

Comparative evaluation of pregnancy diagnosis in sheep using On-site pregnancy test (Pregnostx) 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. This study is aimed to compare and evaluate the accuracy of on-site pregnancy test (pregnostx) of cattle and transabdominal scanning in determining pregnancy status in 30 sheep. The obtained results showed that the sensitivity of on-site pregnancy test (pregnostx) is 96.7% compared to ultrasonography scanner. The results obtained with the two tests are similar in early pregnancy diagnosis (17/17) at 25-35 days of pregnancy and in mid pregnancy (12/13) above 35-55 days of pregnancy.

We recommended that on-site pregnancy test (pregnostx) is a useful, rapid, and accurate tool for small farmers, who do not have the knowledge or experience in using ultrasonography, to detect early pregnancy diagnosis at 25-35 days of pregnancy and in mid-pregnancy above 35-55 days of pregnancy.

Keywords: Pregnancy Specific protein B (PSPB); Pregnancy -Associated Glycoprotein (PAGs); Ultrasonography; On-site Pregnancy test (Pregnostx)

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 sheep and goats is challenging, especially in the early stages. A reliable technique for detection of pregnancy in sheep & goats is emerging and enabling herdsman to separate non-pregnant sheep, 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 trophoblast 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.

Recently Biotracking, USA developed Pregnostx Cattle Pregnancy test (28 days post- breeding) which are ready for on-farm use and deliver fast and accurate pregnancy test results from a simple blood sample and it is also recommended for use on sheep and goats.

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

2. Material and Methods

2.1. Study area and period.

The study was conducted at private sheep farms at Dhurma which is a small town located 73.4 kilometres (45.6 mi) by road northwest of Riyadh, Saudi Arabia. It is the center of the small Dhumra Governorate of Riyadh Province,

2.2. On-site Pregnanact test (Pregnostx)

30 sheep were used in this study. The test was performed as per BioPRYN instructions. : Draw blood sample, shake tube 10 times, place blood into sample port, add 3 drops of chase and results will show after 30 minutes.(Fig.1 &2). Two lines in control and test samples is considered positive, while one line in control is defined as negative.



Figure 1 On-site pregnancy test. (pregnostx)



Figure 2 Pregnostx Test instructions

2.3. Ultrasonography for pregnancy evaluation

Transabdominal digital ultrasonic diagnostic imaging system iScan 2 (Fig.3) 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 sheep. Pregnancy was confirmed by observing gestational sac, fetus, fetal part (s), cotyledon etc.

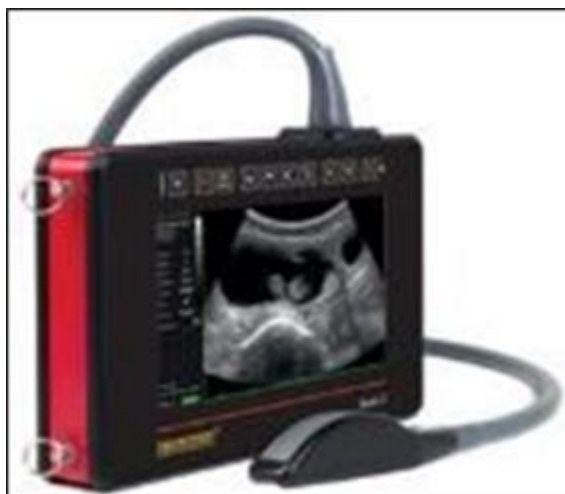


Figure 3 iScan2

3. Results

On-site pregnancy test results were illustrated in Table 1., Figure 4&5). The results showed animals defined as pregnant (P), non-pregnant, (N). Out of 30 tested samples 29 were positive (96.7%) and 1 is negative (3.7%)

Ultrasonography pregnancy results were illustrated in Figures 6,7,8

Comparison between on-site pregnancy test and ultrasound results were illustrated in Table 2 & Table 3

Early pregnancy from day 25 -35 days of pregnancy were detected in 16 out of 30 tested samples. While mid pregnancy above 35 days till 55 days of pregnancy were detected in 13 samples. One sample (No.10) is defined as non-pregnant by on-site pregnancy test, while it is identified as pregnant by ultrasonography.

Table 1 Results of on -site pregnancy rapid test

Sample ID	On-Site Rapid Test Result
1	P
2	P
3	P
4	P
5	P
6	P
7	P
8	P
9	p
10	N
11	P
12	P
13	P
14	P
15	P
16	P
17	P
18	P
19	P
20	P
21	P
22	P
23	P
24	P
25	P
26	P
27	P
28	P
29	P
30	P

Table 2 Comparative results of on-site pregnancy rapid test and ultrasound results

Sample ID	Rapid Test Result	Ultrasound Results
1	P	P (30 days)
2	P	P (45 days)
3	P	P (30 days)
4	P	P (30 days)
5	P	P (30 days)
6	P	P (40 days)
7	P	P (25 days)
8	P	P (30 days)
9	p	P (30 days)
10	N	P (50 days)
11	P	P (40 days)
12	P	P (30 days)
13	P	P (40 days)
14	P	P (25 days)
15	P	P (40 days)
16	P	P (30 days)
17	P	P (30 days)
18	P	P (25 days)
19	P	P (40 days)
20	P	P (50 days)
21	P	P (30 days)
22	P	P (55 days)
23	P	P (25 days)
24	P	P (35 days)
25	P	P (45 days)
26	P	P (45 days)
27	P	P (55 days)
28	P	P (35 days)
29	P	P (40 days)
30	P	P (30 days)

Comparison results of on-site pregnancy test and transabdominal pregnancy scanning is presented in Table 2 & Table 3. Out of the 30 sheep tested samples 29 were positive with the two tests while only one sample (sample No.10) was negative with on-site test and positive with ultrasonography test on day 50 of pregnancy. The pregnancy in on-site was detected by observing 2 lines in the control and test lines (Figures 4 ,5) , while in the ultrasonography test it was detected by observing the fetus (Figures , 6,7,8).

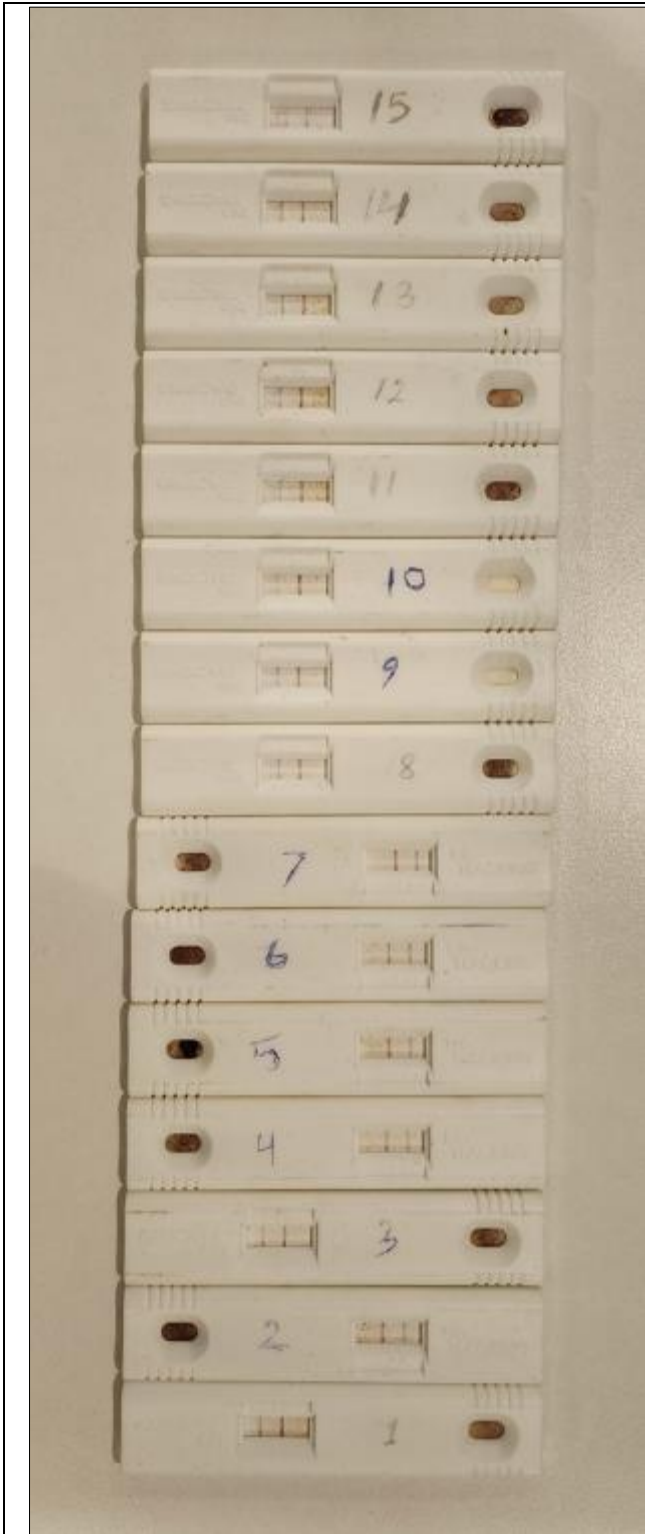


Figure 4 On -site pregnancy test



Figure 5 On -site pregnancy test

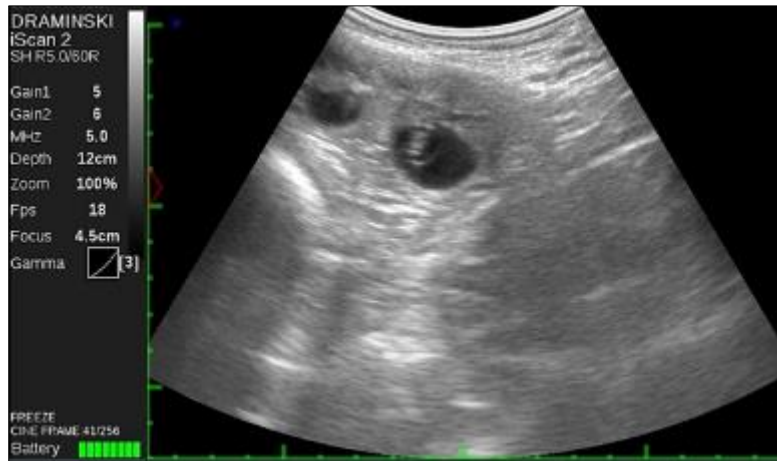


Figure 6 Fetus at 25 days of age

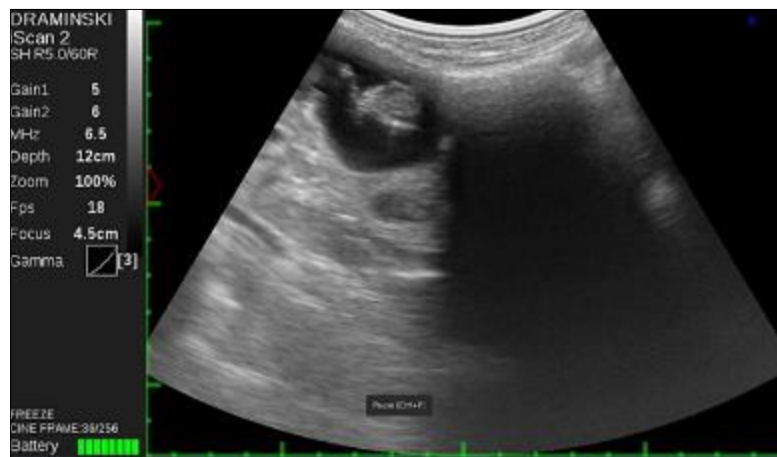


Figure 7 Fetus at 35 days of age

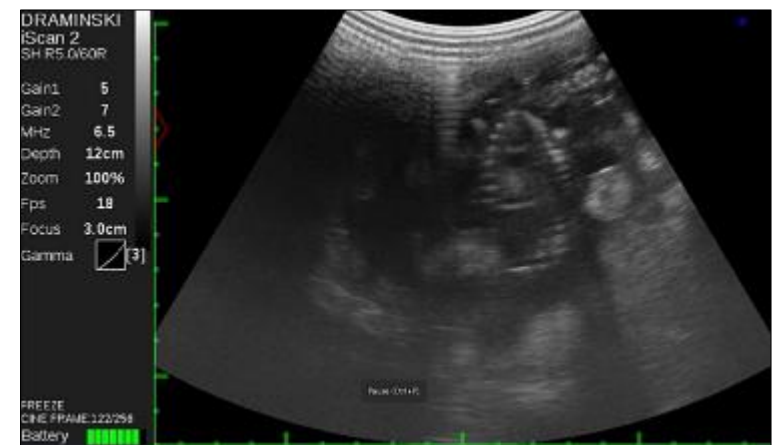


Figure 8 Fetus at 55 days of age

Table 3 Results comparison between on-site pregnancy test and ultrasound scanning at 25-35 days and above 35-55 days of pregnancy.

Days of testing	On -site pregnancy test	Ultrasound
25-35 days of pregnancy	17/17	17/17
Above 35-55 days of pregnancy	12/13	13/13

4. Discussion

On-site pregnancy test (pregnostx) manufactured by Biotracking, USA) was proven and trusted technology. It is recommended to be used 28-days post breeding in cattle for detection of PSPS protein. It was recommended to be utilized in the field and farms without access to laboratories.

Based on the recommendations of the manufacturer we used pregnostx in the field as rapid tool to detect early pregnancy in sheep and compared the results obtained with ultrasound scanning.

The authors showed that the sensitivity of on-site pregnancy test (pregnostx) is 96.7 % compared to ultrasonography scanner. The two tests give similar results at early 25-35 days of pregnancy and at mid-pregnancy above 35-55 days of pregnancy.

5. Conclusion

In conclusion, on-site pregnancy test (pregnostx) is more valuable and accurate in detecting pregnancy at early pregnancy at 25-35 days of pregnancy and in mid-pregnancy at stages above 35-55 days of pregnancy.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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