RESEARCH ARTICLE

Comparison of Different Techniques for Early Pregnancy Diagnosis in Surti Does

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Abstract

Thirty adult Surti goats which did not return to estrus after service by normal fertile bucks at induced estrus following different synchronization protocols were selected for early pregnancy diagnosis by three different techniques, *viz.,* serum progesterone levels on day 22, pregnancy associated glycoprotein (PAG) levels on day 22 and 26, and transabdominal ultrasonography (USG) on day 35 after breeding. The does were considered positive when the concentration of serum progesterone was over 1.0 ng/mL and PAG over 1.5 ng/mL. The mean serum progesterone levels on day 22 after breeding in goats diagnosed pregnant correct, pregnant incorrect and not-pregnant correct were 7.38±0.33, 5.00±0.00 and 0.65±0.00 ng/mL, respectively. The levels of PAG (ng/mL) in diagnosis pregnant correct and non-pregnant correct on day 22 and 26 were 3.09±0.06 and 0.54±0.5; 19.03±0.25 and 0.43±0.02, respectively. The mean level of serum PAG on day 22 in diagnosis not-pregnant incorrect was found to be 0.99±0.03 ng/mL. Out of 30 goats, 26 goats were found pregnant based on observations of foetus and heartbeats during ultrasonography on day 35, but kidding occurred in 28 does. The sensitivity, specificity, +ve predictive value, -ve predictive value and diagnostic accuracy of early pregnancy diagnosis by progesterone assay on day 22 were 100.00, 50.00, 96.55, 100.00 and 96.66 %, respectively. The corresponding percentages for PAG on day 22 were 92.86, 100.00, 100.00, 50.00 and 93.33, respectively. However, all these parameters were found 100 % on day 26. The percentage values of above parameters of USG for early pregnancy diagnosis on day 35 were 92.86, 100.00, 100.00, 50.00 and 93.33 %, respectively. It was concluded that the earliest pregnancy diagnosis can be done by estimation of PAG on day 26 with cent per cent accuracy. However, looking to the Indian goat owners' economic condition, ultrasonography is quick and safe for early pregnancy diagnosis.

Key words: Comparative efficacy, Early pregnancy diagnosis, Goats, Induced estrus, Serum PAGs, Serum progesterone, Ultrasonography,
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Introduction

Pregnancy maintenance is a clear economic factor in dairy animals since most of the pregnancy losses occur during the early stages of pregnancy (Commun *et al.,* 2016). An early and accurate pregnancy diagnosis is essential to improve reproductive efficiency (Cosentino *et al.,* 2018) as well as management of herd (Kaya *et al*., 2016). The economic value of early pregnancy detection is extremely important, since it is advantageous to be able to detect the pregnancy sooner in order to monitor detection of both unsuccessful mating and artificial insemination, identify and cull infertility cases, and help to resolve pregnancy-related problems (Al-Hassan and Al-Samawi, 2017). Successful detection of pregnancy at earliest period shortens the inter-parturition intervals and subsequently increases production, causing huge economic benefits (Dillon *et al.,* 2006; Diskin *et al.,* 2015).

Real-time ultrasonography is recommended as a reliable means of pregnancy detection as early as 26 days after conception in goats (Padilla-Rivas *et al.,* 2005). Transabdominal ultrasound scanning (US) is more accurate at Day 35 when the criterion for positive pregnancy diagnosis is based on the recognition of an embryo proper with movement or beating of the heart. Progesterone assay at day 21 post-mating (cut off value, 1 ng/mL can be used for pregnancy diagnosis in goats (Medan *et al.,* 2004). Gonzalez *et al.* (1999) demonstrated an improved RIA system for the detection of Caprine Pregnancy Associated Glycoprotein

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(caPAG) in the blood which can discriminate between a pregnant and a non-pregnant doe as early as Day 21 after breeding. The PAGs, also known under a variety of other names including pregnancy-specific protein B (PSPB), pregnancy-specific protein 60 and SBU-3 antigen, were first described as placental antigens that were also present in the blood serum of the mother soon after implantation (Sousa *et al.,* 2006). Use of plasma level of PAG as a biomarker for early pregnancy diagnosis is also reported in cattle (Commun *et al.,* 2016), sheep (El Amiri *et al.,* 2015) and goats (Sousa *et al.,* 2006; Shahin *et al.,* 2013). In goats, many methods have been applied for pregnancy diagnosis, including radiography, abdominal palpation, ultrasonography, and hormonal and PAG assays (Al-Hassan and Al-Samawi, 2017). The need for reliable, accurate, simple, inexpensive and easy-to administer methods of early pregnancy diagnosis became more important with the use of new breeding techniques. Therefore, the present investigation was made to compare efficacy of different techniques for early pregnancy diagnosis in Surti does.

Materials and Methods

The study was carried out at Goat farm, of Pashupalan Sanshodhan Kendra, Veterinary and Animal Science Research and Extension Unit, Kamdhenu University, Ramna Muvada-Gujarat, India during September 2021 to February 2022. Adult Surti goats were synchronized using different estrus synchronization protocols and 30 does that did not return to estrus after service by normal fertile Surti bucks were selected for early pregnancy diagnosis by three different techniques, *viz.,* by estimation of serum progesterone levels on day 22, estimation of serum pregnancy associated glycoprotein (PAG) levels on day 22 and 26 and transabdominal ultrasonography (USG) on day 35 post-breeding. Blood was collected from each doe on day 22 and 26. Separated serum was stored at -20°C till analysis.

Progesterone and PAG estimations were done by using RIA and ELISA techniques, respectively. The transabdominal ultrasonography (USG) was carried out as per Hesselink and Taverne (1994) using convex 3.5 MHz transabdominal transducer (Mindray, China). The sensitivity, specificity, +ve predictive value, -ve predictive value and diagnostic accuracy of early pregnancy diagnosis by three methods were calculated using standard formulae (Dogan and Kose, 2022). The data generated were analyzed using descriptive statistics.

Results And Discussion

Early Pregnancy Diagnosis in Surti Goats by Serum Progesterone Profile

The concentrations of serum progesterone on day 22 after breeding in goats diagnosed pregnant correct, pregnant incorrect and not-pregnant correct were 7.38±0.33, 5.00±0.00 and 0.65±0.00 ng/mL, respectively (Table 1). The higher level of progesterone found on day 22 in one goat, which was later on found non-pregnant may be due to an extended lifespan of corpus luteum for reasons other than the pregnancy. Indeed, P_4 levels higher than the threshold (1 ng/mL) on day 22 indicate a functional CL which is associated either with pregnancy, irregular length of the estrous cycle, early embryonic death, hydrometra, or luteal cysts. In the present study, sensitivity, specificity, +ve predictive value, -ve predictive value and diagnostic accuracy of early pregnancy diagnosis by progesterone assay on day 22 were 100.00, 50.00, 96.55, 100.00 and 96.66 %, respectively (Table 2). The sensitivity, positive predictive value, negative predictive value, and accuracy determined by Dogan and Kose (2022) on day 28 after breeding were lower than the current findings, while specificity found by them was higher than the current study.

Early Pregnancy Diagnosis by Serum PAGs Profile

The mean diagnostic values of serum pregnancy associated glycoproteins (PAGs, ng/mL) in diagnosis pregnant correct and diagnosis not-pregnant correct on day 22 and 26 were 3.09±0.06 and 0.54±0.5; 19.03±0.25 and 0.43±0.02, respectively. The mean value of serum pregnancy associated glycoprotein on day 22 in diagnosis not-pregnant incorrect was found to be 0.99±0.03 ng/mL (Table 1). However some workers Gonzalez *et al.* (2004); Chentouf *et al.* (2008) and Al-Hassan and Al-Samawi (2017) reported lower values of PAG on day 21 and day 20 than the current reports. Zamfirescu *et al.* (2011) reported increased level of PAGs upto 20 ng/

Table 1: Serum progesterone and pregnancy associated glycoprotein levels of pregnant/non-pregnant Surti goats on different days after breeding

Diagnostic results	Progesterone (ng/mL)		Pregnancy Associated Glycoprotein (ng/mL)			
	Day 22		Day 22		Day 26	
	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range
Diagnosis pregnant correct (a)	7.38±0.33 (n=28)	4.15 – 10.50	3.09±0.06 (n=26)	2.06 – 3.56	19.03±0.25 (n=28)	16.97 – 21.00
Diagnosis pregnant incorrect (b)	5.00 (n=01)	5.00	-	-	-	-
Diagnosis not pregnant correct (c)	0.65 (n=01)	0.65	0.54±0.05 (n=02)	0.48 – 0.59	0.43±0.02 (n=02)	0.41 – 0.45
Diagnosis not pregnant incorrect (d)	-	-	0.99±0.03 (n=02)	0.96 – 1.01	-	-

mL during first month of pregnancy, which concurred well with the current finding of day 26. The placental binucleate cells, the major source of maternal PAG, are first observed at day 18 of pregnancy and the relative cell number increases rapidly from day 19 (less than 1-16 %) to day 23 (about 22 %) of pregnancy (Wango *et al.,* 1990). In two cases, the lower values of PAG observed on day 22 indicated non-pregnant does, but later values went high on day 26 indicating pregnancy, which were also confirmed at kidding. Lower values of PAG on day 22 may be due to the less synthesis/secretion of pregnancy associated glycoproteins from binucleate cells of trophoblast.

The sensitivity, specificity, positive (+ve) predictive value, negative (-ve) predictive value and diagnostic accuracy of early pregnancy diagnosis by analysis of PAGs on day 22 were 92.86, 100.00, 100.00, 50.00 and 93.33 %, respectively. However, the values of all these parameters found were 100 % on day 26 (Table 2). The sensitivity of pregnancy diagnosis by pregnancy associated glycoprotein obtained by Dogan and Kose (2022) on day 28 was 94.12 %, which was closely in agreement with current findings of day 22 and 26, however, their accuracy of pregnancy diagnosis was lower. The specificity and positive predictive value of current findings of day 22 and 26 were higher, while negative predictive value on day 22 was lower than the finding of Dogan and Kose (2022) on day 28.

Early Pregnancy Diagnosis in Surti Goats by Ultrasonography

Out of 30 scanned goats for early pregnancy by trans-abdominal ultrasonography (Fig.1), 26 goats were found pregnant based on observations of foetus and foetal heartbeats, but kidding occurred in 28 does. Two goats kidded were not determined pregnant during ultrasonographic scanning on day 35 may be due to the poor visibility or folds of uterus during scanning. The sensitivity, specificity, +ve predictive value, -ve predictive value and diagnostic accuracy of ultrasonography for early pregnancy diagnosis on day 35 after breeding were 92.86, 100.00, 100.00, 50.00 and 93.33 %, respectively (Table 2). The observed sensitivity of trans-abdominal ultrasonography for early pregnancy diagnosis on day 35 in present study was higher than the finding of Karen *et al.* (2003), which was 41.90 and 51.70 % on day 36 and 50, respectively. However, in another experiment carried out by them, higher sensitivity reported on day 34 (98.50%) was due to the fasting and lifting of abdomen during transrectal ultrasonographic scanning. In without fasting ultrasound scanning, the intestinal gas or ingesta may have interfered with the visualization of the pregnant uterus. The present sensitivity was almost similar with the observations made by Singh *et al.* (2004) and Khalif *et al.*

Fig. 1: Ultrasonography for pregnancy diagnosis at day 35 post-breeding

Table 2: Sensitivity, specificity and accuracy of early pregnancy diagnosis by profile of progesterone and pregnancy associated glycoprotein and ultrasonography (n=30)

Diagnostic results / Predictive values	Progesterone (Day 22)	Pregnancy Associated Glycoprotein		USG (Day 35)
		Day 22	Day 26	
Diagnosis pregnant correct (a)	28	26	28	26
Diagnosis pregnant incorrect (b)	01	00	00	00
Diagnosis not pregnant correct (c)	01	02	02	02
Diagnosis not pregnant incorrect (d)	00	02	00	02
Sensitivity (%) = 100 x a/(a+d)	100.00	92.86	100.00	92.86
Specificity (%) = 100 x c/(b+c)	50.00	100.00	100.00	100.00
+ve predictive value (%) =100 x a/(a+b)	96.55	100.00	100.00	100.00
-ve predictive value (%) = 100 x c/c+d)	100.00	50.00	100.00	50.00
Diagnostic accuracy (%) = 100 x (A+C)/(A+B+C+D)	96.66	93.33	100.00	93.33

(2014) on days 24-26 and 18-50 of breeding, respectively. The specificity in present study on day 35 was higher than that (88.70%) recorded by Karen *et al.* (2003) on day 36. Specificity obtained by Singh *et al.* (2004) and Khalif *et al.* (2014) also concurred with current findings. Positive predictive value found in current experiment was higher than that (48.10%) reported by Karen *et al.* (2003), however, it corroborated with the finding of Khalif *et al.* (2014). Negative predictive value found in present study was lower than 99.50 and 86.00 % reported by Karen *et al.* (2003) and khalif *et al.* (2014) on day 34 and 18-50, respectively. Khalif *et al.* (2014) and Anya *et al.* (2017) reported 96 and 100 % accuracy of ultrasonography, respectively, for early pregnancy diagnosis, which almost corroborated with our results.

Conclusions

Determination of progesterone on day 22 and pregnancy associated glycoprotein on day 26 after breeding in serum samples provide very accurate pregnancy diagnosis in Surti goats. Estimation of hormone particularly progesterone on day 22 after breeding is cent per cent accurate in detection of non-pregnancy. B-mode transabdominal ultrasonography was found to be useful, reliable, safe, accurate and practicable means in diagnosing pregnancy at day 35 post-breeding.

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