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Comparative evaluation of pregnancy diagnosis in goats using rapid visual pregnancy test (Bio-RPD) and ultrasonography

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Abstract

Early detection of pregnancy gives breeders, veterinarians, and researchers the necessary information to improve prenatal care, improve the condition of postnatal lambs and kids and increases production efficiency in small ruminants. In this study, it was aimed to compare and evaluate the accuracy of Bio-RPD rapid visual pregnancy test and transabdominal scanning in determining pregnancy status in 64 goats. The obtained results showed that Bio-RPD visual is more accurate than ultrasound scanning at 30 days of pregnancy. Both tests have the same results and accuracy at 45 days of pregnancy. We recommended that BioRPD visual test is a useful, rapid, and accurate tool for early pregnancy diagnosis at the 30th day of pregnancy. Transabdominal scanning could be used as a useful tool for pregnancy diagnosis in goats and small ruminants at 45 days of pregnancy.

Key words: BioPRYN Rapid visual Pregnancy test (Bio-RPD); BioPRYN Flex ELISA; Pregnancy Specific protein (PSPB); Pregnancy -Associated Glycoprotein (PAGs); Non returning estrous; Abdominal palpation; Palpation of caudal uterine artery; Laparotomy; Vaginal Biopsy and vaginal smear; Hormonal Assays; Ultrasonography

1. Introduction

Pregnancy diagnosis in small ruminants is a part of reproduction management (Munsi et al., 2017). Failure to detect early pregnancy causes huge economic losses (Lone et al., 2016). Pregnancy diagnosis in goats is challenging, especially in the early stages. A reliable technique for detection of pregnancy in goats is emerging and enabling herdsman to separate non-pregnant goats and thereby save on feed, labor, vaccination, and veterinary cost.

Numerous methods have been used to diagnose pregnancy in small ruminants (sheep & goats). These methods include some less practical ones like not returning of estrous, abdominal palpation, palpation of the caudal uterine artery, laparotomy, vaginal biopsy and vaginal smear and most practical methods such as hormonal assays, pregnancy protein assays and ultrasonography.

In small ruminants, ultrasonography is a safe, fast accurate, cost -effective, and practical method that can be used to detect pregnancies at early stages (Crilly et al., 2017). It also provides other advantages like determining the number of fetuses, fetal age and sex, fetal deaths, and monitoring fetal development (Guller and Kaymaz, 2011, Alkan et al., 2020).

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Early detection of pregnancy by ultrasonography gives breeders, veterinarians, and researchers the necessary information to improve prenatal care, improve the condition of postnatal lambs and kids, and increases production efficiency in small ruminants (Jyothi et al., 2020). Ultrasonography can be used transrectally or transabdominally.

Although in practice, transabdominal scanning is less painful for animals, easier and faster for practitioners and provides a very wide field of view, so it is preferred more (Crilly et al., 2017).

Pregnancy protein assays include detection of Pregnancy -Associated Glycoprotein (PAGs) which belongs to a large family of aspartic peptidase of which Pregnancy -Specific Protein B(PSPB) was the first member to be discovered (Butler et al., 1982). These glycoproteins are produced exclusively by specialized trophoblastic giant cells in ruminant placenta (Ekblad et al., 1985), which migrates from trophectoderm to fuse with maternal uterine epithelial cells and release their granular content containing (PSPB and PAG) into the maternal circulation (Wooding FBP, 1992). Because these proteins are specific to placenta tissue, it is possible to use for detection of PAGs in the maternal circulation as indicator of pregnancy (Gabor G, et al., 2007).

More recently, ELISA assay for PSPB (Gabor G, et al, 2007) and PAG become commercially available (Friedrich M et al, 2010). The PSPB ELISA is available under the trade name BioPRYN Flex ELISA assay and provides a qualitative pregnancy classification based on measurement of PSPB in the serum of pregnant ruminants and has been commercially available since 2003 in USA and applied 25 days post breeding in heifers and 28 days post breeding in adult cows and 30 days in small ruminants while PAGs was developed by IDEEX, USA.

BioPRYN (Biotracking, USA) has recently manufactured Bio-RPD kits to be inspected visually with shorter incubation and utilized on farms or in areas without access to laboratory equipment. Cows can be tested 28 days post breeding or greater, heifers at 25 days post breeding while small ruminants can be tested 30 days post breeding. The manufacturer claimed that the sensitivity is 99.9% and matches with BioPRYN Flex ELISA assay, but with visual interpretation and human subjectivity there is a small percentage 1.5% of non-pregnant animals that were identified as pregnant. Hameed O.A et al., (2020) evaluated the rapid visual pregnancy (Bio-RPD) test for detection of pregnancy specific protein PSPB in cow serum. Their results showed that the accuracy of BioPRYN visual pregnancy test has a sensitivity of 99% compared to BioPRYN Flex ELISA assay. They recommend that BioPRYN visual test could be used as a rapid accurate tool for pregnancy diagnosis on farms levels where laboratory ELISA equipment was not available.

In the present study, we aimed to compare the pregnancy diagnosis results in goats by using Bio PRD rapid test, ultrasonography and to evaluate the level of agreement between the two techniques as an early tool for pregnancy diagnosis in goats.

2. Material and Methods

2.1. Study area and period

The study was conducted at private goat farms at Al Zulfi area (Qassim district, Saudi Arabia) during November 2022.

2.2. Rapid visual pregnancy (Bio-RPD) test

64 goats we used in this study. The test was performed as per BioPRYN instructions. 100 microliters of goat sera were added to the well of the plate. The standards were added after all 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 well 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 high standard is considered pregnant and any sample below the low standard is considered non-pregnant. Any sample between the Hi and Low standards is considered Re-check. This means the animal has a lower-than-expected level protein and should be re-checked in two weeks and the protein will either have risen to definitive pregnant level for pregnant animal to have cleared to where it will read non-pregnant in case of embryonic loss.

2.3. Ultrasonography for pregnancy evaluation

Transabdominal digital ultrasonic diagnostic imaging system iScan 2 (Fig. 1) from DRAMINISKI, POLAND with fixed Convex Recital probe 5.0 MHz and Gain1= 6, Gain= 6, MHz = 5.0 &6.5, Depth= 12 & 10 and Gamma = 3 & 4 was used in

standing position without sedation to diagnose pregnancy in goats. Pregnancy was confirmed by observing gestational sac, fetus, fetal part (s), cotyledon etc.

Figure 1 iScan 2

3. Results

Rapid visually pregnancy test (Bio-RPD) results were illustrated in Table 1 and Figure 2. The results showed animals defined as pregnant, non-pregnant, re-check.

Table 1 Rapid visually pregnancy test (Bio-RPD) results

Hi Standard	Re-check	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant
Hi Standard	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant
Lo Standard	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant
Lo Standard	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant
Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Re-check	Pregnant	Pregnant	Hi Standard
Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Hi Standard
Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Re-check	Lo Standard
Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Pregnant	Lo Standard

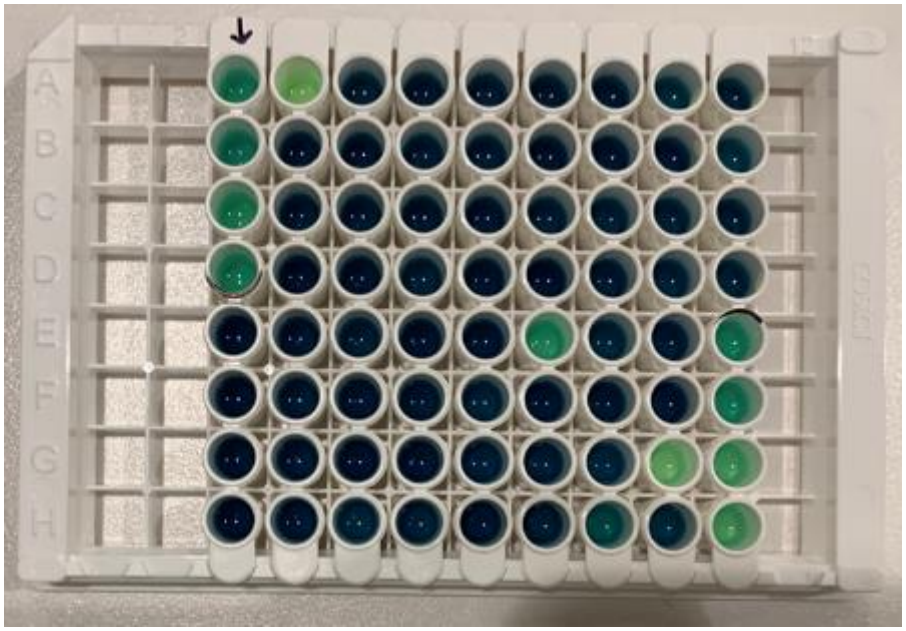

Figure 2 Rapid visually pregnancy test (Bio-RPD) plate results

Out of 64 samples tested 61 samples were read positive. Their color is higher than Hi standard, while 3 samples read re-check as their color is between Hi and low standards Table 1 & Fig 2. The three samples were tested after 2 weeks due to manufacturer instructions. After 2 weeks they read positive which confirms that these animals have lower level of protein (PSPB) to be detected at that time.

The transabdominal pregnancy scanning is presented in Table 2. On the 30th day, the pregnancy was detected by observing gestational sac or fetus or fetal heartbeat. (Fig,3,4). Out of 64 tested goats, 50 goats were positive while 14 goats were negative. After 2 weeks, these 14 goats were retested again with ultrasound scanning as they were positive by Bio-RPD visual test. All of them were positive.

Table 2 Ultrasound transabdominal pregnancy scanning results

Sample #	Result	Sample #	Result	Sample #	Result	Sample #	Result
56	Pregnant	117	Non-Pregnant	157	Pregnant	32	Pregnant
195	Pregnant	211	Pregnant	100	Pregnant	181	Pregnant
128	Pregnant	149	Pregnant	74	Pregnant	102	Pregnant
124	Pregnant	151	Non-Pregnant	302	Pregnant	114	Non-Pregnant
109	Non-Pregnant	65	Pregnant	274	Pregnant	91	Pregnant
141	Non-Pregnant	60	Pregnant	64	Pregnant	322	Pregnant
86	Pregnant	67	Pregnant	126	Non-Pregnant	87	Pregnant
58	Pregnant	20	Pregnant	210	Pregnant	171	Pregnant
116	Pregnant	182	Pregnant	144	Non-Pregnant	55	Pregnant
143	Non-Pregnant	88	Pregnant	16	Pregnant	164	Non-Pregnant
321	Pregnant	207	Non-Pregnant	317	Pregnant	120	Non-Pregnant
97	Pregnant	69	Pregnant	19	Pregnant	38	Pregnant
59	Pregnant	70	Pregnant	8	Pregnant	48	Non-Pregnant
192	Pregnant	53	Non-Pregnant	249	Pregnant	25	Pregnant

9	Pregnant	51	Pregnant	11	Pregnant	43	Pregnant
112	Pregnant	121	Non-Pregnant	235	Pregnant	57	Pregnant

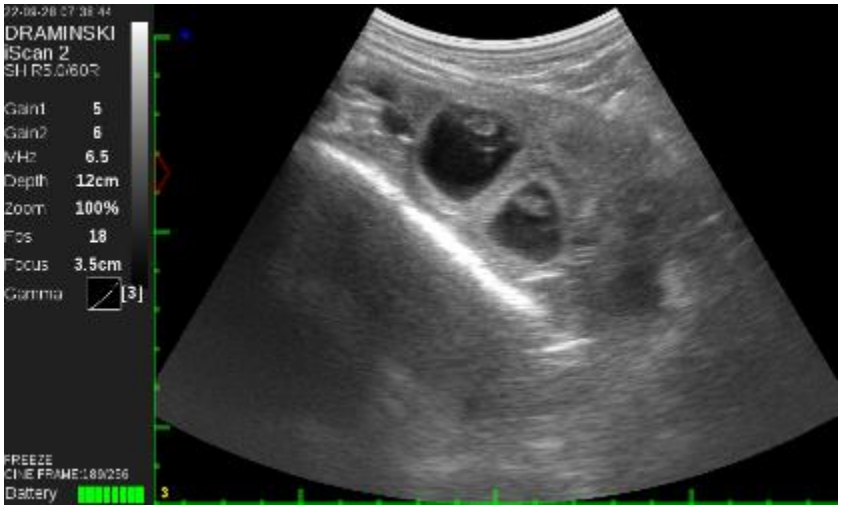

Figure 3 Gestational sac

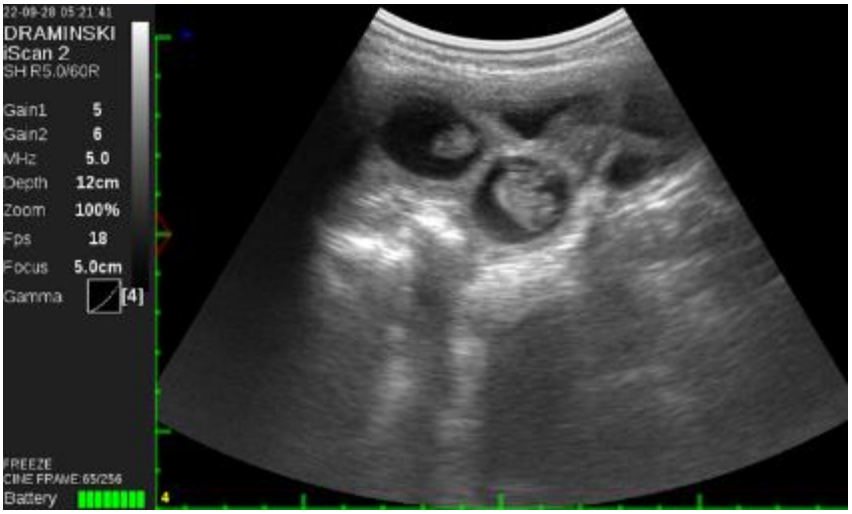

Figure 4 Fetus (twins)

4. Discussion

The rapid visual Bio-RPD assay was recommended based on visual inspection with shorter incubation to determine PSPB. It was recommended by the manufacturer (Biotracking, USA) to be utilized in the field and farms without access to laboratory. It had extremely high and similar sensitivity for early pregnancy diagnosis in dairy cows compared to BioPRYN Flex ELISA for detection of PSPB (Hameed O.A etal.,2022)

The authors showed that the sensitivity of Bio-RPD visual is 99% compared to BioPRYN Flex ELISA.

Based on the recommendations of the manufacture and the data reported by Hameed et al., 2020 we used Bio-RPD visual test in the field as rapid tool to detect early pregnancy in goats and compared the results obtained with ultrasound scanning. In the first run of Bio-RPD visual test, out of 64 tested sera, 61 samples were identified as pregnant (95.3 %) while 3 samples were identified as re-check and 0 sample was identified as non-pregnant. In the second run of Bio-RPD visual test (after 2 weeks as per the recommendation of the manufacture), these 3 re-check samples were identified as

pregnant as the PSPB protein have been risen to definitive pregnant level. The results proved that Bio-RPD visual rapid test identified 100% of pregnant goats at 45 days of pregnancy.

Traditional methods for pregnancy diagnosis in small ruminants are ballottement of the abdomen and noting udder enlargement in late pregnancy. Transabdominal ultrasonography has been used with great accuracy for pregnancy diagnosis and estimation of fetal numbers in goats (Gonzalez et al., 2004). In small ruminants' early diagnosis of pregnancy is possible by detecting gestational sac, fetus, and fetal heartbeat (Garcia et al., 1993). The results of ultrasonography scanning were presented in table 2. It identified 78% of pregnant goats at day 30 of pregnancy. After 2 weeks (45 days of pregnancy) the ultrasound scanning identifies 100% of the pregnant goats.

In our study, the authors demonstrated that Bio-RPD visual is more accurate than ultrasound scanning at 30 days of pregnancy. While both tests have the same results & accuracy at 45 days of pregnancy as shown in table 3. We believe that the two examination tools (Bio-RPD & Ultrasonography scanning) were advantageous in pregnancy confirmation in goats.

Table 3 Results comparison between Bio-RPD visual and ultrasound scanning at 30 and 45 days of pregnancy

Days of testing	Bio-RPD Plate	Ultrasound
30 Days	95.30%	78%
45 Days	100%	100%

5. Conclusion

In conclusion, Bio-RPD visual rapid test is more valuable and accurate in detecting pregnancy at 30 days, while transabdominal ultrasonographic examination should be performed at 45 days to help detecting early pregnancy in small ruminant to improve prenatal care, improve the condition of postnatal lambs and kids and to increase production efficiency.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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