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Popular Article

Pregnancy diagnosis in buffalo with molecular techniques: current status and future benefits

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Abstract

Reproductive efficiency is a primary challenge for livestock producers, as it may have a massive financial impact on any farm animals' operation. Determining pregnancy on its early stages is only important factor with regards to reproductive efficiency in buffaloes and other ruminants. The diagnosis of pregnancy at early-stage aids in shortening the calving interval and will aid in timely rebreeding of open animals. Early detection of non-pregnant buffaloes provides better assessment of conception and effectiveness in semen fertility in artificial insemination programming and it also helps in determining of any possible disease that can cause infection in it. In the Indian subcontinent, a buffalo is the vital dairy animal that suffers from problems like late puberty and high calving interval. Different techniques are used for pregnancy in females including hormonal and biochemical methods. In animals, new techniques have opened up a way for detection of biomarker molecules which can be used in pregnancy detection. Nowadays recent molecular techniques like ELISA, proteomics and NASBA plays a major role in it. This article is to take a look at different pregnancy methods for dairy animals using molecular techniques and detecting pregnancy in early stages for future prospects of research in this area.

Introduction

Animal biotechnology is continually evolving. Advancement had been made for management of livestock for commercial use. High embryonic mortality and low reproductive efficacy is the major failure in milk production of dairy animals. Pregnancy diagnosis in early stages can help to shorten calving interval and detection of open buffaloes for re-breeding the animal in same breading season or in earlier next opportunity [1]. Early re-breeding of non-pregnant buffaloes after pregnancy detection leads to economic benefits to dairy farmers i.e., one calf per annum. For detection, early

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after mating of animal a test is needed which is accurate, safe and able to distinguish between pregnant and non-pregnant females. Different approaches are used for pregnancy diagnosis in female buffaloes. There are two general approaches for pregnancy diagnosis in pregnant buffaloes i.e., clinical and laboratory (direct and indirect method). In today's world research had made development in molecular diagnostics like. PCR, ELISA, DNA and protein array for early diagnosis in dairy animals like buffaloes

Different approaches of pregnancy diagnosis

1) Clinical approaches (direct approach)

(A) **Transrectal palpation:** it is one of the oldest methods to check pregnancy in buffaloes after mating. Although this method give accuracy in pregnancy diagnosis but only after 30 -45 days. This method had its own demerits like unable to provide survival rate of fetus after the risk cause of iatrogenic embryonic mortality

(B) Trans-rectal ultrasonography: This method of detection is better than palpation as it is done in earlier stage as well as do not need an experience operator like previous one. This method is used after 28-31 day of mating and it can provide accurate diagnosis on day 31 as sensitivity is high in animals. Trans-rectal ultrasonography needs a high efficiency of ultrasound machine with rectal transducer which is not cost effective as a result harm the dairy farmers as well as practical implementation in research field [2].

Both of these approaches give accurate results but needs a great amount of skilled and experience veterinarians.

2**)Laboratory approaches (Indirect approach)**

A**) Progesterone**: in many ruminants, progesterone level measurement is indirect approach to diagnose pregnancy. The concentration of progesterone serves as an indication of the corpus luteum function rather than the existence of an embryo or fetus. Therefore, animals undergoing extended cycles can be identify as positive. Decrease amount of progesterone secretion up to twenty-fourth day is an indicator of non-pregnant female while high secretion does not ensure pregnancy due to variances in both the duration of the estrous cycle among buffaloes.

B) **Pregnancy specific proteins**: - PAG, also referred to as PSPB or PSP-60, were initially described as antigens found in the placenta which were also found in the mother's blood serum shortly after the embryo was implanted. In outer placental cells glycoproteins are synthesized, modified, regulated by mono and di nucleated trophoblast cells and this pregnancy associated glycoproteins are secreted

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in blood stream of pregnant female during placentomes formation and firm attachment of blastocyst with uterine walls. Two proteins, PSP-A and PSP-B, that are specific to pregnancy have been extracted from bovine fetal membranes. PSP-A was identified as alpha-fetoprotein, and PSP-B was observed to be only found in the placenta. PSP-B is primarily used for detecting pregnancies in bovines.[3]

C) **Interferons**: - Interferon IFNT is a protein that is expressed and triggers the production of interferon-stimulated genes (ISGs) in the endometrium during the process of recognizing a pregnancy. After conception in bovines, an embryo begins to grow and elongate rapidly. This is necessary for the secretion of a certain amount of IFNT, so the mother can recognize the pregnancy, and also to create a larger area of vascular exchange with the maternal tissues after implantation. This elongation and increase in vascular exchange occur two to three weeks after fertilization. The mother cow is able to recognize when a fetus is present in her uterus between days 12 and 26 of her pregnancy due to the secretion of IFNT by the bovine conceptus.[4] These IFNt may escapes the uterus and can be detected in blood. Leukocytes respond to IFNt by expressing interferon-stimulated genes (ISG) as interferon-stimulated protein such as 15 kDa (ISG15), myxovirus resistance 2 (Mx2) and 2'-5' oligoadenylate synthetase (OAS1), in peripheral blood leukocytes which can be detected during early pregnancy.

D) **Estrone sulphate**: - The concentration of Estrone sulphate is associated with fetal numbers, as it tends to be increased when the number of developing fetuses exceeds one. However, it is important to note that estrone sulphate does not serve as an optimal pregnancy biomarker due to its susceptibility to various influencing factors such as genetic composition, parity status, environmental conditions and body weight [5]. Estrogen 3- sulfate is a natural hormone. Basically, an endogenous steroid and estrogen ester found in female blood, milk and other body fluids. Usually after fourth or fifth month of pregnancy in buffalo there is a sudden increase in its level in blood plasma allows pregnancy detection and identification of functions particularly related to embryonic growth. A negative result may indicate a non-pregnant state, but it does help in early diagnosis of pregnancy. Instead, this assay is valuable in ensuring fetal vitality during the last two months of pregnancy.

E) **Micro RNAs:** Recently, many research groups have focused on identification of circulating nucleic acid and microRNAs. These have important role in pregnancy physiology and can be quantified using in many biological fluids like serum, urine and milk. Currently, research methods are required to find out the selective miRNA which can be used in detection of pregnancy. These

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miRNAs may have role in identification of embryonic viability which need to be explored. Preliminary data from our laboratory indicates some micro-RNA have significant upregulation during 18th days of pregnancy and these can act as biomarker for detection of early pregnancy [6].

Conclusion

It is clear that currently available approaches of pregnancy detection for dairy production do not provide an ideal solution by virtue of various limitations. This indicates necessity for further research to develop novel diagnostics. Instrumentation and molecular techniques, which are still in their infancy, offer promise for discovering molecules related to developmental stages of fetus in buffaloes. It can be the breakthrough needed to effectively diagnose pregnancy in livestock.

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