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Distribution of alpha$_1$-fetoprotein in fetal plasma, allantoic fluid, amniotic fluid and maternal plasma of cows*

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Summary. Maximal concentrations of AFP, measured by RIA, were obtained in fetal plasma and amniotic and allantoic fluid between the 3rd and 4th month of gestation, with levels declining thereafter until term. AFP values in maternal plasma were unchanged. Throughout gestation, AFP values were higher in allantoic than in amniotic fluid and the ratio of allantoic fluid/amniotic fluid AFP was significantly correlated with gestational age.

Introduction

Alpha$_1$-fetoprotein (AFP), a fetus-specific protein synthesized by the fetal liver and yolk sac in a number of mammalian species (Gitlin & Boesman, 1967), is a major protein in fetal serum, but is present only in very low levels in normal adult serum (Ruoslahti & Seppala, 1972; Sell & Gord, 1973). In man, abnormally high amniotic fluid levels of AFP have been associated with fetal neural tube defects (Brock, 1978).

The concentrations of AFP in bovine fetal serum have previously been estimated by a radial immunodiffusion method (Abe, Komatsu, Takeishi & Tsunekane, 1976) and AFP, the most predominant of 3 fetus-specific proteins in cattle, has now been purified and characterized (Lai, Peters & Lorscheider, 1978b) and a radioimmunoassay for it developed (Lai, Smith, Church & Lorscheider, 1979). This assay was used to measure concentrations of AFP in various fluids during gestation in cows.

Materials and Methods

Animals

Twenty-one matched bovine amniotic fluid and fetal blood samples were obtained at a local slaughterhouse. The intact amniotic sac was removed from the uterus and 3–4 ml clear amniotic fluid were withdrawn by syringe. Fetal blood samples (2–4 ml) from the same conceptuses were obtained from the umbilical cord by heparinized syringe. An additional 67 amniotic fluid and 58 allantoic fluid samples (3 ml), 49 of which were matched, were obtained in a similar manner. The fetal crown–rump length and fetal weights were utilized as a basis for estimating the fetal age as indicated by Roberts (1956) and Salisbury & VanDemark (1961).

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Matched allantoic and amniotic fluid samples (2–5 ml) were also obtained at slaughter from 7 conceptuses (43–70 days) of cows with planned mating dates (Animal Reproduction Laboratory, Colorado State University, Fort Collins, Colorado). Fetal blood (0·1–0·2 ml) was obtained from some of these embryos by a 0·5 ml syringe fitted with a 27-gauge needle.

Maternal blood samples were obtained periodically from the caudal vein of cows whose oestrous cycles had been synchronized. All samples were stored at −20°C before assay.

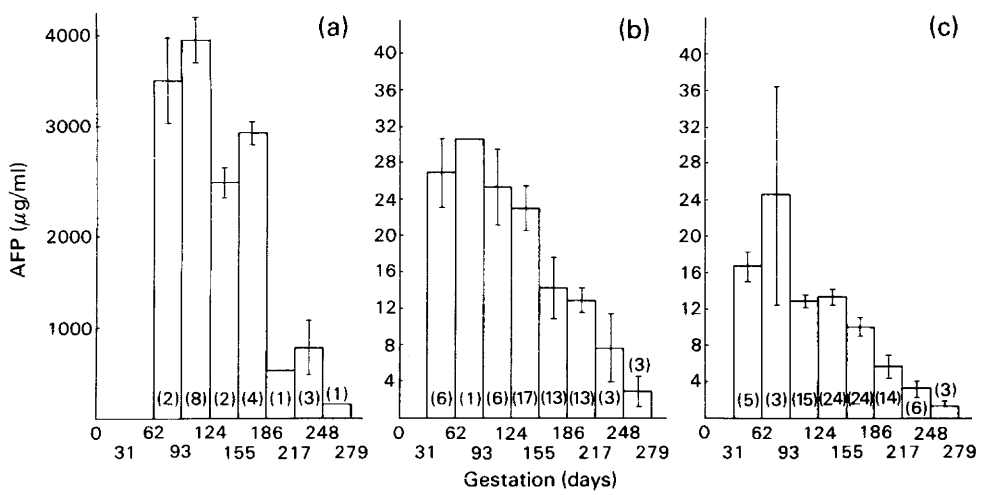
The AFP concentrations were determined by the double-antibody radioimmunoassay described by Lai *et al.* (1979).

Statistical analysis

Analyses of ratios of AFP levels in various fluid compartments in the conceptus were performed by polynomial regression for linear, quadratic, cubic and quartic trends (Dixon, 1974).

Results

The AFP concentration was highest during the 3rd and 4th months of pregnancy (approximately 4000 μg/ml) and declined thereafter until term (Text-fig. 1a). The highest levels of AFP in allantoic fluid were observed during the first third of pregnancy (27–31 μg/ml) and declined thereafter to a minimum of 3 μg/ml in the final month (Text-fig. 1b). Concentrations of AFP in amniotic fluid were highest during the first third of pregnancy (17–25 μg/ml) but they then declined to <1·5 μg/ml during the 9th month (Text-fig. 1c).



Text-fig. 1. Mean concentrations ± 1 s.e.m. of bovine alpha$_1$-fetoprotein (AFP) as a function of gestational age in (a) fetal plasma; (b) allantoic fluid; and (c) amniotic fluid. The number of animals per group is indicated in parentheses.

The matched allantoic fluid/amniotic fluid ratio of AFP as a function of gestational age exhibited a significant linear correlation ($n = 56$, $r = 0{\cdot}42$, $P < 0{\cdot}005$). Other regression trends were not significant. Linear and curvilinear plots of matched fetal plasma/amniotic fluid ratios of AFP against gestational age indicated no significant correlation.

Maternal plasma levels of AFP at various stages of gestation remained low (approximately 0·010–0·021 μg/ml) and were not elevated above the levels of 0·023 ± 0·008 μg/ml found in non-pregnant cows (Lai *et al.*, 1979).

Discussion

In rats (Lai, Forrester, Hancock, Hay & Lorscheider, 1976) and rabbits (Branch, 1972), which have short pregnancies, AFP levels in fetal blood peak near term, whereas in sheep (Lai, Mears, Van Petten, Hay & Lorscheider, 1978a), women (Gitlin & Boesman, 1966) and cows, which have long gestation periods, fetal serum AFP levels are highest in early gestation. Although the timing for the suppression of the AFP gene is different among species, the concentration of fetal blood AFP levels decreases before birth in all species so far studied. The gestational pattern of fetal plasma AFP, as well as the actual AFP levels (0·2–4·0 mg/ml), are in general agreement with results previously reported by a less sensitive radial immunodiffusion method (Abe *et al.*, 1976). Bovine AFP exists as two molecular variants based on their carbohydrate heterogeneity, but both forms are immunologically indistinguishable (Lai & Lorscheider, 1978). The values reported in the present study therefore represent total AFP.

The present study indicates that bovine maternal plasma levels of AFP remain very low throughout gestation, as in sheep (Lai *et al.*, 1978a), but unlike the significant increases during pregnancy in rats (Lai *et al.*, 1976) and women (Hay, Forrester, Hancock & Lorscheider, 1976). A possible explanation for this difference is that cow and sheep placentae have more layers of tissue separating fetal and maternal blood than do the placentae of rat and man (Balinsky, 1975; Eckstein & Kelly, 1977). A possible systemic immunosuppressive function of AFP during pregnancy, as suggested by others (see review, Tomasi, 1977), may not be significant in the cow because the concentration of AFP in maternal blood remains low throughout gestation.

The gestational pattern of AFP concentrations in amniotic fluid also differs in different species. As in sheep (Lai *et al.*, 1978a) and women (Randle & Cumberbatch, 1973), amniotic fluid AFP in cows reaches high values early in pregnancy, whereas in rats levels are highest in late gestation (Lai *et al.*, 1976). Fetal urine is the source of human amniotic fluid AFP (Weiss, Macri & Elligers, 1976), and the source of amniotic fluid AFP in the cow is also likely to be fetal rather than maternal since the AFP concentration in fetal plasma remains much higher than that in amniotic fluid throughout gestation.

The findings in the present investigation indicate that, when the AFP levels in each fluid compartment are treated as independent variables, the allantoic fluid concentration of AFP is higher than the concentration observed in amniotic fluid at all corresponding stages of gestation. However, the ratio of allantoic fluid/amniotic fluid AFP was significantly correlated to gestational age when paired values for individual animals at each gestational age were analysed. It is probable that AFP would enter both fluids as a component of fetal urine and, since other plasma proteins of similar molecular weight do not appear to cross fetal membranes (Hervey & Slater, 1968), the increase in the ratio of allantoic/amniotic fluid AFP with advancing gestation may be explained by factors which regulate the volumes and composition of urine entering the two sacs as a function of gestation (Arthur, 1969; Mellor & Slater, 1972; Reeves, Daoud, Gentry & Eastin, 1972; Eley *et al.*, 1978).

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