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Evaluation of A Rapid Visual Pregnancy (Bio- RPD) test for detection of Pregnancy Specific Protein B (PSPB) in Cow Serum

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Abstract

The early pregnancy diagnosis allows optimizing production and timely management correction, with a greater reproductive output of livestock. The BioPRYN Rapid Visual Pregnancy Test® from Bio tracking USA consists of an ELISA for visual reading which does not require the use of readers in the laboratory, with satisfactory pregnancy diagnoses at 28 days of pregnancy in heifers and 30 days in cows. Therefore, the aim of this study is to determine the accuracy of this rapid test (Bio-RPD) based on visual observation of color change and Optical Density (OD) using BioPRYN Flex ELISA. For this purpose, 92 serum samples from cows were tested. The numbers of positive pregnant samples obtained with Bio-rapid (Bio-RPD) were 63 (68.5 %) while with BioPRYN Flex ELISA was 66 (71.7%). The numbers of negative not pregnant samples were 25 (27.2%) with Bio-RPD while they were 26 (28.2%0) with BioPRYN ELISA. Three samples were recorded as recheck with Bio-RPD and they were pregnant with BioPRYN Flex. Only one sample was identified as pregnant by Bio-RPD (1.1%) while it is not pregnant by BioPRYN Flex ELISA (false positive). Test results were showing that accuracy of the BioPRYN Visual Pregnancy test kit with sensitivity of 99% compared to BioPRYN ELISA Pregnancy test kit. In conclusion, we recommend that the Bio-RPD visual test could be used as a rapid accurate tool for pregnancy diagnosis on farms which has no laboratory ELISA equipment or trans rectal ultrasonography.

Keywords: BioPRYN Rapid visual Pregnancy Test (Bio-PRD); BioPRYN Flex ELISA; Optical Density (OD); Pregnancy Specific Protein B (PSPB); Pregnancy –Associated Glycoprotein (PAG); Prepared Enhancer solution; TMB substrate; Stop solution; Ultrasonography; Rectal palpation; Artificial Insemination (AI).

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Introduction

Early pregnancy diagnosis is critical for maximizing herd productivity in the cattle industry. Current methods for pregnancy diagnosis include: observation for return to estrus, rectal palpation, trans-rectal ultrasonography, and blood tests for specific antigens.

These blood tests include detection of Pregnancy-Associated Glycoproteins (PAGs) which belongs to a large family of aspartic peptidase of which Pregnancy-Specific Protein B (PSPB) was the first member to be discovered [1]. These glycoproteins are produced exclusively by specialized trophoblastic giant cells in ruminant placenta [2] , which migrates from trophectoderm to fuse with maternal uterine epithelial cells, and release their granular content (containing PSPB and PAG) into the maternal circulation [3]. The PAGs consist of several isoelectric and molecular protein variants that can be partially purified from other placental proteins. Antibodies made against the purified proteins can be used to detect the presence of these proteins in the peripheral circulation of cattle. Because these proteins are specific to the placental tissue, it is possible to use detection of PAGs in the maternal circulation as indicator of pregnancy [4].

Trans-rectal palpation, B-mode ultrasonography and measurement of blood PSPB and PAG concentration are commonly used for early pregnancy diagnosis. Serum PSPB and PAG concentrations increase progressively from 30 days of pregnancy, peak 1-5 days

before calving and decline there after [5,6]. The first blood –based pregnancy- specific assay for ruminant was described by Sasser et al. on the basis of radioimmunoassay for PSPB [5,7]. Pregnancy associated glycoproteins such as PBSP B [8, 9] and other PAGs [6,10,11] have been used for early detection of pregnancy in dairy cattle, starting as early as 21 days after AI.

More recently, ELISA assays for PBSB [4] and PAG [10] became commercially available. The PSPB ELISA is available under the trade name BioPRYN and provides a qualitative pregnancy classification based on measurement of PBSB in serum of pregnant ruminants and has been commercially available since 2003 in USA and applied 25 days post breeding in heifers and 28 days in adult cows while PAGs was developed by IDEXX, USA.

BioPRYN (Bio Tracking) has recently manufactured Bio-RPD kits to be inspected visually with shorter incubations and utilized on-farm or in areas without access to laboratory equipment. Cattle can be tested 28 days post breeding or greater. The manufacture claimed that the sensitivity is (99.9%) and matches with BioPRYN-flex ELISA assay, but with visual interpretation and human subjectivity there were a small percentage (1.5%) of none pregnant animals that were identified as pregnant.

Therefore the objective of the present study was to evaluate and compare the results of Bio-RPD with BioPRYN flex ELISA assay as early pregnancy determination in dairy cattle.

Materials and Methods

The study was performed at Al Safi dairy farm Al Kharj, Saudi Arabia. 92 cows sera were used for both Bio-RPD and BioPRYN flex ELISA and the results obtained by both assays were compared.

Bio-RPD Visual test:

The test was performed as per BioPRYN instructions. 100 microliters of cow's sera were added to the well of the plate. The standards were added after all the samples have been loaded in duplicates (Hi and low standards). Gently swirl and allow the plate for at least 10 minutes after the last standard is added. Then add 50 microliters of detector Buffer (#1) in the well with the samples and standards and gently swirl and incubate for 10 minutes at room temperature. Wash 4 times with water and then add 100 microliters of prepared Enhancer solution (#2) to all wells and incubate for 10 minutes. Wash 4 times with water and add 100 microliters of TMB substrate (#3) to all wells and incubate for 10 minutes at room temperature. Add 50 microliters of stop buffer (#4) to all well without removing TMB solution. Then visually read the results of the plate. Any sample above the Hi standard is considered pregnant and any samples below the low standard is considered not pregnant. Any sample between the Hi and Low standard is considered Recheck. This means the animal has a lower than expected level protein and should be rechecked in a week to determine the actual status.

BioPRYN Flex ELISA Assay:

BioPRYN Flex ELISA (Bio Tracking LLC, Moscow, ID, USA) was also used in the study and it was performed according to the instruction of the manufacture. It is provide a qualitative classification based on comparison of a sample optical density with three threshold optical density values (high, low and cut-off) calculated for each plate. The threshold values were calculated from the Optical Density (OD, wave length 450nm) values of duplicates test well for two PSPB (high and low). If the OD exceeded the high threshold the animal was categorized as pregnant. An OD less than threshold, was designated as not pregnant.

Figure 1 BioPRYN visual results.

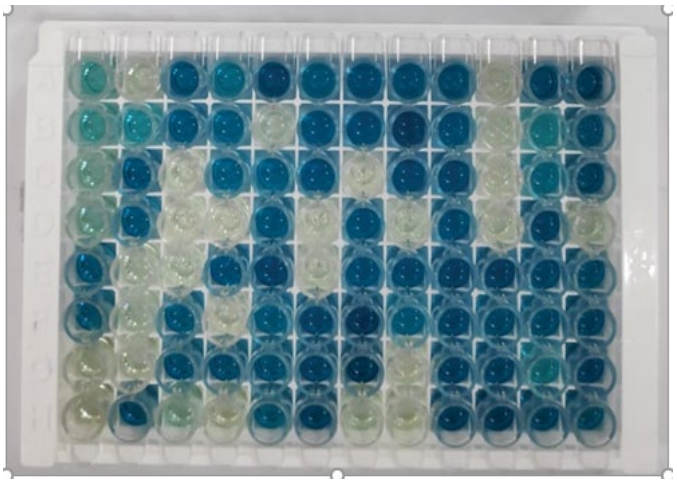

Figure 2 BioPRYN ELISA results.

Table 1: Bovine serum BioPRYN ELISA and BioPRYN visual pregnancy results.

Unit	Samples analyzed	Pregnant		Re-check		Open		False positive		Total
BioPRYN ELISA Pregnancy	92	66	71.70%	0	0%	26	28.30%	0	0%	92
BioPRYN Visual Pregnancy	92	63	68.50%	3	3.30%	25	27.20%	1	1.10%	92

Table 2: Pregnancy results.

Sr. No.	Date of Rep.	ELISA results	ELISA OD	Visual results	Remarks
1	21.09.2020	Pregnant	1.858	Pregnant	
2	21.09.2020	Pregnant	1.861	Pregnant	
3	21.09.2020	Open	0.124	Open	
4	21.09.2020	Open	0.084	Open	
5	21.09.2020	Open	0.109	Open	
6	21.09.2020	Pregnant	0.96	Re-check	
7	21.09.2020	Pregnant	2.191	Pregnant	
8	21.09.2020	Pregnant	2.249	Pregnant	
9	21.09.2020	Open	0.144	Open	
10	21.09.2020	Open	0.185	Open	
11	21.09.2020	Open	0.129	Open	
12	21.09.2020	Pregnant	2.227	Pregnant	
13	21.09.2020	Pregnant	1.961	Pregnant	
14	21.09.2020	Pregnant	2.134	Pregnant	
15	21.09.2020	Open	0.116	Open	
16	21.09.2020	Open	0.107	Open	
17	21.09.2020	Open	0.156	Open	
18	21.09.2020	Pregnant	1.954	Pregnant	
19	21.09.2020	Pregnant	2.033	Pregnant	
20	21.09.2020	Open	0.267	Open	
21	21.09.2020	Pregnant	1.383	Re-check	
22	21.09.2020	Pregnant	1.85	Pregnant	
23	21.09.2020	Pregnant	1.709	Pregnant	
24	21.09.2020	Open	0.106	Open	
25	21.09.2020	Pregnant	2.366	Pregnant	
26	21.09.2020	Open	0.096	Open	
27	21.09.2020	Pregnant	2.199	Pregnant	
28	21.09.2020	Open	0.112	Open	
29	21.09.2020	Pregnant	2.549	Pregnant	
30	21.09.2020	Open	0.157	Open	
31	21.09.2020	Pregnant	1.645	Pregnant	
32	21.09.2020	Pregnant	1.994	Pregnant	
33	21.09.2020	Pregnant	2.56	Pregnant	
34	21.09.2020	Pregnant	1.712	Pregnant	
35	21.09.2020	Pregnant	1.847	Pregnant	
36	21.09.2020	Pregnant	1.781	Pregnant	
37	21.09.2020	Pregnant	1.922	Pregnant	
38	21.09.2020	Pregnant	2.201	Pregnant	
39	21.09.2020	Pregnant	2.129	Pregnant	
40	21.09.2020	Open	0.114	Open	
41	21.09.2020	Open	0.119	Open	
42	21.09.2020	Pregnant	2.247	Pregnant	
43	21.09.2020	Pregnant	2.2	Pregnant	
44	21.09.2020	Pregnant	2.389	Pregnant	
45	21.09.2020	Pregnant	2.178	Pregnant	
46	21.09.2020	Pregnant	2.196	Pregnant	
47	21.09.2020	Open	0.087	Open	
48	21.09.2020	Pregnant	2.235	Pregnant	
49	21.09.2020	Pregnant	2.173	Pregnant	
50	21.09.2020	Pregnant	2.926	Pregnant	
51	21.09.2020	Pregnant	2.988	Pregnant	
52	21.09.2020	Open	0.151	Open	
53	21.09.2020	Pregnant	1.997	Pregnant	
54	21.09.2020	Pregnant	2.882	Pregnant	
55	21.09.2020	Pregnant	2.474	Pregnant	
56	21.09.2020	Open	0.135	Open	
57	21.09.2020	Pregnant	2.323	Pregnant	
58	21.09.2020	Pregnant	1.703	Pregnant	
59	21.09.2020	Open	0.093	Open	
60	21.09.2020	Open	0.109	Open	
61	21.09.2020	Pregnant	2.129	Pregnant	
62	21.09.2020	Pregnant	2.064	Pregnant	
63	21.09.2020	Pregnant	2.422	Pregnant	
64	21.09.2020	Pregnant	2.37	Pregnant	
65	21.09.2020	Pregnant	2.551	Pregnant	
66	21.09.2020	Pregnant	2.275	Pregnant	
67	21.09.2020	Pregnant	2.371	Pregnant	
68	21.09.2020	Pregnant	2.447	Pregnant	
69	21.09.2020	Open	0.098	Open	
70	21.09.2020	Open	0.116	Pregnant	False positive
71	21.09.2020	Open	0.1	Open	
72	21.09.2020	Open	0.113	Open	
73	21.09.2020	Pregnant	2.54	Pregnant	
74	21.09.2020	Pregnant	2.034	Pregnant	
75	21.09.2020	Pregnant	2.204	Pregnant	
76	21.09.2020	Pregnant	2.548	Pregnant	
77	21.09.2020	Pregnant	2.04	Pregnant	
78	21.09.2020	Pregnant	0.965	Re-check	
79	21.09.2020	Pregnant	1.349	Pregnant	
80	21.09.2020	Pregnant	2.498	Pregnant	
81	21.09.2020	Pregnant	2.128	Pregnant	
82	21.09.2020	Pregnant	1.929	Pregnant	
83	21.09.2020	Pregnant	1.025	Pregnant	
84	21.09.2020	Pregnant	2.106	Pregnant	
85	21.09.2020	Pregnant	2.116	Pregnant	
86	21.09.2020	Pregnant	1.92	Pregnant	
87	21.09.2020	Pregnant	2.215	Pregnant	
88	21.09.2020	Open	0.174	Open	
89	21.09.2020	Pregnant	2.05	Pregnant	
90	21.09.2020	Pregnant	2.081	Pregnant	
91	21.09.2020	Pregnant	2.347	Pregnant	
92	21.09.2020	Pregnant	1.823	Pregnant	

Result

Test results of 92 tested sera by Bio-RPD and BioPRYN Flex were illustrated in **(Table 1**, **Table 2**, **Figure 1, Figure 2)**.

The results showed animals defined as pregnant, recheck, not pregnant (open) and false positive. Out of 92 tested sera, 66 were categorized by BioPRYN Flex ELISA as pregnant (71.7%) and 63 were defined by Bio-RPD as pregnant (68, 5%).

Twenty six recorded as not pregnant (open) using BioPRYN Flex (28.3%) and Twenty five were categorized not pregnant using Bio-RPD (27.2%). Three samples were considered recheck by Bio-RPD as their color reading is between the Hi and Low standards.

With BioPRYN Flex none were defined false positive (0%) while with Bio-RPD 1 is diagnosed as false positive 1.1% as it is not pregnant (open) by BioPRYN Flex. The sensitivity of Bio-RPD is 99% when compared with BioPRYN Flex ELISA.

Discussion

Pregnancy-Specific Protein B (PSPB) was first detected in bovine placenta (Butler et al., 1982). Antibodies made against these purified proteins (PSPB) can be used to detect the presence of these proteins in the peripheral circulation of the cows. And because these proteins are specific to the placenta tissue, they become visible in the maternal blood and can be determined with high accurateness from 28 to 30 days post breeding as an indicator of pregnancy.

The rapid visual Bio-RPD and BioPRYN ELISA were used in this study to measure PBSB values in dairy cows under field condition and their results were compared together.

In our study, a higher accuracy and sensitivity of Bio-RPD were recorded (99%) when compared with BioPRYN ELISA. These results seemed consistent with those reported by the manufacture who claimed that the sensitivity is (99.9%) and matches with BioPRYN-flex ELISA assay.

Any samples between the Hi and Low standards mean the animal has a lower than expected protein and should be rechecked in a week to determine the actual status. One limitation of the present study was the fact that 3 cow samples were recorded as recheck, and could not be used for determination of accuracy and sensitivity calculation because no chance for testing them again after one week to check the level of the protein.

In summary, the rapid Bio-RPD assay, based on visual inspection with shorter incubation for determine PSPB and utilized on farms without access to laboratory, had extremely high and similar sensitivity for early pregnancy diagnosis in dairy cows compared with PSPB ELISA.

Conclusion

Test results showed that the sensitivity of Bio-RPD visual test is 99% compared to BioPRYN Flex ELISA. Thus it is recommended to be utilized as rapid tool for pregnancy diagnosis on farms that have no laboratory equipment or transrectal ultrasonography.

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