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Trends in development of pregnancy diagnosis techniques in ruminants

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ABSTRACT

Pregnancy diagnosis is highly important in livestock sector to measure a timeline of reproductive efficiency. An efficient reproductive performance is highly important for sustainable, profitable and economic livestock farming. It measures the incidence of delayed puberty, anoestrous, calving interval, lactation length along with provides a guideline for better reproductive management, treatment and possibility of rebreeding in animals. A profitable reproductive management process with an accurate early pregnancy diagnosis technique is a prerequisite to a decline in the rate of embryonic mortality and accelerates production-based profit for the farmers.Various methods have been adopted for diagnosis of pregnancy at different stages in animals with different specificity and sensitivity depending on reproductive behaviour of ruminants. However, none of the methods of pregnancy diagnosis adopted so far in ruminants found to be an ideal one. The advancement of applied molecular techniques like proteomics,transcriptomics and metabolomics in animal research has paved the path to identify some ideal biomarker molecules related to specific stage of pregnancy in the farm animals. This review explains the common methods available for pregnancy diagnosis infarm ruminants and the possibilities of applying advanced modern technologies in development of diagnostics for detection of pregnancy in different farm animals.

Key words: Glycoprotein, oestrogen, pregnancy diagnosis, progesterone, ultrasound

INTRODUCTION

Efficient reproductive management in farm animals plays important role in contribution to growth of livestock sector. Successful pregnancy in animals is the outcome of scientific reproductive management applied in farming system. Diagnosis of pregnancy plays an important role in evaluating the reproductive managemental procedures applied in farm animals time to time basis. Various methods have been developed and evaluated to detect pregnancy in farm animals in due course of time with different accuracy, specificity and sensitivity. An efficient reproductive management process with an early pregnancy diagnosis technique is a prerequisite to a decline in the rate of embryonic mortality and accelerates production-based profit for the farmers.

PREGNANCY DIAGNOSIS TECHNIQUES

Different techniques have been developed and evaluated in the past for finding out a wide range of markers associated with diagnosis of pregnancy with accuracy. The techniques have been categorised as direct and indirect methods (Fig. 1).

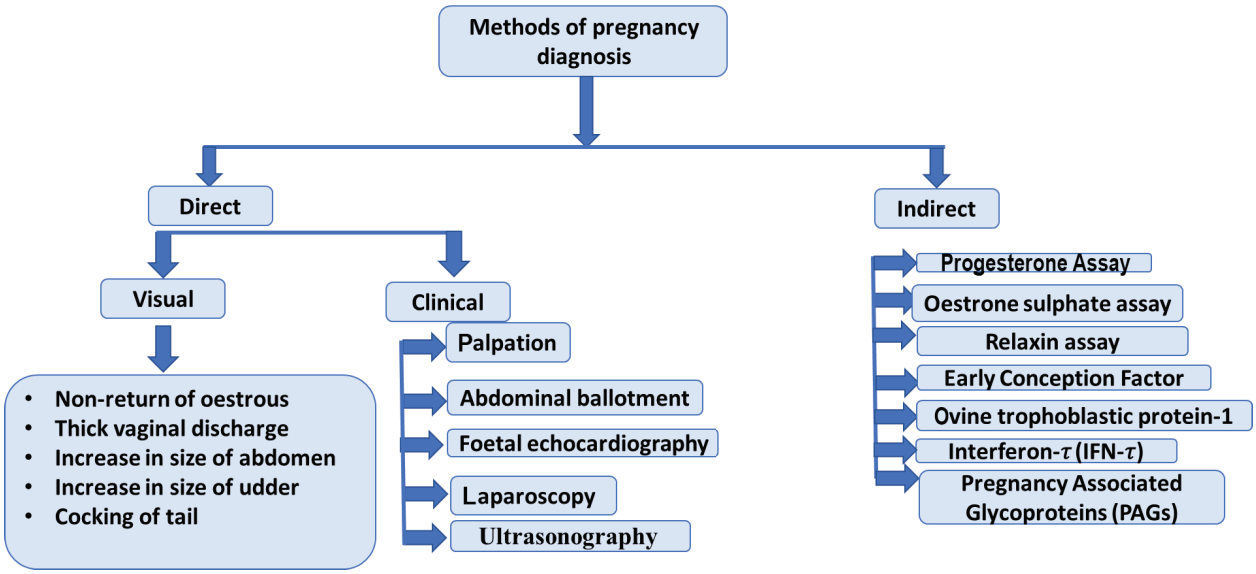

Fig. 1. Different methods used for pregnancy diagnosis in farm animals

Direct method

Visual methods

The method detects some of the external manifestation related to changes in body physiology in relation to pregnancy. Followings are some of the visual changes detected in ruminants following pregnancy.

(i) Non return of oestrous

If an animal does not return to oestrus following mating or artificial insemination (AI), it is usually supposed that the animal has become pregnant. Sustenance of corpus luteum prevents returning to oestrous in farm animals. The symptom is not applicable to seasonal breeders and anestrous conditions. Specifically, silent estrous in case of buffaloes paves the hurdle for determination of pregnancy in the species. In case of sheep, goat and mares oestrous detection should be done in a proper way with high efficiency and accuracy. However, the method has been found suitable in case of cows not returning to oestrous and confirming pregnancy (Kidder et al., 1954). Sometimes prolonged inter-estrus intervals may confuse with pregnancy as reported by Sartori et al. (2004) in case of cows.

(ii) Other physical signs

Apart from the above-mentioned signs, some other symptoms associated with pregnancy are as follows:

- gradual increase in size of abdomen
- gradual increase in size of udder
- thick mucoidal vaginal discharge
- cocking of tail

However, the accuracy of these visual methods has been found to be always low. Further, these visual methods are required to assist other confirmatory methods associated with pregnancy.

Clinical methods

Palpation

(i) Recto-genital palpation

This method is the easiest, cheapest and fastest method of pregnancy diagnosis with little harm to the animal and its foetus as followed in most of the large ruminants like cattle, buffaloes, mares, female camels and also in pigs. But the method is not feasible in case of small ruminants like sheep and goats.

(ii) Per-rectal palpation

Per-Rectal Palpation is the oldest and most common practised method for pregnancy diagnosis in large ruminants like cattle (Cowie, 1948) and buffaloes. Per-rectal palpation is generally performed

to detect presence of amniotic vesicle (Wisnicky and Cassida, 1948) and chorioallantoic membranes (Zemjanis, 1970) of developing foetus in uterus at 30th day of pregnancy in case of cows and in buffaloes the method confirms pregnancy at 45th of pregnancy (Arthur et al., 1966). The method has not been applicable to small ruminants like sheep and goats. There is possibility of iatrogenic embryonic mortality (Franco et al., 1987; Paisley et al., 1978; Vaillancourt et al., 1979; White et al., 1989) in frequent per rectal palpation in large farm animals.

(iii) Abdominal palpation

Abdominal palpation has been adopted as a common and easiest method for detection of pregnancy in animals for a long time but to the later phase of gestation. In cows, the palpation process helps in detection of presence of foetus floating in fluid during late gestation at around 7 months onward. But, in sheep and goats' rectal abdominal palpation can be done at and beyond 4 months of pregnancy for detection of foetus. In small ruminants the palpation is done with glass rod placed in the rectum to lift the uterus which is palpated through abdomen (Ott et al., 1981; Hulet, 1972; Chauhan and Waziri, 1991). Similarly bimanual palpation for pregnancy diagnosis with palpation of uterus through fingers in the rectum and lifting the abdomen has been suggested for small ruminants (Chauhan and Waziri, 1991; Kutty, 1999). However, for small ruminants like sheep and goats, the abdominal palpation methods were found to be inaccurate.

Fetal echocardiography

Fetal echocardiography can be implicated in diagnosis of pregnancy in cattle, sheep and mares but with the advent of ultrasonography its use has been limited.

Vaginal electrical resistance

The method measures the conductivity or vaginal electrical resistance (VER) of the vaginal mucous membranes by ohm meters at estrus. The conductivity is high and VER is low during oestrous because of hydration and high blood supply as observed in case of cows. The VER resistance certainly can be differentiated from pregnant animal vaginal mucous membrane (Foote et al., 1979; Mc Caughey, 1981; Gupta and Purohit, 2001; Meena et al., 2003). However, the blood supply and the hydration status of vaginal mucus membrane vary with different external and internal factors like water intake, feed intake, environment, weather disease condition etc. So, the accuracy of such estimations in diagnosis of pregnancy is limited due to false positive results and the problem related with daily probing of animals.

Laparoscopy

Laparoscopy is an invasive method of pregnancy diagnosis by directly visualizing the internal uterine environment of farm animals with the help of a probe. However, the invasive nature of the technique, the high cost of equipment, requirement of skilled personnel and the availability of non-invasive techniques limits the use of this technique as a means of pregnancy diagnosis in most of the small and large ruminants.

Ultrasonography

Ultrasound scanning is a common, reliable and comparatively cheap method for detection of pregnancy in animals (Gonzales-Bulnes et al., 2010). In case of large ruminants, ultrasonography method has been successfully implicated for pregnancy diagnosis at 28 days of artificial insemination (Curran et al., 1986; Franco et al., 1987; Sharma et al., 2011).In small ruminants like sheep and goats (Kumar et al., 2015; Azizunnesa et al., 2019) trans-abdominal ultrasonography has been followed with the transducer above the udder between the thigh and abdomen preferably the left side and moved in a 'W' shape from one side of the abdomen to the other side. In case of sow's sector scanners (3.5-5.0 MH2 frequency) or doppler instruments are frequently used to detect fetal heart beat, fetal movements and uterine artery pulsations. Transrectal ultrasonography has been routinely used in cattle (Sharma et al., 2011) and buffaloes (Fricke et al., 2001) to get information on ovarian structures, identification of twins, and determination of fetal viability, age, and sex. The method was found to be

least invasive, high accuracy, and efficient technique for early pregnancy diagnosis (Paisley et al., 1978; Vaillancourt et al., 1979) and may minimize the rare incidence of palpation-induced abortions. Most studies on the utility of transrectal ultrasonography for pregnancy diagnosis have been conducted in cattle, but lately it has found utility in buffalo cows as well. In buffaloes, transrectal ultrasonography is most commonly used to determine pregnancy, fetal age, and sex as well as ovarian activity between 19 and 22 days after AI (Pawshe et al., 1994; Ali et al., 2008). The sensitivity of detection of pregnancy was observed to be 97.9% (Bhosreker et al., 2000). Nation et al., 2003 reported that the sensitivity and specificity of pregnancy diagnosis in lactating dairy cows based on ultrasonographic detection of uterine fluid as well as embryonic membranes from 28 to 35 days after AI were 96% and 97%, respectively. Combined direct approach of per-rectal palpation and transrectal ultrasonography have been found to be useful with high accuracy for pregnancy diagnosis in large ruminants like cattle and buffalo. Both the method requires high degree of skill and expertise with high grade ultrasound machineries and expensive modern rectal transducers.

Indirect method

Hormone progesterone

Progesterone is the steroid hormone produced by the corpus luteum present in the ovary (Fig. 2). The hormone is solely responsible for maintenance and sustenance of pregnancy throughout the gestation period. Both serum and urine concentration of the hormone gradually increases with the advancement of pregnancy and the concentration drops to zero level at the onset of parturition or abortion or embryonic death. So, detection and assessment of serum and urine progesterone concentration at 18 to 24 days post breeding provides information about the pregnancy status of animal with around 98 % of specificity and 75 % accuracy that may be due to early embryonic death (Zaied et al., 1979; Laing et al., 1980; Gowan et al., 1982; Pennington et al., 1985; Nebel et al., 1987, Waldman, 1993). Generally different types of ELISA based kits were developed and standardised for estimation of Progesterone level in serum, urine or milk of pregnant animals including cattle, buffaloes, sheep, and goats. In cows the serum and milk hormone estimation has been found to be useful to differentiate pregnant and non-pregnant animal to the earliest of 19 days of post breeding (Laing and Heap, 1971 ; Shemesh et al.,1973).

The progesterone levels in milk found to be comparatively higher than blood level in pregnant buffalo cows at 21 days after insemination (Perera et al., 1980). The hormone assay has also been found to be useful in pregnancy diagnosis in the female camel in which species the CL is required for the entire gestation (Abdel Rahim and El-Nazier, 1987). But this hormone assay cannot be used to diagnose pregnancy in the bitch as no placental progesterone produced in the pregnant bitch (Verstegen-Onclin and Verstegen, 2008)

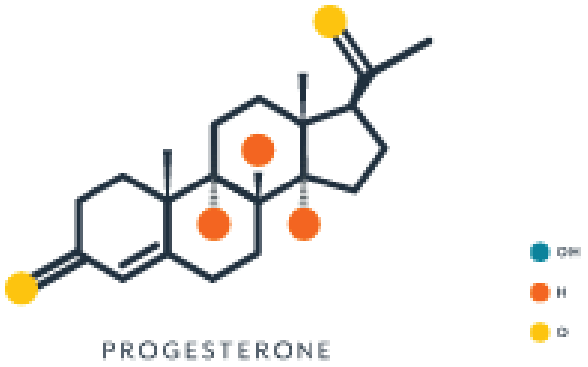

Fig. 2. Structure of progesterone hormone

Estrone sulphate

The estrone sulfate is the derivative of steroid hormone (Fig. 3) oestrogen produced by the placentome (Eley, 1979). The hormone has been detected in amniotic fluid and maternal serum of different ruminants at around 60th day of pregnancy till the end of gestation (Robertson, 1979). The hormone has been found to detect pregnancy in the later or advanced phase of pregnancy in cattle and buffaloes (Prakash and Madan, 1993). The lack of sensitiveness of this test may be attributed to various factors like genetic makeup, weight, parity status, and environment (Lobago, 2009). Usually radioimmunoassay, enzyme immuno-assay are the methods used for assessment of estron sulphate and its metabolites

in urine milk or serum of animals at different time intervals (Bamberg et al., 1991).

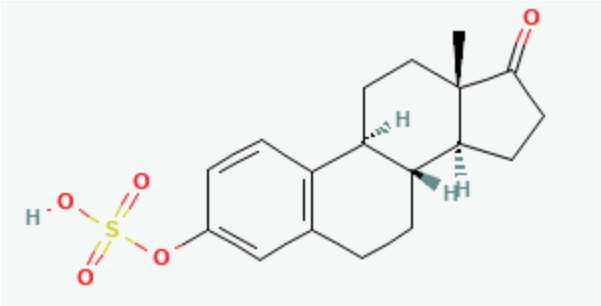

Fig. 3. Structure of estrone sulfate (Source: pubchem)

Relaxin

Relaxin is the hormone produced by Placenta (Fig. 4) and is meant for maintenance of pregnancy by inhibiting contraction and expulsion of foetus. The hormone has significant contribution in differentiating pregnant and non-pregnant animals mostly in case of canines (Steinetz et al., 1989). However, low level of specificity and sensitivity of this hormone has been observed in ruminants in detection of pregnancy.

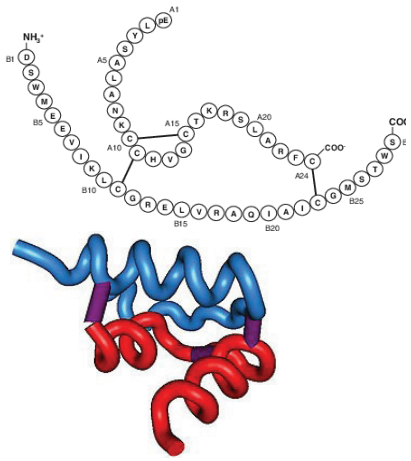

Fig. 4. Structure of relaxin molecule(source: wikipedia)

Early Conception Factor (ECF)

Early Conception Factor (ECF) is a protein produced by the embryo as early as 6 to 24 hours of fertilisation. It plays a vital role in mediating the process of conception/ implantation. Detectable amount is observed within 6 to 24 hours of fertilization (Morton et al., 1976, 1987, 1992) in the serum of pregnant animal and the concentration persists till 14 weeks of pregnancy. Abruptive decline in concentration is observed at early embryonic death or removal of foetus. The factor has been considered as an early marker pf pregnancy in ruminants like sheep (Morton et al., 1979; Wilson et al., 1983), cows (Cordoba et al., 2001; Gandy, 2001; Whisnant et al., 2001), buffaloes (Chanderet et al., 1983), pigs (Grewal et al., 1983), mares (Ohnuma et al., 1996). Various commercial kits based on antibody detection methods have been developed for early estimation of ECF in serum. However, the specificity is very low as the factor can be secreted from tumors too.

Ovine trophoblastic protein-1

Ovine trophoblastic protein-1 (OTP-1) first reported by Godkin et al. (1984) is found to be mediator of pregnancy (Bazer et al., 1992) secreted from blastocyst of 14–16 day in vitro cultured sheep embryo when it is transferred to uterus of ewes. It is an early indicator of maternal recognition and successful establishment of pregnancy in the method of ETT (Bazer, 1992; Roberts et al., 1992). The ovine specific protein is found to be similar to interferon alpha secreted by other ruminants like cows and buffaloes Imakawa et al. (1987).

Interferon-τ (IFN-τ)

Similar to ovine trophoblastic protein-1 (OTP-1), Interferon-τ (IFN-τ) is a type I interferon (Roberts et al., 2007) produced by the conceptus between 14–16 days of insemination in cattle (Mann et al., 1999; Roberts et al., 1999; Thatcher et al., 1995). The biochemical mechanism of the protein is most extensively studied in ruminants (Fig. 5). The protein in ruminants downregulates expression of estrogen receptor alpha and oxytocin receptors in endometrial cells (Bazer et al., 1997) and enzymes like cyclooxygenase2 and prostaglandin F synthase (Spencer et al., 1996) in the uterus. It helps in maintenance of pregnancy preventing PGF release necessary for luteolysis. Though, endometrial concentration of the peptide is significantly higher in case of pregnant cows but the serum and urine concentration if observed to be too low to be considered as marker of pregnancy.

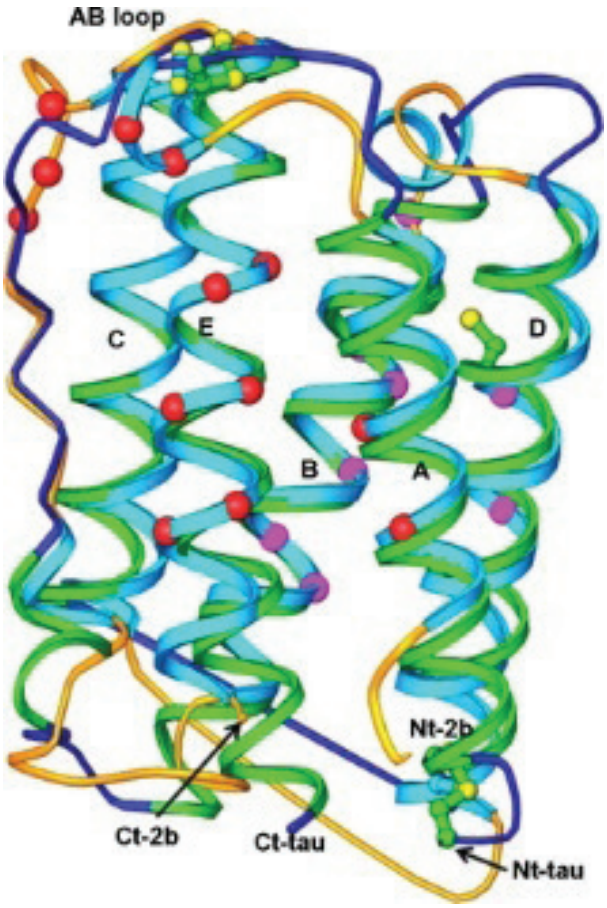

Fig. 5. Structure of bovine interferon (Source: Roberts, 2007)

Pregnancy Associated Glycoproteins (PAGs)

These are the group of carbohydrate associated proteins (Fig. 6) belonging to the aspartic proteinase super family having homology to pepsin, chymosin, cathepsins D, and enzyme renin (Xieet et al., 1992;1994) secreted from trophoblast cells of placenta between days 20 to 28 days of implantation in case of bovines (Zoliet et al., 1992).

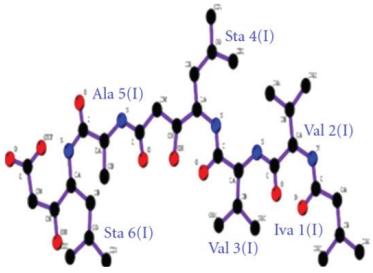

Fig. 6. Structure of buffalo Pregnancy Associated Glycoproteins (Source: Andonissamy, 2012)

Various isoforms of these glycoproteins have been detected in sera of pregnant cows (Butler et al., 1982). The molecule is often referred as "protein B" or the "pregnancy-specific protein B" (PSPB) in (Zoli et al., 1991) bovines. Biochemically these are group of proteins expressed by 22 distinct cDNA libraries (Constanta et al., 2011). In bovines three PAGs isomers having similar N-terminal sequences (Martal et al., 1996) are detected namely PSPB, PAG 67 or bPAG-1 (Xie et al., 1994) and PSP60 (Mialon et al., 1996). Transcription of bPAG-2 and -11 mRNA is seen all through the pregnancy; -4, -5, and -9 mRNAs in early pregnancy and bPAG-1 mRNA are detectable only after day 45 (Green et al., 2000).

Other molecules

Integrative development and advancement of omic approaches like proteomic, genomic, metabolomic, peptidomic studies have paved pathways to find out unique molecules related to pregnancy in different farm animals that could be used as diagnostic. New set of molecules specific to pregnancy like IFN-τ stimulated genes (ISG), interferon-stimulated protein 15 kDa (Isg15), myxovirus resistance 2 (MX2), and 2' -5; oligoadenylate synthetase (OAS1), has been identified and related techniques are under the process of development. Proteomic analysis of urine of pregnant farm animals like cow and buffalo are studied to find out different biomolecule's specific to different stages of pregnancy to be further used as diagnostics (Balhara et al., 2014). Genomic studies have also revealed various genomic markers related to expression upregulation or downregulation of genes like IFN-τ to be used as diagnostics in various molecular techniques (Green et al., 2010; Forde et al., 2011; Kizaki et al., 2013).

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