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Pregnancy Diagnosis in Cattle for Fertility Management: A Review

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Abstract: Pregnancy diagnosis is an essential part of fertility management. Early detection of pregnancy allows early recognition and treatment of problems. The aim of this manuscript is to overview the methods of pregnancy diagnosis in cow and to highlight the advantages of pregnancy diagnosis in relation to fertility management. Methods of pregnancy diagnosis in cow can be classified into direct and indirect methods. Direct methods of pregnancy diagnosis include transrectal palpation and ultrasonography. Indirect methods for early pregnancy diagnosis include measurement of endocrine hormones and pregnancy specific proteins. Visual method is far from perfect. Detecting animals that have been inseminated returning to heat is the most cost-effective method of early detection of non-pregnancy. However, it cannot be relied on as a method of pregnancy detection as a high proportion of cattle (often>30%) which are not seen in heat are actually empty. Transrectal palpation and ultrasonography continue to be the methods of choice for an accurate and early pregnancy diagnosis. Transrectal palpation remains the method of choice for early pregnancy diagnosis. Ultrasound examination is better for aging and sexing the embryo. An accurate, early diagnosis of pregnancy is essential to a successful breeding program. In both beef and dairy cattle, pregnancy diagnosis is an important tool to measure the success of a reproductive management, to allow for early detection of problems and to achieve resynchronization of non-pregnant cows. Generally Pregnancy diagnosis is an important part of good management and helps to maximize reproductive performance. Therefore, the veterinarians and animal production workers should develop an awareness creation about the importance of early pregnancy diagnosis for the farmers and the government should build national laboratories and research centers to provide pregnancy testing for the farmers as a valuable tool for fertility management.

Key words: Pregnancy Diagnosis · Progesterone · Transrectal Palpation · Ultrasonography

INTRODUCTION

Pregnancy diagnosis is essential for profitable animal husbandry particularly in the productive animal species. Early pregnancy diagnosis would help to evaluate the therapies at an early date and devise alternative manipulations in the current systems of planned breeding. Furthermore, early diagnosis of pregnancy is essential in animal management for economic reasons. 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 [1]. Early identification of non-pregnant dairy cows and heifers post breeding can improve reproductive efficiency and pregnancy rate by decreasing the interval between AI services and increasing AI service rate. Thus, new technologies to identify non pregnant dairy cows and heifers early after artificial insemination (AI) may play a key role in management strategies to improve reproductive efficiency and profitability on commercial dairy farms [2]. A changing cattle industry may affect how pregnancy diagnoses are performed in the future. Intensification of reproductive management in beef herds and the implementation of AI are creating the need for more accurate and timely diagnoses of pregnancy [3].

The methods of pregnancy diagnosis are divided into direct and indirect methods or it can be divided in to visual, clinical and laboratory methods. The direct pregnancy diagnosis methods include transrectal palpation and ultrasonography. Indirect methods for early pregnancy diagnosis use qualitative or quantitative measures of reproductive hormones at specific stages

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after AI or detect conceptus specific substances in maternal body fluids as indirect indicators of the presence of a viable pregnancy. Currently available indirect methods of pregnancy diagnosis include measurement of endocrine hormones such as progesterone, estrone sulphate and pregnancy specific proteins such as pregnancy-associated glycoproteins, the early pregnancy factor and interferon-tau [2]. Per-rectal palpation and ultrasonography are direct and accurate methods for pregnancy diagnosis. Both require a great deal of skill and experience. Transrectal palpation does not require expensive equipment or a power supply. It has significant advantages in time for small numbers of cows and for very late pregnancies [4]. It is the oldest and most widely used method for early pregnancy diagnosis in dairy cattle [2]. Veterinary-grade 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]. Practical application of ultrasound by bovine practitioners for routine reproductive examinations of cattle is the next contribution this technology is positioned to make to the livestock industry. The information-gathering capabilities of ultrasonic imaging are far exceed those of rectal palpation [6]. The visual methods of pregnancy diagnosis include non-return to estrus, met estrous hemorrhage and exposure to a bull or artificial insemination [2]. The use of estrous detection following insemination is useful to detect non-pregnant cows and allow for re-synchronization of such a group of cows [4].

Potential economic benefits of pregnancy detection include timely culling, saving costs, maintaining cows which will not provide economic returns and providing information to allow planning for replacement needs [7]. Early pregnancy diagnosis is crucial to shortening the calving interval through enabling the farmer to identify open animals so as to treat and/or rebreed them at the earliest opportunity. Dairy farmers need to recognize non pregnancy at the earliest opportunity so as to rebreed the dam at the very next opportunity [8].

Pregnancy diagnosis is a very important fertility management tool. Due to this the interest of the farmer for pregnancy checking was increasing from time to time to prevent economical loss. But, unfortunately the level of a review on pregnancy diagnosis in cattle is very low and there is scarcity of sources regarding pregnancy diagnosis. Therefore, the objectives of this paper are to review the methods of pregnancy diagnosis in cow and highlight the advantages of pregnancy diagnosis in relation to fertility management.

Pregnancy Diagnosis in Cow: Pregnancy diagnosis in cattle is a widely practiced procedure and has come to be accepted as a highly recommended management technique in beef and dairy cow herds [7]. Efficient livestock production requires accurate and early pregnancy detection so pregnancy diagnosis is a common procedure in most dairy farms. A wide variety of methods have been used and each has its own advantages and disadvantages [4]. The diagnosis of pregnancy is essential for profitable animal husbandry, especially in the productive animal species [1]. Early pregnancy diagnosis is crucial to shorten the calving interval through enabling the farmer to identify open animals so as to treat and/or rebreed them at the earliest opportunity [8].

Methods of Pregnancy Diagnosis: A variety of approaches have been evaluated and developed during the past and recent years, some of which have some limitations to their wide scale use. Generally, the methods of pregnancy diagnosis are divided into direct and indirect methods or it can be divided in to visual, clinical and laboratory methods [9].

By definition, direct methods for early pregnancy diagnosis of dairy cattle involve direct detection of the tissues and/or associated fluids of the conceptus either manually or via electronic instrumentation. Currently available direct methods for diagnosis of pregnancy include transrectal palpation and ultrasonography and both methods are currently used by bovine practitioners to diagnose pregnancy in dairy cattle. Because they are direct methods, the test outcome can be subjective among practitioners. Technical expertise, operator proficiency and the stage post breeding that the technique is performed can affect the specificity and sensitivity of the test [2].

Indirect methods for early pregnancy diagnosis use qualitative or quantitative measures of reproductive hormones at specific stages after AI or detect conceptus specific substances in maternal body fluids as indirect indicators of the presence of a viable pregnancy. Currently available indirect methods of pregnancy diagnosis include measurement of endocrine hormones such as progesterone, estrone sulphate and pregnancy specific proteins such as pregnancy-associated glycoproteins, the early pregnancy factor and interferon-tau [2]. The visual method of pregnancy diagnosis includes non-return to estrus, met estrous hemorrhage and exposure to a bull or artificial insemination [2]. An appropriate method for

pregnancy diagnosis depends on the objectives of the reproductive program and considerations that are unique to each individual farm [10].

Transrectal Palpation: Palpation of the reproductive tract through the rectal wall has been the customary method for pregnancy diagnosis [11]. 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 a trained operator to detect characteristics of the tract that coincide with either pregnancy or non-pregnancy [7]. Currently, rectal palpation is the easiest, fastest, cheapest and most accurate method that meets most of our goals [12].

The goal in rectal palpation is to be 100% accurate at determining the pregnancy status 35 days post breeding [9]. 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. The only positive signs of pregnancy in the cow are fetus, cotyledons/caruncles, amniotic vesicle and fetal membrane slip [7]. 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 [9]. 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 cardinal signs of pregnancy is carefully ascertained [7].

Rectal palpation also, allows an estimation of the stage of pregnancy. The pregnancy status was diagnosed and days in gestation of each cow were determined by per-rectal palpation 70 days after AI [7]. 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, fetal crown-to-nose length and position of the uterus [11]. Because pregnancy in cattle can be terminated by manual rupture of the amniotic vesicle, transrectal palpation induces iatrogenic embryonic mortality [2]. The risk of embryonic mortality is high during the period of gestation when cows are diagnosed by transrectal palpation [9].

Ultrasonography: By per-rectal palpation an expert can accurately diagnose an animal pregnant only after day 35 of gestation, but the application of ultrasonography has made diagnosis possible as early as day 28 after insemination or even earlier [13]. Transrectal ultrasonography provides additional information on ovarian structures, identification of twins and determination of fetal viability, age and sex [5, 13,14]. Transrectal 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 [5]. 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. It requires a great deal of skill and experience [5].

Transrectal ultrasonography is slowly being incorporated into reproductive management schemes in dairies primarily by bovine practitioners who have adopted this technology. The extent to which transrectal ultrasonography will displace transrectal palpation as the primary direct method for pregnancy diagnosis in dairy cattle remains to be seen; however, if current trends continue, transrectal ultrasonography may displace transrectal palpation as the direct method of choice for pregnancy diagnosis among bovine practitioners. Because transrectal ultrasonography reduces the interval from insemination to pregnancy diagnosis by 7 to 9 days [15]. An accuracy 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 [4].

Veterinary-grade 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 main disadvantages of the use of ultrasonography are related to cost and time involved with the use of this technique. Ultrasound machines are expensive and it takes more time to perform a pregnancy diagnosis with an ultrasound machine than by rectal palpation [4].

Progesterone: Measurement of progesterone 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 foetoplacental development [8]. The corpus luteum formed on the ovary subsequent to ovulation produces progesterone for maintenance of pregnancy for entire gestation in cow. 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. 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 [9].

Progesterone concentrations in milk or serum can be quantified using a laboratory RIA or ELISA procedures [2]. Currently, milk progesterone 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 [4].

Although subtle but, significant differences in serum progesterone concentrations have been reported between pregnant and non-pregnant cows early post breeding [16, 17], assessment of plasma progesterone 4 to 8 days after AI was not an accurate method for identifying non pregnant cows.

The main advantage of using milk or plasma progesterone concentrations to diagnose pregnancy is that this method allows detection of non-pregnant cows soon after insemination. In particular, cows can be diagnosed not pregnant as early as 21 days after insemination. There are several disadvantages of using milk or plasma progesterone to diagnose pregnancy. Milk and plasma progesterone samples need to be collected at least two times after insemination. Usually, samples are collected at 21 and 24 days after insemination. If either one of those samples is considered to have low progesterone concentrations, cows are diagnosed as not pregnant. If both samples have high progesterone concentrations, however, there is still a certain probability that the cow is not actually pregnant. The reliability of progesterone tests to detect pregnant cows is estimated to be only around 80 %. That means that 20 % of cows diagnosed pregnant by progesterone are, in reality, not pregnant [18].

Estrone Sulphate: Estrone sulfate is a conjugated estrogen that has been used to diagnose pregnancy using milk samples in cattle. Estrone sulfate is produced specifically by the conceptus (embryo-fetus and associated placental membranes) and, therefore, its presence is a direct indicator of pregnancy [2]. It is a conjugated steroid product of estrone, present predominantly in the bovine placentomes and it is the major estrone present in the fetal (allantoic and amniotic) fluids and maternal peripheral plasma of cows [8]. In cows, concentrations of estrone sulfate 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 sulfate in maternal circulation are not reliably detectable until around 80 days in gestation, estrone sulfate cannot compete with progesterone to assess pregnancy status early post breeding in cattle and therefore this test can only detect late pregnancy [2, 8].

Estrone sulphate is produced by the foetal-maternal unit. It is present in milk and plasma of non-pregnant animals, as well, but its concentration increases as pregnancy advances. Several studies report that after day 100 to 120 of gestation a high proportion of pregnant cows will have estrone sulphate levels above the highest concentration found in non-pregnant animals. The potential value of oestrone sulphate lies, therefore, in late-stage pregnancy [19].

Concentration of estrone sulphate in the maternal body fluids is a useful indicator for the placental functions especially those related to embryonic growth. Estrone sulphate concentrations have also been frequently correlated to fetal numbers [20]. As these are higher when the number of developing fetuses is more than one. Yet, estrone sulphate is not an ideal pregnancy biomarker as the plasma and milk profiles are influenced by many other factors such as genetic makeup, weight, parity status and environment [21].

Pregnancy Associated Glycoproteins: The pregnancy associated glycoproteins (PAGs) are secretory products from the mono and binucleated trophoblastic cells in bovine placentomes [8]. The PAGs consist of a large family of more than 20 closely related proteins that are only produced by the placenta [22]. They can be detected in the blood of pregnant cows beginning at approximately 25 days after insemination [23]. Monitoring the concentrations of PAGs in blood is an effective method of pregnancy detection [24]. By using biochemical

procedures, some molecules of the PAGs were isolated from cotyledons of cow [25, 26]. Although their function during pregnancy or parturition is unclear, [27] demonstrated that PAGs are inactive members of the aspartic proteinase family. If enzymatically active, PAGs could be important in structural remodeling of the placenta during pregnancy or in placental detachment after parturition [28].

Although PAG are presently one of the more promising markers for early detection of pregnancy in cattle, PAG-based pregnancy tests are limited by a number of factors. 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 [2].

Early Conception 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 [8]. Early pregnancy factor (EPF) is a protein that was first detected in the serum of pregnant mice within 4 to 6 hours after mating. 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 [11, 29].

Serum concentrations of the various EPF forms and their components vary from species to species and are dependent on gestational age [29]. 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 cattle 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 [9].

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 [30]. It is the pregnancy recognition hormone in sheep and other ruminants 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 corpus luteum (CL) and posterior pituitary to induce luteolytic pulses of PG F2á. Thus, IFN-tau blocks the ability of the uterus to develop the luteolytic mechanism [31-33].

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 [34]. IFN-tau, acting within the uterine cavity [8]. With extremely low levels in extra uterine tissues and peripheral circulation, prevents direct use of IFN-tau as an early pregnancy diagnosis molecule [35]. The INF-tau secretion is transitory. It reaches a maximum by 20 to 24 days and is completely gone by day 30 of pregnancy. The IFN-tau is unlike hCG because its expression is transitory and it 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 [24].

Visual Methods of Pregnancy Diagnosis in Cow

Exposure to a Bull or Artificial Insemination Records: 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. While fertilization rates are high, only about 50% of inseminations result in a detectable pregnancy. Conversely, unobserved, unplanned, or unrecorded mating are not uncommon. Thus, history is not a reliable indicator of pregnancy status and may sometimes be deceptive [11].

Non Return to Estrous Cycle: Cows are commonly show estrus approximately every 21 days (20 days for a heifer). The actual average length in lactating dairy cows is about 23 days. After insemination, cow can either conceive or fail to conceive to that service. If a cow is pregnant after insemination, the corpus luteum will not regress, progesterone concentrations will remain high and the cow

will not return to estrus. Alternatively, if a cow is not pregnant after insemination, the corpus luteum will regress, plasma progesterone concentrations will decrease and the cow will return to estrus approximately 20-23 days after insemination. Therefore, if a cow is observed in estrus after insemination, it can be concluded that the cow is non-pregnant. The use of estrus detection following insemination is useful to detect non-pregnant cows and allow for re-synchronization of such a group of cows [4]. When an animal is mated and it does not return to estrus, the animal is pregnant. This happens because during pregnancy, the conceptus inhibits the regression of the corpus luteum and thus, prevents the animal from returning to estrus [9]. Bovine embryos signal their presence around day 15 to 17 after ovulation, the corpus luteum is maintained and the maternal estrous cycle is suspended. Thus, failure of a cow to return to estrus 18 to 24 days after breeding suggests that conception has occurred [11].

In beef herds that use natural service, perceptive managers may observe that a greater than expected number of cows return to estrus after mating. This situation suggests an infertile bull, the presence of a venereal disease, or some other cause of infertility and there is an opportunity to take corrective action before the breeding season ends [11].

Besides the non-return to estrus a few of other visual signs of pregnancy appearing in late pregnancy includes increase in the size of the abdomen, development of the udder or mammary glands specially in dairy heifers (4 months onwards), slight vaginal discharge (from 4-5 months onward in dairy cows) and movements of the fetus visible externally (especially in fed cows on the right side of abdomen 6 months onwards). However, the accuracy of these visual diagnostic symptoms is always low and a clinician must use them as a supplement to clinical diagnosis [9].

Metestrous Hemorrhage: A bloody vaginal discharge is common in cows 24 to 48 hours after estrus and is the result of hemorrhage from capillaries in the lining of the uterus due to rapid decline in estrogen that follows ovulation. If metestrus bleeding is observed in a cow that was not seen in estrus a few days previously, it is implied that estrus was unobserved and the animal is not pregnant [11].

Palpable Findings of Pregnancy in Cow: The definite signs of pregnancy in the cow as determined by rectal palpation are palpation of the amniotic vesicle, slipping of the fetal membranes, palpation or ballottement of the fetus and palpation of the placentomes [9].

The Amniotic Vesicle: The amnion 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 cattle [11]. The amniotic vesicle can be palpated with due care between 30-50 days of gestation as a movable oval object within the uterine lumen [9].

Slipping of the Fetal Membranes: It is possible to feel the slipping of the chorioallantoic membrane (fetal membrane) along the greater curvature within the uterus [4]. The fetal membrane slip can be felt between 35-90 days of gestation [9]. The examiner can detect the chorioallantois (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 chorioallantois 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 touch and some experience are required to recognize this sign of pregnancy [11].

Palpation of Placentomes: Placentomes are the structures formed by the union of maternal caruncles and fetal cotyledons by which the placenta is attached to the uterus [4]. 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 [9]. 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 [11].

Palpation of the Fetus: As pregnancy progresses, it becomes possible to feel the presence of the fetus within the 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 [4]. The palpation of the fetus itself is a positive sign of pregnancy. 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) [9].

In the early stages, 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 4 months 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 [11].

Estimation of the stage of pregnancy is performed by rectal palpation and days in gestation of each cow were determined [7]. The size of the fetus as explained in the table below is approximately that of a mouse or rat at 2 and 3 months and it increases to the size of a small cat at 4 months, a large cat at 5 months and a beagle dog at 6 months respectively. Beyond 8 months of gestation, fetal parts (legs, head) are palpable within the pelvic cavity or just cranial to the pelvic brim. Palpation of a fetal extremity is sufficient evidence for pregnancy if other uterine findings are normal [9].

Table 1: Calf fetal size at various stages of pregnancy in relation to the size of some commonly known adult animals

Stage of pregnancy	Calf fetal size in relation to the size of commonly known adult animals
2 months	Mouse
3 months	Rat
4 months	small cat
5 months	large cat
6 months	beagle dog

Source: Purohit, [9]

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 [9].

Fetal Mummification: Fetal mummification occurs most often between the 3rd and 8th month of gestation, without any concomitant luteolysis of the CL or opening of the cervix. It is associated with a persistent CL is observed mainly in cattle. After fetal death, the amniotic and allantoic fluids are resorbed and the fetal tissues are dehydrated. Eventually, the caruncles disappear during the dehydration process. The longer the mummified fetus is retained, the dryer, firmer and more leathery the tissues of the fetus become [36, 37]. A uterus with mummified fetus has thick uterine walls, absence of fluid and placentomes and presence of a hard palpable structure. The fetus is closely apposing the uterine wall. The fremitus is absent [9].

The entire process of mummification takes several weeks, depending on the age of the fetus at the time of death. Once all fluids are completely resorbed, the fetal membranes and uterine wall adhere closely to the fetus and the whole mass becomes brownish black, leathery in appearance and odorless [37-39].

Fetal Maceration: Fetal maceration is defined by 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 [40]. When there is maceration of the fetus there will be copious vaginal pus discharge. The uterine wall is thick and doughy and placentomes are not palpable. The fremitus is absent [9].

Pyometra and Mucometra: Pyometra and mucometra are difficult to differentiate from normal pregnancy especially when the pus or mucus is present in enormous quantity (sometimes 20-40 liters) so that the uterus is largely enlarged and placed on the abdominal floor [9].

In pyometra the uterine wall is thick, uterus is doughy and placentomes or fetus is not palpable and the fremitus is absent. Sonographic evaluation would depict anechoic fluid without cotyledons accumulated inside the uterus. A clinical therapy to terminate the corpus luteum on an assumption that the condition is pyometra could be hazardous and loss to the owner, if unfortunately the condition was a normal pregnancy [9]. Diagnosis of pyometra by ultrasonography had previously been done on the basis of high volume of accumulated anechoic uterine content without fetus and cotyledons, closed cervix and corpus luteum on the ovary [41, 42].

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. A consistent ultrasonographic finding was thin walled uterus which was filled with anechoic fluid. When these two conditions are to be differentiated from early pregnancy a characteristic feature found most often is the bilateral enlargement of both uterine horns, which is not found in pregnancy. The fetus is not palpable in these two pathologies and often there is a history of vaginal discharges [9].

Advantages of Pregnancy Diagnosis in Cow: The diagnosis of pregnancy is essential for profitable animal husbandry especially in the productive animal species. For an economically dairy farm, animals must calve every year and to maintain this, identifying pregnant animals at an early date seems imperative. It therefore, appears that early diagnosis of pregnancy is essential in animal management for economic reasons. 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 [1]. Early identification of non-pregnant dairy cows and heifers post breeding can improve reproductive efficiency and pregnancy rate by decreasing the interval between AI services and increasing AI service rate [2].

Poor fertility costs more money. Effective fertility management is thus a key component of profitable dairy farming. Such management requires regular, accurate evaluation of the fertility status of the herd. To evaluate fertility status properly, requires pregnancy testing [4]. Potential economic benefits of pregnancy detection include timely culling of open cows, aid in the culling decision for cows with other issues, breeding management (move to next calving season, for example), grouping cows for feeding, calving and other managements [7].

CONCLUSION AND RECOMMONDIUTIONS

Pregnancy diagnosis is an important part of good management and helps to maximize reproductive performance. It is an essential part of fertility management. Generally early identification of pregnant and non-pregnant cows post breeding improves reproductive efficiency and pregnancy rate in cattle by decreasing the interval between services. Early pregnancy diagnosis is an important aspect for optimizing dairy production, but unfortunately none of the present day methods qualifies as an ideal diagnostic due to limitations of accuracy, later stages of applicability and requirement for elaborate instrumentation and laboratory setup. The traditional method of manual rectal palpation is widely practiced due to its economic benefits, accuracy and speed of diagnosis. In some areas, ultrasound is performed so that more information is collected and pregnancies are detected sooner after insemination. But unfortunately there are too few skilled practitioners that can perform pregnancy diagnosis by manual palpation or ultrasound. In these places, blood pregnancy tests are a viable option for pregnancy diagnosis. The ability to make an accurate, early diagnosis is required of most successful large animal practitioners. 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.

Based on the above conclusion the following recommendations are forwarded:

- The veterinarians and animal production workers should develop an awareness creation about the importance of early pregnancy diagnosis for the farmers.
- Veterinarians should take care during transrectal palpation to prevent early embryonic mortality and early abortion of the fetus.
- The government should build national laboratories and research centers to provide pregnancy testing for the farmers as a valuable tool for fertility management.
- Records should be kept after insemination or after a natural service with the possible identification of individual animals.
- The government should provide ultrasound access for easy and early determination of pregnancy.
- Dairy or beef farms should use pregnancy testing as a valuable tool for fertility management.

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