

Comparative study of pregnancy diagnosis in ewes (*Ovis aries*) using barium chloride and progesterone-kit

MH Rahman*, MN Munsi and S Akther

Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh

Abstract

One hundred and twenty sheep were used to test a low-cost technique for early diagnosis of pregnancy in sheep from July 2017 to June 2018. Different age groups were randomly categorized under four groups (15 to 30 days, 31 to 45 days, 46 to 60 days & 61 to 75 days). One mL barium chloride (1, 1.5 & 2%) was mixed with one mL of urine and kept for 5 to 10 minutes at room temperature: precipitation indicated non-pregnant. Three to five drops of serum were added to Bovipreg kit (progesterone-based kit) and left at room temperature for 5 minutes. One red line was interpreted as negative and two red lines positive for pregnancy. Barium chloride solution gave 67 - 70.5% accuracy. The accuracy for progesterone-based early pregnancy diagnostic kit was 80 - 85%. Progesterone-based early pregnancy diagnostic kit is much more accurate. (*Bangl. vet.* 2020. Vol. 37, No. 1 - 2, 36 - 41)

Introduction

In Bangladesh, sheep population is about 3.7 million (DLS, 2020) and farmers are interested to increase their productivity (Munsi *et al.*, 2018; Broom *et al.*, 2013). Pregnancy diagnosis is a part of reproductive management (Munsi *et al.*, 2018). Failure to detect early pregnancy causes huge economic losses (Lone *et al.* 2016). The early pregnancy diagnosis allows timely management correction (Camila de Miranda *et al.*, 2019). Due to poor management many lambs die (Commun *et al.*, 2016; Garcia-Ispierto *et al.*, 2013). Pregnancy diagnosis in small ruminants is difficult. Most of the techniques require sophisticated instrument and skilled personnel. Estimation of pregnancy based on hormonal assays of blood plasma, serum or milk, and estimation of pregnancy-specific antigens or proteins give a higher degree of accuracy within 30 days of pregnancy (Goel and Agrawal., 1992). Ultrasonic pregnancy detectors can detect pregnancy at 50-70 days post-breeding with over 90% accuracy. By laparoscopy it is possible to diagnose pregnancy at 30 - 40 days of gestation (Sing *et al.*, 2004; Goel and Agrawal., 1992). Between 60 and 80 days of gestation, an A-scan instrument is approximately 95% accurate (Haibel, 1990; Ishwar., 1995) and Doppler system is up to 100% accurate in diagnosing pregnancy from 60 days post-breeding (Keane, 1969; Shone and Fricker., 1969; Fraser and Robertson., 1968; Lindahl., 1968).

*Corresponding author:- E-mail: ratan.bau67@gmail.com

Determination of estrone sulphate provides a highly sensitive and specific pregnancy test for ewes 70- and 50-days post-breeding, respectively (Refsal *et al.*, 1991). Palpation of the uterus via laparotomy after Day 28 and Day 42 of gestation in ewes provides 92-100% accuracy, but has limited field application (Smith., 1980; Hulet and Foote., 1968). Abdominal palpation and ballottement are only effective during late pregnancy and do not give reliable information (Pratt and Hopkins., 1975). A low-cost method for detecting early pregnancy is the use of barium chloride solution in ewes (Ohazurike, 1990), pigs (Ndu *et al.*, 2000) and cattle (Elpakov and Tsyganok., 1966; Maslov and Smorno., 1965). This is a simple method where urine sample can be easily tested by use of barium chloride solution in short time. Considering the scenario of Bangladesh, barium chloride and serum progesterone assay kit were compared for early diagnosis of pregnancy in ewes.

Materials and Methods

Study area and period

This study was conducted at Sheep Research Farm and Small ruminants research laboratory of Goat and Sheep Production Research Division at Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh from July 2017 to June 2018.

Animal grouping

About 120 ewes were grouped into four according to their gestation length as group 1: 15 to 30 days gestation, group 2: 31 to 45 days gestation, group 3: 46 to 60 days gestation and group 4: 61 to 75 days gestation.

Sample collection

Urine samples of ewes were collected in the morning using collection bags. Blood (5 mL) was collected by jugular venepuncture and serum was separated from the clotted blood.

Pregnancy detection

The barium chloride solution of 1%, 1.5% and 2% were mixed with equal parts of urine and kept for 5 and 10 minutes at room temperature. Precipitates in the mixture indicated non-pregnancy.

Three to five drops of serum were poured into the Bovipreg kit (progesterone-based kit, TWil Canada, 25 South Bonnington Ave, Toronto, ON, Canada, M1N 3M2) and stood for five minutes at room temperature. A red line indicated negative pregnancy and two red lines confirmed pregnancy.

Results and Discussion

The study was undertaken to compare diagnosing pregnancy in ewes by barium chloride solutions, and a progesterone-based kit. In ewes 15 to 30 days after mating the accuracy of pregnancy diagnosis was 67%, 67.8% and 67% with 1%, 1.5% and 2%

barium chloride solution, respectively (Table 1). These results were compared with the Bovipreg kit where accuracy was 80%. At 31 to 45 days after mating 1%, 1.5% and 2% barium chloride solution showed 67.8%, 68% and 67.8% accuracy compared to 82% with progesterone kit (Table 2). In ewes 46 to 60 days after mating accuracy with 1%, 1.5% and 2% barium chloride solution were 68.8%, 69.3% and 67% compared to 83% with progesterone kit (Table 3). In ewes 61 to 75 days after mating accuracy with 1%, 1.5% and 2% barium chloride solution were 68.5%, 70.5% and 67% compared to 85% with progesterone kit (Table 4).

The barium chloride method showed 70.5%, 68.8% and 67.8% accuracy in pregnancy diagnosis in ewes, with 1.5%, 1% and 2%, respectively, which are inconsistent with Khora and Kaikini (1992) and Munsi *et al.* (2018), where 89% and 93.5% accuracy of pregnancy were confirmed. Ndu *et al.* (2000) used 1% barium chloride with 95% accuracy at about 39 days of pregnancy in sows. Balbin *et al.* (2020) found 62% accuracy in diagnosing pregnancy using 5% barium chloride solution in does. However, the accuracy of this test in cows was 70-95% (Maslov and Smirnov., 1965; Elpakov and Cyganok., 1966; Akmadeev and Vasilev., 1967) from 15 to 210 days of pregnancy. Kavani (1976) noticed only 64% accuracy with a high level of false positive and negative results. In camels, the test was considered to be 85% accurate between days 50-90 of pregnancy (Banerjee, 1974). Few studies had encouraging report of using barium chloride for pregnancy diagnosis in sheep and goat (Balbin *et al.*, 2020; Munsi *et al.*, 2018; Khora and Kaikini., 1992).

Table 1: Comparative results of Barium Chloride and Progesterone-based Kit pregnancy diagnosis in sheep 15 to 30 days after mating

Number of ewes	Barium chloride concentration and accuracy of pregnancy diagnosis						Progesterone-Based Kit (Diagnostic accuracy)
	1%		1.5%		2%		
30	5 min.	10 min.	5 min	10 min	5 min.	10 min.	5 min.
	66.5%	67.5%	68%	67.5%	67.5%	66.5%	80%
Mean	67%		67.8%		67%		-

Table 2. Comparative results of Barium chloride and progesterone-based Kit for the diagnosis of pregnancy in ewes 31 to 45 days after mating

Number of ewes	Barium chloride concentration and accuracy of pregnancy diagnosis						Progesterone-based Kit (Diagnostic accuracy)
	1%		1.5%		2%		
30	5 min.	10 min.	5 min	10 min	5 min.	10 min.	5 min.
	68%	67.5%	68.5%	67.5%	68.5%	67%	82%
Mean	67.8%		68%		67.8%		-

Table 3. Comparative results of Barium chloride and progesterone-based Kit used to diagnose pregnancy in ewes 46 to 60 days after mating

Number of ewes	Barium chloride concentrations and accuracy of pregnancy diagnosis						Progesterone-based Kit (Diagnostic accuracy)
	5 min.		10 min.		5 min.		
30	1%		1.5%		2%		5 min.
	70%	67.5%	70.5%	68%	67.5%	66.5%	83%
Mean	68.8%		69.3%		67%		-

Table 4. Comparative results of Barium chloride and progesterone-based Kit for pregnancy diagnosis in ewes 61 to 75 days after mating

Number of ewes	Barium chloride concentration and accuracy of pregnancy diagnosis						Progesterone - based Kit (Diagnostic accuracy)
	5 min.		10 min.		5 min.		
n = 30	1%		1.5%		2%		5 min.
	69%	68%	70%	71%	66.5%	67.5%	85%
Mean	68.5%		70.5%		67%		-

Conclusions

Barium chloride can be used for pregnancy diagnosis in ewes, but the progesterone-based kit is much more accurate.

References

- Akmadeev AN, Vasilev GT 1967: Results of a field trial of methods of diagnosing pregnancy in cows. *Veterinariya Moscow* **431** 77-78.
- Balbin AJ, Nayga JN, Gaffud OM, Marcos MJ, Sotelo EO, Marcelo DS 2020: Preliminary trial on the use of barium chloride for pregnancy diagnosis in goats. *Philippine Journal of Veterinary and Animal Sciences* **46** 82-86.
- Broom DM, Galindo FA, Murgueitio E 2013. Sustainable, efficient livestock production with high biodiversity and good welfare for animals. *Proceedings of the Royal Society B: Biological Sciences* **280** 2013-2025.
- Commun L, Velek K, Barbry JB, Pun S, Rice A, Mestek A, Egli C, Leterme S 2016. Detection of pregnancy-associated glycoproteins in milk and blood as a test for early pregnancy in dairy cows. *Journal of Veterinary Diagnostic Investigation* **28** 207-213.
- Department of Livestock Service (DLS) 2020: Livestock economy at a glance, Dhaka, Bangladesh pp. 1.

- de Miranda e Silva Chaves C, da Costa RL, Duarte KM, Beltrame RT, Quirino CR 2020: Evaluation of a cattle rapid test for early pregnancy diagnosis in sheep. *Tropical Animal Health and Production* **52** 1345-1349.
- Elpakov KA, Cyganok NS 1966: Pregnancy diagnosis in cows by the barium chloride reaction with urine. *Veterinariya* **43** 96-97.
- Fraser AF, Robertson JG 1968: Pregnancy diagnosis and detection of foetal life in sheep and pigs by an ultrasonic method. *British Veterinary Journal* **124** 239-44.
- García-Ispuerto I, S Almería, B Serrano, NM De Sousa, JF Beckers and F López-Gatius 2013: Plasma concentrations of pregnancy-associated glycoproteins measured using anti-bovine PAG-2 antibodies on day 120 of gestation predict abortion in dairy cows naturally infected with *Neospora caninum*. *Reproduction in Domestic Animals* **48** 613-618.
- Goel AK, Agrawal KP 1992: A review of pregnancy diagnosis techniques in sheep and goats. *Small Ruminant Research* **9** 255-264.
- Haibel GK 1990: Use of ultrasonography in reproductive management of sheep and goat herds. The veterinary clinics of North America. *Food Animal Practice* **6** 597-613.
- Hulet CV, Foote WC 1968: A rapid technique for observing the reproductive tract of living ewes. *Journal of Animal Science* **27** 142-145.
- Ishwar AK 1995: Pregnancy diagnosis in sheep and goats: a review. *Small Ruminant Research* **17** 37-44.
- Kavani FS 1976: Comparative study on the efficiency of different tests for early pregnancy diagnosis in cattle; MVSc Thesis; University of Udaipur, India.
- Keane M 1969: Pregnancy diagnosis in the sheep by an ultrasonic method. *Irish Veterinary Journal* **13** 67-72.
- Pratt MS, Hopkins PS 1975: The diagnosis of pregnancy in sheep by abdominal palpation. *Australian Veterinary Journal* **36** 57-66.
- Khora SC, Kaikini AS 1992: Studies on certain laboratory test for pregnancy diagnosis in buffaloes. *Indian Journal of Animal Reproduction* **13** 58-60.
- Lindahl IL 1968: Early pregnancy diagnosis in ewes in continual breeding. *Journal of Animal Science* **27** 1511.
- Lone SA, Gupta SK, Kumar N, Prakash K, Ganaie BA, Rather HA, Kumar S 2016: Recent technologies for pregnancy diagnosis in sheep and goat: An overview. *International Journal of Environmental Science and Technology* **3** 1208-1216.
- Maslov N, Smirnov A 1965: The simplest method for the early diagnosis of pregnancy. *Mol Mjasn Skotovod* **10** 24-25.
- Munsi MN, Akther S, Rahman MH 2017: A comparative study on pregnancy diagnosis in goats (*Capra hircus*) using barium chloride and progesterone based- kit. *Proceedings of the Annual Research Review Workshop, BLRI, Savar, Dhaka, Bangladesh*, December 06-07, pp. 359-366.
- Ndu A, Bratte, Eyoufe SU 2000: A preliminary trial on the use of barium chloride for pregnancy diagnosis in sows. *Tropicultura* **18** 161-163.

- Ohazurike EE 1990: Pregnancy diagnosis in ewes and goat does using urine and 2% barium chloride solution. *B Agric Research Project, Obafemi Awolowo University, Ile-Ife, Nigeria*, pp. 24.
- Refsal KR, Marteniuk JV, Williams CS, Nachreiner RF 1991: Concentrations of estrone sulfate in peripheral serum of pregnant goats: relationships with gestation length, fetal number and the occurrence of fetal death in utero. *Theriogenology* **36** 449-461.
- Singh NS, Gawande PG, Mishra OP, Nema RK, Mishra UK, Singh M 2004: Accuracy of ultrasonography in early pregnancy diagnosis in doe. *Asian-Australasian Journal of Animal Sciences* **17** 760-768.
- Shone DK, Fricker JW 1969: The diagnosis of pregnancy in the ewe with an ultrasonic foetal pulse detector. *Journal of the South African Veterinary Association* **40** 377-378.
- Smith MC 1980: *Caprine Reproduction*. In: Morrow, DA (Ed.), *Current Therapy in Theriogenology* (1st edn.). WB Saunders, Philadelphia, PA, pp. 975-977.