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A Comparative Study on Certain Blood Metabolites and Hormonal Indices for Detection of Pregnancy of Crossbred Cows of High Altitude: Mizoram

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ABSTRACT

Background: Early detection of pregnancy can perk up the reproductive competence of dairy animals and decreasing the interval between two successive AI. Several methods of pregnancy diagnosis both direct and indirect are being practiced; but to date, none of these technologies qualifies as the ideal pregnancy detection method due to its limitation. The present study was designed to learn about the changes of hormonal and blood metabolites during early bovine pregnancy.

Methods: A total of 30 numbers of crossbred cows at 2nd to 5th lactation was selected for the study. Clinico-gynaecological examination was carried out in all the cows and those have devoid of ill health, having a regular estrous cycle and estrus period were inseminated at mid to late estrus. Ten milliliter of blood samples were collected aseptically from all the cows (6 samples from each cow and a total of 180 samples) on day 1, 10, 20, 30, 60 and 90 of post-A.I. From the obtained serum, levels of glucose, cholesterol, BUN, total protein and hormonal profile estrone sulphate, progesterone were analysed. All the cows were observed for the absence of external signs of estrus after AI and pregnancy was confirmed by using ultrasonography and per rectal examination on day 30 to 40 of post insemination.

Result: It was observed that level of glucose, BUN, cholesterol and progesterone was significantly increased during early pregnancy (P<0.01). While, the serum level of estrone sulphate significantly decreased while advancing the pregnancy. There was positive correlation between the level of progesterone (P<0.01) and negative correlation of estrone sulphate (P<0.05) with serum glucose, BUN, cholesterol at different days of post A.I. The blood biochemical constituents *i.e*. glucose, BUN, cholesterol and hormone estrone sulphate, progesterone possibly will used as an ideal marker of early pregnancy in cattle. Thus blood biochemical constituents like glucose, BUN, cholesterol and hormone estrone sulphate, progesterone possibly can be used as a tool for diagnosis of non pregnant dairy cattles with very high accuracy at around 30 days of post insemination.

Key words: Blood biochemical, Cattle, Hormonal profiles, Pregnancy.

INTRODUCTION

Mizoram is one of the mountainous state of India with a inhabitants of about 900 000 and it under the tropics of latitude 21.19°C to 24.35°C in north and longitude 92.15°C to 93.29°C in east. As a hilly state with almost 80% forest coverage, Mizoram having small number of bovine populations which mostly reared for milk as well as meat production. As per the 20th Livestock Census (2019-20), Govt. of India, Mizoram recorded 43,928 numbers of total cattle population out of which exotic mature female cattle is 11,000 and indigenous female cattle is 10,000 in numbers, which is one of the major earning sources of marginal farmers. A total of 25782 tonnes of milk produced in Mizoram per annum and the per capita availability of milk was 54.86 grams per day which was very low compared to the Indian Council of Medical Research (ICMR) recommendation of 240 gms of milk per day per individual (Economic Survey Report, 2019-20, Govt. of Mizoram, India). The profit of dairy farmers mainly depends on the sustainability of milk production which in turn depends on the sustained reproductive capacity of dairy animals.

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Identifying non-pregnant animals at an early age and preparing them for subsequent artificial insemination (AI) at the proper time is important, which can improve the reproductive efficiency of dairy animals and decreasing the

interval between two successive AI (Fricke, 2002). To maintain the optimum growth of the embryo or fetus and to prevent pregnancy loss; progesterone (P_4) and glucose is required (Lucy *et al*., 2012; Lucy *et al*., 2014; Talukdar *et al*., 2015). Proteins are the building blocks of living organisms, which can help in the growth of the fetus and this is why pregnancy considerably influences the protein content of serum in cattle (Zvorc *et al*., 2000). The common blood metabolites are positive indicators of body energy balance, namely glucose, cholesterol and urea (Reist *et al*., 2002; Talukdar *et al*., 2016). A study by Bathla *et al*. (2015) revealed that the presence of several proteins in the urine of cow could be helpful for the early detection of pregnancy. The relative level of progesterone, blood urea nitrogen (BUN), cholesterol, protein and glucose in the blood might be indicator of pregnancy (Zvorc *et al*., 2000; Alameen and Abdelatif, 2012; Talukdar *et al*., 2015). A piece of better knowledge regarding the expression and role of each blood metabolite during early or late pregnancy will help to study the relationship between mother and fetus during gestation for overall economic benefit (Nandre *et al*., 2012). While several studies have been reported that progesterone supplementation is beneficial, other studies have shown no benefit in the pregnancy rate (Talukdar *et al*., 2013). Studies on the levels of progesterone, estrone sulphate, bPSPB, PAGb, interferon tau and early pregnancy factor are some of the common clinically practised pregnancy diagnosis methods in bovines. Several methods of pregnancy diagnosis both direct and indirect are being practised, but to date, none of this technique qualifies as the ideal pregnancy detection method due to its limitation. The progression of molecular diagnostic techniques like proteomics and their application in animal research has unlocked the opportunities among the research communities to look for new. Keeping these in view, the present work has been designed to study the changes of blood metabolites and hormonal profiles like serum levels of glucose, cholesterol, BUN, total protein, estrone sulphate and progesterone during early pregnancy of crossbred cows in the foothill of Mizoram.

MATERIALS AND METHODS

The experimental plan of study was duly approved by the Institution Animal Ethics Committee of College of Veterinary Sciences and Animal Husbandry, Aizawl, Mizoram, India. The study was conducted on 30 number of crossbred cows at 2nd to 5th lactation at Instructional Livestock Farm Complex, College of Veterinary Sciences and Animal Husbandry, Aizawl and private farms in and around the Aizawl district of Mizoram, India. All cows were kept on standard feeding as well as managemental practised. The clinico-gynaecological examination was carried out in all the cows and those cows devoid of ill health, having a regular estrous cycle and estrus period were inseminated at mid to late estrus. Ten (10) ml of blood sample were collected aseptically from all the cows (6 samples from each cow and a total of 180 samples) on day 1, 10, 20, 30, 60 and 90 of post-A.I. in a vacuum clot activator vial containing no additives by jugular puncture with a sterile 18 gauge needle fitted with a plastic syringe. Following standing at room temperature for 20 minutes, the clot activator vials were centrifuged at 3000 rpm for 10 minutes. Then obtained sera were kept at -20°C until analysis. The serum levels of glucose, cholesterol, BUN and total protein were analysed through FUJIFILM (DRI CHEM 4000i) autoanalyzer and blood hormonal profile estrone sulphate, progesterone were analysed by commercial ELISA kit. All the cows were observed for the absence of external signs of estrus after AI and pregnancy were confirmed by using ultrasonography and per rectal examination on day 30 to 40 of post insemination.

The data collected from the study were subjected to statistical analysis using a suitable formula as per Snedecor and Cochran (1994) for meaningful and accurate comparison and interpretation. Analysis of variance of complete data was carried out by one-way ANOVA. The correlation of blood metabolites with progesterone and estrogen was also analyzed to identify the metabolites for early pregnancy diagnosis.

RESULTS AND DISCUSSION

In the present study, the serum levels of glucose, cholesterol, BUN and total protein and blood hormonal profile estrone sulphate, progesterone of pregnant cows were depicted in Table 1.

The mean serum glucose, BUN and cholesterol levels of cows at day 1, 10, 20, 30, 60 and 90 of post-A.I. was significantly differed (P<0.01) from one another showed

Table 1: Blood-biochemical and hormonal indices of crossbred cows during different stages of pregnancy (mean±SE) (*n* =30).

Parameters	Day 1	Day 10	Day 20	Day 30	Day 60	Day 90	F value
Glucose (mg/dl)	69.40 ± 2.24^{AB}	65.43 ± 2.05^{A}	71.83 ± 2.31^{BC}	76.50 ± 2.39^{CD}	78.56 ± 2.08^{D}	74.00 ± 1.87^{BCD}	4.895**
BUN (mg/dl)	26.23 ± 1.37^{A}	26.96 ± 1.92^{A}	29.23 ± 1.20^{AB}	30.73 ± 1.00^{B}	29.73 ± 1.23^{AB}	32.00 ± 1.02^{B}	3.485**
Cholesterol (mg/dl)	177.07 ± 6.73^{A}	183.80 ± 5.50^{A}	182.73 ± 7.26^{A}	174.77 ± 8.47^{A}	218.53 ± 5.81^{B}	206.23 ± 4.68^{B}	7.344**
Total protein (mg/dl)	6.29±0.19	7.17±0.18	6.83±0.20	7.18±0.23	8.64±2.03	6.87±0.21	0.868^{NS}
Estrone sulphate (pg/ml)	5.21 ± 0.38^{D}	3.07 ± 0.41^{C}	2.32 ± 0.31^{C}	1.56 ± 0.16^{B}	0.70 ± 0.08^{A}	0.35 ± 0.04^{A}	42.574**
Progesteroe (ng/ml)	1.44 ± 0.10^{A}	4.95 ± 0.53^{B}	$7.66\pm0..37^{C}$	7.82 ± 0.36^{C}	8.36 ± 0.36^{C}	8.25 ± 0.28^{C}	57.663**

**P<0.01; ^{NS}Non significant.

Means bearing different superscripts in upper case in a row differed significantly.

an increasing trend of serum glucose level from day 1 to day 90 with peak level on day 60 of post A.I. excepting cholesterol which was highest on the day 60 of post A.I. When the total serum protein level of cows at day 1, 10, 20, 30, 60 and 90 of post-A.I. was depicted in Table 1 and there was no significant difference between them but it was highest on the day 60 of A.I.

Glucose plays an important role by enhancing the optimum growth of the embryo or fetus and decrease the chance of pregnancy losses in dairy cows (Lucy *et al.,* 2012; Lucy *et al*., 2014). In the present study, glucose level at different stages of pregnancy was significantly differed (P<0.01) from one another and increased gradually during early pregnancy from 65.43 to 78.56 (mg/dl) which was higher than the findings of Alameen and Abdelatif (2012). The higher level of blood glucose during early pregnancy might be due to more demand for energy for embryonic development (Mir *et al*., 2008).

Urea is the major outcome of the catabolism of proteins (Coles, 1996). BUN is a good indicator of an energy protein balance and its higher level might be due to various physiological and hormonal changes during early pregnancy (Muhamad *et al*., 2000). Again, insufficient availability of dietary energy increased rumen ammonia and blood urea concentrations by limiting the microbial synthesis of protein (Murphy, 1999). It was also reported by Greenwood *et al.* (2002) that during periods of energy restriction, the shortfall in energy might be met by catabolism of body proteins which results in increased urea concentrations in the blood. In the present study, BUN level at different stages of pregnancy was significantly differed (P<0.01) from one another and increased gradually during early pregnancy from 26.23 to 32.00 (mg/dl) which was in the same line up with Alameen and Abdelatif (2012). In certain cases, it was reported that the BUN level might be increased when the animal suffered from water deprivation and thirst or had diseases such as urinary diseases, diarrhoea, acidosis and pregnancy toxaemia (Djokovic *et al*., 2013). However, these conditions were not observed in this study.

Cholesterol plays an important role in the manufacture of bile acids, steroids, hormones and vitamin D. It also is a key constituent of blood plasma and an essential constituent of cell membranes (Edfers-Lilja *et al*., 1980). Cholesterol level in the blood was influenced by diet, age and sex of the animal (Abou-Tarboush and Dawood, 1993). Macial *et al*. (2001) reported that a decrease in plasma cholesterol concentration led to a reduction of plasma concentrations of IGF-I and progesterone which resulted in the suppression of luteal function and eventually delayed ovulation in dairy cows (Talukdar *et al*., 2013). In the present study, cholesterol level at different stages of pregnancy was significantly differed (P<0.01) from one another and they increased gradually during early pregnancy from 174.77 to 218.53 (mg/dl) which was higher than the reported value of Alameen and Abdelatif (2012), which indicates that animals used in the present research were in a positive energy balance.

Proteins requirement is very essential for reproduction, development and growth of the embryo as well as various metabolic activities in living organisms. Hormonal changes in the body might interfere with plasma protein concentrations. The pregnancy rate might be decreased because of a high protein diet during pregnancy (Butler *et al*., 1996 and Dhali, 2001). The animal kept on a high protein diet showed higher urea levels in the blood, which might reduce fertility by interfering with the normal inductive effects of progesterone on the uterine microenvironment, thereby providing non-feasible conditions for embryonic development (Butler, 2001). In the present study, the total serum protein level at different stages of pregnancy was not significantly different and showed ranges between 6.29 to 8.64 (mg/dl) which was similar to Mir *et al*. (2008). The higher levels of total serum protein in the present study might be due to an increased demand for proteins as pregnancy significantly influences the serum proteins present in the pregnant cow.

The mean estrone sulphate and progesterone level of cows at day 1, 10, 20, 30, 60 and 90 of post-A.I. were significantly differed (P<0.01) between different stages of pregnancy which is depicted in Table 1, which showed a decreasing trend of serum estrone sulphate level from day 1 to day 90 of post-A.I., however, an increasing trend of serum progesterone level was noticed from day 1 to day 90 with peak level on day 60 of post A.I. There was positive correlation between the level of progesterone (P<0.01) and negative correlation of estrone sulphate (P<0.05) with serum glucose, BUN, cholesterol at different days of post A.I.

It was reported that estrone sulphate is a conjugated steroid product of estrone, which is predominantly present in the bovine placentomes (Eley *et al*., 1979) and its concentration in the maternal body fluids is a useful indicator for the placental functions especially those related to embryonic growth (Prakash and Madan, 1993). Hu *et al*. (2010) found that estrone sulphate concentrations have also been commonly interrelated to fetal numbers as these are higher when the number of developing fetuses is more than one. It is not a perfect pregnancy biomarker as the plasma profiles are prejudiced by factors like genetic make-up, weight, parity status and environment (Lobago *et al.,* 2009). In the current study, estrone sulphate level at different stages of pregnancy was significantly differed (P<0.01) from one another and they decreased gradually during early pregnancy from 5.21 to 0.35 (ng/dl) which was also recorded by Lobago *et al.* (2009).

It was reported by Romano *et al*. (2007) that the progesterone (P4) hormone is essential for implantation of the embryo, optimum growth and maintenance of pregnancy. Negative energy balance was recorded to be associated with reduced circulating P4 in dairy cows, thereby, reducing the conception rate in dairy cows (Butler, 2003). So, the energy balance is positively associated with plasma. Furthermore, P_4 enhance the optimum growth of the embryo

or fetus and decrease the chance of pregnancy losses in dairy cows (Lucy *et al*., 2012; Lucy *et al*., 2014). In the present study, the progesterone value of pregnant cows increased gradually from day 0 to day 90 and they are significantly differed (P<0.01) from one another which corroborated the finding of Bhoraniya *et al*. (2012) and Astiti and Panjaitan (2013). The present study revealed that the hormonal status of estrone sulphate and progesterone can be used as a biomarker for the diagnosis of different stages of pregnancy.

CONCLUSION

In summary, the complexity of the blood metabolites and hormonal profile and its impact on the establishment of pregnancy is highlighted in our study. Based upon the evaluation of blood metabolites and hormonal indices, it could be used as an ideal indicator of early pregnancy in dairy cattle. More research is warranted to identify the most accurate indicator with specific early predictive value along with the greatest sensitivity and specificity.

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Conflict of interest

All authors declare that they have no conflict of interest.

REFERENCES

Abou-Tarboush, H.M. and Dawood, A.A. (1993). Cholesterol and fat contents of animal adipose tissues. Food Chemistry. 46(1): 89-93.

Alameen, A.O. and Abdelatif, A.M. (2012). Metabolic and endocrine responses of crossbred dairy cows in relation to pregnancy and season under tropical conditions. Am. Eurasian. Journal of Agriculture and Environmental Sciences. 12: 1065-1074.

Astiti, L.G.S. and Panjaitan, T. (2013). Serum progesterone concentration in Bali cow during pregnancy. Open Science Repository Veterinary Medicine, (open-access). pp. 70081943.

Bathla, S., Rawat, P., Baithalu, R., Yadav, M.L., Naru, J., Tiwari, A., Kumar, S., Balhara, A.K., Singh, S. and Chaudhary, S. (2015). Profiling of urinary proteins in Karan Fries cows reveals more than 1550 proteins. Journal of Proteomics. 127: 193-201.

Bhoraniya, B.L., Dhami, A.J. and Ankita, K. (2012). Influence of estrus synchronization protocols on fertility plasma progesterone and biochemical constituents in Kankrej cows. Indian Journal of Animal Reproduction. 33(2): 14-18.

Butler, W.R. (2001). Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. Anim. Sci. Occas. 26: 133-145.

Butler, W.R. (2003). Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Production Science. 83: 211-218.

Butler, W.R., Calaman, J.J. and Beam, S.W. (1996). Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. Journal of Animal Sciences. 74: 858-865.

Coles, E.H. (1996). Veterinary Clinical Pathology, 4th Edition, W.B. Saunders Company, London.

Dhali, A. (2001). Studies on the effect of feeding management system on blood and milk urea nitrogen concentration in dairy cattle. Ph.D. Thesis, submitted to National Dairy Research Institute, Karnal, India.

Djokovic, R., Vladimir Zoran Ilic, K., Cincovic, M., Petrovic, M., Fratric, N. and Jasovic, B. (2013). Evaluation of metabolic status in Simmental dairy cows during late pregnancy and early lactation. Veterinary Archiv. 83: 593-602.

Edfers-Lilja, C.C., Van Amburgh, M. and Butler, W.R. (1980). Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. Journal of Animal Sciences. 71: 702-706.

Eley, R.M., Thatcher, W.W. and Bazer, F.W. (1979). Hormonal and physical changes associated with bovine conceptus development. Journal of Reproduction and Fertility. 55: 181-190.

Fricke, P.M. (2002). Scanning the future-Ultrasonography as a reproductive management tool for dairy cattle. Journal of Dairy Science. 85: 1918-1926.

Greenwood, P., Hunt, A., Slepetis, R., Finnerty, K., Alston, C., Beermann, D. and Bell, A.W. (2002). Effects of birth weight and postnatal nutrition on neonatal sheep. III. Regulation of energy metabolism. Journal of Animal Sciences. 80: 2850-2861.

Hu, J., Zhang, Z., Shen, W.J. and Azhar, S. (2010). Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Journal of Nutrition and Metabolism. 7: 47.

Lobago, F., Bekana, M., Gustafsson, H., Beckers, J.F., Yohannes, G., Aster, Y. and Kindahl, H. (2009). 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. 44(4): 685-692.

Lucy, M.C., Butler, S.T. and Garverick, H.A. (2014). Endocrine and metabolic mechanisms linking postpartum glucose with early embryonic and foetal development in dairy cows. Animal. 8(Suppl. 1): 82-90.

Lucy, M.C., Green, J.C., Meyer, J.P., Williams, A.M., Newsom, E.M. and Keisler, D.H. (2012). Short communication: Glucose and fructose concentrations and expression of glucose transporters in 4 to 6 week pregnancies collected from Holstein cows that were either lactating or not lactating. Journal of Dairy Science. 95: 5095-5101.

Macial, S.M., Chamberlian, C.S., Wettemann, R.P. and Spicer, L.J. (2001). Dexamethasone influences endocrine and ovarian function in dairy cattle. Journal of Dairy Science. 84: 1998-2009.

Mir, M.R., Pampori, Z.A., Iqbal, S., Bhat, J.I.A., Pal, M.A. and Kirmani, M.A. (2008). Hemato-biochemical indices of crossbred cows during different stages of pregnancy. Inter. Journal of Dairy Science. 3: 154-159.

Muhamad, F., Sarvar, A., Hayat, C.S. and Anwar, M.I. (2000). Peripheral plasma progesterone concentration during early pregnancy in Holstein Friesian cows. Pakistan Veterinary Journal. 20: 166-168.

Murphy, J.J. (1999). The effects of increasing the proportion of molasses in the diet of milking cows on milk production and composition. Animal Feed Science and Technology. 79: 189-198.

Nandre, R., Joshi, C. and Derashri, H. (2012). Pregnancy proteins: Early indication of pregnancy diagnosis in farm animals. International Journal of Life Science. 2: 1-8.

Prakash, B.S. and Madan, M.L. (1993). Influence of gestation on oestrone sulphate concentration in milk of zebu and crossbred cows and murrah buffaloes. Tropical Animal Health and Production. 25(2): 94-100.

Reist, M., Erdin, D., Von, E.D., Tschuemperlin, K., Leuenberger, H., Chilliard, Y., Hammon, H.M., Morel, C., Philipona, C. and Zbinden, Y. (2002). Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. Journal of Dairy Science. 85: 3314-3327.

Romano, J.E., Thompson, J.A., Kraemer, D.C., Westhusin, M.E., Forrest, D.W. and Tomaszweski, M.A. (2007). Early pregnancy diagnosis by palpation per rectum: Influence on embryo/fetal viability in dairy cattle. Theriogenology. 67(3): 486-493.

Snedecor, G.W. and Cochran, W.G. (1994). In: Statistical Methods. 8th edn. Oxford and IBH. Pub. Cp. New Delhi.

Talukdar, D.J., Nath, K.C., Bonia, K.K. and Goswami, J. (2013). Hormone levels in crossbred cows treated for induction of ovulation. Indian Veterinary Journal. 90(5): 135-137.

Talukdar, D.J., Talukdar, Papori and Ahmed, K. (2016). Minerals and its impact on fertility of livestock: A review. Agricultural Review. 37(4): 333-337. doi: 10.18805/ag.v37i4.6464.

Talukdar, D.J., Nath, K.C., Bonia, K.K. and Deori S. (2015). Cyclic changes in blood biochemical profiles following induction of ovulation in anovulatory crossbred cows. The North-East Veterinarian. XV(1): 22-24.

Twentieth (20th) Livestock Census-2019, All India Report, Ministry of Fisheries, Animal Husbandry and Dairying Department of Animal Husbandry and Dairying, Animal Husbandry Statistic Division, Krishi Bhawan, New Delhi.

Zvorc, Z., Matijatko, V., Beer, B., Forsek, J., Bedrica, L. and Kucer, N. (2000). Blood serum proteinograms in pregnant and non-pregnant cows. Veterinary Arhiv. 70: 21-30.