www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i9.61

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

# Analysis of Specific Proteins for the Detection of Early Pregnancy in Cattle, A Review

Authors
**Er. S. Shekhar Mohapatra[1], Ms. Anshurekha Dash[2], Dr Manoj Kumar Jena[1]**
[1]School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab India 144411
[2]Department of Bioscience and Biotechnology, Fakir Mohan University, Balasore, Odisha, India756020

**Abstract**
*Cattle play important role in economy of India as it is the largest milk producer in world. Different dietary products can be derived from Cattles which are rich in different nutrients. Not only for food but Cattles are also used for ploughing. Keeping all these insight the numbers (quantity) as well as quality of cattle is also needed to be improved. More numbers of Cattles can be found among live stocks where the fertility of live stocks is good. Different factors affect the fertility of them. Their nutrition, environment, disease resistance power, different proteins and enzymes and hormones. Protein, enzymes and hormones are plays the key role in efficiency of pregnancy in cattle. The estrous cycle of cattle also plays a very important role in highly fertile cattle. Cows having a perfect estrous cycle can give birth more qualitative off springs. Difference in different proteins can also be found in pregnant cows and non pregnant cows. During estrous cycle and also during gestation period fluctuations of different proteins takes place at high levels. The proteins are expressed by different genes which get activated during the pregnancy period. During different stages of gestation period also difference in protein levels are found by experiments. But now a day due to several dysfunctioning early pregnancy diagnoses is needed to avoid the unwanted miscarriage or loss of pregnancy. Due to unusual repeated crossings the loss of pregnancy and abnormality in offspring are found. After fertilization and implantation of embryo chances the loss of pregnancy becomes less and productivity will be increased. The early pregnancy detection can be done by examining different proteins dubbed as pregnancy specific proteins like PAGs, PSPB, uteroglobins, interferon-induced GTP binding proteins etc. Comparison must be done between different proteins, from histotroph and endometrium at estrous stage, pregnant cow and non pregnant cow. After this precaution should be taken to avoid the loss of pregnancy and fertility can be obtained which will come under live stocks management.*
***Index Terms****— IFNT, PAGs, PDBU, PSPB, Progesterone, Uteroglobin, Interferon Induced GTP Binding Proteins.*

## Introduction

The Duration of pregnancy varies in different cows but usually falls in between 276-283 days. Due to different reasons the gestation period varies like gender of the offspring, age of the mother and various physiological factors. Cows need to be pregnant and give birth to a healthy off spring is one of the important factors for them who have livestock farming. Most pregnancy loss takes place in first 21 days the chances reduced by 42 days only to come extent. So the first 3 months are crucial as the foetus becomes mouse sized. During 5-6 month pregnancy the foetus becomes cat sized. The mother needs proper nutrition for

proper fetal growth, for a healthy calf and maximum milk production. Food requirement increases as the gestation period increases. During all these periods cow can give milk but before a month of delivery the cow should not be milked. During 7-9 months the calf has grown 70% of its total growth. The unborn offspring is largely protein, the need for protein is increases tremendously. After implantation of embryo the conceptual is designed to secrete interfera tou (IFNT) that is the signal for the D16 pregnancy. This secretion maintains progesterone secretion and also for the endomertin cells shows antagonistic response to phorbol 12,13-dibutyrate (PDBU) which activates protein kinase C. Implantation in uterus takes about 30-40 days. For the secretion of different proteins and hormones in pregnant cattle various parts of the body plays key role, those are,

**Trophoblast:** The cellular mass covered to blastocyst which provides nutrition to the embryo and develops in to a large part of the placenta.

**Endometrium:** The mucous membrane that lines the inside of womb. This undergoes several changes throughout the estrous cycle and pregnancy. It becomes rich and thick with blood vessels to prepare for pregnancy.

**Corpus luteum-** It is a temporary endocrine structure involved in ovulation and early pregnancy.

The early pregnancy can be diagnosed in dairy catle by measuring the different hormone concentration in milk

**Progesterone:** This is a steroid hormone but plays a very crucial role in maintenance of pregnancy. Progesterone is secreted from corpus luteum(CL) after ovulation in pregnant cows. Otherwise cl degrades by 17 days. If pregnancy takes place then the amount of progesterone increase gradually in 4 to 6 days and reach up to maximum between 10 to 17 days. If pregnancy does not occur after estrous cycle then the decrease of progesterone level is seen between 18 to 19 days and the cow can have estrous cycle after 21 to 23 days. This hormone is most important for establishment and maintenance of fetus.

Progesterone is found in both milk and blood and remains high just prior to calving and higher in milk than blood. So by detecting the milk the pregnancy can be detected.

**Table 1.** Different conc. Of Progesterone in different samples

| | | | conc.(ng/mL) | | |
|---|---|---|---|---|---|
| Bovine Species | Sample Type | day after Insemination | Pregnant(ng/ml) | Nonpregnant(ng/ml) | Reference |
| Cow. | Milk. | (i),0 or 1.<br>(ii),9 or 10.<br>(iii),21 or 22.<br>(iv),27 or 28. | 1.5<br>11.1,<br>12.0,<br>12.5, | 1.2,<br>10.3,<br>3.0,<br>6.8, | Zaied et al.,1979 |
| Cow | Milk. | 18 | >8, | _ | Simersky et al., 2007 |
| Buffalo | Milk, Plasma, | 21-35 each | 16.1 and 3.61, | 0.41- 2.67 | Kamboj and Prakas 1993 |
| Buffalo. | Plasma. | 0,13 | 0.1 and 3.6, | 0.6 | Batra et al.1979 |
| Buffalo. | Plasma, | 21 or 22 | 1.0 | <0.7 | Perera et al.1980 |
| Cow. | Milk. | 20 | >11 | <8 | Penington et al.1985 |
| Buffalo. | Milk. | 18-22 | 24.83 | 2.89 | Singh.et al. 1980 |
| Cow. | Faces. | 18-24 | >50 as compared to nonpregnant | _ | Isobe et al.2005 |

| Sl.No | Hormones | Level | Secretion |
|---|---|---|---|
| 1 | Progesterone. | Increase. | Corpus leuteum, maternal adrenal and placenta. |
| 2 | Oestrone sulphate. | Increase from mid-pregnancy. | Produced from progesterone for parturition. |
| 3 | PAGs. | Increase during the last 10 days prior to parturition. | Trophoblastic binucleate cells. |
| 4 | PSPB. | Secreted during early pregnancy d-15. | Placenta , trophoblast. |
| 5 | Uteroglobin | Increase. | Endomatrium |

PSBE levels in different sample types of bovine species is shown in Table 4.

**PAGs:** These are secreted from the mono-and bi-nucleated trophoblastic cells in cattle placenta. The amount of PAGs increase gradually in pregnant cow serum by 25 day of pregnancy. Butler et al, detected two pregnancy specific proteins in the pregnant cows sera these are 65-70 kDa. And 47-53 kDa. of different PI. Secretion of PAGs from the embryonic trophoblastic cells layer of the endometrium between 20-28 days helps in the successful implantation and continuation of pregnancy. The PAG with 47-53 kDa is also known as protein B or pregnancy specific protein B (PSPB) in cattles.PAG-4 and b-PAG-1 mRNA of Cattles are transcribed highly till 250 days and become in active at the end of gestation.

**Table- 3**. showing the PAGs type and time of detection

| Sl.No. | PAGs. | Time of detections. |
|---|---|---|
| 1 | b-PAG- 2 and- II mRNA. | All through the pregnancy. |
| 2 | b-PAG-4,-5,and -9 mRNA. | Early pregnancy. |
| 3 | b-PAG-1 mRNA. | After 45 days. |
| 4 | PAG-4 and b-PAG-1 mRNA. | Highly transcribed throughout pregnancy till 250 days. |

Very recent experiment shown that the defect of placenta mostly seen in somatic nuclear transfers is complemented by very high plasma PAGs level. Three most studied PAGs in cattle are PSPB, PAG 65 kDa or b-PAG-1 , and PSP 60 are isomers.

**PSPB:** PSPB is a type PAG in bovine having molecular weight 78,000.later purification and functional analysis of those proteins from bovine foetal cotyledons shows that protein B is 65 kDa PAG. Biochemical analysis shows that these proteins are enzymatically inactive and members of the aspartic proteinase super family homologous to pepsin, chymosin, cathepsin D etc. PSPB is found both in milk and serum of pregnant cows for a long period of gestation starting from the fourth week of gestation to several weeks and can be seen after few weeks of parturition also. These proteins have high circulating levels on days 80-100.post partum restrict their use as a pregnancy diagnosis test.

**Characteristics of pregnancy specific protein B during pregnancy:** Double antibody radioimmunoassay was developed by Sesser and co-workers for the detection of serum PSPB for pregnancy detection in cattle and found that the level of serum PSPB increases gradually.

**Table 4** showing the various PSPB conc

| Time. | Concentrations. |
|---|---|
| After 30 days. | 1 ng/ml. |
| After 3 months. | 9 ng/ml. |
| After 6 months. | 35 ng/ml. |
| After 9 months. | 150 ng/ml. |

Further another scientist Green et al. developed a sandwich ELISA method using monoclonal antibodies against PAGs in all pregnant animals and found very high during the week of parturition and very low levels within 4 weeks postpartum.

**Uteroglobin**

This is a secretory protein of super family secretoglobin. This is a steroid inducible immune-modulatory so known as blastokimin. This is a multi functional protein showing anti-inflammatory properties. Its expression is regulated by both steroid and non-steroid hormones express in uterus. Another reason for name blastokimim is its activity in the growth of pre implamentation in embryo. It has been progesterone binding capacity.

**Conclusion**

Though Cattles are very important part of economy so their management is very important which not only include their health and nutrition but also their reproductive quality and a healthy calf. Only by this good reproductively dairy productivity can be increased. To maintain this loss of pregnancy in early days of about 30% should reduce to minimum. For this the detection of several pregnancy specific proteins and their importance is most necessary to be studied. Only scientific study or researches cannot help this but also awareness among the farmers are most popular and proper methodology should be used. Different test can be done by using those proteins taken from both pregnant cows and estrous cows and a large variation can be seen. Amount of different proteins may increase or decrease as pregnancy times goes on. Different test can be done as kit development by ELISA, RIA, Immunoprecipitetion. By these test different pregnancy diseases and deficiencies can be diagnosed.

**Reference**


1. A. A. Zaied, C. J. Bierschwal, R. G. Elmore, R. S. Youngquist, A. J. Sharp, and H. A. Garverick, "Concentrations of progesterone in milk as a monitor of early pregnancy diagnosis in dairy cows," Theriogenology, vol. 12, no. 1, pp. 3–11, 1979.
2. R. Simersky, J. Swaczynova, D. A. Morris, M. Franek, and M. Strnad, "Development of an ELISA-based kit for the on-farm determination of progesterone in milk," Veterinarni Medicina, vol. 52, no. 1, pp. 19–28, 2007.
3. M. Kamboj and B. S. Prakash, "Relationship of progesterone in plasma and whole milk of buffaloes during cyclicity and early pregnancy," Tropical Animal Health and Production, vol. 25, no. 3, pp. 185–192, 1993.
4. S. K. Batra, R. C. Arora, N. K. Bachlaus, and R. S. Pandey, "Blood and milk progesterone in pregnant and nonpregnant buffalo," Journal of Dairy Science, vol. 62, no. 9, pp. 1390–1393, 1979.
5. B. M. Perera, N. Pathiraja, S. A. Abeywardena, M. X. Motha, and H. Abeygunawardena, "Early pregnancy diagnosis in buffaloes from plasma progesterone concentration," Veterinary Record, vol. 106, no. 5, pp. 104–106, 1980.
6. A. Singh and R. Puthiyandy, "Estimation of progesterone in buffalo milk and its application to pregnancy diagnosis" Journal of Reproduction and Fertility, vol. 59, no. 1, pp. 89–93, 1980.
7. N. Isobe, M. Akita, T. Nakao, H. Yamashiro, and H. Kubota, "Pregnancy diagnosis based on the fecal progesterone concentration in beef and dairy heifers and beef cows," Animal Reproduction Science, vol. 90, no. 3-4, pp. 211–218, 2005.
8. "Committee on Reproductive Nomenclature Recommendations for standardizing bovine reproductive terms," The Cornell Veterinarian, vol. 62, no. 2, pp. 216–237, 1972.
9. N. Ayalon, "A review of embryonic mortality in cattle," Journal of Reproduction and Fertility, vol. 54, no. 2, pp. 483–493, 1978.
10. D. Morris and M. Diskin, "Effect of progesterone on embryo survival, " Animal, vol. 2, no. 8, pp. 1112–1119, 2008.
11. E. S. E. Hafez, Reproduction in Farm Animalsedition, Lea and Febiger, Philadelphia, Pa, USA, 6th edition, 1993.
12. T. A. Cowie, Pregnancy Diagnosis Tests: A Review, Commonwealth Agricultural Bureaux Joint Publication no. 13, Commonwealth Agricultural Bureaux, Oxford, UK, 1948.
13. W. Wisnicky and L. E. Cassida, "A manual method for diagnosis of pregnancy in cattle," Journal of the American

Veterinary Medical Association, vol. 113, p. 451, 1948.
14. R. Zemjanis, Diagnostic and Therapeutic Techniques in Animal Reproduction, Williams and Wilkins, Baltimore, Md, USA, 2nd edition, 1970.
15. G. H. Arthur, D. E. Noakes, H. Pearson, and T. J. Parkinson, "Reproduction in the buffalo," in Veterinary Reproduction and Obstetrics, WB Saunders, London, UK, 1966.
16. O. J. Franco, M. Drost, M.-J. Thatcher, V. M. Shille, and W. W. Thatcher, "Fetal survival in the cow after pregnancy diagnosis by palpation per rectum," Theriogenology, vol. 27, no. 4, pp. 631–644, 1987.
17. R. K. Sharma, J. K. Singh, S. K. Phulia, S. Khanna, and I. Singh, "Fetal sex determination with ultrasonography in buffaloes," Indian Veterinary Journal, vol. 88, no. 10, pp. 105–107, 2011.
18. S. Curran, R. A. Pierson, and O. J. Ginther, "Ultrasonographic appearance of the bovine conceptus from days 20 through 60," Journal of the American Veterinary Medical Association, vol. 189, no. 10, pp. 1295–1302, 1986.
19. P. M. Fricke, "Scanning the future—ultrasonography as a reproductive management tool for dairy cattle," Journal of Dairy Science, vol. 85, no. 8, pp. 1918–1926, 2002.
20. L. G. Paisley, W. D. Mickelsen, and O. L. Frost, "A survey of the incidence of prenatal mortality in cattle following pregnancy diagnosis by rectal palpation," Theriogenology, vol. 9, no. 6, pp. 481–491, 1978.
21. D. Vaillancourt, C. J. Bierschwal, D. Ogwu et al., "Correlation between pregnancy diagnosis by membrane slip and embryonic mortality," Journal of the American Veterinary Medical Association, vol. 175, no. 5, pp. 466–468, 1979.
22. A. Ali and S. Fahmy, "Ultrasonographic fetometry and determination of fetal sex in buffaloes (Bubalus bubalis)," Animal Reproduction Science, vol. 106, no. 1-2, pp. 90–99, 2008.
23. R. Han, H. R. Kim, K. Naruse et al., "Identification of bovine pregnancy-specific milk proteins using proteomics," Biology of Reproduction, vol. 78, p. 143, 2008.
24. D. I. Jin, H. R. Lee, H. R. Kim, H. J. Lee, J. T. Yoon, and C. S. Park, "Proteomics analysis of pregnancy-specific serum proteins in bovine," Reproduction, Fertility and Development, vol. 18, no. 1-2, p. 183, 2005.
25. A. Karen, S. Darwish, A. Ramoun et al., "Accuracy of ultrasonography and pregnancy-associated glycoprotein test for pregnancy diagnosis in buffaloes," Theriogenology, vol. 68, no. 8, pp. 1150–1155, 2007.
26. A. Ayad, N. M. Sousa, J. Sulon, J. L. Hornick, M. Iguer-Ouada, and J. F. Beckers, "Correlation of five radioimmunoassay systems for measurement of bovine plasma pregnancy-associated glycoprotein concentrations at early pregnancy period," Research in Veterinary Science, vol. 86, no. 3, pp. 377–382, 2009.
27. E. Silva, R. A. Sterry, D. Kolb et al., "Accuracy of a pregnancy-associated glycoprotein ELISA to determine pregnancy status of lactating dairy cows twenty-seven days after timed artificial insemination," Journal of Dairy Science, vol. 90, no. 10, pp. 4612–4622, 2007.
28. J. A. Green, T. E. Parks, M. P. Avalle et al., "The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers," Theriogenology, vol. 63, no. 5, pp. 1481–1503, 2005.
29. R. G. Sasser, C. A. Ruder, K. A. Ivani, J. E. Butler, and W. C. Hamilton, "Detection

of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation," Biology of Reproduction, vol. 35, no. 4, pp. 936–942, 1986.

30. A. P. Zoli, L. A. Guilbault, P. Delahaut, W. B. Ortiz, and J.-F. Beckers, "Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnancy diagnosis," Biology of Reproduction, vol. 46, no. 1, pp. 83–92, 1992.
31. G. H. Kiracofe, J. M. Wright, R. R. Schalles, C. A. Ruder, S. Parish, and R. G. Sasser, "Pregnancy-specific protein B in serum of postpartum beef cows," Journal of Animal Science, vol. 71, no. 8, pp. 2199–2205, 1993.
32. P. Humblot, S. Camous, J. Martal et al., "Diagnosis of pregnancy by radioimmunoassay of a pregnancy-specific protein in the plasma of dairy cows," Theriogenology, vol. 30, no. 2, pp. 257–267, 1988.
33. B. P. V. L. Telugu, A. M. Walker, and J. A. Green, "Characterization of the bovine pregnancy-associated glycoprotein gene family—analysis of gene sequences, regulatory regions within the promoter and expression of selected genes," BMC Genomics, vol. 10, article 185, pp. 1–17, 2009.
34. K. Klisch, A. Boos, M. Friedrich et al., "The glycosylation of pregnancy-associated glycoproteins and prolactin-related protein-I in bovine binucleate trophoblast giant cells changes before parturition," Reproduction, vol. 132, no. 5, pp. 791–798, 2006.
35. S. Xie, J. Green, J. B. Bixby et al., "The diversity and evolutionary relationships of the pregnancy-associated glycoproteins, an aspartic proteinase subfamily consisting of many trophoblast-expressed genes," Proceedings of the National Academy of Sciences of the United States of America, vol. 94, no. 24, pp. 12809–12816, 1997.
36. J. A. Green, S. Xie, X. Quan et al., "Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy," Biology of Reproduction, vol. 62, no. 6, pp. 1624–1631, 2000.
37. J. Martal, N. Chêne, S. Camous et al., "Recent developments and potentialities for reducing embryo mortality in ruminants: the role of IFN-τ and other cytokines in early pregnancy," Reproduction, Fertility and Development, vol. 9, no. 3, pp. 355–380, 1997.
38. F. Constanta, S. Camousa, P. Chavatte-Palmera et al., "Altered secretion of pregnancy-associated glycoproteins during gestation in bovine somatic clones," Theriogenology, vol. 76, pp. 1006–1021, 2011.
39. M. M. Mialon, S. Camous, G. Renand, J. Martal, and F. Ménissier, "Peripheral concentrations of a 60-kDa pregnancy serum protein during gestation and after calving and in relationship to embryonic mortality in cattle," Reproduction, Nutrition, Development, vol. 33, no. 3, pp. 269–282, 1993.
40. S. Xie, B. G. Low, R. J. Nagel, J.-F. Beckers, and R. M. Roberts, "A novel glycoprotein of the aspartic proteinase gene family expressed in bovine placental trophectoderm," Biology of Reproduction, vol. 51, no. 6, pp. 1145–1153, 1994.
41. J. E. Butler, W. C. Hamilton, R. G. Sasser, C. A. Ruder, G. M. Hass, and R. J. Williams, "Detection and partial characterization of two bovine pregnancy-specific proteins," Biology of Reproduction, vol. 26, no. 5, pp. 925–933, 1982.
42. F. W. Bazer, W. W. Thatcher, P. J. Hansen, M. A. Mirando, T. L. Ott, and C. Plante, "Physiological mechanisms of pregnancy recognition in ruminants," Jour-

nal of Reproduction and Fertility. Supplement, vol. 43, pp. 39–47, 1991.

43. N. Forde, F. Carter, T. E. Spencer et al., "Conceptus-induced changes in the endometrial transcriptome: how soon does the cow know she is pregnant?" Biology of Reproduction, vol. 85, no. 1, pp. 144–156, 2011.
44. J. C. Green, C. S. Okamura, S. E. Poock, and M. C. Lucy, "Measurement of interferon-tau (IFN-τ) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18-20d after insemination in dairy cattle," Animal Reproduction Science, vol. 121, no. 1-2, pp. 24–33, 2010.
45. C. S. Whisnant, L. A. Pagels, and M. G. Daves, "Case study: effectiveness of a commercial early conception factor test for use in cattle," Professional Animal Scientist, vol. 17, pp. 51–53, 2001.
46. M. C. Cordoba, R. Sartori, and P. M. Fricke, "Assessment of a commercially available early conception factor (ECF) test for determining pregnancy status of dairy cattle," Journal of Dairy Science, vol. 84, no. 8, pp. 1884–1889, 2001.
47. H. Morton, A. C. Cavanagh, S. Athanasas-Platsis, K. A. Quinn, and B. E. Rolfe, "Early pregnancy factor has immunosuppressive and growth factor properties," Reproduction, Fertility, and Development, vol. 4, no. 4, pp. 411–422, 1992.
48. F. Lobago, M. Bekana, H. Gustafsson et al., "Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate and progesterone during gestation and some factors influencing the profiles in ethiopian borana and crossbred cattle," Reproduction in Domestic Animals, vol. 44, no. 4, pp. 685–692, 2009.
49. M. Hirako, T. Takahashi, and I. Domeki, "Peripheral changes in estrone sulfate concentration during the first trimester of gestation in cattle: comparison with unconjugated estrogens and relationship to fetal number," Theriogenology, vol. 57, no. 7, pp. 1939–1947, 2002.
50. H. A. Robertson and G. J. King, "Conjugated and unconjugated oestrogens in fetal and maternal fluids of the cow throughout pregnancy," Journal of Reproduction and Fertility, vol. 55, no. 2, pp. 463–470, 1979.
51. T. H. Wimpy, C. F. Chang, V. L. Estergreen, and J. K. Hillers, "Milk progesterone enzyme immunoassay: modifications and a field trial for pregnancy detection in dairy cows," Journal of Dairy Science, vol. 69, no. 4, pp. 1115–1121, 1986.