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Molecular Cloning and Characterization of Pregnancy Specific Protein Uteroglobin can Lead to Pregnancy Detection in Cattle- A Review

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Abstract
Various kinds of approaches are treated of early pregnancy diagnosis for further aggravates the situation. Several methods of pregnancy diagnosis are being practiced in bovine species, yet none qualifies as the ideal pregnancy diagnosis method due to the inherent limitations of sensitivity, accuracy, specificity, speed, and ease of performing the test. One concern with previous assessments of this test is that animals with viable embryos early during pregnancy that subsequently undergo embryonic loss before pregnancy diagnosis increase the rate of false-positive results and bias the assessment. Assessment of a Commercially Available Early Conception Factor (ECF) Test for Determining Pregnancy Status of Dairy Cattle, while assessing the usefulness of the modern technologies in detecting novel pregnancy markers and designing future strategies for research in this area. Uteroglobin is a small progesterone-binding protein expressed in various organs of the cattle. Uteroglobin (UG) is an anti-inflammatory protein secreted by the airway epithelia of all mammals. A critical role of UG in preventing the development of PF and the of principle that recombinant UG may have therapeutic potential. The early pregnancy detection can be done by examining different proteins dubbed as pregnancy specific proteins like PAGs, PSPB, Uteroglobin, interferon-induced GTP binding proteins etc.
Keywords: *Urine, Biomarker, DIGE, LFQ, Mass spectrometry, Gene Ontology, Network construction, Bovine serum, Plasma, Milk.*

Introduction

Cattles are acted the main roles in increasing the dairy fields for economical market. An early and precise pregnancy diagnosis is an important criterion for better reproductive management in livestock like cows and buffaloes. Ideally a 60-day post parturient barren interval in dairy animals is recommended for breeding. Dairy farmers need to recognize no pregnancy at the earliest opportunity so as to rebreed the dam at the very next challenge[1]. The early embryonic period in cattle has been described to be long lasting for approximately 42 days post insemination, encompassing a series of events starting with fertilization and culminating in implantation. Cattle are the most important dairy animal of the Indian subcontinent, they experience problems related to reproduction especially high calving

interval, late puberty, and high incidence of anestrus[2,3]. Uteroglobin (UG) is a conserved protein which is induced by progesterone and secreted by the epithelia of various mammalian reproductive and respiratory organs. Recombinant bovine Uteroglobin (recb UG), consisting of 80 amino acids with a C-terminal His6 tag, was over expressed in Escherichia coli and purified. The protein was crystallized in two geometric forms, rhomboid and cognate (wedge-shaped), by the hanging-drop vapor-diffusion method at 295 K [4]. Rhomboid crystals are diffracted to a maximum resolution of 1.6 A ° using synchrotron radiation.

These crystals belong to space group P21212, with unit cell parameters a = 81.42, b = 82.82, c = 45.26 A °, and contain four monomers per asymmetric unit[5]. The cognate crystals diffracted to 2.35 A ° resolution using a rotating-anode generator. These crystals belong to space group C2221, with unit cell parameters a = 43.39, b = 93.94, c = 77.30 A °, and contain two molecules per asymmetric unit.

Purified protein were crystallized using the hanging-drop vapor diffusion method at 295 K. Thin crystalline plates of recb UG were obtained using a mixture of 2-propanol, sodium acetate and CaCl2 as the reservoir solution. The hanging-drops consisted of 1.5 ml protein solution and 1.5 ml reservoir solution[7]. By adding PEG 4000 or 8000 to concentrations between 3 and 5%, two different crystal forms, rhomboid and cognate (wedge-shaped), appeared reproducibly after about three weeks[8]. The reservoir solution (1 ml) for the rhomboid crystals was 100 mM sodium acetate pH 5.1, 200 mM CaCl2, 20% 2-propanol and 5% PEG 8000. For the connate crystals, the reservoir solution (1 ml) was 100 mM sodium acetate pH 4.9, 200 mM CaCl2, 17% 2-propanol and 3% PEG 4000 [9, 10]. Both crystal forms were mounted in small loops using glycerol as a cry protectant (final concentration 30% (v/v)).After mounting the crystals, they were immediately flash-cooled to 100 K in a nitrogen stream. X-ray data were collected in house and at beam line X13 of the EMBL/DESY facility in Hamburg .We collected a complete data set with a maximum resolution of 1.6 A ° using synchrotron radiation [11]. The data were processed and scaled with XDS and the self-rotation functions were calculated with CCP4 (Collaborative Computat-ional Project, Number 4, 1994). The rhomboid crystal form was shown to belong to space group P21212, with unit-cell parameters a = 81.42, b = 82.82, c = 45.26 A°. Assuming the presence of two molecules in the asymmetric unit, a Matthews coefficient of 4.3 A ° 3 Da_1 was calculated[12].

Since recb UG forms a dimer in solution and four molecules resulted in a Matthews's coefficient of 2.2 A ° 3 Da_1, we concluded that there were four molecules per asymmetric unit [13]. Analysis of the crystal form indicated an orthorhombic centered space group C2221, with unit-cell parameters a = 43.39, b= 93.94, c= 77.30 A °.Assuming the presence of two molecules in the asymmetric unit, a Matthews coefficient of 2.2 A ° 3 Da_1 was calculated. Again, a twofold axis was shown in the self-rotation function. Additionally, a strong denaturing agent such as mercaptoethanol (C2H6OS) was required to reduce its dimeric structure completely[14]. There is no visible difference between these samples and the others which were not subjected to boiling. Structural determinations are in progress [15,16]. The structure of rec –bUG will be compared with existing UG structures in order to provide more information about the physiological functions of this conserved protein [17].

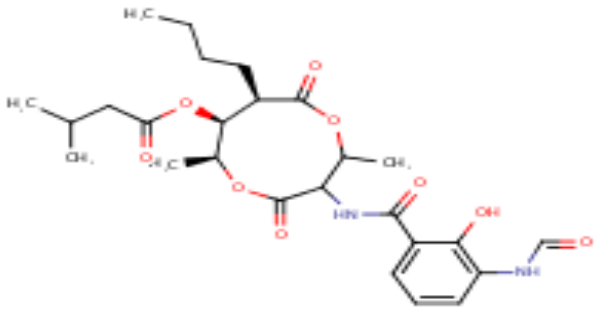
Recognized potential protein biomarkers treated for early detection of pregnancy in cow urine using 2D DIGE and label free quantitation. This method of early pregnancy diagnosis is prerequisite for efficient reproductive management in dairy industry[18]. The early detection of pregnancy also helps in to reduce the calving interval and rebreeding time which is beneficial for industries as well as farmers. The aim of this work is to identify potential biomarker for pregnancy detection at earlier stages (16–25 days) [19].

DIGE experiment shows that a total of eleven differentially expressed proteins out of which nine were up regulated having fold change ≥1.5 in all time points. The LFQ analysis revealed 195

differentially expressed proteins (DEPs) out of 28 proteins were up-regulated and 40 down regulated having significant fold change ≥1.5 and ≤0.6 respectively[21]. Bioinformatics analysis of DEPs showed that a majority of proteins were involved in regulation of leukocyte immunity, end peptidase inhibitor activity, regulation of peptidase activity and polysaccharide binding. This research report on differentially expressed protein during various time points of pregnancy in cow to our best knowledge.

In this research work declared that some proteins such MBP, SERPIN, IGF which were different-ially expressed and actively involved in various activities related to pregnancy such as embryo implantation, establishment and maintenance of pregnancy [22,23]. Due to their involvement in these events, these can be considered as biomarker for pregnancy but further validation of is required .Conceptualizing' the Endometrium: Identification of Concepts-Derived Proteins During Early Pregnancy in Cattle1.byNiamh Forde,2,3 Fuller W. Bazer, 4 Thomas E. Spencer,5 and Pat Lonergan3[24].

Image (Chemical Structure of Uteroglobin)



Methods for Early Pregnancy Detection

This new technology must be practically integrated into a systematic on-farm reproductive management strategy and empirically demonstrated to exceed the status quo of the industry in reproductive performance. Finally a future direction for research and technology in the area of early pregnancy diagnosis in dairy cows is presented, and the overall conclusions of the ideas presented herein are drawn, assessing the usefulness of the modern technologies in detecting novel pregnancy markers and designing future strategies for research in this area.

- Pregnancy Detection Methods
- Direct Method
- Per-Rectal Palpation

Cow first described trans rectal palpation of the uterus as a method for pregnancy diagnosis in cattle which makes it the oldest and most widely practiced method for early pregnancy diagnosis in large dairy animals even today. It confirm that the 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. In buffaloes too, palpation per rectum is a simple, economic, and the most widely practiced method for pregnancy diagnosis; however, this method is only accurate from day 45 of pregnancy. This study has suggested that examining pregnant cows early in gestation by trans rectal palpation increases the risk of iatrogenic embryonic mortality.

Ultrasonography

Ultrasound 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[25]. The first visible changes appearing by day 21 after breeding, when fetal heartbeat can be visualized, also helped confirm a viable pregnancy though it is not a routinely assessed parameter for pregnancy diagnosis. Trans rectal ultrasonography have the added advantages of providing additional information on ovarian structures, identification of twins, and determination of fetal viability, age, and sex. Trans rectal 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 and buffaloes. Ultrasounds have minimally invasive, accurate,

and efficient technique for early pregnancy diagnosis and may minimize the rare incidence of palpation-induced abortions [26].

These case studies on utility of trans rectal ultrasonography for pregnancy diagnosis conducted in cattle, but lately it has found utility in buffalo cows as well. In buffalo, Tran's rectal ultrasonography is most commonly used to determine pregnancy, fetal age, and sex as well as ovarian activity. In early 1990s, various workers started using trans rectal ultrasonography in buffaloes with visualization of the embryonic vesicle and embryo proper in pregnant buffalo cows between 19 and 22 days after AI. The study on 260 buffaloes between 30 and 45 days after AI, sensitivity of detection of pregnancy was observed to be 97.9%[27]. The accuracy in selecting pregnant buffaloes at day 21 after AI to be about 50%, which increases to almost 100% by day 30. In cattle Tran's rectal ultrasonography for pregnancy diagnosis between days 21 and 25 after breeding has sensitivity and specificity of 44.8% and 82.3%, respectively, which further increase to 97.7% and 87.7%, respectively, when conducted between 26 and 33 days after AI [28,29].

Similarly, Nation et al. Direct observation of a fetus with ultrasonography was found more accurate than assays for the presence of pregnancy-specific proteins in plasma but resulted in more false negative diagnoses[30]. Per rectal palpation (The digital rectal examination) and transracial ultrasonography are direct and accurate methods for pregnancy diagnosis. Both require a great deal of skill and experience [31]. 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.

Table 1: Important components play the role during the early embryonic period.

S. No.	Days of pregnancy	Events
1.	Days 0-1	Fertilization, single cell embryo
2.	Day 2	Early cleavages in oviduct,
3.	Days 3-4	Embryo enters the uterus
4.	Days 5-6	16-32 cells zonal area enclosed
5.	Days 7-8	Formation of blastocoel
6.	Days 9-10	Blastocysts expansion
7.	Days 11-15	Blastocysts elongation
8.	Days 14-19	Maternal recognition
9.	Days 19-20	Implantation begins
10.	Day 21	Carbuncles cotyledons appear
11.	Days 22-41	Implantation progresses
12.	Day 42	Implantation completed

Indirect Method

Progesterone

The major 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 first documented this in milk to diagnose cows in early pregnancy. The examine of progesterone is an indirect method for pregnancy diagnosis in many livestock species including cattle, buffaloes, sheep, and goats. 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 regulates the uterine endometrium in a state which supports embryonic development, implantation, and foetoplacental development. Progesterone concentrations vary with the stage of the estrous cycle which makes it one of the most commonly studied reproductive hormones in bovine ruminants for pregnancy detection and ovarian activity [32].

Study of the bovine estrous cycle show 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 and beyond [33]. Increase the level of progesterone in serum or milk between days 18 and 24 after insemination forms the basis of establishment of pregnancy in cattle. Interferon exerts its antiluteolytic effect by inhibiting the endometrial expression of oxytocin receptors, through which oxytocin stimulates pulsatile release. Although low progesterone concentrations at 18 to 24 days after breeding can accurately predict nonpregnancy, high progesterone concentrations during this period are not the specific indicators of pregnancy due to variations among

cows in duration of the estrous cycle as well as the incidence of early or late embryonic mortality[34].

In buffaloes and cows, dictate the quite evident that the progesterone levels in milk are four to five times higher than those in blood plasma just like cattle; buffaloes too can be accurately diagnosed as non-pregnant by determination of plasma progesterone concentrations 21 days after insemination. A major constraint in using progesterone assay for pregnancy diagnosis is its use only in cases where AI or breeding dates are known or recorded and not randomly in the herd. Nevertheless, progesterone analysis remains the most common clinical use of any of the reproductive hormones.

Table 2 Describes the work in different labs on the level of progesterone in pregnant and non-pregnant bovines.

Progesterone levels in different sample types in bovine species.

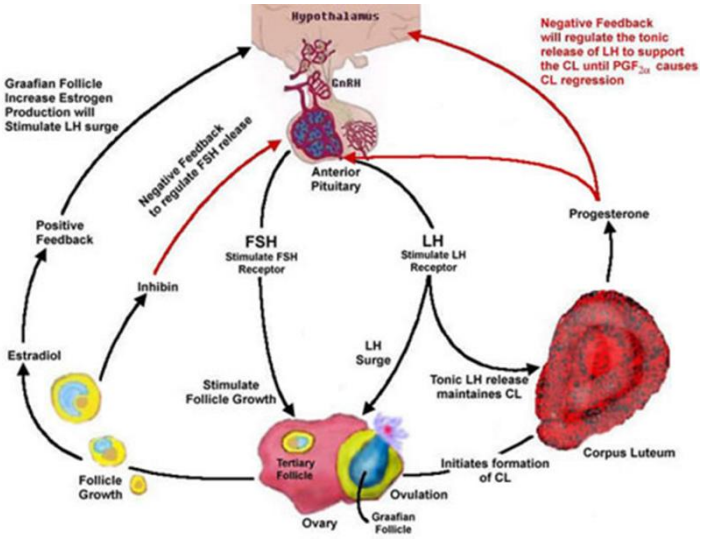
P4 Concentration (mg/ mL)				
Bovine	Sample Type	Day After Insemination	Pregnant (Mg/mL)	Non Pregnant (Mg/mL)
Buffalo	Milk	18-22	24.83	2.89
Buffalo	Plasma	21 or 22	1.0	<0.7
Cow	Milk	18-22	24.83	2.89
Cow	Milk	0 or 1 9 or 10 21 0r 22 27 or 28	1.5 11.1 12.0 12.5	1.2 10.3 3.0 6.8
Cow	Milk	18	78	---
Cow	Faces	18-24	750 Compared to Nonpregnant	---

Estrogen Sulphate

Estrogen sulphate is a conjugated steroid product of estrogen, present predominantly in the bovine placentomes and it is the major estrogen present in the fetal (allantois and amniotic) fluids and maternal peripheral plasma of cows with measurable quantities detectable by day 52 onwards till the end of gestation. Its concentrations increase from day 60 and plateau around day 150 after insemination. Early pregnancy detection is more possible only after day 100 of gestation and therefore this test can only detect late pregnancy. Concentration of estrogen sulphate in the maternal body fluids is a useful indicator for the placental functions especially those related to embryonic growth [35].

In zebu and crossbred cattle and Murray buffaloes in detection levels (<50 pg./mL) of estrogen sulphate 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[36]. Levels of estrogen 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. Estrogen sulphate concentrations have also been frequently correlated to fetal numbers, as these are higher when the number of developing fetuses is more than one. Yet, estrogen 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 [37].

Hormonal Changes During Detection of Early Pregnancy



(Fig: Showing different hormonal changes during pregnancy)

Concepts and Placenta Secreted Products

The very fact that pregnancy brings about numerous physiological changes in the female body through secretion/altered secretion of various biomolecules, which often are proteins or their metabolites, supports research endeavors aimed at identifying novel proteins as the candidate molecules for pregnancy detection. Human chorionic gonadotropin

(hCG), discovered for the first time by Ascham and Zondek in the urine of pregnant women in 1927, is perhaps the best example of a placental protein hormone used for pregnancy diagnosis[38]. With the advancement of biotechnological tools, hCG based pregnancy diagnosis has become the simplest, cheapest, and most commonly practiced test for humans to diagnose pregnancy as early as 8–10 days after conception.

Homologous to the human protein, only higher primates produce a chorionic gonadotropin (CG) for maintaining luteal activity during early pregnancy, while ruminants produce type I interferon as an antiluteolytic factor during this period.

Early Conception Factor (ECF)

Early pregnancy detecting factor (EPF also known as early conception factor ECF) a 10.84 kDa protein, is present in the sera of pregnant mammalian females, detectable within 6 to 24 hours of fertilization and disappearing within 24 to 48 after death or removal of the embryo , EPF is present in the serum up to two-thirds of the gestation. EPF due to the earliest serum benchmark for positive fertilization and hence successful conception. This novel pregnancy-specific protein has high immunosuppressive ability which is demonstrated by rosette inhibition test, a bioassay first demonstrated inpregnant mice [39, 40]. Laleh et al. demonstrated significant differences inrosette inhibition titers of pregnant and open cows with values being 8–10 and 3–5, respectively [41].

EPF is reported to be present in the pregnant sera of most mammalian species including humans, mice, sheep, cows, pigs, mares, and some wild animals. In buffalo pregnancy, Chandler demonstrated decreased E-rosette formation but failed to demonstrate the presence of a rosette inhibiting factor (RIF, which probably would have been EPF) in the serum. Antibodies raised against a cow serum glycoprotein were used to detect EPF leading to development of a lab method, which has been commercialized in the USA as Early Conception Factor (ECF) test claiming detection within 48 hours. Extensive study on the effectiveness of the commercial ECF test for diagnosing non pregnancy revealed a high degree of non-reliability of the test wherein only 44.4% and 55.6% of the confirmed non pregnant heifers were identified correctly by serum ECF analysis at days 1 to 3 and days 7 to 9 after AI, respectively[42]. Similar conclusions were drawn by and. EPF is through secreted in early pregnancy detection, It is not strictly pregnancy specific because of its secretion from non-placental sources such as tumors and transformed cell lines, which makes it an erroneous pregnancy detection method. EPF belongs to a family of heat shock proteins, though detected extracellular and having immunosuppressive and growth factor properties. These morphological properties are crucial to avoid rejection of an antigenically alien embryo and support its development. Therefore, with the advent of modern biosciences, there is hope that these changes could be identified and used as diagnostics for very early detection of pregnancy[43]. According to this research study of such an early test may still remain low due to high incidence of losses during the first 15 days of conception.

Interferon- (IFN-)

Moor and Rawson, the pioneers of sheep embryo transfer, transferred embryos on days 12, 13, and 14 to unmated ewes and suggested interactions between the embryo and uterus that influence the luteal function and result in establishment of pregnancy. Godkin et al. purified ovine trophoblastic protein-1 (oTP-1), an early secretory protein of the sheep blastocyst, from in vitro cultured day's 14–16 conspectuses [44]. They revealed that oTP1 acts on the maternal endometrium thereby eliciting maternal responses which contribute to the maintenance of pregnancy. Imakawa et al. reported the primary amino acid sequence of oTP-1 to demonstrate that the protein is most probably an interferon-alpha. Later research proved that the secretions from the conceptus are, in fact, responsible for the maternal recognition of pregnancy.

Interferon-, a novel type I interferon is first produced by the conceptus between days 12-13 after insemination in sheep and days 14–16 in cattle.

High ovine IFN- levels are attained on days 12-13 before luteolysis could actually be triggered[45]. In ruminants, IFN-, a 172 amino acid polypeptide, blocks transcription of estrogen receptor alpha and oxytocin receptors in endometrial cells, while down regulating the expression of enzymes cyclooxygenase-2 and prostaglandin F synthase, thus preventing PGF release necessary for luteolysis. IFN-, acting within the uterine cavity with extremely low levels in extra uterine tissues and peripheral circulation, prevents direct use of IFN- as an early pregnancy diagnosis molecule. Rapid advancement of molecular techniques in the last two decades has opened new avenues for exploring this unique molecule as a pregnancy marker for ruminants through studies on IFN stimulating genes (ISG), namely interferon-stimulated protein 15 kDa (Isg15), myxovirus resistance 2 (MX2), and 2′-5′ oligoadenylate synthetase (OAS1), in peripheral blood leukocytes during early pregnancy. Microarray analysis study shows that many genes, including IFN- stimulated, are upregulated during early pregnancy. Green et al. Have however shown that the differential expression of such genes is influenced by the parity of the animal, being more definite in heifers as compared to multiparous animals[46]. All these experiments have suggested IFN- stimulated genes to be potential pregnancy detection biomarkers; still there is no field level test available based on these markers.

Pregnancy Associated Glycoproteins (PAGs)

Relocation of the extra embryonic trophoblastic cell layers to the endometrium between days 20 to 28 and secretions from the conceptus lead to successful implantation and continuation of pregnancy in ruminant species. Pregnancy associated glycoproteins (PAGs) are produced products from the mono- and bi nucleated trophoblastic cells in bovine placentomes. Among these glycoproteins, Butler et al. Detected two pregnancy-specific proteins in the sera of pregnant cows, a 65–70 kDa and a 47–53 kDa protein at pI 4.6–4.8 and 4.0–4.4, respectively[47]. The former showed an immune reaction similar to that of -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. Further purification and characterization of several isoforms from bovine fetal cotyledons found that protein B is actually a 67 kDa PAG. The Biochemical and functional research established these proteins to be enzymatically inactive members of the aspartic proteinase super family having homology to pepsin, chymosin, cathepsins D, and enzyme renin. PAGs are a very complex group of proteins, a fact proven by the already documented 22 distinct cDNA libraries[48]. The three most studied bovine PAGs PSPB, PAG 67 kDa or bPAG-1, and PSP60 are isomers of the same protein having similar N-terminal sequences. Transcription of bPAG-2 and -11 mRNA is seen all through the pregnancy; -4, -5, and -9 mRNAs in early pregnancy and bPAG-1 mRNA are detectable only after day 45. The bovine PAG-4 and bPAG-1 mRNA are highly transcribed till day 250 of gestation but become indiscernible at the end. The six N-glycosylation sites are responsible for the variations in molecular weight and half-life of PAGs and is also the reason for expression of different PAGs during different stages of gestation.

It has been observed that placental defects, commonly seen during somatic nuclear transfers in cattle, are complemented by unusually high plasma levels of PAGs, probably due to diminished clearance of PAGs proteins following changes in the glycosylation patterns[49]. PSPB is detectable for serum of pregnant cows over a long period of gestation starting at about the fourth week of gestation to several weeks after parturition. High circulating levels of glycoproteins on days 80 to 100 postpartum restrict their use as a pregnancy diagnosis test.

Sasser and coworkers was developed double antibody radioimmunoassay for the serological detection of PSPB for pregnancy detection in cattle and found serum levels increasing progressively from 1 mg/mL after day 30 to 9 mg/mL, 35 mg/mL, and 150 mg/mL after three, six, and nine months of pregnancy, respectively[51]. This study claimed

PSPB detection to be more accurate than the traditional rectal palpation method for pregnancy detection. Green et al. developed a sandwich ELISA, using anti-PAG monoclonal antibodies, which were able to detect PAG in all pregnant animals with concentrations of 8.75 mg/mL on day 28, the highest at 588.9 mg/mL during the week of parturition, and very low levels within 4 weeks postpartum. Silva et al. Predicted 93.7, 95.4, and 96.2% accuracies for first, second, and third postpartum timed artificial inseminations, which were in agreement with other commonly practiced pregnancy detection methods. Various kinds of homologous (RIA-497) and heterologous radioimmunoassay systems (RIA-706, RIA-780, RIA-809, and RIA-Pool) developed for measure-ment of ruminant blood PAG concentrations are highly correlated and can be used for pregnancy detection of 30–80 days. Radioimmunoassay of pregnant sera of zebu cattle established PAG concentrations to be 6.0 mg/mL, 196.0 mg/mL, 1095.6 mg/mL, and 348.4 mg/mL at 8 weeks, at 35 weeks, at term, and at 2 weeks postpartum, respectively, a pattern similar to other breeds of cattle [52, 53, and 54]. Results of PAG-RIA based pregnancy diagnosis in buffaloes have also been encouraging with a high degree of accuracy of diagnosis as early as day 31 with 100% sensitivity and 90–100% specificity. PAGs are used in development of bench-top pregnancy detection methods, which are now commercially available as BioPRYN, DG29, and IDEXX. BioPRYN blood test is the most extensively used PAG based kit for pregnancy detection in ruminants. By May 2010, already there were 2 million cattle blood tests conducted for pregnancy detection [55].

Proposed Research Objectives

- Isolation and cloning of Uteroglobin gene in suitable cloning vector.
- Expression of Uteroglobin in suitable expression vector and characterization.
- This work will have value in developing a kit for early diagnosis of pregnancy in cattle.
- An early, reliable and noninvasive method of early pregnancy diagnosis is prerequisite for efficient reproductive management in dairy industry. The early detection of pregnancy also helps in to reduce the calving interval and rebreeding time which is beneficial for industries as well as farmers.

Current Research in Biomarkers for Pregnancy

The Trends of sequential changes in blood proteome profile from the day of estrus to successful conception and through progression of gestation can lead to discovery of molecules, which will perhaps be novel and specific to the physiological stage of the animal. However, to qualify as a marker for pregnancy, the candidate molecule should be able to accurately determine the pregnancy status as early as possible with minimum false positives or false negatives.

Additionally, the biological marker for pregnancy should have the following desired characteristics:

- Specifically upregulated or down regulated during pregnancy,
- Least affected by non-animal factors like feed, environment, and drug interactions,
- Having the ability to reflect age as well as viability of the concepts,
- Present in easily accessible body fluids like serum, milk, urine, and vaginal discharge,
- It expressed over a considerable period of time to give sample time for diagnosis,
- Revealing the result immediately.

Proteomics is broad study of protein functions, protein expression, protein-protein interactions, or posttranslational modifications in a particular cell, tissue, or organism and is intended for identification of all the proteins present. Proteomics often more challenges to simultaneously analyse thousands of proteins in a single experiment from a complex mixture of proteins in various body fluids [55].

This will help in identifying specific and sensitive biomarkers fulfilling the characteristics of uniqueness for a pregnancy diagnosis molecule. Our

purpose of the proteomics research includes documentation of biomarkers, altered protein expression patterns indicative of pathophysiological changes, and therapeutically important drug targets [56]. Easily body fluids like blood serum and milk have a wide range of abundant proteins and these few proteins make up about 97% of the total serum and thereby interfere in the proteomic analysis[57]. It takes the low abundance proteins which have the highest prospect of being the novel biomarkers of changes in internal milieu of body. To sort the problem of high abundance proteins, two approaches are suggested: removal of abundant proteins (usually by immune affinity) and concentration of the low abundance/scarce proteins with simultaneous removal of high abundance proteins, technically known as combinatorial peptide ligand libraries, CPLL[58,59]. Commercially available Proteo Miner kit from M/s BioRad is CPLL based. Both approaches, however, lead to loss of a significant portion of the low abundance proteins along the high abundance proteins, yet the later approach is preferred [60].

According to this is study, it keep short information on the bovine proteome in relation to pregnancy. Jin et al [61]. It hence the proteomics analysis using blood serum samples of pregnant and non-pregnant Holstein dairy cattle at 21 and 35 days after AI and reported composite profiles of key proteins conjugated in early pregnancy and suggested the potential use of identified proteins to detect early pregnancy in bovines [62, 63]. These included nine pregnancy specific spots in day 21 and day 35 serum samples. "Early pregnancy detection proteins are recognized like albumin, IgG2a heavy chain constant region, and immunoglobulin gamma heavy chain variable region" [64,65,67]. Further, differential proteomic analysis of milk samples from pregnant and non-pregnant cows revealed 16 protein spots, 14 pregnancy specific and 2 spots down regulated in the pregnant milk sample. Pregnancy specific detection proteins can be identified as serum albumin precursor,IgG1 heavy chain constant region, conglutinin precursor, epithelial keratin 10, and ketch like ECH-associated protein. Though some identified spots were abundant milk or serum proteins, their molecular weights and pI values were different from main milk or serum proteins [68,69]. This study suggest that these proteins could be pregnancy-specific subunits or fragments of albumin and IgG or carrying differentially expressed small proteins, which may ultimately have potential for pregnancy detection.

Expected Research Outcome

- Development of cloned vector in the E.coli host.
- Developed of expression vector in E.coli.
- Protein expression and use for further experiment.
- The purified protein will be used for further characterization.

Future Prospects

This work for molecular cloning and characterization of pregnancy specific protein i.e. Uteroglobin(UG) will lead to develop a pregnancy specific biomarker for the early pregnancy detection in cattle.

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 is 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, and Immunoprecipitetion. By these test different pregnancy diseases and deficiencies can be diagnosed.

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