Ovine fetal growth curves in twin pregnancy: ultrasonographic assessment

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Summary

Purpose of investigation: The fetal-lamb model is a fundamental tool for developing clinical applications for the treatment of human fetal pathology (e.g., open fetal surgery). Accurate estimation of gestational age is important to avoid size-related problems during surgery.

Methods: To evaluate the effect of twinning on ovine fetal growth, we followed seven twin pregnancies in Comiso ewes from mating through parturition. Fetal growth indexes (muzzle-occipital and mean abdominal diameters, abdominal circumference, femoral and humeral length) were measured weekly using perimammary ultrasonography and analyzed with a linear quadratic regression model based on natural logarithms of each parameter and fetal age.

Results: The model explained > 90% of the variability observed, with determination coefficients of 95% (femoral length, abdominal circumference), 94% (abdominal diameter, humeral length), and 89% (muzzle-occipital diameter).

Conclusion: Mean birth weight was lower than that reported for singleton fetal lambs, as it is in bigeminal pregnancies in humans, despite the uterine and placental differences between these two species. With the exception of slightly earlier growth deceleration, curves for head and long-bone growth resembled those for singleton ovine fetuses. Ovine fetal growth patterns (like those of humans) in singleton and twin pregnancies are similar.

Key words: Experimental animal; Ovine fetal growth curves; Ultrasonography; Fetal surgery.

Introduction

The ovine fetus is the animal model most frequently used for studies of fetal physiology, and as such it is a fundamental tool in research aimed at the development of clinical applications for the treatment of human fetal pathology, in particular open fetal surgery [1-5]. Accurate estimation of gestational age is highly important in these studies because problems can arise during surgery if the fetus is found to be larger or smaller than expected on the basis of the breeder's reports. Ultrasonography (US) was first used in veterinary medicine in the early 1980s. Its use became widespread particularly in veterinary obstetrics, where it provided a relatively easy means for observing the physiological and pathological events of pregnancy for research purposes and a practical and economic tool for breeders. Ultrasonography has been reported to be the method of choice for early diagnosis of pregnancy in sheep and other species. Pregnancy can be verified as early as 20-30 days after mating using transrectal or even transcutaneous probes [6, 7], and the number of fetuses present in the uterus can be identified by the 40th-45th day [8]. Various US methods for estimating ovine fetal growth have also been described in the literature. These techniques are based on serial measurements of specific somatic parameters in the fetus such as the biparietal diameter of the skull, head circumference, abdominal circumference, and the lengths of the femur, tibia, and metatarsal bone [1, 9-12].

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As part of a broader research project aimed at the application of modern diagnostic methods in commercially bred animals, the present study was conducted to obtain ovine fetal growth curves in twin pregnancies. The model used was the fetal lamb of the Comiso breed, which is known for its high rate of twinning.

Materials and Methods

Animals. The initial phase of the study was conducted at the Experimental Institute of Zootechnics (Monterotondo, RM - Italy) on seven adult Comiso ewes. This highly prolific breed has a reproductive rate of 180% and a body weight at maturity of 45-50 kg. The animals were grazed and fed with legume hay together with a vitamin-mineral supplement. Estrus was induced with polyurethane vaginal suppositories impregnated with fluorogestone acetate (40 mg), which were left in place for 14 days. The ewes were housed with rams that had been fitted with colored chest tampons, which stained the backs of the ewes during mating. The ewes were checked every four hours and isolated from the rams immediately after labeling was observed (day 0 in our study).

Pregnancy was diagnosed between the 25th and 28th days after mating by means of a transvaginal ultrasonographic scan with a 6.5 MHz transducer (details provided below). The seven ewes with twin pregnancies were transferred to the Center for Animal Breeding and Use at the Medical Faculty of the Catholic University of the Sacred Heart in Rome and used for the present study.

The animals were housed in an area with a pro-capita surface area of 0.7 m^2. The floor was covered with straw and sawdust, which were changed daily. The temperature was kept between

10°C and 24°C with a relative humidity of 55 ± 10%. Ventilation was provided at an approximate rate of 3 m^3/hr/kg of body weight to prevent the buildup of intestinal gas (methane) and ammonia. The animals were fed legume hay (alfalfa, clover) (1.7 + 0.3 kg/day) and a cereal mixture specially formulated for pregnant ewes (300 ± 50 g/day) [13, 14].

Sonographic studies. All ultrasonographic examinations (including the initial scan to diagnose pregnancy) were performed between 1 November 1998 and 20 March 1999. The following scanners used were:

– Ansaldo AU 440 with convex (3.5 MHz and 5 MHz) and transvaginal (6.5 MHz) transducers.

– Sonomed Concept MCV with convex (3.5 MHz) and transvaginal (6.5 MHz) transducers.

The seven ewes with twin pregnancies were examined with 3.75 MHz and 5.0 MHz perimammary transducers from the 40[th] through the 140[th] day of gestation. These examinations were carried out at intervals of approximately seven days, with the exception of the last two, which were separated by an interval of ten days. The ewes were examined in the supine position with two assistants restraining the front and back legs. Each study lasted 15-30 minutes.

The examinations included determination of fetal number, viability, and heart rate. Crown-rump lengths were checked against growth curves elaborated by our group (not yet published) to verify the presumptive gestational age of each fetus at the first examination. For each fetus, the following parameters were recorded:

– Muzzle-occipital diameter (MOD) (Figure 1)
– Mean abdominal diameter (MAD) (Figure 2)
– Abdominal circumference (AC) (Figure 3)
– Femoral length (FL) (Figure 4)
– Humeral length (HL) (Figure 5).

The MOD was measured by placing the calipers over the external surface of the maxillary and occipital bones. The MAD and AC were measured at a level just above the insertion of the umbilical cord. The FL and HL were measured by placing the calipers on the external surfaces of the bones. An image of each parameter was recorded with a Sony video graphic printer UP890 CE, and the value recorded was the mean of two consecutive measurements. A total of 315 examinations were made (18 per animal) by two operators (85% by D.R., 15% by G.N.). Intra- and inter-operator variability were within acceptable limits (≤ 4 mm for MOD, FL and HL: ≤ 10 mm for MAD and AC).

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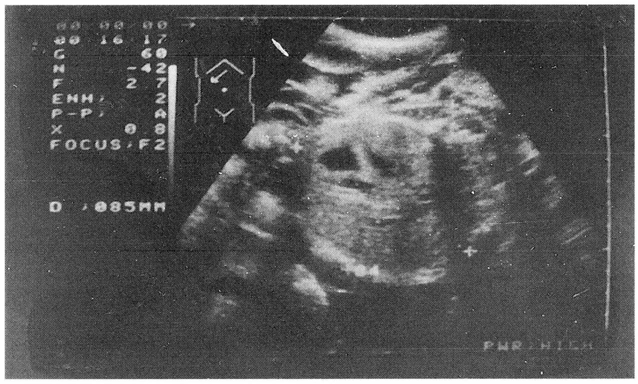

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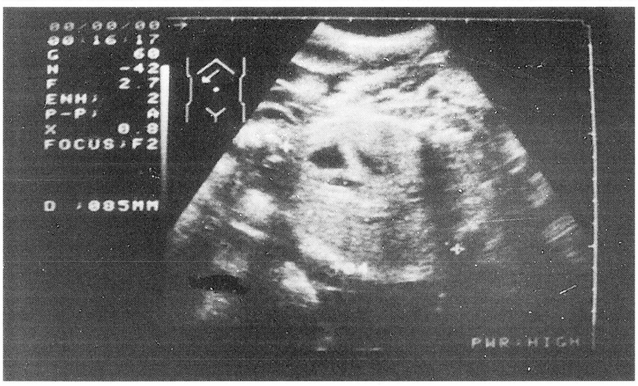

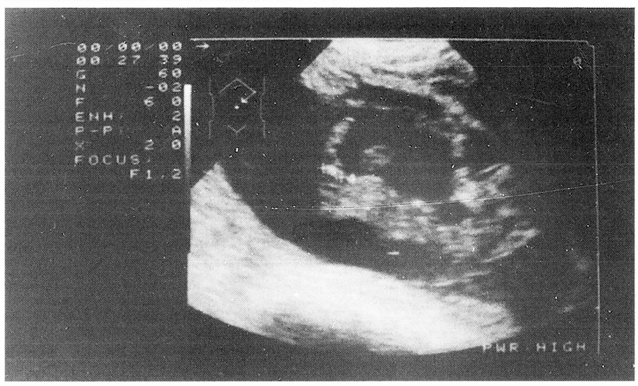
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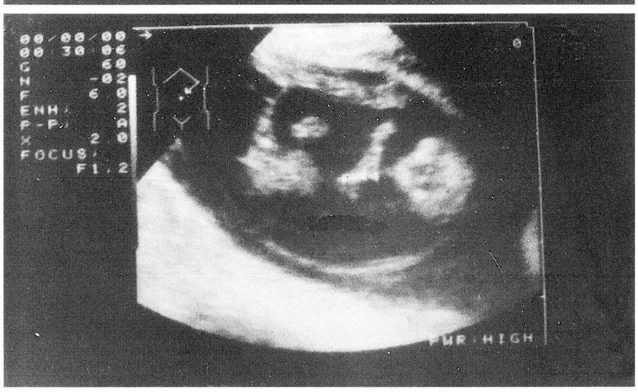

Figure 1. — Muzzle-occipital diameter (MOD).
Figure 2. — Mean abdominal diameter (MAD).
Figure 3. — Abdominal circumference (AC).
Figure 4. — Femoral length (FL).
Figure 5. — Humeral length (HL).

The 14 fetuses were weighed at birth, and the mean weight was 3,300 ± 200 g.

Statistical analysis and growth curve construction. The possible effect of the fetus's location (right vs left uterine horn) on the variability of each parameter was evaluated in a preliminary phase of the study, and no significant effects were observed. The data were inserted into a single data base and analyzed with an appropriate linear quadratic regression model based on the natural logarithms of each fetal parameter and of fetal age. The linear model utilized was the following:

$$ln\ yi = a + b\ lnx_i + c(lnx_i)^2 + ei$$

where $ln\ yi$ is the measurement in millimeters, a is the intercept, b and c are the regression coefficients, x_i is the gestational age in days, and e_i is the residual error of the first observation.

Fetal development was thus estimated over the course of gestation, and predicted values for each parameter were calculated for each day of the gestational interval (days 40 through 140). Ninety-five percent confidence intervals (minimum or maximum) were calculated for each predictor or expected value. The confidence interval provides an index of the maximum and minimum variations that can be expected in each parameter each day, i.e., the range of variations that should not lead to gross errors in prediction. The expected values with their respective confidence intervals were plotted on a graph to represent the growth trend for each parameter considered. All analyses were performed on an AST (IBM-compatible) computer equipped with SAS/STAT software [15, 16].

Results

The equations that describe the growth curves and the relative statistics for each parameters are shown in Table 1. The simple regression coefficients that express the slopes of the curves were highly significant ($p < 0.001$; $p < 0.01$); the same was true of the quadratic coefficients although the slope of the HL curve displayed greater variability (s.e. = 0.19) than any of the other parameters. The values of the intercept expressing theoretical development at time 0 of gestation were also highly significant ($p < 0.001$; $p < 0.01$). For all of the parameters examined, the hypothesized model explained over 90% of the variability observed: the determination coefficient (R^2), which expresses the ratio of inter-animal variability to total variability, was 95% for FL and AC, 94% for AD and HL, and 89% for MOD.

An analysis of the curves constructed from the estimated values (Table 2) and reproduced in Figures 6, 7, 8, 9, and 10 revealed that the MOD curve displayed convexity toward the abscissa (gestational age), while the curve for FL was characterized by marked concavity. The remaining curves (AC, AD, HL) were virtually linear.

By the 70th day of gestation the MOD had attained 52.8% of its estimated length at 140 days, i.e., near parturition. Corresponding figures were 34.4% for the AC, 35.3% for the AD, 24.4% for the FL and 22.9% for the HL (Table 3).

Table 1. — *Regression coefficients and statistics for somatic parameters of ovine fetal growth measured with ultrasonography.*

	BONE PARAMETERS			ABDOMINAL PARAMETERS	
	Muzzle-occipital diameter	Humerus length	Femoral length	Abdominal circumference	Mean abdominal diameter
No. of observations	75	69	70	75	75
Model					
– intercept	–19.74**	–15.23**	–20.38**	–10.58**	–11.44**
– age	9.56**	6.08*	8.47**	5.49**	5.36**
– age^2	– 0.94**	–0.43	–0.70*	–0.43*	–0.42*
R^2	0.89	0.92	0.95	0.95	0.94
s. e.	0.14	0.19	0.14	0.11	0.12

Legend: **$p < 0.001$; *$p \leq 0.01$

Table 2. — *Estimated values for ovine fetal growth parameters from 6 to 40 weeks of gestation.*

GESTATIONAL WEEK		Mean abdominal diameter (mm)	Abdominal circumference (mm)	Muzzle-occipital diameter (mm)	Humerus length (mm)	Femoral length (mm)
6	(35-42 days)	15.3	51.0	17.5	4.4	4.5
7	(42-49 days)	21.3	71.7	25.2	6.8	7.2
8	(49-56 days)	28.0	94.8	33.5	9.7	10.7
9	(56-63 days)	35.0	119.6	41.8	13.2	14.8
10	(63-70 days)	42.6	145.9	49.8	17.1	19.5
11	(70-77 days)	50.3	173.1	57.4	21.5	24.6
12	(77-84 days)	58.2	201.0	64.3	26.2	30.2
13	(84-91 days)	66.2	229.3	70.6	31.4	36.0
14	(91-98 days)	74.2	257.8	76.1	36.9	42.1
15	(98-105 days)	82.1	286.2	80.8	42.6	48.3
16	(105-112 days)	90.0	314.5	84.8	48.7	54.6
17	(112-119 days)	97.8	342.5	88.1	54.9	61.0
18	(119-126 days)	105.5	370.0	90.8	61.4	67.4
19	(126-133 days)	113.0	397.2	92.9	68.0	73.7
20	(133-140 days)	120.4	423.7	94.4	74.8	80.0

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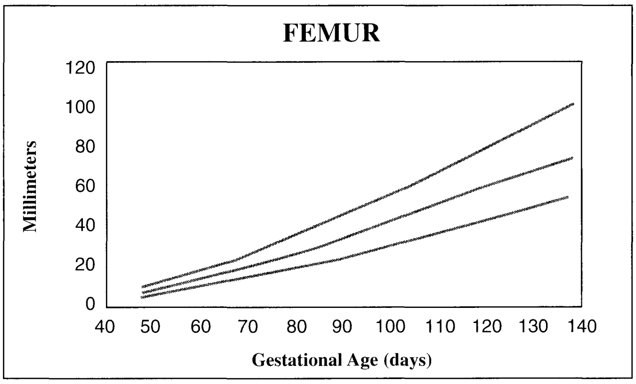

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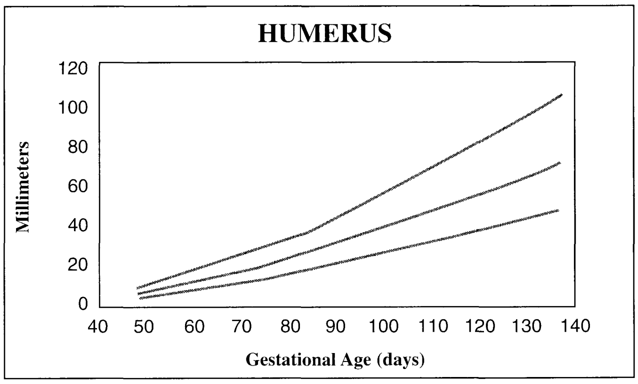

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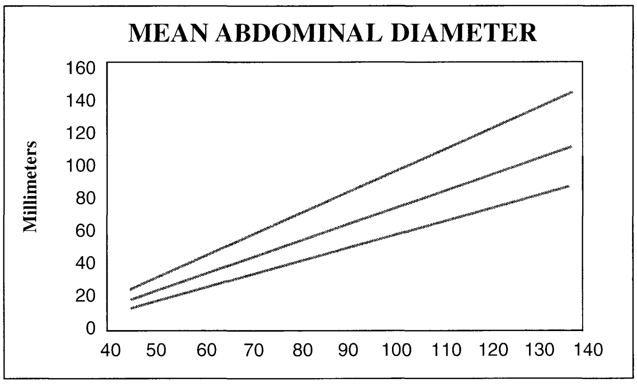

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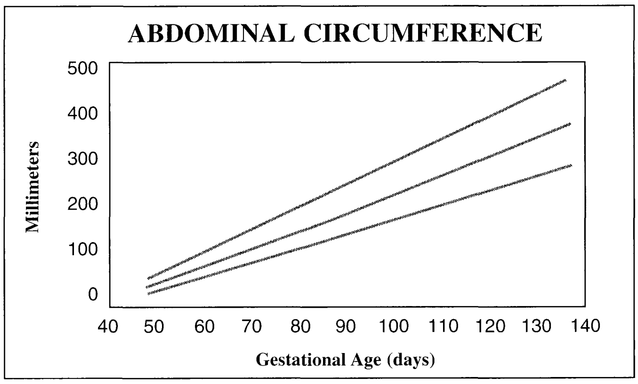

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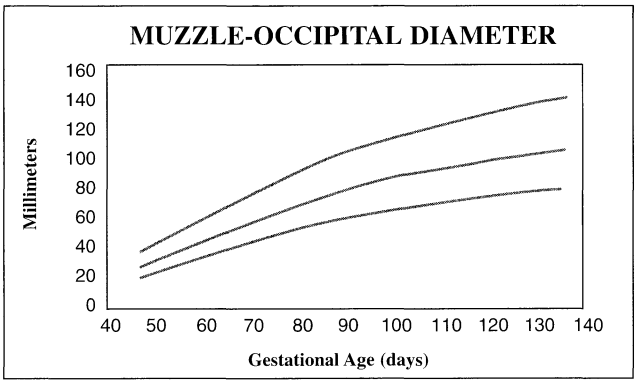

Figure 6. — Femoral length (FL).
Figure 7. — Humeral length (HL).
Figure 8. — Mean abdominal diameter (MAD).
Figure 9. — Abdominal circumference (AC).
Figure 10. — Muzzle-occipital diameter (MOD).

Discussion

Use of ultrasonography in experimental animals has two important objectives, both of which depend on the development of techniques with a high level of reproducibility:

1) Validation of biometric curves for the study of open fetal surgery.

2) The study of certain models of fetal physiopathology for extrapolation to clinical practice in humans [6, 9, 10, 17].

Barbera *et al.* [9] have examined several biometric parameters in fetal lambs in terms of their correspondence with those of human fetuses. Their findings can be summarized as follows:

1) The growth pattern of the biparietal diameter in both species displays a linear trend, but in the fetal lamb there is a precocious deceleration in growth during the final phases of pregnancy.

2) Abdominal circumference follows a linear growth trend that begins earlier and is more rapid in the ovine fetus.

3) Long-bone growth (the tibia and femur) also displays a linear trend in both species. These authors maintain that tibial length is the more sensitive of the two in terms of somatic growth in the ovine fetus, whereas the femur is considered to be of greater value in assessing human fetal growth [9].

Others have assessed the validity of biometric parameters in experimental animals for defining gestational age and fetal weight [11], such as metatarsal length or ventrodorsal diameter [1].

Various experimental approaches have been developed for the study of fetal physiopathology, including a model of environmental stress capable of provoking intrauterine growth retardation to identify the regional impact of such insults on the fetus and their onset time with respect to stress exposure [10]. Other groups have measured oxygen

Table 3. — *Percentage of total growth (i.e., at term: 40 weeks) achieved at the end of gestational weeks 6-20 for fetal somatic parameters.*

GESTATIONAL WEEK		Mean abdominal diameter (%)	Abdominal circumference (%)	Muzzle-occipital diameter (%)	Humerus length (%)	Femoral length (%)
6	(35-42 days)	12.7	12.0	18.5	5.9	5.6
7	(42-49 days)	17.7	16.9	26.7	9.1	9.1
8	(49-56 days)	23.2	22.4	35.5	13.0	13.4
9	(56-63 days)	29.1	28.2	44.3	17.6	18.5
10	(63-70 days)	35.3	34.4	52.8	22.9	24.4
11	(70-77 days)	41.8	40.9	60.8	28.7	30.8
12	(77-84 days)	48.3	47.4	68.2	35.0	37.7
13	(84-91 days)	54.9	54.1	74.8	42.0	45.0
14	(91-98 days)	61.6	60.8	80.6	49.3	52.6
15	(98-105 days)	68.2	67.5	85.6	57.0	60.4
16	(105-112 days)	74.8	74.2	89.5	65.1	68.3
17	(112-119 days)	81.2	80.8	93.4	73.4	76.3
18	(119-126 days)	87.6	87.3	96.2	82.1	84.3
19	(126-133 days)	93.9	93.7	98.4	90.9	92.2
20	(133-140 days)	100.0	100.0	100.0	100.0	100.0

uptake using a transducer that measures blood transit time in the umbilical artery [2], and computerized models of placental insufficiency have also been described [18]. A number of elegant models have been developed to identify the Doppler-flowmetric indexes most closely correlated with acute changes in pO_2 [4, 19, 20, 21].

In the present study the calculation of biometric curves in a species with a high reproductive rate and a high frequency of twinning was aimed at evaluating the fetal growth trend as a function of gestational age and twinning, considering above all the anatomical differences in the uteri of sheep and humans and the differences in placental implantation, which affects oxygen delivery to the fetuses.

As Barbera *et al.* have pointed out, the birth weight of singleton fetuses is the same in humans and sheep even though the duration of pregnancy in the latter species is approximately half that of humans. One of the first observations that can be made on the basis of our findings is that in twin pregnancies in sheep, the birth weight of each fetus is inferior to that of a singleton fetus (3,300 ± 200 g vs 4,000 ± 250 g), as it is in bigeminal pregnancies in humans. In essence, this finding demonstrates that prenatal growth of both singleton and twin fetuses is not significantly different in the two species in spite of their divergence in terms of the anatomic and morphological characteristics and the contractile complicance of the uterus and the formation/implantation of the placenta, which affects fetal nutrition.

We compared our data for twin fetuses with those reported by Barbera *et al.* for singleton ovine fetuses [10]. The specific growth indexes and the measurement schedule used in our study were slightly different from those used by the latter group. At the mid-point of gestation (70 days in our study vs 75 days in that of Barbera *et al.*), the indexes of twin fetal head (MOD) and long-bone (HL) growth were more or less consistent with the corresponding regional figures for singleton fetuses (BPD ant TL, respectively) although growth deceleration in both the head and limbs occurred slightly earlier in the twin fetuses. The most substantial difference was found in head growth: while the BPD of a singleton fetus has completed 49% of its total growth by day 75, the MOD of the twin fetus has already achieved 54% of its total growth by the 70th day of gestation. It is entirely possible, however, that this divergence is merely a reflection of the methodological differences cited above and/or the reproductive rates and birth weights of the two breeds of sheep (Comiso vs Columbia-Rambouillet).

A final comment is warranted on the growth indexes chosen for this study, in particular on the reproducibility of measurements of the humerus. This parameter was the one associated with the greatest variability in our study, but the regression coefficient was still highly significant (R2 = 0.92). It is also interesting to note that development of the maxillary and occipital bones showed linear correlation with that of the parietal bones based on the figures of Barbera *et al.* [9]. This finding suggests that the MOD can be used as an alternative index of fetal growth in cases where accurate measurement of the BPD is not feasible.

Conclusion

In conclusion, our findings demonstrate that:

1) District biometry in the ovine fetus follows a pattern of growth that is similar regardless of whether the pregnancy is singleton or bigeminal.

2) The growth trend for the various parameters follows a well established evolutionary plan in which attainment of fetal birth weight at term is related to the animal's adaptation to its environment according to species-specific needs.

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