Prediction of fetal sex in cattle by testosterone levels in allantoic fluid

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Summary. The testosterone concentration in allantoic fluid between 90 and 150 days of gestation in cattle can be used to determine the fetal sex; values were 442 ± 20.3 (S.E.M.) pg testosterone/ml for male fetuses and 215 ± 8.2 pg/ml for female fetuses.

In man, the levels of 17β -hydroxyandrogens in the amniotic fluid vary according to the sex of the fetus (YoungLai, 1972; Dörner *et al.*, 1973; Giles, Lox, Heine & Christian, 1974), but chromosomal analysis of abortuses showed that the difference could not be used for accurate prediction of fetal sex (YoungLai & Lin, 1973). A sex-related difference in the hormone levels of fetal fluid could be of practical use to the livestock industry as an aid to prenatal sex determination and to complement chromosomal analysis of cells in the amniotic fluid (Bongso & Basrur, 1975).

In the present paper the levels of 17β -hydroxyandrogens in allantoic and amniotic fluid were measured as testosterone equivalents and related to the sex of the fetus.

The 128 fetal fluid samples (64 amniotic, 64 allantoic) were obtained from cows at various stages of gestation immediately after slaughter at the abattoir. Each sample was centrifuged and the supernatant was stored at -20°C until testosterone levels were measured by radioimmunoassay (YoungLai, 1972). Levels of free testosterone were <40 pg/ml in all samples. The antiserum had 35% cross-reaction with dihydrotestosterone and results are expressed as testosterone equivalents. The coefficient of variation of replicate analysis was about 7%. The crown-rump (C-R) length of each fetus was measured to determine the gestational age (Arthur, 1964), which ranged from 45 to 193 days. The gonadal sex of each well-developed fetus (>60 days) was recorded and the sex of fetuses <60 days (C-R=6.4 cm) was confirmed by chromosomal analysis on muscle biopsy cultures.

Table 1. The mean ($\pm$ S.E.M.) concentrations of testosterone (pg/ml) in the amniotic and allantoic fluid of bovine fetuses (no. in parenthesis) at different stages of gestation

Stage of gestation (days)	Amniotic fluid		Allantoic fluid	
	Males	Females	Males	Females
<60	120.7 ± 7.2 (3)	$*62.0 \pm 6.98$ (4)	114.0 ± 13.3 (3)	77.5 ± 8.7 (4)
61-90	155.5 ± 3.5 (2)	111.7 ± 10.7 (3)	190.0 ± 30.0 (2)	129.0 ± 19.2 (3)
91-120	237.0 ± 16.1 (9)	169.6 ± 16.7 (9)	457.8 ± 32.3 (9)	$\dagger 199.0 \pm 11.4$ (9)
121-150	217.3 ± 15.5 (9)	191.8 ± 13.7 (10)	426.7 ± 27.3 (9)	$\dagger 229.4 \pm 11.4$ (10)
151-180	276.3 ± 16.3 (4)	$*176.2 \pm 13.4$ (6)	311.3 ± 37.7 (4)	225.0 ± 20.9 (6)
181-210	175.0 ± 32.9 (4)	80 (1)	341.8 ± 40.1 (4)	225 (1)

Significantly different from concentration in males; * $P<0.01$; $\dagger P<0.001$.

Thirty-three of the fetuses were females and 31 were males. The levels of testosterone in amniotic fluid samples did not show any differences between male and female fetuses at the gestational stages examined ($t=1.09$; $P>0.01$). There was a significant difference between sexes ($t=2.75$; $P<0.01$) in testosterone levels in allantoic fluids throughout gestation. During the period 91-150 days of gestation (Table 1) testosterone levels were 442 ± 20.3 pg/ml in males and 215 ± 8.2 pg/ml in females ($t=3.96$;

$P < 0.001$). This period overlaps that which Bongso & Basrur (1975) found best for aspiration of amniotic fluid for sex prediction. If arbitrary levels of 240 and 320 pg testosterone/ml are taken as the limits to predict female and male fetuses respectively, the testosterone concentration of the allantoic fluid alone can be considered indicative of fetal sex. A double-blind study on a number of samples confirmed the validity of this procedure.

The high androgen levels in the allantoic fluid of male fetuses are consistent with the higher serum levels of testosterone and androstenedione found in male fetuses or mothers carrying males (Mongkonpunya, Lin, Noden, Oxender & Hafs, 1975).

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