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Review on Pregnancy Diagnosis in Dairy Cows

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Abstract: Pregnancy diagnosis of cows is important tool to know whether or not an animal has become pregnant after a service and it is an essential part in fertility management. It assists in dairy farm management by identifying non-pregnant animals, which can be served again or culled with minimum necessary delay. Such procedure will improve the breeding efficiency and greatly contribute towards the economy of the farm. Pregnancy diagnosis is helpful to categorize pregnant and non-pregnant animals for proper care and feeding. It is also very useful in selling; buying, registration and insurance of animals. Therefore, our objectives are to review the methods of pregnancy diagnosis in cows and to highlight the importance of pregnancy diagnosis in relation to fertility management. The methods of pregnancy diagnosis in cows can be classified into clinical, laboratory and visual methods. Clinical method of pregnancy diagnosis includes trans-rectal palpation and ultrasonography. Laboratory test for early pregnancy diagnosis include measurement of endocrine hormones and pregnancy specific proteins. Visual methodsare not reliable indicator of pregnancy in cows. Rectal palpation remains the method of choice for pregnancy diagnosis. Ultrasound examination is better for early diagnosis, aging and sexing of the embryo. In both dairy and beef cows an accurate, early diagnosis of pregnancy is essential to a successful breeding program and allows for early detection of problems and to achieve resynchronization of non-pregnant cows. Generally, early identification of pregnant and non-pregnant cows after breeding improves reproductive efficiency and pregnancy rate in cows by decreasing the interval between services. Therefore, there is a need to educate farmers to get their animals checked for pregnancy at an early date.

Key words: Endocrine hormones · Pregnancy diagnosis · Trans-rectal Palpation · Ultrasonography

INTRODUCTION

Pregnancy diagnosis means to determine whether or not an animal has become pregnant and to detect health of maternal and the fetus after service in the early and later stage of pregnancy. It is vital for profitable animal husbandry particularly in the productive animal species. For a cost effective dairy farm, cows must calve every year and to maintain this sequence, identifying pregnant animals at an early date seems necessary. Early pregnancy diagnosis would help to evaluate the dairy farms therapies at an early date and devise alternative manipulations in the current systems of planned breeding. There is a need to check cows for pregnancy at an early date as it has been shown that earlier the pregnancy diagnosis performed, the more profitable is the return for dairy cows [1].

Early recognition of non-pregnant dairy cows and heifers post breeding can improve reproductive efficiency and pregnancy rate by decreasing the interval between Artificial insemination (AI) services and increasing AI service rate. Hence, new technologies to identify non pregnant cows and heifers early after AI may play a key role in management strategies to improve reproductive efficiency and profitability on commercial a changing cattle industry [2]. The ideal pregnancy test should have high sensitivity, correctly identify pregnant animals, high specificity, correctly identify non pregnant animals, simple, inexpensive to conduct under field conditions and provide additional information, such as embryonic or fetal viability and sex of the fetus, are increasingly beneficial [3]. The methods of pregnancy diagnosis (PD) are divided into direct and indirect methods or it can be categorized into visual, clinical and laboratory methods.

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Direct methods of pregnancy diagnosis include rectal palpation and ultrasonography. Indirect methods for early pregnancy detection use qualitative or quantitative measures of reproductive hormones at specific stages after AI service or detect conceptus specific substances in maternal body fluids as indirect indicators of the presence of a viable pregnancy [4].

Ultrasound machines equipped with a rectal transducer are expensive in developing countries and therefore the high initial cost of this technology partly limits its practical implementation [5]. The information-gathering capabilities of ultrasonic imaging are far exceed those of rectal palpation [6]. The visual methods of pregnancy diagnosis are used to examine external indicators of pregnancy [7].

Potential economic benefits of pregnancy diagnosis include: timely culling, saving costs of maintaining cows which will not provide economic returns and providing information to allow planning for replacement needs [8]. Pregnancy diagnosis is a very important fertility management tool. In this regard, the interest of the farmer for pregnancy checking was increasing from time to time to prevent economic loss [9]. However, the level of a review on pregnancy diagnosis in cows is low and there is scarcity of sources regarding pregnancy diagnosis in Ethiopia.

Therefore, the objectives of this seminar paper were to review the methods of pregnancy diagnosis in cows and to highlight the importance of pregnancy diagnosis in relation to fertility management.

Importance of Pregnancy Diagnosis in Cows: Pregnancy diagnosis is of great importance to know whether or not a female animal has become pregnant after a service. It assists in herd management by identifying non-pregnant animals, which can be served again with minimum necessary delay [10]. For cost effective dairy farm, cows must calve every year and to maintain this, identifying pregnant animals at an early date seems necessary. There is a need to check animals for pregnancy at an early date as it has been shown that earlier the pregnancy diagnosis performed, the more profitable is the return for dairy cows. Early identification of non-pregnant dairy cows and heifers post breeding can improve reproductive efficiency, pregnancy rate and greatly contribute towards the economy of the enterprise by decreasing the interval between AI services and increasing AI service rate [4].

Poor fertility costs more money. Effective fertility management is thus a key component of profitable dairy farming. Such management requires regular and accurate evaluation of the fertility status of the herd. To evaluate fertility status properly, requires pregnancy testing [11]. There are various problems responsible for infertility in cows. The more common of these include: cystic ovaries, repeat breading, anestrus and uterine infection. Diseases and management problems affecting the whole dairy or beef herd can be identified much earlier if cattle are pregnancy tested [12].

Other Potential economic benefits of pregnancy detection include: timely culling of non-pregnant cows, aid in the culling decision for cows with other issues, breeding management and move to next calving season for example, grouping cows for feeding, calving and other managements. Pregnancy diagnosis is also very useful in selling, buying, registration and insurance of cows and helpful in proper care and feeding of pregnant animals [8].

Methods of Pregnancy Diagnosis in Cows: Due to the importance of early pregnancy diagnosis in farm animals and specially dairy ones, various pregnancy diagnosis methods have been evaluated and developed during the past and recent years, some of which have some limitations to their wide scale use. The methods of pregnancy diagnosis could be divided into direct and indirect methods or it could be divided into visual, clinical and laboratory methods [13].

Direct methods (Clinical examinations) for early pregnancy diagnosis of dairy cows involve direct detection and monitoring the conceptus, the fetal membranes and/or associated fluids of the conceptus either per rectal palpation or via ultrasonography. Direct methods for diagnosis of pregnancy include: trans-rectal palpation and ultrasonography and both are used to examine internal indicator of pregnancy. Because they are direct methods, the test outcome can be subjective among practitioners. Both require a great deal of skill and experience [3].

The current available indirect methods (Laboratory tests) of pregnancy diagnosis include: measurement of endocrine hormones such as progesterone, estrone sulphate and pregnancy specific proteins such as: pregnancy associated glycoproteins, early pregnancy factor and interferon tau. They are used to examine internal indicator of pregnancy [4].

The visual method of pregnancy diagnosis includes: non-return to estrus, exposure to a bull or AI service, external ballottement and other physical changes related to pregnancy. It is used to examine external indicator of pregnancy [14].

Table 1: Methods of PD in cows in chronological order after service/AI:

Days of gestation	Pregnancy diagnosis methods
First week	Early pregnancy factor
18-24 days	Failure to return to estrus and persistence of CL
22-26 days	Milk or plasma progesterone assay
24-30 days	Ultrasonography
24-30 days	Pregnancy associated glycoproteins
30-65 days	Palpation of amniotic vesicle
35-90 days	Asymmetry of horn, fluctuation of uterine content and membraneslip
65-150 days	Palpation of fetal bump
70 days to term	Palpation caruncles/cotyledons
90 days to term	Fremitus in middle uterine artery
105 days to term	Esterone sulphate in milk assay and fremitus in non-gravid horn

Source: Sharma *et al*. [24]

Visual Methods of Pregnancy Diagnosis

External Indicator of Pregnancy: A number of physical changes may be observed in the later stages of pregnancy, including of the distention of the abdomen occurs at about the same rate as the rate of development of the fetus, which is greatest towards the end of the period [15]. Enlargement of the udder especially in dairy heifers (4 months onwards), pluriparous cow (Last 2-3 weeks of gestation) and slight vaginal discharge (From 4-5 months onward) are external indicator of pregnancy [16].

Furthermore, edema and relaxation of vulva at 5thand 7th months onward in heifers and cows respectively [17] movements of the fetus visible externally (On the right side of abdomen 6 months onwards) is external indictor of pregnancy. However, the accuracy of these visual diagnostic symptoms is always low particularly at early stage of pregnancy and these indicators are used as a supplement to clinical diagnosis [18].

Cessation of Estrum (Non- Return to Estrus): Failure to return to heat is commonly used as a cheap and simple method of pregnancy diagnosis which does not require veterinary involvement. Detection of non-return to estrus is an essential part of any fertility management plan. If cows fail to get pregnant, then detecting their next heat will ensure that they get a chance to become pregnant as soon as possible; i.e. non-pregnant cows will be identified at an early stage (18 to 24 days after service) and can be mated without delay. However, non-return has a major disadvantage in that wrongly classifies a large number of cows as being pregnant when they are not. As the calculation in figure 1 shows, using non-return almost 30% of cows assumed to be in-heat 21 days after service will actually be empty and around 20% of cows thought to be pregnant are actually not. So although detecting non-return to estrus is a useful part of fertility management, it needs to be backed-up by additional measures [11].

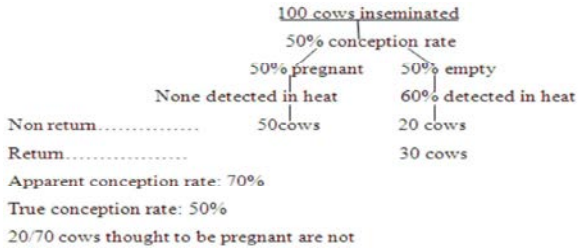

Fig. 1: Calculation of the accuracy of detecting non-return to heat

Source: National Animal Disease Information Service [11]

Exposure to a Bull or Artificial Insemination Service: A history of cohabitation with a bull, the observation of mating, or artificial insemination is used by some to suggest that a cow is pregnant. Conversely, unobserved, or unrecorded mating are common. Thus, history is not a reliable indicator of pregnancy status and may sometimes be deceptive [19].

External Ballottement: Abdominal palpation and abdominal ballottement of the fetus is possible to some extent in cows during late gestation. It is performed by placing the first over the lower right abdominal wall and pushing it in an intermittent manner in a dorsal medial direction deeply. The fetus can be felt as a hard solid object floating in fluid. This is usually possible in lean cows after the 7th month of gestation. The fetal movements can be seen at the same place by careful visual observation however, the method is applicable too late in diagnosis [13].

Clinical Examinations

Internal Indicator of Pregnancy: There are different internal symptoms of pregnancy and can be felt by examination of the genital tract by the expert having adequate knowledge in anatomy and physiology of the livestock [3].

Early sign of pregnancy (1-3 month) includes combination of the following: asymmetry of the uterine horns, decrease in the tone of the pregnant horn, fluctuant contents in the pregnant horn, a firm, round and palpable corpus luteum on the ovary, membrane slip and appreciation of an amniotic vesicle. In later stage of pregnancy (>3 months) , cervix is located anterior to the pelvic brim and the uterus cannot be retracted and is flaccid, placentomes and sometimes the fetus, are palpable and the median uterine artery increases in diameter and fremitus can be detected [20].

Rectal Palpation of Pregnancy in Cow: Palpation of the reproductive tract through the rectal wall has been the customary method for pregnancy diagnosis [19]. Rectal examination of cows is a very time-honored technique for the diagnosis of pregnancy. The proximity of the reproductive tract of the cow to the rectum and its elasticity allows detecting characteristics of the tract that coincide with either pregnancy or non-pregnancy [8].

Currently, rectal palpation is the easiest, fastest and cheapest and mostoperators trained accurate method of pregnancy diagnosis. The goal in rectal palpation is to be 100% accurate at determining the pregnancy status 35 days post breeding. The golden rules of rectal pregnancy examination includes examination of the entire tract before declaring the cow is open and find one of the positive signs of pregnancy before you call a cow is pregnant. If you are not sure, recheck the cow. Technique of pregnancy diagnosis by rectal palpation of a retractable uterus, after retracting the uterus fully and accurately, first feels the uterus for asymmetry. At 35 days of pregnancy the pregnant horn will feel slightly larger. Non-pregnancy is determined by a thorough examination of the uterus, usually after it is retracted onto the floor of the pelvis and the absence of the positive signs of pregnancy is carefully ascertained [13].

Rectal palpation also, allows an estimation of the stage of pregnancy [8]. The stage of gestation can be estimated on the basis of palpable characteristics of the uterus and fetus. In early pregnancies, stage of gestation can be estimated on the basis of the size of the pregnant horn and the size of the amniotic vesicle. In more advanced pregnancies, age of the fetus is estimated based on determination of the size of the fetus and position of the uterus [19]. Because pregnancy in cows can be terminated by manual rupture of the amniotic vesicle, trans-rectal palpation induces iatrogenic embryonic mortality [4].

The four positive signs of pregnancy in the cow are amniotic vesicle, fetal membrane slip, cotyledons/ caruncles and fetus. The amniotic vesicle is a portion of the placenta that contains the developing conceptus and the amniotic fluid, is palpable as early as 28 days after conception in heifers and by 32 to 35 days in older cows. The vesicle is recognized as nearly spherical, turgid, fluid-filled structure that is approximately 1 cm in diameter at 28 days and increases in size as pregnancy advances. Intentional rupture of the amniotic vesicle has been used in the past as a method to intentionally provoke abortion in cow [19]. The amniotic vesicle can be palpated with due care between 30-50 days of gestation as a movable oval object within the uterine lumen [13].

Slipping of the fetal membranes is one internal definitive sign of pregnancy and it is possible to feel the slipping of the fetal membrane along the greater curvature within the uterus [11]. The fetal membrane slip can be felt between 35-90 days of gestation. The examiner can detect the chorio-allantois (Developing placenta) within the lumen of the pregnant uterus by compressing the uterine horn between the thumb and forefinger, lifting the uterus and then allowing the horn to slowly slip from the grasp. If the cow is pregnant, the chorio-allantois can be felt to slip through the fingers just prior the uterine wall. The membranes can be slipped in the pregnant horn as early as 30 days and can be reliably detected by day 35. During early pregnancy, the fetal membranes are thin and a delicate to touch and some experience are required to recognize this sign of pregnancy [21].

Placentomes are the structures formed by the union of maternal caruncles and fetal cotyledons by which the placenta is attached to the uterus and can be felt through rectal palpation [11]. The presence of placentomes is another positive sign of pregnancy and is detectable from about 75 days to term. The period of pregnancy when the uterus has descended into the abdominal cavity and the fetus is not palpable, palpation of a placentome is the surest indication that the cow is pregnant. In general, they can be detected as soft, thickened lumps in the uterine wall and are more easily detected as pregnancy advances [13]. The size of placentomes varies with the stage of gestation and their location in the uterus. They are most consistent in size just in front of the cervix and are palpated at that location to estimate the stage of gestation [19].

Palpation of the fetus is also surest indicator of pregnancy in cows. As pregnancy progresses, it becomes possible to feel the presence of the fetus within the

Table 2: Uterine position and structures during pregnancy

Days of gestation	Uterine position	Palpable Structures
35-40	Pelvic floor	Uterine asymmetry/membrane slip
45-50	Pelvic floor	Uterine asymmetry/ membrane slip
60	Pelvis/abdomen	Membrane slip
90	Abdomen	Small placentomes/fetus (10-15 cm)
120	Abdomen	Placentomes/fetus (25-30 cm)/fremitus
150	Abdomen	Placentomes/fetus (35-40 cm)/fremitus

Source: Broaddus and Vries [22]

pregnant horn. After about day 150, the fetus is too far forward in the body cavity to palpate the entire fetus although fetal structures can be palpated [11]. Depending on the skill of the examiner and the location of the fetus, the fetus can be palpated from the time of amniotic softening (65 to 70 days) to term. The whole of the fetus is palpable many times only during early gestation (2 to 4 months) [13].

In the early stages of pregnancy, the fetus can be grasped directly. Later, the fetus is detected by ballottement; the examiner sets the fetal fluids in motion by rocking the hand against the uterine wall and recognizes the fetus as it rebounds against the hand. The fetus is identified as a free-floating firm object within the fluid-filled uterus during the first 4months of gestation. As pregnancy advances, increased weight of the fetus and fluid pulls the uterus downward and forward until the fetus comes to rest on the abdominal floor during the fifth and sixth months. Continued growth of the fetus positions it closer to the maternal pelvis during the last trimester and palpation of the fetus is facilitated [19].

Ultrasonography: Trans-rectal ultrasonography is the more reliable and relatively simple method of diagnosing pregnancy not only in cows but all farm and domsiticated animals by allowing the monitoring of the early conceptus and its associated membranes. It may displace trans-rectal palpation as the direct method of choice for pregnancy diagnosis among bovine practitioners [23]. Some pregnancy associated structures can be seen as early as 9 days after conception. However, reliable pregnancy detection is generally accepted after 26 to 30 days. At this stage a fetal heartbeat can usually be seen assuring the viability of the fetus [8].

By this method accuracy of over 99% can be achieved, enabling fertility problems to be identified rapidly. Two factors affect the speed at which ultrasound examination can be conducted in dairy farm: operator proficiency, availability and restraint of animals. When both factors are optimized, the speed of ultrasonography approach that of rectal palpation, while exceeding rectal palpation in the amount of information gathered from each animal. Pregnancy can be detected earlier with ultrasound compared with rectal palpation [11].

Trans-rectal ultrasonography has the added advantage of providing additional information: on ovarian structures, identification of twins and determination of fetal viability, age and sex [24].

The genital tubercles (the future vulva or the prepuce) have a different appearance and are in different locations for the male and the female fetus by day 50. Knowing the gender of a fetus may aid in culling, selection and sale decisions [8].

Moreover ultrasonography made a thorough examination of the reproductive health of the animal possible and, therefore, it has now become an established research tool to study bovine reproductive biology in cattle. It is a direct and accurate method for pregnancy diagnosis. The outcome of the test is known immediately at the time the test is conducted. But ultrasound machines equipped with a rectal transducer are expensive in developing countries and therefore the high initial cost of this technology partly limits its practical implementation [5]. Furthermore, Ultrasound can detect the viability of the embryo/fetus by detecting their beating heart and count their numbers.

Laboratory Test

Hormonemeasurements

Progesterone Assay: Measurement of progesterone (P4) is an indirect method for pregnancy diagnosis in many livestock species including cattle. Conception extends the life of the corpus luteum (CL) by preventing the luteolytic mechanism from being triggered, thus prolonging and maintaining its functional characteristics, ensuring continued high progesterone levels. Progesterone maintains the uterine endometrium in a state which supports embryonic development, implantation and feto placental development [7]. In normally cycling cows the CL is lysed because of the effects of prostaglandins from the uterus, if the animal is not pregnant and thus, the progesterone level goes down.

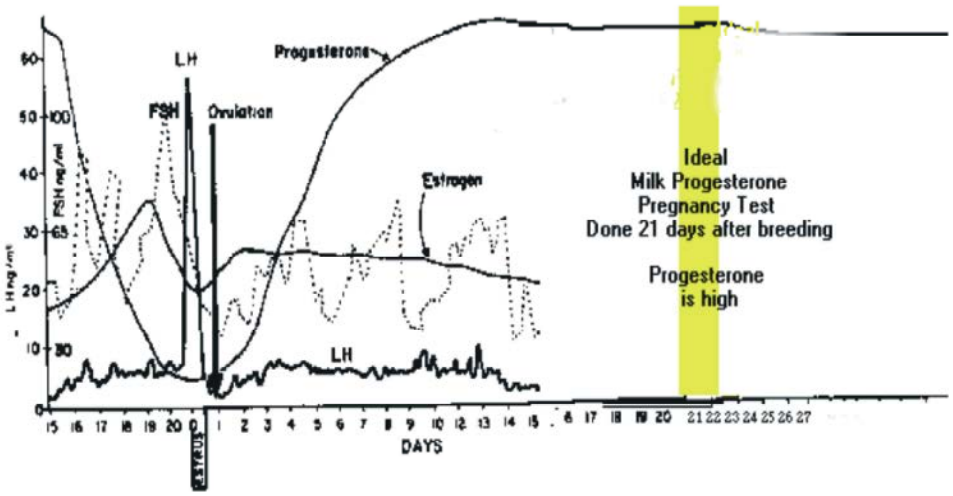

Fig. 2: If Progesterone is high 21 days after breeding the cow 'probably'be pregnant
Source: Eilts [26]

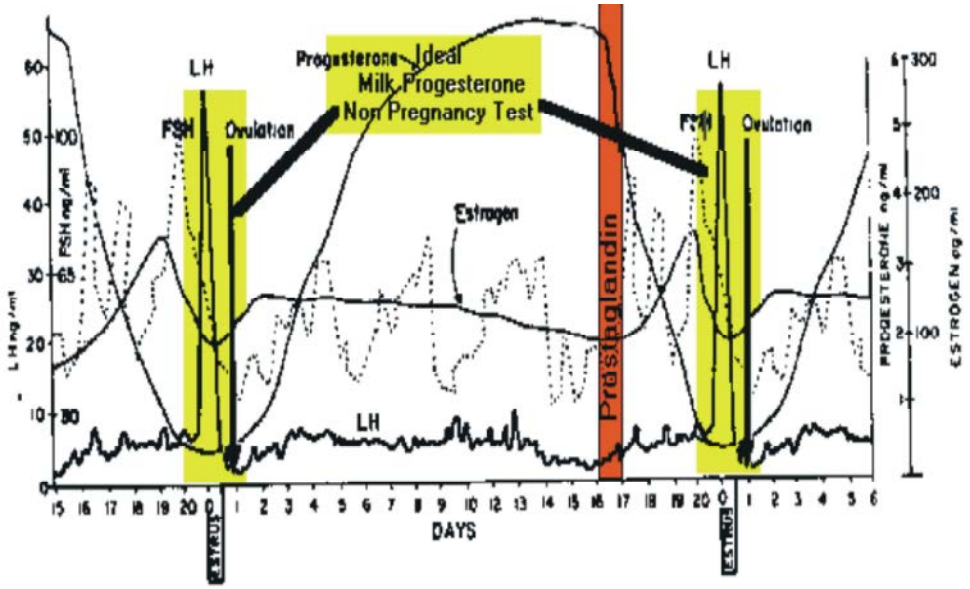

Fig. 3: If Progesterone is low 21 days after breeding the cow cannot be pregnant.
Source: Eilts [26]

Therefore, low progesterone concentrations in maternal blood at 18 to 24 days post breeding can predict that the animal is non-pregnant and high progesterone gives an insight that probably the animal is pregnant [13]. The drawback of the test is the presence of some animals with long interestrous intervals or those with short estrous cycles could increase the false positive results.

Progesterone concentrations in milk or serum can be quantified using a laboratory RIA or ELISA procedures [4]. Currently, milk P4 is the only test that is likely to be a competitor to rectal palpation. A single sample taken 24 days after service will accurately identify non-pregnant cows; > 95% of cows with low progesterone will not be pregnant. However, a significant proportion of cows that are non-pregnant will have high progesterone 24 days after AI, so with a single milk progesterone test around 20 to 25% of cows identified as pregnant will not be [11, 25].

Estrone Sulphate: Estronesulphate is a conjugated estrogen that has been used to diagnose pregnancy using milk samples in cow. Estrone sulphate is produced specifically by the embryo-fetus and associated placental membranes and, therefore, its presence is a direct indicator of pregnancy [4].

In cows, concentrations of estrone sulphate detectable in the whey fraction of milk are similar to those in maternal plasma and increase from about 60 days in gestation to the end of lactation. However, because concentrations of estrone sulphate in maternal circulation are not reliably detectable until around 80 days in gestation, estrone sulphate cannot compete with progesterone to assess pregnancy status early post breeding in cow and therefore this test can only detect late pregnancy [7]. Concentration of estrone sulphate in the maternal body fluids is a useful indicator for the placental functions especially those related to embryonic growth [27].

Pregnancy Associated Substances

Pregnancy Associated Glycoproteins: The pregnancy associated glycoproteins (PAGs) are secretory products from the mono and binucleated trophoblastic cells in bovine placentomes [28]. The PAGs consist of a large family of more than 20 closely related proteins that are only produced by the placenta [29]. They can be detected in the blood of pregnant cows beginning at approximately 25 days after insemination [30].

Monitoring the concentrations of PAGs in blood is an effective method of pregnancy detection [31]. By using biochemical procedures, some molecules of the PAGs were isolated from cotyledons of cow [32, 33]. Although their function during pregnancy or parturition is unclear, but it is demonstrated that PAGs are inactive members of the aspartic proteinase family [34]. If enzymatically active, PAGs could be important in structural remodeling of the placenta during pregnancy or in placental detachment after parturition [35].

Although PAGs are presently one of the more promising markers for early detection of pregnancy in cow, a number of factors limit PAG-based pregnancy tests. First, PAG is not detectable in milk or urine and therefore requires that a blood sample be collected, a procedure that is difficult on some farms. Second, because PAG reaches high concentrations in maternal circulation during the periparturient period and has a long serum half-life, cows inseminated too early postpartum are not eligible for testing [36].

Interferon Tau: Bovine interferon tau (BoIFN- tau) is one of the principal proteins secreted by the bovine conceptus from 16 to 25 days. Interferon-tau, a novel type I interferon, is produced in large amounts by the embryo after day 14 to signal the mother and establish the pregnancy [37]. It is the pregnancy recognition hormone in sheep and cows that acts to silence expression of estrogen receptor alpha (ESR1) and, in turn, oxytocin receptor (OXTR) to prevent development of the luteolytic mechanism that required oxytocin (OXT) from the CL and posterior pituitary to induce luteolytic pulses of PGF2á. Thus, IFN-tau blocks the ability of the prostaglandin to develop the luteolytic mechanism [38].

A primary function of IFN-tau is to abrogate development of the uterine luteolytic mechanism to ensure maintenance of ovarian CL to produce P4, the hormone essential for establishment and maintenance of pregnancy [36]. IFN-tau, act within the uterine cavity [7]. With extremely low levels in extra uterine tissues and peripheral circulation, prevents direct use of IFN-tau as an early pregnancy diagnosis molecule [39].

The INF-tau secretion is transitory. It reaches a maximum by 20 to 24 days and is completely gone by day 30 of pregnancy and does not accumulate in the blood or urine. Thus, IFN-tau cannot be used for a pregnancy test in the blood or urine of the cow [31].

Early Conception Factor/early Pregnancy Factor: Early pregnancy factor (EPF, also known as early conception factor-ECF) which is present in the sera of pregnant mammalian females, detectable within 6 to 24 hours of fertilization and disappearing within 24 to 48 hours after death or removal of the embryo.

EPF remains the earliest serum benchmark for positive fertilization and hence successful conception [7]. EPF is made of two components (EPF-A and EPF-B). EPF-A is secreted by the oviduct as well during gestation but EPF-B is secreted by the ovary. Production of EPF-B requires a signal from the fertilized egg (Ovum factor). EPF is an attractive marker for pregnancy in that it appears within hours after conception and disappears rapidly after death or removal of the embryo [19].

Serum concentrations of the various EPF forms and their components vary from species to species and are dependent on gestational age [40]. It is recommended that milk or serum samples be tested at 7 days after insemination. This molecule was supposedly present in the blood of pregnant cows within two days after conception. The exact nature of this molecule and how it got into circulation were not well defined but nonetheless, it could be assayed by using a rosette inhibition test [13].

Other Method of Pregnancy Diagnosis

Milk Ejection by Non-Luteolytic Low Dose of Prostaglandin: A method tested some years ago was the injection of low dose prostaglandin F2 alpha (Non-luteolytic dose) in animals two weeks after breeding resulting into milk ejection. The intrajugular administration

of a sub luteolytic dose of PGF2 alpha induces a large increase in intramammary pressure when given during the luteal phase. However, due to potential dangers of inducing luteolysis by accidental over dosage, the use of this technique of pregnancy diagnosis, could not gain wide popularity [13].

In ruminants, a non luteolytic dose (125μg) of PGF2á administered intrajugularly causes release of oxytocin from corpus luteum and thereby contraction of the milk alveolus resulting in milk let down or milk ejection or increase in intra mammary pressure. Administration of PGF2á causes the milk ejection following disappearance of skin folds over the teats, engorgement of teats and/or oozing of milk from the teats and thus cows are considered as pregnant. By 15 minutes after administration of PGF2á, if engorgement of teats is not noticed, then the animal is considered as non-pregnant [41].

Differential Diagnosis of Pregnancy

Urinary Bladder: Only rarely does a urinary bladder full of urine creates confusion for the presence of an early pregnancy (2-3 months) in dairy cows. This can be easily differentiated by the absence of palpation of both uterine horns and the ease with which the animal urinates when the bladder is gently massaged leading to disappearance of the enlargement [13].

Rumen: The shape of the dorsal and ventral sacs the rumen or paunch may be mistaken for the head or rear quarters of a calf. The difference can be determined by mashing on these large objects. This indicates that the paunch is full of feed and does not have the watery feel like a pregnant uterus and also the paunch will indent when mashed, while a well-developed calf may move away from the pressure of the touch. Also at these late stages of pregnancy, palpator can easily distinguish fetal features (e.g., ribs, hooves and ears) while touching them [42].

Kidney: The kidneys are suspended directly under the spinal column at about a 30-degree downward angle. In cattle, the left kidney is located more toward the rear of the animal than is the right kidney. For this reason, the left kidney is often touched during palpation. It is elliptically shaped and is sometimes mistaken for a calf's nose. Practice will allow you to distinguish the difference, but inexperienced palpators can avoid the left kidney by feeling at a steeper angle into the abdominal cavity. It is usually at this steeper angle that large fetuses are located [42].

Hydrometra: The presence of fluids in the uterus is always associated with absence of estrus cycle specially in the presence of progesterone and a functional corpus luteum, which confuses with pregnancy? There is absence of fetus and placentomes. And also there is failure of progressive development of the fetus [43].

Metritis: It is a non-specific infection of the uterus characterized by the presence of visible pus. The pus may be seen on the lips of the vulva and on the tail where it rubs across the vulva. The pus may also be seen in estrus mucus as cloudiness or yellow or white flakes. The uterine wall is thickened and spongy to feel. The condition might be confused with the 35 to 40 day stage of pregnancy [43].

Mummified Fetus: A uterus with mummified fetus has thick uterine walls, absence of fluid and placentomes and a hard palpable structure. The fetus is closely opposing the uterine wall. The fremitus is absent [13].

Maceration of the Fetus: Fetal maceration is defined as fetal death, regression of corpus luteum but abortion fails to occur. It stays in uterus and starts getting putrefied. This is due to infection present in uterus or may be introduced from outside. If it occurs before bone formation then, it is reabsorbed, only pus is expelled out. But if it occurs after bone formation, the muscles will be destroyed and bone will remain there. Two factors for maceration are fetal death and open cervix [44]. There is copious vaginal pus discharge. The uterine wall is thick and doughy. There is no dorsal bulging of the uterus and placentomes are not palpable. Parts of bones are sometimes palpable separately floating tacked up. The fremitus is absent [13].

Pyometra and Mucometra: These two conditions are difficult to differentiate from normal pregnancy especially when the pus or mucus is present in enormous quantity. So that, the uterus become enlarged and placed on the abdominal floor. In pyometra the uterine wall is thick, uterus is doughy and placentomes or fetus is not palpable and the fremitus is absent [45, 46]. In mucometra, the positive findings of pregnancy are absent, but, contrary to pyometra the uterine wall is thin. Ultrasonography can easily differentiate the condition from pregnancy. These two conditions are differentiated from early pregnancy; by a characteristic feature, found most often is the bilateral enlargement of both uterine horns, which is not found in pregnancy. There is also a history of vaginal discharges [13].

CONCLUSION AND RECOMMENDATIONS

Pregnancy diagnosis is a significant part of good management and helps to enhance reproductive performance. It is an essential part of fertility management. Besides, early identification of pregnant and non-pregnant cows after breeding improves reproductive efficiency and pregnancy rate in cows by decreasing the interval between services. However, most of the present day methods of pregnancy test qualify as an ideal diagnostic due to limitations of accuracy and requirement for elaborate instrumentation and laboratory setup. Rectal palpation is widely practiced due to its economic benefits and speed of diagnosis. Moreover, ultrasonography provides more information and detects pregnancies sooner after insemination than rectal palpation. Blood and milk pregnancy tests are another option for pregnancy diagnosis. Many currently available methods for early pregnancy diagnosis is questionable and require that all animals diagnosed pregnant early after insemination be scheduled for rechecks at later times during gestation to identify animals experiencing embryonic mortality. Careless early rectal palpation can induce embryonic mortality. In developing countries farmer bring their animal late for pregnancy check and have no good record keeping after service.

Based on the above conclusion the following recommendations are forwarded:

- Veterinarians should promote an awareness creation about the importance of early pregnancy diagnosis for dairy farm owners.
- Veterinarians should take care during trans-rectal palpation to prevent early embryonic mortality.
- Records should be kept after insemination or after a natural service with the possible identification of individual animal.

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List of Abbreviations:

AI	Artificial Insemination
CL	Corpus Luteum
ECF	Early Conception Factor
ELISA	Enzyme Linked Immunosorbent Assay
EPF	Early Pregnancy Factor
EPF-B	Early Pregnancy Factor B
ESR1	Estrogen Receptor Alpha
FSH	Follicle Stimulating Hormone
IFN-tau	Interferon tau
LH	Luteinizing Hormone
MSD	Merck Sharp and Dohme
NADIS	National Animal Disease Information Service
NIOS	National Institute of Open schooling
OXT	Oxytocin
OXTR	Oxytocin Receptor
P4	Progesterone
PAG	Pregnancy Associated Glycoprotein
PD	Pregnancy Diagnosis
PGF2α	Prostaglandin F2á
RIA	Radioimmunoassay

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