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RESEARCH ARTICLE

Temporal Changes in Physical Signs of Estrus and Validation of Fetal Parameters for Estimation of Gestational Stage through B-mode Ultrasonography in Beetal goats

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ABSTRACT

The current study evaluates changes in the physical signs of estrus (vulvar hyperemia, edema and mucous discharge) from their onset until ovulation, and validates fetal parameters for the estimation of gestational stage in Beetal goats. In experiment 1, temporal changes in estrus signs were monitored subjectively after the administration of two injections of $PGF_{2\alpha}$ at 10-days interval and a cumulative behavioural index (BI) was calculated in goats that showed estrus response (n=7). Ovulation occurred after 18.8±2.3 h relative to peak vulvar hyperemia and edema; ovulation coincided with maximum mucous discharge (0±2.4 h). Maximum BI was observed between 7 to 31 h after the onset of estrus and was highly correlated with vulvar hyperemia (r=0.94), followed by vulvar edema (r=0.90) and mucous discharge (r=0.85). In experiment 2, fetal parameters were monitored in pregnant goats (n=7) via ultrasonography for estimation of gestational stage, and for validation in a separate set of goats (n=20; experiment 3). Gestational stage correlated significantly (P<0.05) with the uterine diameter (UD), crown-rump length (CRL), trunk diameter (TD), intercostal space (ICS); diameter of amniotic vesicle (AV), biparietal diameter (BPD), placentome diameter (PD) and umbilical cord (UC) diameter. Validation revealed the difference between the actual and estimated day of gestation was less for TD than BPD (4.1±0.6 vs 6.8±1.0 days; P<0.05). In conclusion, vulvar hyperemia, edema, and mucous discharge are highly correlated with BI. Fetal parameters are strongly associated with the gestational stage, and the estimation of the actual gestational stage is less variable through TD than BPD in Beetal goats.

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INTRODUCTION

In domestic animals, onset of estrus is marked by behavioural and physical signs. Goats exhibit behavioural (standing estrus, tail wagging, bleating, male seeking, restlessness, frequent urination) and physical signs (vulvar hyperemia, vulvar edema, and mucous discharge) during estrus (Rahman *et al.,* 2008). Depending upon the season, geographical location and herd size, intensity of behavioural and physical signs of estrus vary in goats. In general, the onset of standing estrus is considered a benchmark and has been related to ovulation and insemination in goats (Hameed *et al.,* 2020; Murtaza *et al.,* 2020). Likewise, consistency of vaginal mucus has been related to the onset of estrus and pregnancy rate in goats (Siqueira *et al.,* 2009). In general, there is limited information available about the association of various behavioural and physical signs of estrus with time of ovulation in goats. Consequently, goat breeding becomes a challenging task in countries where the majority of farmers have fewer stocks of animals. In Pakistan, 46% of the total goat population is reared by small and marginal farmers having a flock of fewer than 15 animals (Khan and Usmani, 2005). Therefore, understanding the association of physical signs of estrus with the standing estrus and ovulation will benefit these farmers for optimizing goat breeding plan.

Alternatively, random mating occurs in large flocks, and owners are unaware of the gestational stage of a goat. Hence, early pregnancy diagnosis and estimation of fetal number and gestational stage become critical benchmarks for efficient goat breeding and management (Haibel, 1990). In this context, monitoring of fetal age and growth through real-time, B-mode ultrasonography is employed for accurate estimation of kidding dates and devising an optimum feeding plan for pregnant goats (Jones and Reed, 2017). Besides, it minimizes the chances of neonatal loss and obstetrical complications in goats. Several goat breeds like Shiba (Kandiel *et al.*, 2015), Bulgarian (Karadaev *et al.*, 2018) and cross-bred goats (Gosselin *et al.*, 2018) have been studied for the estimation of gestational stage through B-mode, real-time ultrasonography. For this purpose, various fetal parameters have been monitored in small ruminants (Reichle and Haibel, 1991; Santos *et al.*, 2018) to determine the most reliable estimator of gestational stage. However, marked variations exist in reports regarding the appraisal of gestational stage or days to kidding because of differences in breeds and regression equations used (Gosselin *et al.*, 2018).

Therefore, objectives of the current study were: 1) to evaluate changes in the physical signs of estrus (vulvar hyperemia, vulvar edema, and mucous discharge) from their onset until ovulation, 2) to monitor growth of different fetal parts as a potential estimator of gestational stage, and 3) to validate various fetal parameters for accurate estimation of gestational stage in Beetal goats.

MATERIALS AND METHODS

Experiment 1: Physical signs of estrus relative to the onset of standing estrus: Physical signs of estrus relative to the onset of standing estrus were evaluated during the breeding season (September-October) in Beetal goats kept at the Small Ruminant Training and Research Center, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan. All procedures performed during the study were as per the guidelines of the Animal Care and Ethical Review Committee of the University. Eight nulliparous Beetal goats, 2.3±0.1 years old with average body condition score of 2.1±0.2 (scale 1-5) were given two doses of $PGF_{2\alpha}$ (Dalmazine®, FATRO, Italy) i.m., 0.075 mg each, 10-days apart during the breeding season. Physical signs of estrus (vulvar edema, vulvar hyperemia, and mucous discharge) and duration of standing estrus were monitored every 12 h after the administration of 2nd $PGF_{2\alpha}$ until 144 h.

Vulvar hyperemia, vulvar edema and mucous discharge were scored subjectively by a single person. Grading of vulvar hyperemia (1=pale; 2=pink; 3=reddish), vulvar edema (1=absent; 2=slight; 3=intense) and mucous discharge (1=absent; 2=scanty, 3=wet; 4=flowing) was done (Fig. 1), as reported previously (Ola and Egbunike, 2004). Single buck was used to determine the onset of standing estrus and for goat breeding. B-mode, real-time ultrasonography was performed every 12 h after the onset of estrus until ovulation which was characterized by sudden disappearance of the largest follicle seen at the previous ultrasound examination. The mean diameter of preovulatory follicle, time of ovulation and pregnancy rate (20-35 days after breeding) were monitored by a 7.5 MHz transrectal probe (HS 1500®; Honda, Japan). Behavior index (BI) was calculated based on the cumulative score of physical signs from 12 to 144 h after 2nd $PGF_{2\alpha}$ injection.

Experiment 2: Estimation of gestational stage through fetal biometry: The pregnant goats (n=7) of the first experiment were used in the second experiment. Goats were naturally bred and the day of breeding was considered as Day 0. During the initial 20-35 days after breeding, goats were scanned on every fifth or seventh day by using a transrectal transducer (7.5MHz) for early pregnancy diagnosis and embryonic development. Pregnant goats were scanned on every fifth-seventh day from 35 days post-breeding using a transabdominal transducer (3.5-5.0 MHz) until kidding. Fetal amniotic vesicle (AV) diameter, uterine diameter (UD), biparietal diameter (BPD), crown-rump length (CRL), femur length (FL), placentome diameter (PD) (Karen *et al.,* 2009), intercostal space (ICS), umbilical cord (UC) diameter and trunk diameter (TD) (Gosselin *et al.,* 2018) were measured using B-mode; heart rate (HR) was measured using M-mode ultrasonography. All parameters were correlated with the duration of pregnancy.

Experiment 3: Validation of fetal parameters for the estimation of gestational stage: Based on the values of coefficients of determination (R^2) of significantly correlated fetal parameters, a highly correlated (R^2>0.90) and a moderately correlated (R^2<0.90) estimator (Karen *et al.,* 2009) of gestational stage were further validated by entering the equations of each estimator into the ultrasound machine. For this purpose, the single-blind approach was adopted where the insemination dates of goats (n=20) were unknown to the operator. A comparison of the estimated and known day of gestation was made for the validation of the estimators.

Statistical analysis: Data were analyzed by using statistical software (SPSS, version 20.0, IBM Corp. Armonk, USA). A correlation was determined between each physical sign and BI using the correlation matrix. A regression equation was derived for the calculation of gestational stage based on the changes in fetal parameters over gestational days. The relationship between the actual and estimated day of gestation was determined using a correlation matrix. The comparison between the actual and estimated day of gestation for a given estimator was done through an independent student's t-test.

RESULTS

Experiment 1: Estrus response, signs of estrus and time of ovulation: On the average, the goats exhibited estrus after 41.0±4.4 h of 2nd $PGF_{2\alpha}$ injection (estrus response: 88%; n=7/8), while duration of standing estrus averaged 17.0±2.4 h. The interval to ovulation from 2nd $PGF_{2\alpha}$ injection and end of standing estrus averaged 78.8±4.4 h and 20.5±7.2 h, respectively. The mean diameter of preovulatory follicle was 7.0±0.3 mm and on an average 1.4±0.2 follicles ovulated.

A marked increase in vulvar hyperemia (score: 2.6; reddish) and edema (score: 2.6; intense) was first observed at 7.0 h after estrus onset; the mean interval was 19±7.2 h. Similarly, marked mucous discharge (score: 3.7; flowing) was first observed at 31 h after estrus onset, the mean interval being 37±7.7 h. The overall duration of vulvar edema and hyperemia was 24 h, whereas that of mucous discharge was 36 h. Relative to mean time of maximum vulvar hyperemia and edema, ovulation occurred after 18.8±2.3 h; while ovulation coincided with mean maximum mucous discharge (0±2.4 h; Fig. 2). Maximum BI was observed between 7 to 31 h after estrus onset. The vulvar hyperemia (r= 0.94), vulvar edema (r=0.90) and mucous discharge (r=0.85) correlated significantly (P<0.05) with BI.

Experiment 2: Estimation of gestational stage through fetal parameters: Initially, there was a slight change in uterine diameter (UD) by 22 days post-breeding without the presence of embryonic fluids. However, the mean UD increased significantly (P<0.05) from day 27 onwards until the day 65 (~ 3 months; Table 1) post-breeding and was undetectable beyond this point. The uterine diameter was significantly (R^2=0.91, P<0.05) correlated with gestational stage for the observed period (Table 2). Similarly, the amniotic vesicle (AV) diameter was significantly (R^2 = 0.83, P<0.05) correlated with gestational stage of fetus from day 27 to 65 post-breeding (Table 2). However, it was not often detectable beyond 48 days of gestation (Table 1). The fetal heart rate (HR) decreased along with the gestational stage from 3rd month onward and had a medium value of the coefficient of determination (R^2 = 0.76, P<0.05; Table 2). HR was detectable through transrectal transducer as early as 27 days post-breeding onwards until parturition (~5 months; Table 1). The biparietal diameter (BPD) was measurable from days 34 to 125 post-breeding (~4 months) and increased (P<0.05) from 2 to 4 months of gestation; it was occasionally detected during the last month of pregnancy in two goats (Table 1). BPD was significantly (P<0.05) correlated with gestational stage and the coefficient of determination (R^2) was 0.83 (Table 2).

The crown-rump length (CRL) was only measurable between day 34 to 65 post-breeding (Table 1). Regression equation (R^2=0.93; Table 2) of CRL showed significantly high (P<0.05) correlation with gestational stage. The trunk diameter (TD) increased linearly and showed a significantly high (P<0.05) correlation (R^2= 0.95; Table 2) with gestational stage. The period of detection ranged from days 34 to 132 post-breeding (Table 1). The placentomes could be detectable by day 41 post-breeding until kidding and their diameter increased significantly (P<0.05) with gestational stage (Table 1). There was significant correlation between PD and gestational stage (R^2=0.84, P<0.05) and the linear equation was derived by plotting polynomial curve (Table 2). The diameter of umbilical cord (UC) correlated significantly with gestational stage (R^2=0.87; P<0.05, Table 2); it increased along with gestational stage and was measurable between days 41 to 120 post-breeding (Table 1).

The intercostal space (ICS) and gestational stage were significantly correlated (R^2=0.93; P<0.05) and the linear equation was derived by plotting polynomial curve (Table 2). ICS was measurable from day 48 onwards until kidding and it increased linearly (Table 1). The relationship between the width of six consecutive fetal intercostal spaces and gestational stage was highly significant (R^2=0.95; P<0.05; Table 2). An increase in the width of consecutive intercostal spaces was measurable from day 48 onwards until kidding (Table 1). The femur length (FL) increased along with gestational stage and had a moderate value of coefficient of determination i.e., R^2=0.83; P<0.05 (Table 2). The FL was measurable between days 48 and 100 post-breeding (Table 1). Representative sonograms of various fetal parameters have been shown in Fig. 3.

Table 1: Fetal parameter (mean ± SEM) relative to the stage of gestation (months) in Beetal goats

Fetal parameters (mm)	Stage of gestation (months)				
	1	2	3	4	5
Uterine diameter	17.0±10.0^a	47.0±15.7^b	95.3±5.3^c		
Amniotic vesicle diameter	10.5±1.6^a	34.9±11.9^b			
Heart rate (beats/min)	201.2±27.4^{ab}	216.4±11.6^a	205.0±13.9^b	178.1±15.8^c	148.8±7.2^d
Biparietal diameter		15.3±4.6^a	29.4±4.9^b	41.5±6.2^c	48.1±5.7^c
Crown-Rump length		45.2±27.3^a	104.4±11.7^b		
Trunk diameter		15.6±5.6^a	36.5±8.9^b	56.7±6.5^c	68.4±3.4^d
Placentome diameter		11.4±3.1^a	22.2±3.6^b	27.9±3.8^c	32.1±2.4^d
Umbilical cord diameter		4.9±2.7^a	10.1±1.9^b	13.5±2.0^c	
Inter costal space		2.6±0.9^a	5.7±1.5^b	12.8±3.9^c	20.9±2.8^d
Width of 6 consecutive intercostal spaces		10.0±3.1^a	24.3±7.1^b	43.7±10.5^c	65.4±3.5^d
Femur length		8.5±3.6^a	15.9±15.0^b	30.1±4.7^c	

Values with different superscripts within row show significant difference (P<0.05).

Table 2: Regression coefficients of the various fetal parameters indicating the relationship with gestational stage in Beetal goats

Fetal Parameters	Regression Equation	R^2	Obs.
Uterine diameter*	y = 1.7383x - 27.79	0.91	35
Amniotic vesicle diameter *	y = 1.4699x - 26.71	0.83	23
Heart rate*	$y = 9E\text{-}05x^3 - 0.0311x^2 + 2.3678x + 163.8$	0.76	99
Biparietal diameter*	y = 0.4874x - 8.29	0.83	61
Crown-Rump length*	y = 2.894x - 85.02	0.93	29
Trunk diameter*	y = 0.6495x - 13.39	0.95	91
Placentome diameter*	$y = -0.0021x^2 + 0.6022x - 12.16$	0.84	86
Umbilical cord diameter*	$y = -0.001x^2 + 0.3273x - 9.01$	0.87	55
Intercostal space*	$y = 0.0017x^2 - 0.0829x + 1.99$	0.93	83
Width of 6 consecutive* intercostal space	y = 0.6953x - 28.65	0.95	80
Femur length*	y = 0.501x - 19.33	0.83	30

y = Fetal parameter (mm); x = gestational age (days); R^2 = coefficient of regression/determination; Obs. = no. of observations; *Indicates the significant (P<0.05) correlation with gestational stage.

Fig. 1: Representative pictograms of the changes in vulvar hyperemia, edema and mucous discharge in Beetal goats after 2nd $PGF_{2\alpha}$ injection. For mucous discharge "score 2" is not shown but was subjectively characterized as scanty.

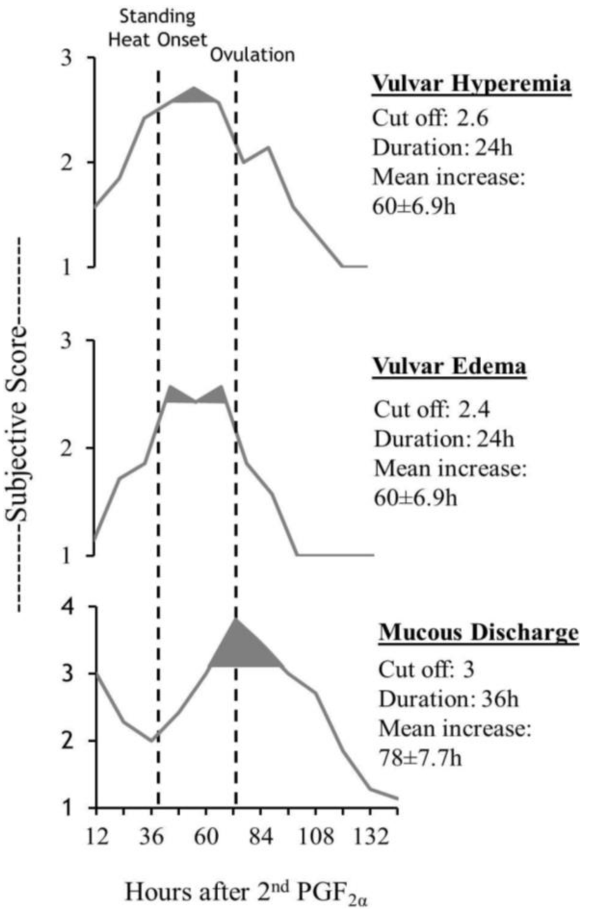

Fig. 2: Initial marked increase (shaded area) in vulvar hyperemia, edema and mucous discharge in Beetal goats (n=7) relative to 2nd $PGF_{2\alpha}$, the onset of standing estrus, and ovulation. The base of the shaded area represents cut off value for the given physical sign. Ovulation occurred 18.8±2.3 h after mean marked vulvar hyperemia and edema; ovulation corresponded (0±2.4h) with mean marked mucous discharge.

Experiment 3: Validation of fetal parameters (TD and BPD): The correlation between actual and estimated day of gestation was greater for the TD as compared to BPD (R^2=0.97; P<0.05 vs R^2 = 0.88; P<0.05, respectively). For each estimator, mean value between the actual and estimated day of gestation did not differ (P>0.05); however, the difference between the actual and estimated day of gestation was less for TD than BPD (4.1±0.6 vs 6.8±1.0 days; P<0.05, respectively). The variation in the estimation of actual gestational stage for TD and BPD during different periods of gestation is shown in Fig. 4.

DISCUSSION

There are limited studies on the intensity of various physical signs during estrus in goats; documenting equivocal findings (Ola and Egbunike, 2004; Kenfack *et al.*, 2013). The current study evaluates temporal changes in physical signs of $PGF_{2\alpha}$ induced estrus in Beetal goats. The behavior index (BI) was highly correlated with vulvar hyperemia, edema and mucous discharge in does. In comparison to the West African Dwarf does, where maximum mucous discharge was seen 12 h after the onset of estrus (Kenfack *et al*., 2013), the mucous discharge in Beetal goats attained peak score after the end of standing estrus coinciding with ovulation. These variations in physical signs of estrus may be related to the breed differences and subjective assessment. Although the subjective assessment of physical parameters of estrus is considered less reliable, it could provide useful information about the limited number of closely observed animals kept as pets for domestic consumption in periurban areas. To eliminate the subjective bias and least relevant physical parameter of estrus in goat, BI was calculated as an alternative indicator of estrus. Consequently, mucous discharge turned out to be a highly variable and least correlated parameter with BI in Beetal does during estrus. These findings are concurrent with a previous report in Beetal goats, where the cumulative mucus index was calculated excluding the volume of mucous discharge due to the variability and subjective bias (Murtaza *et al.,* 2020). Therefore, we recommend BI as a reliable measure of estrus detection in Beetal does based on vulvar hyperemia and edema.

The current study also evaluated the scope of various fetal structures as an estimator of gestational stage in Beetal goats. Although there have been reports documenting the various fetal parameters for the estimation of gestational stage through ultrasonography (Gosselin *et al.,* 2018; Karadaev *et al.,* 2018); however, none of these studies validated the fetal parameters for the estimation of gestational stage in goats. In the current study, we validated two fetal parameters i.e., TD and BPD for the estimation of gestational stage in a set of goats where dates of inseminations were unknown to the operator (a single-blind approach). Both TD and BPD were selected based on their high and moderate value of the coefficient of determination with gestational stage, respectively. In comparison to BPD, TD had a maximum window to assess the stage of gestation. Despite this fact, the previous study had considered BPD as the most common indicator of gestational stage in goats (Karadaev *et al.,* 2018). However, the data obtained in the current study revealed that TD was a comparatively more reliable estimator of gestational stage than BPD in Beetal does as the difference between the actual and estimated day of gestational stage was minimum for TD than BPD. This difference was expected as scanning of the fetal head becomes difficult in later stages of gestation, resulting in variability in measurements (Karadaev *et al.,* 2018).

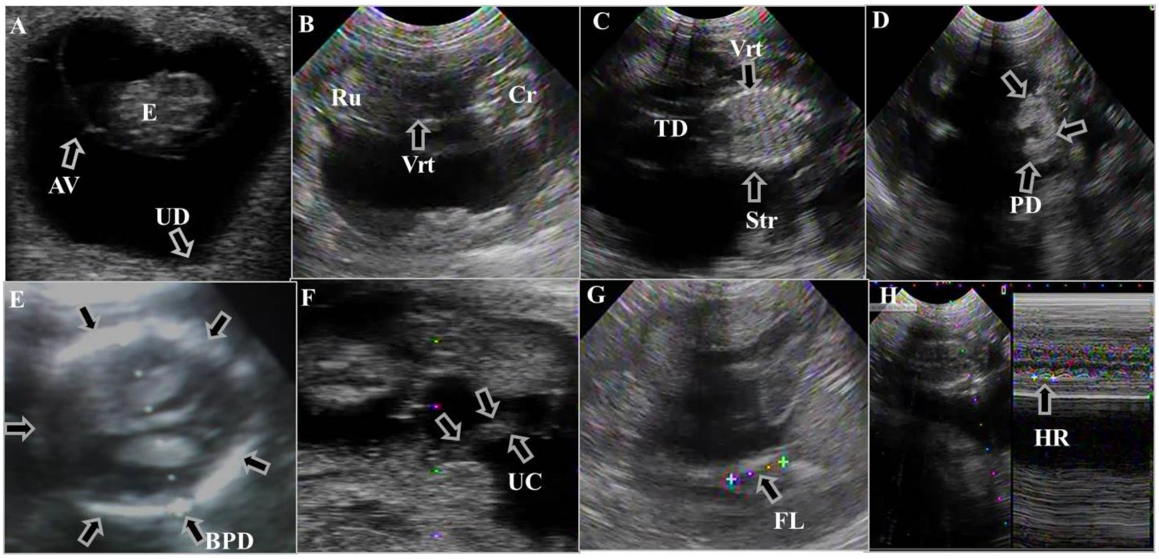

Fig. 3: Ultrasound images showing different fetal parameters: A) UD = Uterine Diameter, E= Embryo, AV= Amniotic vesicle; B) CR= Crown, Ru= Rump length, Vrt= Vertebral column; C) Vrt= Vertebral column, Str= Sternum, TD = Trunk diameter; D) PD = Placentome diameter; E) BPD= Biparietal diameter; F) UC= Umbilical cord diameter; G) FL= Femur length; H) HR= Heart rate.

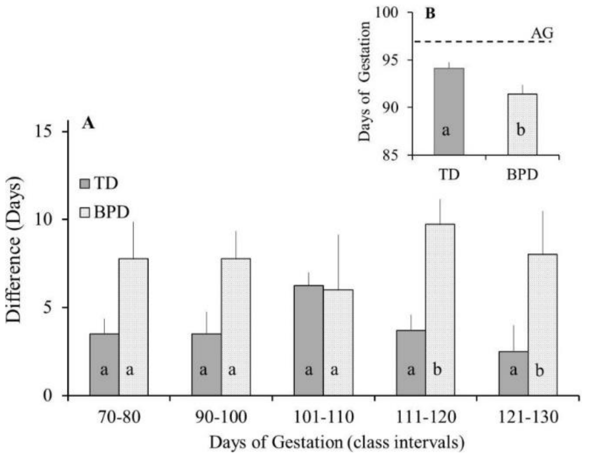

Fig. 4: Difference in the estimation of gestation using trunk (TD) and biparietal diameter (BPD) at different intervals of gestational stage (A). The estimation of actual gestational day (AG; dotted line) was more accurate ($P<0.05$) through TD than BPD (B). Different letters within each class interval of gestational days indicate significant difference ($P<0.05$) between TD and BPD.

In the present study, CRL, ICS, and TD had a high coefficient of determination ($R^2>0.90$) in Beetal goats. These findings are in agreement with the previous reports documenting a higher coefficient of determination ($R^2>0.90$) for such parameters in goats (Martinez *et al.*, 1998; Abdelghafar *et al.*, 2011). Despite a strong correlation with gestational stage, CRL has limited chances of detection after 65 days of gestation, as the fetal size exceeds the viewing plane. On the other hand, ICS and TD are detectable for a longer duration of gestation; hence, both parameters can be used as reliable estimators of gestational stage in goats. The BPD, UC and PD had moderate coefficient of determination ($R^2<0.90$) in Beetal goats. These findings are in agreement with previous reports documenting coefficient of determination ($R^2<0.90$) for BPD, UC, and PD in goats (Lee *et al.*, 2005; Nwaogu *et al.*, 2010). Previously, BPD has been considered as the most common estimator, having a strong correlation with gestational stage (Karadaev *et al.*, 2018); however, it's efficacy during later stages is highly variable (Lee *et al.*, 2005). Similar to BPD, PD has also been found to have a variable correlation with gestational stage (Karen *et al.*, 2009), in part due to variation of their sizes at different portions of uterus (Kandiel *et al.*, 2015). In the current study, FL ($R^2=0.83$) and AV ($R^2=0.83$) had moderate coefficients of determination and were comparable to a previous report documenting a moderate coefficient of determination for FL ($R^2=0.78$) and AV ($R^2=0.76$) in ewes (De-Bulnes *et al.*, 1998). In contrast, other researchers have reported a high coefficient of determination of FL ($R^2=0.90$) in goats (Karen *et al.*, 2009). Apart from the breed difference, the disagreement between the findings could be due to the difficulty in visualizing the transversal or longitudinal images of FL at later stages of gestation.

In the current study, HR had least coefficient of determination ($R^2=0.76$) in Beetal goats, partly due to a decrease in heart rate with the advancement of gestation. A similar decreasing trend in HR was reported in another goat breed between Days 21 and 40 of gestation (Martinez *et al.*, 1998). Moreover, a low value of coefficient of determination for HR has been reported in goats (Karen *et al.*, 2009), cattle ($R^2=0.49$; (Kähn, 1989) and sheep ($R^2=0.27$; (Aiumlamai *et al.*, 1992). Nonetheless, the detection of the embryo with a heartbeat is considered a positive sign of early pregnancy in goats. In the current study, embryonic heartbeat was detected relatively later (on day 27 post-breeding) than previous studies reporting the detection of heartbeat as early as days 22.9 (Padilla-Rivas *et al.*, 2005) and 24 of gestational stage (Medan *et al.*, 2004). This variability may be due to the differences in frequency of ultrasound probe, operator's skills, and goat breed.

Conclusions: In conclusion, vulvar hyperemia, edema, and mucous discharge are highly correlated with BI. Beetal goats can be accurately diagnosed for early pregnancy through ultrasound on Days 27-30 of gestation. Moreover, all fetal parameters are strongly associated with gestational stage, and variation in the estimation of actual gestational stage is low for TD than BPD in Beetal goats.

Authors contribution: MIRK, MEH, NH designed the study, analyzed the data and wrote the manuscript. MEH, TS, QA, and IM executed the study. AR and all authors critically reviewed the manuscript.

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