitivity and 74.6% specificity which increased to 91% and 81% respectively after removing noise files. Even this method can detect pregnancy non-invasively from 30 days of conception but this approach is not accepted due to the problems of apparatus in coupling of audio sensors to the skin and failure of weak signal detection in the field [24].

Progesterone is a steroid hormone present in both pregnant and non-pregnant animals. Progesterone levels in serum and milk are related to luteal activity of the ovum which is being evaluated during the luteal phase of the ovarian cycle with low concentration at 4-5 days of estrous cycle. But monitoring of progesterone levels in between 18-24 days showed a sharp increase of progesterone levels in pregnant animals. Schwarzenberger et al. [26] proved that Progesterone is metabolized to pregnanediol and hydroxylated pregnanes which are excreted through feces. By using group specific antibodies they analyzed these metabolites to study ovarian cycles and pregnancy detection [26]. Till now most of the researchers worked on progesterone and its metabolites to detect pregnancy. As the progesterone and its metabolite concentrations in milk, serum and feces are related to ovarian luteal activity which affected the efficiency of these techniques, N isobe et al., [19] found an answer for this problem and found that progesterone metabolite concentration increases in between 18-20 days. Depending on the progesterone increasing level, they differentiated pregnant and non-pregnant animals. Sensitivity and specificity of pregnant animals is 70% and non-pregnant animals are 100% for these assays. As the progesterone has huge number of metabolites and the assays were developed against specific metabolites there might be a chance for missing real progesterone concentration which is impacting the sensitivity and specificity of these assays. Pregnancy detection by progesterone is a secondary indicator for pregnancy detection and it needs repetitive and serial sample collection and its analysis. So these techniques are not succeeded well in the field.

**Conclusion**

As there is no reliable specific molecule found to denote pregnancy status in cattle. Researchers tried to evaluate the pregnancy status with physiological and biochemical markers which have limited applications in the field. Due to the difficulties in sampling or less specificity and sensitivity of the developed assays, they are not succeeded well in the market to produce a commercial kit for pregnancy detection in cattle. So there is a need to increase the sensitivity and specificity of pregnancy detection assays in pregnant animals to improve easy, non-invasive, economical and accurate method which will work even with single sample. Despite of working with limited application markers, research has to be progressed to find the pregnancy specific hormones or proteins to develop new assays for pregnancy detection. Otherwise instead of working with specific metabolite of progesterone it is advisable to evaluate total progesterone level or the metabolite which will represent the maximum level of progesterone concentration to differentiate pregnant and non-pregnant animals which may be useful to design an efficient kit to detect pregnancy.

**Reference**

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