The production of chorionic somatomammotrophin in sheep

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Summary. The production of ovine chorionic somatomammotrophin (OCS) was demonstrated in the trophoblast from Days 16–17 of pregnancy. Concentrations in the placenta rose slowly until about Day 100 when there was a rapid increase to reach 70 ± 5 μ g prolactin equivalent/g fresh placental tissue and 15 ± 2 mg/placenta on Day 120. After Day 140, the concentrations decreased. It is suggested that OCS may be luteotrophic and have an effect on fetal growth.

Introduction

A placental hormone with somatotrophic and lactogenic activities has been recently purified and characterized for the sheep (Handwerger, Maurer, Barrett, Hurey & Fellows, 1974; Martal & Djiane, 1975, 1976; Martal, Djiane & Delouis, 1975, 1976; Chan, Robertson & Friesen, 1976). The hormone, ovine chorionic somatomammotrophin (OCS) or ovine placental lactogen (OPL), is homologous with the CS (or PL) of women, and its concentration, expressed as prolactin-like activity, has been measured in the blood but not in the placenta by a radioreceptor assay (Kelly, Robertson & Friesen, 1974; Djiane & Kann, 1975). In women, the relationship between the level of serum HCS and its placental production is not fully understood (Sciarra, Sherwood, Varna & Lundberg, 1968; Saxena, Emerson & Selenkow, 1969; Singer, Desjardins & Friesen, 1970; Seppälä & Ruoslahti, 1970; Rolschau, Date, Kristoffersen, Pedersen & Ulrich, 1975).

With the isolation of OCS, however, the sheep would appear to be a good experimental model for study of the relationships between the synthesis and excretion of OCS, and the present study was designed to investigate the appearance of OCS and its placental variations during pregnancy.

Materials and Methods

The studies were carried out on 146 pregnant ewes of the Préalpes du Sud breed which were mated during the oestrus following removal of a progesterone-impregnated vaginal sponge (Roberts, 1966). Trophoblast material was surgically obtained at different stages of pregnancy by flushing the uterine horns with sterile saline. Placentae were also obtained at various stages of pregnancy after slaughter of the ewes.

The number of cotyledons associated with single fetuses was about 70. Fetal cotyledons were separated from maternal cotyledons, thoroughly blotted, weighed and frozen at -15°C . The weight of the fetal cotyledons was determined because they have been reported to secrete OCS (Forsyth, 1972; Dubois, Martal & Djiane, 1976). Equal amounts of fetal cotyledons were thawed and homogenized in 0.01 M-phosphate buffer, pH 7.6 with 0.3 M-KCl and filtered through cheese-cloth. Trophoblasts were similarly thawed and homogenized. After stirring at pH 9.5 for 4 h the samples were centrifuged at 12,000 g for 30 min and the lactogenic activity of supernatant fluids was measured by the radioreceptor assay described by Shiu, Kelly & Friesen (1973) with small modifications (Djiane & Kann, 1975). Membrane proteins were obtained from the mammary gland of lactating rabbits treated with 2 α -bromocryptine (CB 154; Sandoz). The standard curve was established by incubation of membrane receptors, labelled hormone and different concentrations of unlabelled ovine prolactin (NIH-P-S7, 24 i.u./mg) for 16 h at 4°C . The placental lactogenic activity was determined by incubating

the placental extracts or blood samples instead of the unlabelled prolactin. The specificity of the assay has been verified (Djiane & Kann, 1975): only hormones which have lactogenic activity in the rabbit (prolactins of different species, placental lactogens, human growth hormone) are able to compete with ovine prolactin at the receptor sites. Serial dilutions of placental extracts gave a displacement curve parallel to that obtained with increasing amounts of ovine prolactin. The use of previously desaturated membrane preparations by removal of endogenous prolactin after bromocryptine treatment *in vivo* was found to improve the accuracy of the assay ($\pm 5\%$) (P. Durand & J. Djiane, unpublished observations). The affinity constant for the hormone receptor interaction was $K_a = 3.2 \cdot 10^9 \text{ M}^{-1}$, and the sensitivity of the assay (20 ng ovine prolactin equiv./ml serum or placental extract) was therefore lower than with radioimmunological methods.

Serum prolactin was measured by radioimmunoassay (Kann, 1971) and the value subtracted from the total serum lactogenic activity obtained by the radioreceptor assay.

Results

OCS activity (μg prolactin equiv./trophoblast) first appeared about Days 16–17 (0, 1, 0.3, 0.2 and 1 on Days 15, 16, 17, 18 and 20, respectively) and increased to 4.5 on Day 25 and 9.5 on Day 30 of pregnancy.

The weight of the fetal cotyledons increased sharply until Day 70 of pregnancy but remained almost constant thereafter (Table 1), until a decrease began about Day 140. At parturition, the fetal cotyledonary mass was only about one-third of the weight recorded between Days 80 and 130 of pregnancy.

Table 1. Changes (mean $\pm$ S.E.M.) of placental weight during a singleton pregnancy in Préalpes ewes

Day of gestation	50	62	75	85	100	110	115	120	130	140	At term
No. of ewes	1	5	9	12	14	14	13	24	5	6	6
Wt of fetal cotyledons (g)	51	182 $\pm$ 27	211 $\pm$ 32	232 $\pm$ 20	228 $\pm$ 11	228 $\pm$ 14	206 $\pm$ 20	209 $\pm$ 11	229 $\pm$ 8	175 $\pm$ 24	74 $\pm$ 5

Table 2. Changes (mean $\pm$ S.E.M.) in OCS concentrations in the placenta of the sheep during singleton pregnancy

Day of pregnancy	70-75	80-85	90-95	100	110-120	140	At term
No. of samples	9	12	3	6	13	5	6
OCS concentration μg/g fresh tissue	22 ± 4	22 ± 5	20 ± 9	38 ± 7	70 ± 5	17 ± 2	10 ± 1
mg/placenta	5.2 ± 3.2	4.5 ± 1	5 ± 3	8 ± 4	15 ± 2	3 ± 1	0.8 ± 0.2

The concentration of OCS per g fresh cotyledonary tissue increased about Day 100, reached a maximum at Day 120 and then dropped (Table 2). A similar result was obtained when the OCS concentration was expressed as mg/placenta, but the peak at Day 120 was less pronounced (Table 2). The OCS concentration/g fresh placental tissue was similar regardless of whether 1, 2 or 3 fetuses were present, and therefore the amounts of OCS/sheep were 2- to 3-fold higher in twin and triplet pregnancies (Table 3) and a similar relationship was seen for the serum OCS concentrations. Thus, the number of fetuses in any pregnant ewe can be predicted.

Table 3. Differences in the mean $\pm$ S.E.M. placental weight and OCS concentrations in sheep with 1, 2 or 3 fetuses on Day 110 of pregnancy

	No. of fetuses		
	1	2	3
No. of ewes	11	7	1
Placental weight (g)	188 $\pm$ 10	361 $\pm$ 15	615
OCS concentration			
ng/ml maternal serum	512 $\pm$ 65	1374 $\pm$ 199	1989
μ g/g fresh tissue	72 $\pm$ 5	82 $\pm$ 18	65

In 3 sheep, 1 fetus and 2 corpora lutea were observed on Day 120 of pregnancy. For example, one ewe showed a placental weight of 318 g (corresponding to a twin pregnancy) and serum OCS levels of 562 ng/ml (single pregnancy), suggesting that resorption of one fetus had taken place. A second ewe had a placental weight of 387 g and an OCS level of 1207 ng/ml, indicating the occurrence of resorption after maximal production of OCS had been achieved. The third ewe had a placental weight of 164 g and an OCS level of 392 ng/ml, suggesting that resorption had occurred early in pregnancy during the phase of placental growth.

Until Day 130 of pregnancy, the corpus luteum remained functional, weighing 693 $\pm$ 26 (S.E.M.) mg (N = 6). On Day 140 the corpus luteum weight was 375 $\pm$ 46 mg (N = 6), indicating regression. In hypoprolactinaemic pregnant ewes (injected twice daily between Days 70 and 140 with 1 mg 2 α -bromocryptine/animal), the corpus luteum weight was only slightly lower (561 $\pm$ 19 mg, N = 30) than in control pregnant ewes until Day 130, at Day 140 the weight was 462 $\pm$ 34 mg (N = 6).

Discussion

Chorionic somatomammotrophin is secreted in the ewe by large binucleate PAS-positive cells in the epithelium of the chorionic villi (Dubois *et al.*, 1976). These cells were not observed before Days 16–17 of pregnancy by Boshier (1969). Moreover, the life-span of the corpus luteum of the cycle could not be extended by intrauterine infusion of purified OCS (40 μ g prolactin equiv./day) from Day 12 (unpublished observations). These findings suggest that OCS is not the substance associated with the presence of the embryo on Day 12 known to be essential for inhibiting regression of the corpus luteum (Moor & Rowson, 1966a,b).

It is possible that OCS is luteotrophic, like human placental lactogen (Josimovich, 1963) and prolactin in sheep (Denamur, 1973; Denamur, Martinet & Short, 1973). In normal pregnancy, the weight of the corpus luteum remains constant until Day 130; there is only a slight (present study) or 25% (Denamur, Kann & Short, 1971) reduction in luteal weight in sheep rendered hypoprolactinaemic or hypophysectomized after Day 60 of pregnancy, respectively, suggesting a placental luteotrophin. However, after Day 50 the ovaries are no longer required for the maintenance of gestation (Denamur & Martinet, 1955) because the placenta secretes large amounts of progesterone (Bassett, Oxborrow, Smith & Thorburn, 1969). Nothing is known about the relationship between OCS and progesterone secretion in the placenta.

The total weight of the placenta increases rapidly until Day 80 of pregnancy and then remains constant until Day 130 (Alexander, 1964). The concentration of OCS is therefore not proportional to placental weight because it is low before Day 80 and rises between Days 80 and 130. Nothing is known about the molecular mechanism of OCS synthesis for comparison with information available for HCS (Boime, Boguslawski & Caine, 1975; Hubert & Cédart, 1975).

After Day 140 of pregnancy, placental weight and OCS production decrease rapidly, the latter slightly earlier than the former, and both are much reduced at parturition. The mechanism of placental regression is not known, but the spectacular involution of the placenta before parturition suggests lysosomal activity. Lysosomes are known to be labilized under the influence of oestrogens (Szego,

1974; Gustavii, 1975) and maternal oestrogens are rapidly increasing at term (Challis, 1971). Prostaglandins may also be involved since the concentration of PGF-2 α in the maternal cotyledons rises before parturition (Liggins & Grieves, 1971).

The high concentrations of OCS between Days 90 and 130 of pregnancy could be related to mammogenesis and lactogenesis. Mammogenesis in ewes rendered hypoprolactinaemic is apparently normal (Djiane, Kann, Delouis & Martal, 1975; Martal & Djiane, 1977) and can be attributed to OCS. Lactose synthesis in the sheep mammary gland begins after about Day 95 of pregnancy (Denamur, 1965), the time at which there is a large increase in OCS production. Purified OCS (Martal & Djiane, 1975) has been shown to have lactogenic activity *in vitro* by its effects on the histology of mammary gland tissue of pseudopregnant rabbits and on lactose synthetase activity and casein synthesis (Martal *et al.*, 1976).

The hormonal regulation of fetal growth is not clear (Charrier, 1973) but growth is only delayed after hypophysectomy of fetuses after Day 93 (Liggins & Kennedy, 1968), whereas growth hormone has been found in the fetal pituitary (Stokes & Boda, 1968) and blood (Bassett, Thorburn & Wallace, 1970) from Day 50 of pregnancy. It seems possible that a growth hormone-like substance is secreted by the placenta. OCS binds to growth hormone receptors from liver (Handwerger *et al.*, 1974; Chan *et al.*, 1976; Martal & Djiane, 1977) and has been shown to increase body weight and the width of the epiphyseal cartilage of the tibia in hypophysectomized rats (Chan *et al.*, 1976; Martal & Djiane, 1977). In the first half of pregnancy, therefore, OCS may regulate fetal growth, while in the second half OCS and fetal growth hormone may have complementary effects.

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