

Application of Radioimmunoassay for Livestock Fertility Management

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Abstract

Reproductive performance is one of the important factors for determining the economics of livestock production. Reproduction is a complex luxurious process affected by environmental factors and involves a series of physiological processes and supported by endocrine system through release of various hormones. A sound knowledge of reproductive functioning in terms of interplay of hypothalamic, gonadotropic and gonadal hormones, with synergistic and antagonistic influences from other hormones and factors involved in the regulation of various reproductive stages, accurate oestrus detection, timely pregnancy diagnosis and early detection of non-conceived stock can be expected to lead to an improvement of the reproductive efficiency. The different phases of reproductive cycle and pregnancy are regulated by intricate sequential events and interactions between hypothalamic releasing hormones, hormones secreted from the pituitary and sex steroids secreted by the ovary. Lack of integration or synchronization or endocrine imbalances at any phase of the sequence may result in reproductive failure. This paper focuses on use of various hormones for detection of estrus and early pregnancy in livestock.

Keywords: Reproductive Efficiency; Gonadotropic Hormones; Gonad Hormones; Reproductive Failure.

Introduction

Assessment of hormones in livestock is widely used to achieve the improvement of both production and reproduction. Conventional ways of (genetic) improvement of livestock are based on selection and crossbreeding programme. These programmes have proved to be very effective, but in general they are time consuming and expensive. Furthermore, heritability estimates are generally very low. For these reasons, much of the current research is directed towards the development of alternative methods of selection, which are based on the correlation between biochemical parameters such as hormones, enzymes, metabolites etc and economically important traits. Much of this type of research has initially been directed to the study of blood types and protein polymorphism in which markers are most likely linked to the action of a single gene or a few genes only. These marker genes may be coupled to other genes which are relevant to economically useful traits, and therefore can be used, in principle, as a basis for selection. Some hormones considered to be possible markers for production traits are thyroid hormones, growth hormone, insulin, prolactin, corticosteroids and androgens. The hormones used for markers of reproductive status are LH, testosterone, progesterone, estrogen,

estrogen sulphate etc. An important step towards an improved understanding of reproductive physiology was the introduction of highly specific and sensitive radioimmunoassay technique in the early 1960s. Earlier bioassay methods of hormone estimation were not so much reliable and accurate. Radioimmunoassay (RIA) and other related techniques now a day's allow measurement of hormones and other substances with a sensitivity up to the level of pico gram. Therefore RIA techniques become so popular over the others.

Monitoring Of Fertility By Measurement Of Hormones:

Measurement of progesterone, estrogen, oestrone sulphate, pregnancy specific protein and pregnancy associated glycoprotein has found practical application as a method for improving reproduction in farm animals.

Expression of estrus

Poor expression of estrus is one of the major factors hampering the efficient utilization of tropical cows and buffaloes. Estrus is traditionally observed by behavioral symptoms which, however is practically very difficult because the overt signs are of low intensity

and of short duration. Occurrence of ovulation which is not preceded by overt behavioral estrus symptoms is quite common in tropical animals during stressful summer and non-stressful winter months. Progesterone serves as a marker for determination of functional status of corpus luteum and diagnostic tool for identifying ovarian condition such as estrus confirmation, differentiating types of cysts, silent estrus and lack of cyclicity in cattle and buffaloes. Estrogen is essential for expression of estrus whereas progesterone is required for preparation of uterus for implantation and maintenance of pregnancy. This is because progesterone levels are low around the time of ovulation and high during luteal phase of estrous cycle or during pregnancy and declined to basal level on the day of parturition. Mean plasma progesterone concentration declined from 1.14 ng/ml on day 4 prior to estrus to less than 0.4 ng/ml on the day of estrus and then rose to 2.73, 1.84 ng/ml on day 10 and 8 of the cycle in cattle and buffaloes, respectively [1-3].

Mean progesterone level was lowest on the day of estrus and rose to a peak level during mid luteal phase of cycle which then declined to a basal level on the day of next estrus in cattle and buffaloes that exhibited overt and silent estrus, respectively [4,5]. The overall plasma progesterone levels in cattle and buffaloes that exhibited silent estrus were lower compared to those in overt estrus and might be responsible for poor expression of estrus. Estradiol levels are maximum on the day of estrus and declines gradually with a minimum concentration on day 12-14 of the cycle. In males, testosterone is responsible for sexual maturity and sperm production. In infertile animals or in case of oligospermic animal, testosterone levels remain low or undetectable. The economic benefit of progesterone based estrus confirmation has not been quantified. Since the proportion of cows not in or near estrus when inseminated varies among herds from 0 to 60% [6], a substantial benefit might be expected, especially on the farms with a high error rate for estrus detection. This benefit should result from reduction in calving interval and the number of inseminations per conception [7] demonstrated a strategy of on farm milk progesterone testing on day 19 after insemination, followed by prostaglandin treatment of non pregnant cows will be profitable, but only if the efficiency of detection of estrus among cows diagnosed non pregnant is increased by more than 20% and if the error rate in pregnancy is less than 3%. To achieve the latter, the cows which are non pregnant on the basis of on farm progesterone test should be checked again for pregnancy by other methods.

Diagnosis of Pregnancy

Early detection of pregnant and non-pregnant livestock has become a key to good breeding management because it is an essential factor for monitoring and controlling fertility. Many new and old technologies are available to identify pregnant and non-pregnant animals early post service. The methods of pregnancy diagnosis are divided into direct and indirect methods. The direct pregnancy diagnosis methods include - transrectal palpation and ultra sonography. Pregnancy diagnosis by rectal palpation have been reported during early pregnancy in cattle [8,9], buffalo [10], sheep [11] and pig [12]. Traditionally, to confirm pregnancy at about day 30 of gestation onwards, the practitioners have relied on the

palpation of the amniotic vesicle and slipping of the chorioallantoic membranes between the thumb and forefinger. Palpation technique detects pregnancy with an accuracy of 66 to 100% from Days 49 to 109 of gestation, however it has low accuracy (17 to 57%) for determining multiple fetuses.

Transrectal ultrasonography has been used for pregnancy diagnosis in cattle [13], buffalo [10-14], sheep [8] and goat [14-17]. Transrectal ultrasonography identifies the embryonic vesicle as early as Day 12 after mating but the sensitivity of this technique for pregnancy is very low (12%) earlier than 25 days after mating. Transrectal ultrasonography for pregnancy diagnosis offers some advantages over palpation per rectum: earlier diagnosis of pregnancy/non-pregnancy, determination of embryo/fetus viability, reduction of misdiagnosis and reduction of "potential" iatrogenic embryo/fetal attrition. The currently available indirect methods of pregnancy diagnosis include measurement of hormones such as progesterone [18-20], estrone sulphate [21,22] and pregnancy specific proteins such as pregnancy-associated glycoproteins [23,24] the early pregnancy factor [25] and interferon-tau [26,27]. Early pregnancy diagnosis will assist dairy producers in managing open cows and improving reproductive performance and economics of their herd.

Progesterone

Measurement of progesterone by RIA has been widely used for verifying whether cows are in estrus at the time of insemination or early diagnosis of pregnancy in cattle, buffaloes, goats and pigs. Progesterone levels elevate during the mid cycle of each reproductive cycle and during the entire gestation period. If the cow is not pregnant, the corpus luteum regresses and progesterone levels decline to low levels about 2 days before the cow comes into heat again. However, if the cow becomes pregnant, the corpus luteum continues to function and progesterone levels remain high throughout gestation. In pregnant cow, progesterone values in peripheral plasma increase with the development of corpus luteum up to conception (5-10 ng/ml) on days 15-20 after conception; these concentrations remain constant until shortly before parturition. Using progesterone assay pregnancy can be predicted between 68 to 95% and this test gives best accuracy after three weeks of pregnancy. Studies in the bovine estrous cycle indicate that the milk or serum progesterone concentrations reach a maximum value 13-14 days after estrus, and if the animal is pregnant, these continue to remain elevated up to day 21 after fertilization [28] and beyond.

These high levels of progesterone in serum or milk between days 18 and 24 after insemination form the basis of establishment of pregnancy in cattle [29,30]. Since Laing and Heap 1971 first described the use of milk progesterone measurements as an early indicator of the reproductive status of the lactating dairy cow, there has been considerable research on the development of sensitive and reliable assays for progesterone in milk Shemesh et al. [30] proposed that the difference in peripheral plasma progesterone levels between pregnant and non-pregnant cows, 19 days after insemination, can form the basis for a very early pregnancy test. Laing and Heap 1971 first documented this in milk to diagnose cows

in early pregnancy. 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 [31]. In buffalo cows, the progesterone levels in milk are four to five times higher than those in blood plasma [32,18]. Like cattle, buffaloes too can be accurately diagnosed as non-pregnant by determination of plasma progesterone concentrations 21 days after insemination [33] also reported that progesterone concentration in the milk of pregnant buffaloes was significantly higher than that in non-pregnant animals on Day 20 and the difference between the two increased with time after insemination. The detection of non-pregnant animals was 100% successful at all times but the diagnosis was correct for 66, 68, 81 and 83% of animals tested on Days 20, 24, 28 and 40 respectively and predicted as pregnant.

Shemesh[30] 18-22 day reported that plasma and milk progesterone concentrations in pregnant sheep 18 22 days after mating were similar, about 3.7 ng/ml whereas values in non-pregnant sheep were less than 1 ng/ml. The accuracy was 92-100% for ewes diagnosed non-pregnant in the breeding season, but for ewes tested in the non-breeding season the diagnosis of non-pregnancy according to milk progesterone levels was only 50% accurate. Progesterone concentrations decrease sharply during regression of the corpus luteum (CL) in the non pregnant doe (one or two days prior to estrus) and return to higher levels within four days following estrus [34-36]. The pregnant doe has high progesterone concentrations during the same time period since the CL does not regress. Recently, Mondal, et al. [37] observed that plasma progesterone concentration declined from day 25 pre partum abruptly to the day of kidding and remained at basal level up to day 25 postpartum in Black Bengal goats.

Estrone sulphate

Estrone sulphate is a conjugated estrogen that can be detected in maternal plasma, serum, milk and urine. It is produced by the fetomaternal axis or the conceptus and its presence is indicator of pregnancy. Actually the viability of fetus is determined by measurement of estrone sulphate. It is detected at day 72 in plasma and between 105-112 days of gestation in milk of cows. Estrone sulphate test can reliably be used to diagnose the pregnancy beyond day 100 of gestation. This test can also be used to diagnose both singlet and multiple calf pregnancies after 110 days of pregnancies. Estrone sulphate test can reliably be used to diagnose the pregnancy beyond day 100 of gestation. This test can also be used to diagnose both singlet and multiple calf pregnancies after 110 days of pregnancies. Estrone sulphate in milk of cows rises from 30 pg/ml to 151 pg/ml in whey between days 41 and 60 of gestation to reach a maximum concentration of about 1000 pg/ml at days 220-240 of pregnancy [37]. Examination of the ranges of ES concentrations in milk sampled from non-pregnant and pregnant cows indicated that all non-pregnant cows and 46% of cows <120 days pregnant had milk ES concentrations <125 pg/ml. However, only 4% of cows ≥ 120 days pregnant had milk ES concentrations <125 pg/ml. The levels of estrone sulphate in different maternal body fluids, namely, milk and blood plasma, can be utilized as the

criteria for confirming pregnancy by after 110 day insemination in bovine species [38].

In Murrah buffaloes the levels of estrone sulphate were below detection levels (<50 pg/mL) during the first two months, followed by sharp increase in the fourth month and values stabilized after reaching the highest levels in the sixth month of pregnancy [39]. Hung and Prakash recorded a progressive increase in estrone sulfate concentrations in buffalo plasma after the 4th or 5th month of pregnancy. In another study by Tsang [40], estrone sulphate was detectable around Day 70 of gestation with value ranging between 0.1 to 0.7 ng/ml, then its level increased steadily till 2 days before parturition when an upsurge was seen (15-50 ng/ml). On Day 85 of gestation, there was a significant difference in the level of estrone sulphate between pregnant and non-pregnant ewes. In goat, oestrone sulphate concentrations began to rise from about Day 40 and reached a plateau of about 19nmol/l by Day 120 [41]. This value was maintained until about 20 days pre partum when there was a slight decline in concentration, rising by 2-fold about 24 h before parturition. The accuracy for detection of non-pregnancy was only 44 % whilst for detection of pregnancy it was 87.9% using the cut-off value of 0.1ng/ml [42]. Estrone sulphate based on farm pregnancy diagnosis is also possible in foetal sample of pigs. The concentration of total unconjugated oestrogens and estrone are consistently higher between days 24 and 30 in pregnant sows compared to non pregnant sows [43].

Pregnancy associated glycoprotein (PAG)

Pregnancy-associated glycoproteins (PAG) belong to a large family of inactive aspartic proteinases expressed by the placenta of domestic ruminants including cows, ewes, and goats [44]. PAG are synthesized by the mono- and binucleate trophoblastic cells of placenta and some of them are secreted in maternal blood from the moment when the conceptus becomes more closely attached to the uterine wall and formation of placentomes begins [45]. Among these glycoproteins, Butler, et al. [46] detected two pregnancy-specific proteins in the sera of pregnant cows - 65-70kDa and a 47-53kDa protein. Of these, the former showed an immune reaction similar to that of a 1-fetoprotein, while the latter showed no reactivity with known proteins and it was given the name "protein B" or the "pregnancy-specific protein B" (PSPB) in bovines. Pregnancy-specific protein-B (PSPB) was the first pregnancy-specific marker identified in cattle [46] and was later found to have the same N-terminal amino acid sequence as bovine PAG-1. The PAG family were isolated from cotyledons of cow [47-49], ewe [47,50], goat [51] and buffalo [52].

Development of specific RIA and EIA for the presence of pregnancy associated proteins of fetomaternal origin in the maternal serum 3-4 wks after conception has been used as a serological marker for pregnancy diagnosis in cattle [53], sheep and goats [54]. During gestation of cow, the concentrations of PAG are detectable as early as from the 19th to 22nd days after the conception, to reach concentrations from 3 to 6 ng/ml in the neighborhoods of the 33rd to 37th days of gestation [55,56] reported that PAG concentrations increased continuously from day 20 of

pregnancy until day 240 followed by a dramatic increase in the last ten days of pregnancy with maximum concentrations between day 5 and day 1 prepartum. In the bovine species, two different patterns of expression were found – those PAG that are expressed predominantly in binucleated cells (PAG 1-subgroup) are invariably absent in term placenta, whereas those PAG expressed more uniformly throughout trophoectoderm (PAG 2-subgroup) are detectable at all stages of pregnancy [57]. For example, boPAG-9 is expressed predominantly in early pregnancy, being detectable at Day 25 and declining as pregnancy progresses until being undetectable at term. On the contrary, boPAG-1 is not detectable at Day 25 but becomes prevalent at later stages, although it is absent in term placenta. Bovine PAG-2, -8, -10, and -11 are detectable throughout gestation and they are the only PAG present in term placenta. PAG measurement has also been used in several studies to monitor pregnancy failure during the late embryo and early foetal period [53,58].

In sheep the plasma PAG profiles are characterized by an initial increase between the 3rd and 4th week, followed by further gradual rise up to the 9th week of pregnancy [59]. Between the 9th and the 19th week the level remained constant, thereafter a drastic surge occurs, reaching a peak at parturition. Recently Rovani et al. [54] detected pregnancy accurately using bovine ELISA kit in sheep 33 days following mating, while PAGs levels from the previous gestation are no longer detected from 21 days postpartum. The accuracy of the ELISA test was 96.1% from 33 days of pregnancy until lambing. In goat's perusal of literature revealed a different profile of PAG with a significant first increase between day 21 and day 28 and maximum levels between the 5th and 8th week of pregnancy. Thereafter, PAG levels decreased slowly until parturition [17] reaching basal levels in the 4th week postpartum. Recently [57] was able to diagnose pregnancy in Boer goats from day 28 of pregnancy onwards using an ELISA based on antibodies raised against caprine or ovine PAG. With the antibody rose against caprine PAG a steep increase to a peak level of 69±9ng/ml on day 56 of pregnancy was followed by a gradual decline to 16±3 ng/ml at parturition and 0.3±0.07 ng/ml four weeks postpartum. With anti-bovine PAG, the PAG level increased to a maximum of 3.1±0.2 ng/ml on day 105 of pregnancy and fluctuated around 3ng/ml until the end of pregnancy suggesting its ability to diagnose early pregnancy and its possible use to evaluate feto-placental well-being [60,61].

Interferon-Tau

In ruminants, the anti luteolytic hormone for pregnancy recognition and maintenance of functional corpora lutea (CL) is interferon tau (IFN- τ) [62,63]. It is secreted in large quantities by the mononucleate cells of the trophoectoderm as the blastocyst begins to elongate at about days 13 in cattle with a peak production when the conceptus reaches its maximal size [64]. The secretion of IFN- τ by mononuclear cells of the ovine trophoectoderm is developmentally regulated with onset of secretion occurring as large spherical blastocysts transition to tubular and elongated filamentous forms between days 10 and 21 of pregnancy. In sheep, it is secreted between days 10-21 by the mononuclear trophoblast cells. On days 11-16, the PGF₂ concentrations are the same

in pregnant and non-pregnant animals, but pregnant animals administered PGF₂ on day 19 or 20 do not return to estrus. It is the pregnancy recognition hormone in sheep and other ruminant that acts to silence the transcription of estrogen receptor alpha (ESR1) and, therefore, ESR1-dependent expression of the oxytocin receptor (OXTR) gene in both uterine LE and superficial glandular epithelium (sGE), hereafter referred to as LE/sGE.

This abrogates development of the endometrial luteolytic mechanism that requires oxytocin-induced release of luteolytic pulses of prostaglandin F_{2 α} (PGF) by uterine LE/sGE; however, circulating concentrations of PGF are greater in pregnant than cyclic ewes due to continued expression of prostaglandin endoperoxide synthase 2 (PTGS2). IFN- τ is a member of the Type I IFN family that acts differentially on the endometrial luminal epithelium (LE), glandular epithelium (GE) and stroma to regulate expression of a number of IFN-stimulated genes (ISGs) that are hypothesized to play roles in the endometrial differentiation and conceptus implantation [65-68]. It has been demonstrated that bIFN- τ has several effects on the endometrium that result in decreased PGF_{2 α} secretion in the pregnant cow and maintenance of the CL. For example, IFN- τ reduces estradiol receptor number and thus prevents an estrogen-induced increase in oxytocin receptor number [69-74]. Moreover, rbIFN- τ inhibits the oxytocin- and/or phorbol ester-induced increase in phospholipase A2, cyclooxygenase-2 and prostaglandin F synthase expression in bovine endometrial cells.

Conclusion

Exploration of fundamental endocrine involvement in reproductive processes is essential for optimum reproductive management strategies and paradigm to overcome the infertility in domestic ruminants. Reliable techniques for early detection of pregnancy aid in culling or rebreeding of animals and provide a valuable tool for controlled breeding programs. Traditional methods of visual observation, abdominal palpation, service records and non-return to estrus are not reliable means of diagnosing early pregnancy. The review has lime lighted the use of hormonal assays for detection of estrus and early pregnancy in cattle, buffalo, sheep, goat and pig with their accuracy. It is concluded that micro quantitation of hormones has come as a revolutionary breakthrough in understanding animal reproduction function and is an important research tool for augmenting both production and reproduction of domestic animals.

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