Pregnancy Diagnosis with Progesterone ELISA Kit in Farm Animals, Its Accuracy and Application

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ABSTRACT

Pregnancy is a special condition where a female lodges one or more young ones within her uterus. It is maintained by various endocrine physiology and metabolic changes between maternal and fetal circumstances. Space dine *secreted by corpus luteum increases extraordinarily throughout the pregnancy, estrogen increases rapidly during first trimester and prolonged lifespan of corpus luteum and small quantity of estrogen prevents prostaglandins pulsatic secretion. We reviewed available literature to evaluate the accuracy of progesterone ELISA test kit in pregnancy diagnosis in farm animals. We found varying accuracy in global and Nepalese context but found that this is an important tool for early pregnancy diagnosis and infertility monitoringwith high accuracy which contributes to increase economic efficiency of a farm. This kit has been used for qualitative and quantitative study of progesterone to understand the reproductive status of animals. Therefore, its use is increasing in globally and in Nepal. This kit has been used at various breeding centers, and livestock service centers of Nepal. However, wider level use is still difficult due to lack of infrastructuresand subsistence farming.*

Keywords*: ELISA Kit, Pregnancy diagnosis, Progesterone

INTRODUCTION

Pregnancy or gestation is the condition or time of a female while one or more young ones are developing within her uterus, maintained by various endocrines physiology and metabolic changes between mother and fetal boundary.Pregnancy diagnosis is a matter of curiosity for farmers since long time (Purohit, 2010). Early pregnancy diagnosis is associated with profitability of the farm due to factors like amount of milk or meat or fleece produced per day per herd, decisions of keeping or culling, feeding schedules and formulations (Dionysius, D.A.,1991) as well as the identification of reproductive diseases for their early treatment and rebreeding of the healthy non-pregnant ones (Engeland, Ropstad and Eik, 1997).

Per cow per day loss will be approximately US$ 2 due to variable costs like feeding costs, breeding costs, labor costs, veterinary costs etc. (Ali, 2011); if the animal delays conception beyond 85 days postpartum which will increase the calving interval of the animal (Stevenson and Call, 1986). Pregnancy diagnosis is a key for shortening the calving interval by reducing the postpartum barren interval in farm animals (Balhara, Gupta, Singh, Mohanty, and Singh, 2013). So, the main target in a herd is not to allocate the pregnant but to identify the non pregnant animals and re-inseminating her as early as possible (Stevenson and Call, 1986)

Animal farm management will be vague and economically boredom without early diagnosis of pregnancy in farm animals. Accurate and early gestation diagnosis is essential which helps in proper management and identification and rebreeding of the non pregnant at the earliest. An early pregnancy diagnosis is essential in mares to tease them if non pregnant, and try to get them pregnant in the same season (Sah *et.al.*, 2017).

Progesterone (P4), an endogenous steroid is a progestogen sex hormone (Sah *et.al.*, 2017). It targets the uterine epithelium, mammary tissue, myometrium and hypothalamus causing endometrial secretion, reducing cervical mucus production, inhibition of GnRH release and promotes maintenance of pregnancy. Its main source is corpus luteum or yellow body of ovary (Stevenson and Call, 1986). But, during pregnancy of farm animals, there is wide species variation in source of progesterone. In case of sow and goat, corpus luteum is very essential source throughout the pregnancy. In sheep and mare, placental production of progesterone in sufficient quantity starts in about 50 and 70 days of pregnancy respectively. P4 gets secreted into blood and subsequently into milk. But its level is higher in milk than in blood due to having more affinity of steroids towards milk fat.

Progesterone at low level stimulates ovulation by promoting release of LH in coordination with estrogen, while at high level of progesterone; it hinders the secretion of FSH and LH by negative feedback mechanism. But, FSH is released sufficiently so that follicles may progress in luteal phase. In a normally cycling cow, levels of P4 are relatively low in both blood and milk around estrus. Then P4 increases in both fluids. On day 17 or 18 of the estrous cycle, it declines as a new follicle develops. If a cow is pregnant, P4 levels would not decrease on day 17 or 18, but would remain elevated (Petersson, 2007).
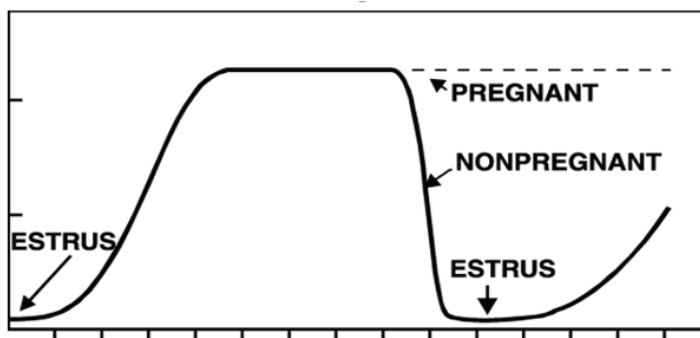

Figure 1. Changes in progesterone levels throughout the estrous cycle (adopted from Silvia and Heersche)

Progesterone quantification in blood and milk in laboratory can be obtained by Radioimmunoassay (RIA) or Enzyme-Linked Immuno-Sorbent Assay (ELISA) methods (Sah *et .al.*, 2017). This quantification could help in pregnancy examination as early as 21 days after natural or artificial insemination (Fricke *et.al.*, 2016). This possibility that progesterone assay in plasma and milk could guide early pregnancy diagnosis in farm animals was proposed in 1971 by Laing and Heap (Rioux and Rajotte, 2004)

METHODS FOR PREGNANCY DIAGNOSIS

The ideal pregnancy diagnosis methods should have high sensitivityto detect pregnant animals and high specificity to detect non-pregnant ones. In addition, they need to be convenient to be used in field conditions and inexpensive for farmers to adopt it. The additional qualities such as embryonic or fetal viability, sex determination for the future benefit would add value to it (Fricke *et.al.*, 2016).

There are various direct and indirect methods for rapid and early gestation diagnosis in animals (Fricke *et.al.*, 2016). Repeat heat in 18/24 days post insemination, rectal palpation to find uterus and its contents (expert clinician can detect as early as in 35 days), ultrasonography and radioimmunoassay or enzyme linked assay of Progesterone hormone in blood serum, plasma and milk of lactating animals are some of them. There are mainly five chemical test kits available for detecting the hormone in milk and one for the blood serum in heifers (Stevenson and Call, 1986). And, the various ELISA kits available are Progesterone ELISA kit from the Abraxis LCC USA Company (used in A.K. Sahs' experiment), Ovucheck product no.C006 purchased from the North American distributor Biovet, Saint-Hyacinthe, QC, Canada (in Stevenson and Calls' experiment), Noctech Progesterone EIA (used in Dionysius' experiment), and Enzygnost Milk Progesterone Vet$ (in Dionysius experiment).

Cattle has an estrous cycle of 21days. Estrus duration is 18hours on average and ovulation starts nearly 11hours later. The size of corpus luteum increases significantly from day 3 to 12 and same does the level of progesterone in blood and milk (Rioux and Rajotte, 2004). Fluctuation in level of milk and blood progesterone implies the changes in the reproductive condition of farm animals (Romangolo and Nebel, 1993). These changes thus can monitor the manager to assess the optimal time of artificial insemination of the animals to obtain the better establishment of pregnancy. Significantly less than 10% of all herds are currently using milk progesterone as a diagnostic aid.

SCENARIO OF PREGNANCY DIAGNOSIS IN LIVESTOCK IN NEPAL

In the context of Nepal, farmers need to wait for more than a month to know the status of their cattle's pregnancy when cattle return to heat in about 21-22 days of estrous cycle. Only the trained and skilled expert can detect the pregnancy by rectal palpation in one month, the accuracy of which is highly variable (Sah *et.al.*, 2017).Annual report of NCRP, 2016 have highlighted that early pregnancy diagnosis to maintain the reproductive efficiency offarmanimals have been a challenge in Nepal due to the lack of infrastructures such as the Ultrasonography (USG) machine, trained personnel to use it and poor economic status of farmers in rural areas (Sah *et.al.*, 2017).

RESULT

We reviewed availablescientific articles to identify the accuracy of progesterone kit in Nepal for pregnancy diagnosis in differentfarm animals such as sheep, goat, cattle of various breeds. We found varying percentage for accuracy of progesterone kits ranging from 60 to 100% for pregnant and 80 to 100% for non-pregnant with varying degree of sensitivity and specificity. In a

research of Chitwan, Nepal, 80% accuracy with 92% sensitivity and 57% specificity was found (Sah *et.al.*, 2017). The results of progesterone kit in pregnancy diagnosis in different farm animals are presented in Table 1.

Table 1. Results of progesterone kit accuracy in the pregnancy diagnosis in different farm animals

SN	Farm animals	Number of animals	Types of samples	Days of sampling (Post-insemination)	Accuracy percentage		Remarks	Reference
					Positive	Negative		
1	Crossbred Jersey and Holstein dairy cows	20	Blood (serum)	37-40	80		92% sensitivity 57% specificity	Sah, *et.al.*, 2017
2	Lactating holstein + Guernsey cows	200	Milk	21-22	77	100		Zaied, *et.al.*, 1979
			Milk	27-28	95	100		
3	Lactating Friesian cow	25	Blood (Periphe-ral plasma)	21-22	85			(Skemesh, *et.al.*, 1973)
4	Lactating ewes of local breed	62	Blood (Periphe-ral plasma)	16-21	93	100		(Skemesh, *et.al.*, 1973)
				31-35	100			
5	Lactating Holestein cows	91	Milk	20	67.3	87.2		(Cox *et.al.,* 2010)
6	Cows	476		21-24	88.6	93.9		(Reimers *et.al.,* 2010)
7	Zebu cattle	64	Milk	20	84	100		Alam, and Ghosh, 1994
			Plasma		84			
			Milk	24	90			
					92			
8	Holstein Friesian cows (Multi-parous + Heifers)	10+8	Blood (plasma)	22	71.4	100		Muhammd, *et.al.*, 2000
				25	83.3			
9	Italian Friesian cows	82		19-23	92.3	68.2		Romangolo, and Nebel, 1992
10	Dairy goats	73	Milk+ Blood	20	82	88		Engeland, *et.al.*, 1996
11	3 commercial dairy goats	28	Milk	21-24	96.4	100		Dionysius, 1991
12	Cows of 4 commercial dairies	42	Milk	22	65.5	90.9	Ovuch-eck EIA was used	
					68.2	90	Enzygn-ost vet was used	
13	Dairy cattle		Milk	21-24	84	90-100		Stevenson and Call
14	Cows of 14 dairy farms	752	Milk				97% heat detection accuracy ; 90% sensitivity 98.7% specificity	Ingenhoff, *et.al.*, 2013
15	Zebu cattles	46	Plasma	20	84	100	RIA was used with [125] I isotope.	Alamand Ghosh, 1994
				24	90			
		58	Milk	20	84			
				24	92			

DISCUSSION

The ELISA kit has not gained popularity due to its low specificity and less accuracy. Progesterone kits are not used in NLBC, Pokhara and NCRP, Rampur, Chitwan currently for pregnancy diagnosis. Ultrasonography is the gold standard test for pregnancy diagnosis in Nepal (Personal communication Dr. A.K Sah). Various pregnancy specific proteins kits like BIOPRYN, with 100% accuracy, have also been used for rapid test (Karki *et.al.*, 2018). Even in

IAAS farm, no provision of early pregnancy diagnosis is practiced using USG or progesterone assays. Rectal palpation only after 3 months is being practised, which is surely to increase the farm cost if the animal is detected to be open cow.

Accuracy of pregnancy positive is repeatedly lower than that of negative (non- pregnant), mostly due to various causes like embryonic mortality, inaccurate sampling, ovarian luteal cysts, prolonged luteal lifespan and breeding during luteal phase. Presence of some animals having long estrus intervals, or short estrous cycles increases the false positive results of this test. Cost of P4 testing and the labor involved in obtaining multiple samples also incur loss. Early pregnancy loss will also limit the accuracy of many direct and indirect methods of pregnancy diagnosis.

Potential uses of progesterone testing in a reproductive management program according to Nebel, R.L. are in identifying errors in detection of estrus. Animals with questionable signs of estrus can be verified by on-farm milk progesterone tests**.** Low P4 concentration of milk alone cannot be a single positive indicator of estrus. But, high P4 level confirms the non-estrus state of animals, even if they have any behavioral heat symptoms. Likewise, it may used to predict time of estrus. Level of progesterone will be very low at estrus and then will go increasing. If the animal gets fertilized, the level will remain elevated throughout the gestation but if not, it will be reduced rapidly in 17/18 days of estrous cycle (2-4 days before estrus) due to the decrease in the size of the corpus luteum in the absence of fertilization and again will come in heat. Likewise, the possibility of establishing a rapid gestation diagnosis in domestic animals by means of a progesterone assay in plasma and milk was introduced from 1971 by Laing and Heap (Rioux and Rajotte, 2004). This is also helpful in differentiating types of ovarian cysts. Ovarian cysts mainly follicular and luteal cysts can be differentiated by the quantification of progesterone where follicular cyst is characterized by low progesterone level whereas luteal cyst is characterized by very high level of progesterone, sometimes up to ml in milk or blood. In addition, this test has been used in evaluating response to endocrine therapy. Any endocrine treatment affecting the ovarian status can be evaluated by the progesterone test. Example: for follicular cyst for leutinization, if GnRH is administered, Progesterone testing 10 days after GnRH administration could guide accurately if retreatment is needed or not.

CONCLUSION AND RECOMMENDATION

Progesterone (P4) is very crucial component of pregnancy and uterine biology, as well as for mammogenesis and lactogenesis. Due to having less accuracy and specificity, progesterone ELISA cannot be used for confirmatory diagnosis, but can be used for screening purpose after 21 days post insemination. In agro-veterinary sciences, one can demonstrate the usefulness and efficiency of this test to predict estrus (heat), gestation, and to evaluate pathological conditions and responses to different hormone-based medicines. However, further research is needed to determine the relative milk progesterone concentrations to improve the reproductive efficiency and economy of dairy farms.

We recommend that due to less accuracy and specificity of progesterone assay, it should not be used for confirmatory pregnancy but may be used as a part of the reproductive management program.

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