GESTATIONAL AGE ESTIMATION IN KARI SHEEP USING NON-CONVENTIONAL ULTRASONOGRAPHIC PARAMETERS

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ABSTRACT

The present study was designed to determine gestational age in Kari ewes measuring of non-conventional foetal growth parameters i.e., Abdominal Circumference (AC), Foetal Heart Beat Rate (FHBR) and Femur Length (FL).The experiment conducted at different gestational age in Kari sheep with   a B-mode ultrasonograph and a 3.5 MHz transducer. The ewes were scanned in dorsal recumbency over a restraining chute with post-scanning shaving of the inguinal regions.Data were collected from 49 ewes. Based on varying length of gestation, Kari ewes were divided into seven categories A, B, C, D, E, F and G representing gestation length of <100, 101-110, 111-120, 121-130, 131-140, 141-150 and > 150 days, respectively. Abdominal Circumference (AC), Femur Length (FL) and Foetal Heart Beat Rate (HBR) were observed at weekly interval throughout gestation. Regression equations for each parameter against the Gestational age of the foetus presented in trend lines using bi-nomial regression analysis. Mean Abdominal Circumference for all categories in week 4 was 54 mm whereas the same for B was 66 mm. The correlation between gestational age and AC was 0.68 which was significant (P< 0.05. Mean Femur Length for all categories in week 3 was 17 mm whereas the same for D was 17 mm. The correlation between gestational age and FL was 0.58 which was very highly significant (P< 0.001). Mean Foetal Heart Beat Rate was 155.5 throughout the gestational life. The earliest Heart Beat was observed in the third week of gestation in category A. Early Heart Beat Rate detection indicated life when bestowed into the embryo / foetal viability. The higher coefficients of determination (R^2) and correlations for AC and FL with gestational age (wk) suggest its reliability to estimate foetal age in Kari sheep.

Key Words: **Kari Sheep, Gestation Period, Ultrasonography, Abdominal Circumference, Femur Length and Foetal Heart Beat Rate**

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INTRODUCTION

Kari is a newly discovered mini size, fine-wool, thin tail sheep breed, inhabiting to Chitral region located in the North of Pakistan, as a result of a recent study (Ahmad and Khan, 2010). The study highlighted some interesting information about the gestation length (GL) and lambing interval (LI) at field conditions at Chitral District.Kari, extraordinary to others in the same species, is variable pregnancy length, the most significant of which was the unusual shorter GL. Majority of the ewes lambed in less than 5 months. Showing poor oestrus symptoms or no return to oestrus in a breeding season after mating is one of the pregnancy diagnosis techniques. Generally, ewes after every 16-17 days shows oestrus if not pregnant, however, research investigation indicates subsequent oestrus detection as poor indicator for pregnancy diagnosis (Senger, 1994). It is one of the needs of today researchers and producers, to image the reproductive position (stage of gestation, ovulation time and ovarian dysfunction) and health evaluation of breeding females through ultrasound. It is for the reason that false positive breeding are most likely when ovaries became acyclic, develop cyst on the ovary or become pseudo-pregnant.

Skill now has developed to visualize foetus between d 28 and 45 of pregnancy, and to diagnose any physiological and pathological disorder of the reproductive tract. The ultrasonographic scanning is harmless and provides immediate results mostly if the examination is performed transcutaneously. Different methods particularly trans-abdominal, trans-vaginal and trans-rectal can be used without risks to the operator or the animal. Ultrasonography is one of the ideal choice for pregnancy diagnosis with an accuracy of 93 to 99% ,unless it is performed too early (Kähn *et al*, 1993). Extensive knowledge and accurate instrument is required for correct prediction of litter size, number and ovulation time; although, pregnancy diagnosis and estimation of foetal age for normal lambing date are amongst the possibilities.

Ultrasongraphy can be used with a high degree of efficiency for pregnancy detection in sheep and goats. Results from pregnancy diagnosis with real-time ultrasonography can be obtained one to two weeks earlier compared to other methods currently used in the ovine industry. Ultrasonographic imaging has its boundaries

beyond the detection of foetus in the reproductive tract. It can assist in detecting function and state of different reproductive organs and age of the foetus besides physiological or pathological examination of visceral organs. Age estimation in human is helpful in avoiding obstetrical complications. In animals, although it is not yet common, however, interests among the farmers are growing to obtain sufficient information regarding the pregnant and non-pregnant stock for rebreeding plan and avoiding unattended births. Owing large variation in size within the ovine specie (8-200 kg body weight), there is a need for specific information available on each breed. Little pertinent research literature is available to provide age-estimation in local sheep breeds from developing countries based on measurement of their feotal-organs.

Trans-abdominal ultrasonography can diagnose pregnancy from day 25 to 30 post-breeding onwards (Gonzalez de Bulnes*et al*., 2010). Accurate foetal age determination is important for prediction of lambing date (White and Russel, 1984). Conventional parameters predict feotal age as earlier as 40 days (Haibel 1988; Richelle and Haibel 1991), however, non-conventional (Abdominal Circumference, Foot Length and Heart Beat Rate) are equally effective (Mercer *et al.*, 1987). Foetal Foot Length (FFL) and Foetal Heart Beat Rate (FHBR) are reliable parameters for the assessment of gestational age particularly when other ultrasonic parameters don't accurately predict gestational age, e.g., hydrocephalus, anencephaly, short limb dysplasia (Mercer *et al.*, 1987; Wilkins and Fowler 1984; Haibel 1990). HBR has been detected as earliest as day-18 or 19 in embryo indicating its viability both in dairy and crossbred sheep (Scrick and Inskeep, 1993). Real-time, B-scan yields two-dimensional image which is accurate, rapid safe for practical means of diagnosing pregnancy. It also determines foetal numbers, moving image of the uterus, uterine fluids, foetus, foetal heart beat and placentomes. Trans-abdominal scanning is performed by scanning the inguinal region cranial to the pelvic girdle. Little pertinent literature regarding HBR and abdominal Circumference (AC) is available. This study aimed to estimate gestational age and viability of embryo at periodic ultrasonographic observations i.e., AC, and HBR; along with FL, using trans-abdominal, B-Mode, Real-Time ultrasonography. Observations AC and FL were obtained and regressed for determining the gestational age in Kari ewes.

The article focuses on studying rate of foetal development in Kari ewes of different gestation length and their age estimation using transabdominal Ultrasonography.Thus, specific objectives of the present study were 1) to determine abdominal circumference and femur length development in Kari ewes throughout pregnancy, 2) to establish the relationship between gestational age and abdominal circumference and femur length measurements and 3) to advise a formula for the accurate prediction of parturition at the time of pregnancy diagnosis.

MATERIALS AND METHODS

A trial under controlled breeding program for examining foetal development in three different sub-types of Kari ewes (Small, Medium Large) and another local breed called Madakhlasht were performed at Chitral. Sheep were acclimatized in an animal house in Civil Veterinary Dispensary Shoghore, has an elevation above 2500 meters. The house comprised of four functional breeding-pens reserved for Large Size-Kari, Medium Size-Kari, Small Size-Kari and Madakhlasht. The trial lasted in 25 months (2008-2011).

Kari Sheep

A total of 49 ewes and four rams representing large, medium, small and Madakhlasht were employed in the trial. Their respective number was 16, 13, 12 and 08 respectively. Six ewes were killed in wolf attacks and other five died due to other reasons at different stages during the trial, and were excluded from the data. Two animals remained

unbred throughout the breeding trial for unknown reasons. Few ewe-lambs born during the trial were included in respective breeding when attained puberty for increased data-bulk. The prediction equation has been computed against the gestational age across different categories of sheep gestated at variable period of lengths i.e., A: 85 – 100d; B: 101 - 110d: C: 111 – 120d; D: 121 – 130d; E: 131 – 140 d: F: 141 – 150 d and G: above150d.

Measurement of Ultrasonographic Parameters as Predictors of Foetal Age

Upon the confirmation of pregnancy and the number of foetuses it carried, Foetal biometry was performed. Three parameters including: Abdominal Circumference (AC), Femur Length (FL) and Heart Beat Rate (HBR) were measured. Parameters, specific to certain stage of Foetal development were recorded and used for regressing/estimating the age of pregnancy.

Abdominal Circumference (AC)

Abdominal Circumference is the widest distance between the parietal bones just behind the proximal end of the zygomatic process and the frontal bone of the foetus. It was measured as a cross-section through the abdominal cavity at the level of the 12th thoracic vertebra, lying just above the umbilical cord.

Femur Length (FL)

This observation became possible upon ossification of Femur which became possible to observe at the end of fourth week of gestation in Kari ewes. Initially, this bone appeared cylindrical, with spherical-shaped centres of ossification at both ends in early gestation within the first 4 weeks. Gradually, the centres broaden out and assume the characteristic shape of adult bone. The length of the femur bone of the foetus was observed and measured.

Heart Beat Rates (HBR)

It is the number of Foetal Heart Beats (HBR) per unit of time, observed as beats per minute. This parameter was observed for the first time at third week (after day-20th) of gestation when the foetuses become live.

Statistical Analysis

The data was subjected to polynomial regression analysis to establish prediction equations for estimation of gestational age in different Kari sheep categorised on the basis of varying gestational lengths. The independent variables of AC and FL were plotted as binomial regression for estimation of the Gestation age in Kari sheep. The accuracy of the estimate was evaluated by coefficient of determination (R^2). The correlation between the gestational age and AC and FL were worked out for estimating the reliability of prediction.

RESULTS AND DISCUSSION

Foetal Age and Growth Rate Prediction

The Foetal development was estimated through measuring Abdominal Circumference and Femur Length using ultrasonogpraphic B-mode weekly examinations in Kari ewes from all sub-types in Chitral. Heart Beats Rates (beats / min) of foetus at gestation were recorded.

Abdominal Circumference (AC)

Mean (±SE) Abdominal Circumference is given in table 3.1.The earliest AC measurements became possible on week 4th in group B and D, whereas the last observation was recorded on week-20th in group G was.

The data shows that Abdominal Circumference remained almost similar across the groups (Slide-1). Abdominal Circumference tended to increase in size with advancement in gestational age. Mean Abdominal Circumference for all categories in week -4 was 54 mm whereas the same for B was 66 mm. The A and B had higher values and thus remained at variance from other categories (Figure 3.1).

Table1. *Average (SE) of abdominal circumference (mm) of different gestation categories of Kari sheep*

Week	A	B	C	D	E	F	G	Overall
4		66.0		42.0				54.0 (12.00)
5				43.0				43.0
6			71.0	48.5 (14.50)		39.0		51.8 (09.01)
7				75.0	75.0			68.7 (06.33)
8	58.0 (32.00)				90.0 (10.47)	42.0 (21.00)		70.0 (11.73)
9	76.0	89.0	104.0	79.0 (1.00)	80.3 (16.51)		60.0	83.7 (06.16)
10	117.0		134.0 (23.54)		107.0 (7.21)	82.7 (8.47)	95.0 (16.00)	103.3 (07.24)
11		165.0	125.5 (21.50)	82.0 (17.00)	121.2 (14.52)	109.0 (19.00)	98.3 (17.29)	113.8 (08.25)
12	126.0		136.7 (17.74)	110.3 (15.45)	139.8 (11.60)	116.0 (6.08)	92.0 (19.00)	120.5 (06.88)
13	136.0			128.5 (10.97)		132.5 (2.50)	116.0 (39.25)	124.1 (08.25)
14				135.0 (11.33)	135.0 (6.97)	131.3 (6.68)		129.7 (04.66)
15			163.0	146.8 (16.00)		165.7 (10.35)	125.6 (8.33)	142.1 (07.89)
16				139.0	153.0			149.0 (05.03)
17				135.0	140.0 (5.00)	189.0	142.5 (9.50)	148.2 (08.70)
18				150.0	159.5 (32.50)		143.0	150.6 (11.00)
20							157.0	150.0 (07.00)

The regressed curved line of Abdominal Circumference against gestational age is given below. The high coefficient of determination (R^2-values) presents their respective reliability of the prediction equations for estimation of gestational age.

A (GP= <100 d), $y = 0.4603x^2 + 4.9459x$ $R^2 = 0.8566$
B (GP= 101-110 d), $= 0.2224x^2 + 11.044x$ $R^2 = 0.7361$
C (GP=111-120d), $y = -0.12x^2 + 12.174x$ $R^2 = 0.7416$
D (GP= 121-130 d), $y = -0.1425x^2 + 11.061x$ $R^2 = 0.8949$
E (GP= 131-140 d), $y = -0.2199x^2 + 12.901x$ $R^2 = 0.8597$
F (GP = 141-150 d), $y = 0.0664x^2 + 8.6522x$ $R^2 = 0.7958$
G (GP= >150 d), $y = -0.0301x^2 + 8.7217x$ $R^2 = 0.8585$

Slide 1.   Abdominal Circumference of a foetus

The correlations between measurements of gestational age and AC were calculated for subtypes of Kari sheep. The correlation between gestational age and AC was 0.68 which was significant ($P < 0.05$).
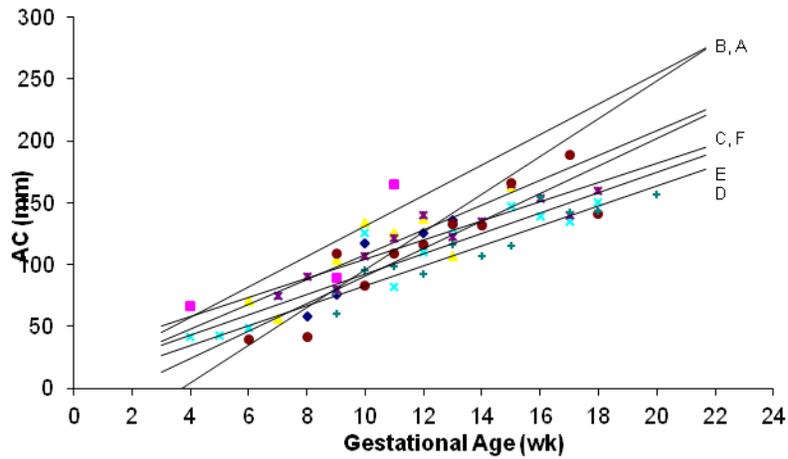
Fig: 3.1 Trend lines (Linear regression lines) of Abdominal Circumference (mm) of foetuses at corresponding gestational ages of different gestational categories (A-G) of Kari ewes (A = <100 d; B = 101-110 d; C = 111-120 d; D = 121-130 d; E =131-140 d; F =141-150 d; G >150 d)

Femur Length (FL)

Femur Length, being postural and stage specific parameter, few observations became possible (Table 3.2). The data indicates the earlier observation was recorded in week-6 in group B until week-18 in group G. The minimum and maximum femur length observed was 17 and 45 mm. The trend indicates that the group A, B and C had higher values at early Foetal life, than other groups (Slide-2).

Slide 2.   Femur Length of Kari foetus

Femur Length showed tendency of increase in size with gestation advancement. Mean Femur Length for all categories in week three was 17 mm whereas the same for D was 17 mm. The comparatively high values of Femur Length at group A and C implies faster Foetal development in these categories (Figure 3.2).

Table2. *Average (SE) of femur length (mm) of different gestation categories of Kari sheep*

Week	A	B	C	D	E	F	G	Overall
6		18.0		17.0				17.5 (5.50)
7			24.0		7.0			15.5 (8.50)
8	17.0		34.0					25.5 (8.50)
9	25.0				19.0	14.00		19.3 (3.18)
10	39.0			25.0	17.0			26.5 (4.57)
11					28.5 (4.50)	17.5 (0.50)		23.0 (3.67)
12							28.0	28.0
13							38.0	38.0
14			34.5 (1.50)		25.0			31.3 (3.28)
15						32.0	45.0	38.5 (7.50)
16						40.5 (6.50)		40.5 (6.50)

The high coefficient of determination (R^2-values) presents their respective reliability of future prediction using following estimating gestational age in ewes. The correlation between gestational age and FL was 0.58 which was very highly significant (P< 0.001).The regression equation for each category is given as:

A (GP= <100 d), $y = 0.9051x^2 - 5.2136x$ $R^2 = 0.9882$
B (GP= 101-110 d), $y = 4.8667x$ $R^2 = 0.881$
C (GP=111-120d), $y = 10x - 46$ $R^2 = 1$
D (GP= 121-130 d), $y = 0.0469x^2 + 1.25x + 7.8125$ $R^2 = 1$
E (GP= 131-140 d), $y = 0.3295x^2 - 1.0705x - 1.1409$ $R^2 = 0.8755$
F (GP = 141-150 d), $y = 0.6042x^2 - 11.554x + 69.789$ $R^2 = 0.9859$
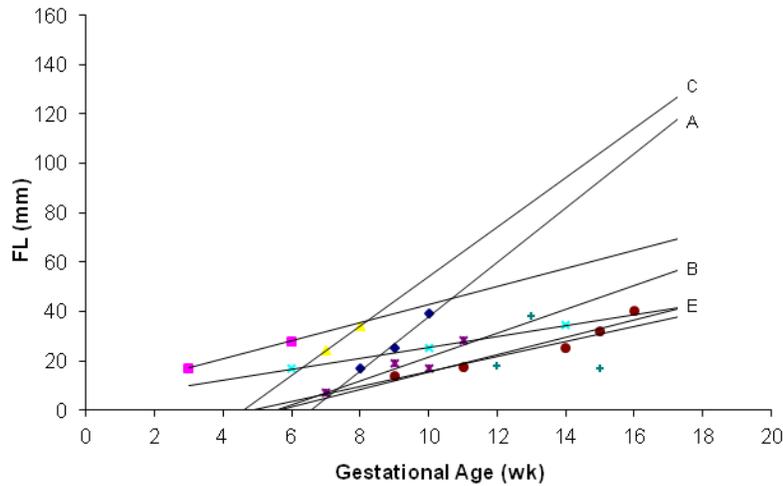G (GP= >150 d), $y = -0.7222x^2 + 26.167x - 192$ $R^2 = 1$

Fig: 3.2 Trend lines (Linear regression lines) of Femur Length (mm) of foetuses at corresponding gestational ages of different gestational categories (A-G) of Kari ewes (A = <100 d; B = 101-110 d; C = 111-120 d; D = 121-130 d; E =131-140 d; F =141-150 d; G >150 d)

Heart Beat Rate (HBR)

Kari foetuses were observed for Heart Beat Rate (per minute) during their gestational life. Mean HBR was 155.5 throughout the gestational life. The lowest HBR was 128 and the highest as 180 observed during the entire trial. The earliest heart beat was observed in the third week of gestation in category A. It was easy to observe and were recorded mostly during examinations. It seems that life came in to foetus at the sixth week of their gestational age in majority of the embryos except one at the third week belonging to group A. The HBR was continuously recorded until the termination of pregnancy (Table 3.3).

Table 3. *Average (SE) foetal heart beat in Kari sheep*

Week	A	B	C	D	E	F	G	Overall
3	146.0							146.0
5					156.0			156.0
6	180.0	144.0	150.0 (6.00)					156.0 (08.50)
7	168.0	130.0	192.0		144.0	160.0		158.8 (10.60)
8	162.0 (18.00)	144.0 (0.00)	154.5 (14.50)	168.0	138.7 (10.40)			150.5 (05.60)
9	174.0 (6.00)		156.0 (12.00)		128.0	168.0		159.3 (07.90)
10	156.0	161.3 (10.40)	165.6 (7.00)	156.0 (6.90)	156.00 (0.00)	144.0 (0.00)	162.0 (6.00)	158.5 (02.60)
11	168.0 (12.00)	158.0 (2.00)	157.0 (5.00)	162.0 (6.00)	168.6 (4.20)	156.0 (8.50)	177.3 (11.60)	164.4 (02.80)
12	144.0	164.0 (8.00)	165.0 (3.00)	158.0 (7.90)	151.2 (6.10)	150.0 (6.00)	156.0	156.5 (02.80)
13	144.0	150.0 (6.00)	166.4 (6.80)	154.5 (6.20)	162.8 (4.90)	160.0 (4.00)	150.0 (6.00)	158.6 (02.70)
14		144.0 (0.00)	157.6 (7.40)	155.0 (5.30)	162.0 (4.10)	158.4 (7.00)	146.7 (4.80)	155.9 (02.50)
15		156.0	156.0 (6.90)	154.9 (3.20)	162.4 (2.40)	150.0 (3.50)	159.0 (7.60)	156.5 (01.90)
16			144.0 (0.00)	157.5 (27.50)	151.0 (4.10)	148.5 (8.40)	150.0 (6.00)	150.1 (04.00)
17			168.0	144.0 (13.90)	144.0 (4.00)	152.0 (8.00)	150.0 (6.00)	148.3 (03.70)
18				144.00	137.0 (5.10)	148.8 (2.90)	153.3 (4.80)	144.7 (02.90)
19					144.0 (13.00)	156.8 (5.40)	144.0	150.4 (05.80)
20							162.0 (6.00)	144.0 (18.30)
21							144.0 (0.00)	144.0 (00.00)
22							156.0	156.0

The pertinent data regarding Abdominal Circumference are lacking in the literature. The abdominal circumference was detectable at the age of 28 days. The overall growth pattern of this parameter was highly linear

and increases as the gestational age of foetus advances. However in our study these measurements were highly variable at the corresponding age of gestation within the categories of Kari ewes.

The data regarding the femur length (FL) showed linear and uniform progression as the gestational age of the foetus advances. The observations were obtainable (17mm) at 3rd week of gestation until the later stages of gestation i-e 18th week. The coefficient of determination for each category was high. This parameter can be used as a highly reliable parameter for the prediction of gestational age in Kari foetuses. Our findings are parallel to those reported by Mercer *et al.,* (1987).

Literature shows different findings on heart beat detection in Sheep/Goats. The finding our study revealed that heart beat was detected for the first time at third week (after day-20th). This finding is similar to those obtained in Baladi Doe, JezerscoSolcavsca-Romanov cross ewes and Shiba goats (Haibel, 1988; Reichel and Haibel 1991; Strmsnik, *et al.,* 2002; Medan *et al.,* 2004).

No scientific observations of the heart beat rate at different weeks of gestation were found in literature. We could detect foetal heart rate ranging from 128bpm (Category E) to 180bpm (Category A). On average the heart beat was detected in all categories at week-6 and was continuously detectable in category G at week 24.

The higher value of coefficients of determination (R^2), and higher correlations (>0.58) for these parameters (FL and AC with the gestational age (wk) suggests actual estimation of GL in Kari sheep. Measurements on AC and FL collectively may provide a precise prediction of gestational age. The wide range of variation and non-uniformity in measurements of Abdominal and Head Circumference between and within the subtypes can be attributed to the fact the rate of variable rate of foetal development and position / posture at the time of examination.

CONCLUSION AND RECOMMENDATIONS

- The Ultrasonographic parameters (FL, AC and HBR) the first four parameters had higher coefficient of determination for estimating the gestational age

- Conservation of the breed should be carried out to protect the breed purity and the traits of extraordinary importance for future use

- The breed may be studied under different ecological zones having different temperature, altitudes and photoperiodic period

- Gene/s responsible for short gestation and the environmental factors triggering their function may be investigated

- Hormonal profile especially the estrone-sulphate should be assessed to study the effect of season and birth weight

- Local pastoral shepherds may be assisted to adopt the breeding strategy and practices concentrating upon accelerated lambing for mutton production through increased focus on shorter gestation

- Mutton from the Kari may be promoted for local and export consumption in order to obtain boost for promotion of the breed for Mutton and Patti (Hand-woven woolen product obtained from Kari-fleece) handicrafts in Chitral

List of Abbreviations

AC .... abdominal circumference

FHBR... foetal heart beat rate

FFL------------------------------------foetal foot length

FL .... femur length

GL... gestation length

LI .... lambing interval

MHz.. mega hertz

Wk .... week

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