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Comparison of a commercial bovine pregnancy-associated glycoprotein ELISA test and a pregnancy-associated glycoprotein radiomimmunoassay test for early pregnancy diagnosis in dairy cattle

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ABSTRACT

The present study aimed to compare the accuracy of a commercial PAG-ELISA test (Bovine Preg Test 29) and bovine pregnancy-associated glycoprotein radioimmunoassay (PAG-RIA) for diagnosing pregnancy at Day 28 after insemination in dairy cows.

Transrectal ultrasonography (TRUS) was performed in 100 Holstein-Friesian cows at Day 28 after artificial insemination (AI; Day 0) to diagnose pregnancy. After TRUS examination, blood sample was collected from the coccygeal vessels of each cow to measure the concentrations of bPAGs by PAG-RIA test and Bovine Preg Test 29. Milk samples were collected at Days 0, 21 and 28 for measurement of progesterone (P4) by ELISA test. The cows were re-examined by TRUS at Day 42 to confirm the pregnancy diagnoses. The actual gold standard was based on TRUS outcomes at Day 28 that agreed with the outcomes of PAG-RIA test or PAG-ELISA test. If the outcomes of TRUS at Day 28 and PAG-RIA test and PAG-ELISA test did not agree, the gold standard was based on the outcome of TRUS at Day 42.

Out of 100 inseminated cows, 41 were confirmed pregnant at Day 28 after AI. Based on the actual gold standard, the sensitivity of TRUS, PAG-ELISA and PAG-RIA tests for diagnosing pregnant cows at Day 28 were 92.7%, 90.2% and 100%, while the specificity of the three tests for diagnosing non-pregnant cows were 91.5%, 98.3% and 94.4%, respectively. The overall accuracy of the three tests were 92%, 95% and 97%, respectively. The degree of agreement (Kappa ± S.E.) between PAG-RIA and PAG-ELISA test was 0.90 ±0.04. The degrees of agreement between PAG-RIA and PAG-ELISA and TRUS at Day 28 were 0.80 ± 0.05 and 0.76 ± 0.06, respectively.

In conclusion, the commercial PAG-ELISA test is a highly accurate method for diagnosing early pregnancy in dairy cows on Day 28 after AI and may be used as an alternative method to the TRUS and the PAG-RIA test.

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1. Introduction

Early pregnancy diagnosis is a management tool required for shortening the calving interval in dairy cows. In addition identification of non-pregnant cows as early as possible after insemination reduces inter-insemination interval for herds performing synchronization and resynchronization without estrus detection (Lucy et al., 2011).

The diagnostic method used for early pregnancy diagnosis should be accurate, practical, fast, and inexpensive. Transrectal palpation (TRP) is widely used for pregnancy diagnosis in cows; however; with a moderate amount of experience, a reliable diagnosis of pregnancy is possible after Days 35–39 (Youngquist, 2007). Transrectal ultrasonography (TRUS) is an accurate method for early pregnancy diagnosis from Day 29 after insemination of dairy cows (Romano et al., 2006), however, it requires a specialized equipment and a trained person to perform the scanning (Lucy et al., 2011). Alternatively, blood or milk chemical tests can be used before Day 29 after insemination. These tests are: progesterone (P4) and bovine pregnancy-associated glycoproteins (bPAGs). Progesterone-ELISA test (cow-side test) can be applied in the milk at Days 21–24 after insemination with a 100% negative predictive value; however the test has relatively low (60–94%) positive predictive value (Nakao et al., 1982; Sauer et al., 1986).

Bovine pregnancy-associated glycoproteins (bPAGs) or pregnancy specific protein B (bPSPB) are mainly secreted by trophoblastic binucleate cells (Wooding et al., 2005) and they have been detected in the blood of some pregnant cows as early as Days 15–22 after AI (Sasser et al., 1986; Zoli et al., 1992; Green et al., 2005). Therefore, these glycoproteins have been used as a marker of pregnancy and feto-placental well-being in cattle (Breukelman et al., 2012). Radioimmunoassays (RIA) for bPSPB or bPAG-1 have a high sensitivity (95–100%) for identifying pregnant cattle from Day 30 after breeding onwards. However, the specificity of these tests for identifying non-pregnant cows in most studies is greatly variable ranging from 69.3% to 93% (Sasser et al., 1986; Humblot et al., 1988; Zoli et al., 1992; Skinner et al., 1996; Szenci et al., 1998) due to the difference in the incidence of embryonic mortalities which might have occurred between days of blood test and day of confirmation of pregnancy by TRUS or transrectal palpation (gold standard) among studies. More recently, ELISA assays for bPSPB and bPAG have been developed and became commercially available. These commercial ELISA assays eliminate the hazard of radiation and enable the producer to apply the test on the farm. The sensitivity of these assays ranged from 93.9% to 100% and their specificity ranged from 66.7% to 95.5% at Days 27–38 after breeding (Silva et al., 2007; Paré et al., 2008; Howard et al., 2007; Romano and Larson, 2010).

To evaluate a new method of pregnancy diagnosis, a contemporary accurate method should be used as a gold standard since a substantial percentage of dairy cows experienced late embryonic mortalities. TRUS has been used as a gold standard in previous studies since it is safe, non-invasive and give immediate results. However, its accuracy for pregnancy diagnosis has not reached 100% before Day 30 in most field studies (Fricke, 2002). The accuracy of the RIA and ELISA tests for bPSPB or bPAG for early pregnancy diagnosis have not been compared in a same study. Therefore, the present study aimed to compare the accuracy of a commercial PAG-ELISA test (Bovine Preg Test 29) with the PAG-RIA test for diagnosing pregnancy at Day 28 after insemination in dairy cows.

2. Materials and methods

2.1. Animals and management

One-hundred Holstein Friesian cows belonging to a dairy farm in Hungary were used in the present study from March to June 2010. The parity of the cows ranged between 1 and 8 with an average of 2.4 ± 1.4. The cows were between 55 and 475 days in milk with an average of 163 ± 92.7 days. The animal protocols were approved by the local committee (Faculty Committee for Animal Experiments, Faculty of Veterinary Science, Szent Istvan University, Hungary).

According to the routine reproductive management, each non-pregnant cow with a mature corpus luteum (diameter $\geq$17 mm, Repasi et al., 2003) determined by TRUS, received 500 μg of cloprostenol sodium (2 mL of PGF Veyx® Forte A.U.V. inj, Veyx-Pharma GmbH, Schwarzenborn, Germany) intramuscularly. The cows were free from mastitis, laminitis and clinical endometritis. The cow was considered as free from clinical endometritis by absence of purulent or mucopurulent vaginal discharge and absence of uterine fluid by TRUS (Sheldon and Lewis, 2006). Three days after PGF2α administration, all of these cows, not inseminated during the first and second day after PGF2α treatment, were examined by TRP by one of the inseminators and if the cow showed clinical signs (erected uterus, estrus discharge, and having a palpable follicle) of estrus was inseminated and then 100 μg of gonadorelin [6D-Phe] acetate (2 mL of GonavetVeyx®, Veyx-Pharma GmbH) was administered intramuscularly. The semen used for artificial insemination (AI) was chosen by the herd managers as part of the routine management of the herd. The day of AI was considered as Day 0 for calculating the gestation period.

2.2. Ultrasonographic examinations

Transrectal ultrasonography was carried out by the same operator using a real time B-mode ultrasound scanner (Falco Vet, Esaote/Pie Medical, Maastricht, The Netherlands) equipped with a 6–8 MHz linear endo-rectal transducer. Transrectal ultrasonography was performed for each cow at Day 28 after AI. Positive diagnoses of pregnancy by transrectal ultrasonography depended on the detection of anechoic allantoic fluid and/or the embryo proper (Szenci et al., 1998). The ovaries were scanned for presence of the corpus luteum. The cows were re-examined by TRUS at Day 42 to confirm the pregnancy diagnoses on the basis of detection of an embryo with a beating heart.

2.3. Milk and blood sampling

Milk samples were collected from each cow at Days 0, 21 and 28 after morning milking. After discarding the first 5 squirts of milk from each quarter, an equal amount