Early Pregnancy Diagnosis in Bulgarian Murrah Buffaloes by PAGs

I. Fasulkov [(1)]*, Y. Ilieva [(2)], P. Penchev [(2)], M. Karadaev [(1)], B. Ivanova [(1)], R. Nenova [(2)] & N. Vasilev [(1)]

[(1)] *Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University (TrU), Stara Zagora, Bulgaria.*

[(2)] *Agriculture Academy (AA), Agricultural Institute, Shumen, Bulgaria.*

** Corresponding author: i.fasulkov@gmail.com*

Timely and reliable pregnancy diagnosis in productive animals is essential for improving reproductive efficiency by reducing calving intervals and allowing rapid rebreeding of non-pregnant females. Pregnancy-associated glycoproteins (PAGs), produced by the ruminant placenta and measurable in maternal circulation, have become an important tool for early pregnancy detection in cattle and buffaloes. The present study evaluated the diagnostic performance of two commercial ELISA kits for detecting PAGs in blood serum and milk of Bulgarian Murrah buffaloes, aiming to determine their suitability for early pregnancy diagnosis under farm conditions.

Blood samples were collected from 57 buffalo cows and 27 heifers on the 28th day following artificial insemination. Additionally, 55 milk samples were obtained from lactating buffaloes on the same day. Pregnancy status was confirmed seven days later, on day 35 post-insemination, through transrectal ultrasonography. PAG concentrations in serum and milk were measured using a commercial ELISA designed for early pregnancy diagnosis. The results were analysed using a non-parametric Chi-square test to compare proportions, employing Statistica 7 statistical software.

PAG concentrations in serum were significantly higher than those measured in milk ($P < 0.05$). Based on serum S–N values, 17 buffalo cows were diagnosed as pregnant and 40 as non-pregnant, while in heifers 7 were classified as pregnant and 20 as non-pregnant. Both serum and milk tests showed acceptable diagnostic characteristics, but serum PAG analysis consistently outperformed the milk test.

In buffalo cows, sensitivity reached 94.4% for serum and 87.5% for milk, while specificity was 100% for both sample types. In heifers, however, sensitivity was considerably lower (55.6%), although specificity for the serum ELISA remained high (88.9%). Accuracy values reflected similar trends: in buffalo cows the serum and milk tests achieved 98.2% and 96.4% accuracy, respectively, while in heifers the serum assay provided 77.8% accuracy.

Positive predictive values (PPV) in buffalo cows were 100% for both serum and milk tests, indicating that all positive diagnoses corresponded to confirmed pregnancies. Negative predictive values (NPV) were also high, reaching 100% for serum and 95.1% for milk. In heifers, PPV was lower at 71.4%, while NPV reached 80.0%. These results show that PAG-based ELISA testing is a reliable method for early pregnancy diagnosis in buffaloes, with serum testing providing the highest overall performance. However, milk sampling offers a valuable, non-invasive alternative that avoids the stress associated with blood collection. The presence of PAGs in milk also makes routine screening feasible in dairy buffalo operations without interrupting daily milking procedures. Although performance in heifers was lower, especially regarding sensitivity, both tests delivered acceptable specificity and practical value for early herd-level pregnancy monitoring.

Overall, the findings indicate that ELISA tests for PAGs in serum and milk are sufficiently sensitive, specific and accurate for diagnosing pregnancy as early as day 28 after artificial insemination. Their use can support more efficient reproductive management in Bulgarian Murrah buffalo herds.

Keywords: pregnancy-associated glycoproteins, serum and milk, ruminants, buffaloes, ELISA.

Reference: https://seer.ufrgs.br/index.php/ActaScientiaeVeterinariae/article/view/148745