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# Immunodetection of bovine chorionic somatomammotrophin (bCS)

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Abstract. Bovine chorionic somatomammotrophin (bCS) was detected in the bovine placenta by using an immunoenzymatic procedure. This hormone appears mainly located in the binucleate cells of the foetal cotyledons and to a lesser extent in some localized superficial areas of the foetal and maternal epithelium. These areas may correspond to foetal binucleate cells migrating into the maternal caruncles.

Placental lactogens or chorionic somatomammotrophins are placental polypeptide hormones with somatotrophin-like and prolactin-like activities, that have been detected in numerous mammals. Both activities are carried by the same molecule but their respective potencies are quite different among the studied species (Blank et al. 1977; Porter 1980). Somatotrophin-like activity affects mainly the foetus; whereas, prolactin-like activity is generally considered to be responsible for maternal mammary gland growth.

The exact origin of these hormones is generally not known except for the human and ovine species: human placental lactogen (hPL) is essentially located in the syncytiotrophoblast (Sciarra et al. 1963; Watkins 1978) and ovine placental lactogen (oPL) is detected in the binucleate cells that are abundant in the placental villi (Martal et al. 1977; Reddy & Watkins 1978) and also in the sheep syncytium (Wooding 1981).

In the bovine, a placental lactogenic activity was first demonstrated by Buttle & Forsyth (1976) as early as the 36th day of gestation. In the same year, a polypeptide hormone, the bovine placental lactogen (bPL), was purified by Bolander & Fellows (1976). However, although this molecule (bPL) was purified to physicochemical homogeneity, it had virtually no biological activity. More recently, Beckers et al. (1980) have described the isolation of a bovine placental hormone from trophoblastic caruncles which had a high prolactinic and somatotrophinic activity. This hormone named bovine chorionic somatomammotrophin (bCS) was purified about 1500 times to electrophoretic homogeneity. Subsequently, using a highly specific and sensitive RIA, Beckers et al. (1982) confirmed the higher concentration of hormone in foetal caruncles when compared with the maternal ones. Moreover the hormonal levels in plasma are quite different in mother and in foetus: in the latter they are high in early pregnancy (about 25 ng/ml) and decrease progressively, reaching about 5 ng/ml near birth. In the mother, the levels are far lower, the hormone is barely detectable (detection limit corresponds to 50 pg/ml) from the 26th to the 110th day of pregnancy. Thereafter, the level increases progressively to reach maximum values of about 2 ng/ml around parturition.

Using the same highly specific antiserum, we report here the localization of bCS-like activity with immunohistochemical techniques in the bovine placenta.

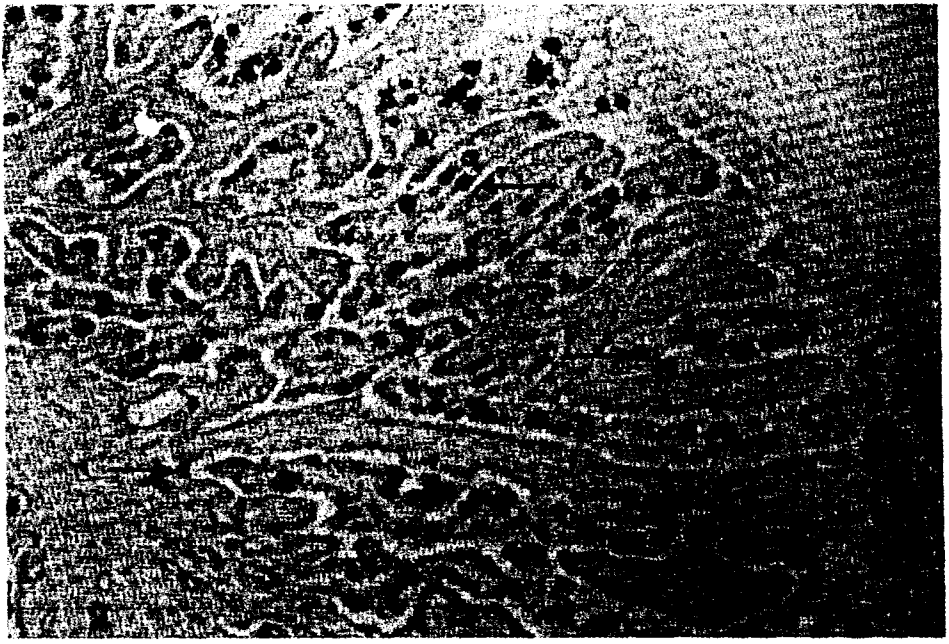

*Fig. 1.*
Cells staining with the anti-bCS in the foetal villi. Black arrows show some stained cells which are clearly binucleate. Red arrows show stained cells in maternal epithelium. Animal No. 1885; 6th month of pregnancy. A blue filter was used for photography.

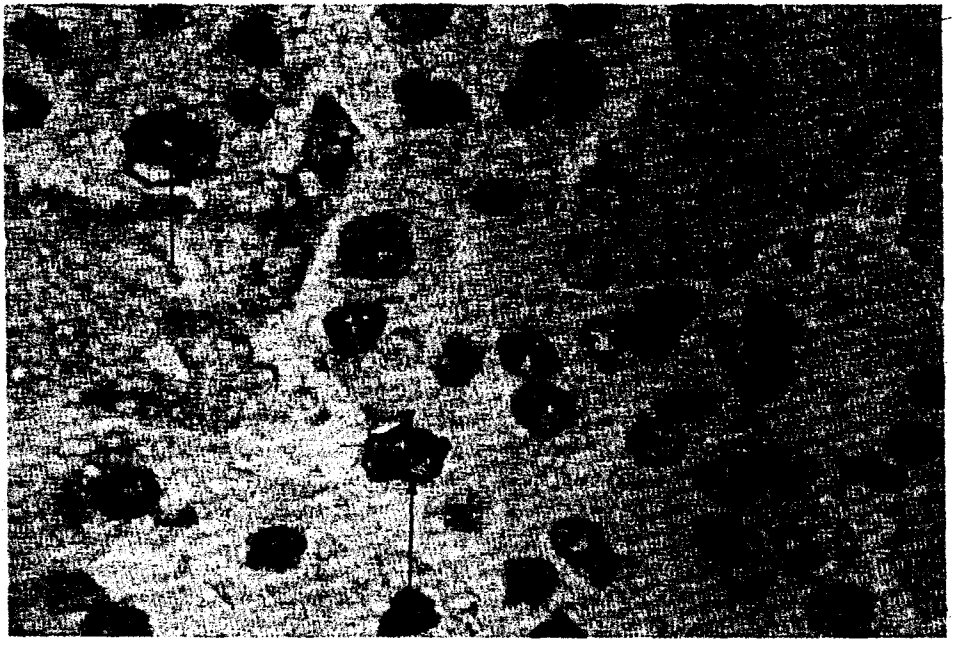

*Fig. 2.*
Showing stained cells in foetal villi. Black arrows show stained binucleate cells. A blue filter was also used. Same animal as Fig. 1.

*Fig. 3.*
Cells stained for bCS in foetal (black arrows) and maternal parts (red arrows). No counterstaining. No filter used for micrography. Animal No. 1843; 5th month of pregnancy.

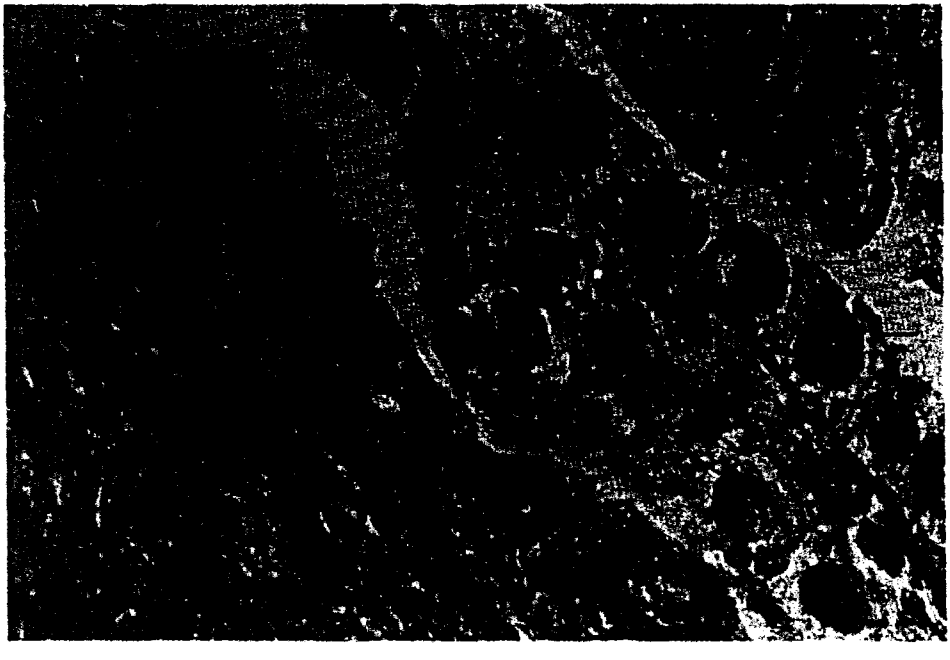

*Fig. 4.*
Serial section to that in Fig. 3. Section stained with PAS. Black arrows indicate the same cells as seen on Fig. 3 in foetal villi. They are PAS positive. Red arrow shows that the immunostained cell seen in the maternal epithelium is also PAS positive.

## Materials and Methods

Thirty-eight placentas from 'Bleu Blanc Belge' breed (BBB) were collected at the local slaughterhouse. Gestational ages were (Table 1) estimated from the crown-rump lengths according to Rexroad et al. (1974) and adapted to BBB by our laboratory (unpublished results). Placentas were dissected out and fragments (0.5 × 0.5 × 0.5 mm) of cytoledons were immersed in Gerard's fixative without delay. Cotyledons of less than 2 cm were cut in 2 mm thickness slices. To preprare the fixative 2.5 g of neutral cupric acetate was dissolved in 100 ml distilled water and then 4 g picric acid added. After solubilisation and filtration, 10 ml formaline (40%) and 1 ml acetic acid were added. One hundred ml of this liquid was mixed just before use with 10 ml of a saturated aqueous solution of mercuric chloride (Gape 1968). After 2 weeks in fixative at room temperature, the fragments were embedded in paraffin and cut into serial 5 µm sections.

Classic histological techniques using PAS, Mayer's Carmalun and toluidine blue were performed to stain the cytoplasm, the nucleus of the placental cells or the foetal and maternal connective tissue. We counted on each section the total number of binucleate cells and also of stained binucleate cells in foetal and maternal parts, respectively.

bCS immunodetection was performed on dewaxed (1-1-1 trichloroethane, 3 × 20 min) sections using Sternberger's (1979) PAP procedure modified as follows:

1) aspecific binding sites were saturated by incubating the section with non-deactived normal sheep serum (NSS-5% in phosphate buffered saline) prior to the antiserum treatment (Polak & Van Noorden 1983),

2) anti-bCS was diluted (1:10 000) in phosphate buffered saline containing 0.1% BSA and 0.01% sodium azide, and applied overnight at 4°C.

The sections were then washed (3 × 20 min) with phosphate buffered saline, pH 7.6 containing 0.1% BSA and 1% NSS,

3) anti-rabbit immunoglobulins as well as PAP complexes (UCB Bioproducts, Brussels) were diluted (1:100) in the same phosphate buffered saline (without BSA and NSS) except for the PAP stage where the buffer did not contain sodium azide (an inhibitor of peroxidase enzymatic reactions); incubation times were 1 h at room temperature.

Between each step, the sections were washed 3 times (20 min) in phosphate buffered saline, pH 7.6, and

4) peroxidase was revealed by the diaminobenzidine reaction −12.5 mg DAB (Sigma) in 10 ml phosphate buffered saline, pH 7.6 and 50 µl of 3% $H_2O_2$ solution (1 Perhydrit tablet 'Merck' in 10 ml $H_2O$). The revelation was stopped with distilled water after 2 to 5 min.

The preparation of the bCS antiserum as well as radioimmunological tests for specificity were previously reported (Beckers et al. 1982). No cross-reactivity was noticed with prolactin, somatotrophin or with other hypophyseal hormones.

Immunocytochemical controls for specificity involved:

1) omision of one of the various steps in the PAP procedure,

2) replacement of bCS antiserum by normal sheep and preimmune rabbit serum (1:100 to 1:10 000), and

3) saturation of the antiserum with purified bCS. A mixture of the antibody (1 ml − 1:10 000) and bCS (10 µg) was left overnight at 4°C and subsequently applied to the test section. Precipitation of Ag.−Ab. complexes as seen in the classical precipitin reaction, was avoided because the concentration of both components was very low. No subsequent precipitate interference with staining was found (Polak & Van Noorden 1983).

Saturation of the antiserum with adenohypophyseal hormones (10 µg) Prl-NIH-B4, GH-NIH-B18, and bFSH, bLH prepared in our laboratory.

## Results

bCS immunoreactivity was detected in all placentas examined (detailed in Table 1). The earliest used was 90 days post coitus (dpc). Because it was very difficult to obtain placentas of less than 90 days in our working conditions, we did not study the possible existence of bCS in younger conceptuses.

bCS is located in the cytoplasm of the foetal chorionic binucleate cells (Fig. 1). These cells have a regular ovoid shape and often possess 2 or 3 nuclei (Fig. 2). Nearly all (90 to 95%) the binucleate cells are intensely stained by the bCS as early as 90 dpc. Frequency, morphology or staining intensity of the bCS-positive cells did not change significantly during the period studied. As can be seen on Figs. 3 and 4 (serial section) all the immunostained cells are PAS positive.

*Table 1.*
Number of placentas studied. Age determined by measurement of crown-rump length (Rexroad et al. 1974),

| Age (month) | Crown-rump length (cm) of foetus | Number of placentas |
|---|---|---|
| 3–4 | 12–20 | 3 |
| 4–5 | 20–30 | 8 |
| 5–6 | 30–42 | 7 |
| 6–7 | 42–56 | 11 |
| 7–8 | 56–72 | 9 |
| 8–9 | 72 | 1 |

On some sections, a few areas of the surface of the foetal villi were stained (Fig. 5, green arrow). In the maternal placenta (red arrows on Figs. 3, 5 and 6), the uterine epithelium also shows some similar stained zones. These zones represent 1 or 2% of the total stained cells.

In the maternal epithelium, these stained areas, like the superficial areas of the foetal villi, could correspond to migrating binucleate cells or portions of the cytoplasm of binucleate cells whose major part lies outside this plane of section. However, probably due to fixation and sectioning artefacts that separate foetal from maternal tissue, we never observed clear evidence of cellular migration towards the maternal placenta.

No staining occurred in the other parts of the placenta, or in the amniotic or allantoic membranes. The staining described above was absent when one step of the PAP technique was omitted, when bCS antiserum was either replaced by normal sheep or rabbit serum or saturated with bCS (Fig. 7). Immunostaining was not modified when bCS antiserum was pre-incubated with hypophyseal hormones.

## Discussion

Results presented here show for the first time, that bCS is found only in the binucleate cells characteristic of the bovine trophectoderm and in a few areas of the foetal and maternal epithelium. This staining of the binucleate cells corresponds to the results obtained in the sheep with anti-oPL antiserum by Martal et al. (1977) but not with the results of Carnegie et al. (1982) who claimed a localisation of oPL only in the uninucleate trophoblastic cells.

In the sheep, immunostaining of granules in the foetomaternal syncytiotrophoblast (Wooding 1981) is said to be the result of binucleate cells migrating from the trophoblast to the syncytiotrophoblast. There, these binucleate cells merge into the syncytium and their content is then released by exocytosis to the maternal connective tissue (Wooding 1982).

In the bovine, the stained cells for bCS that we observe in the maternal epithelium and superficially in the foetal trophectoderm are probably migrating binucleate cells. The 1 or 2% of positive cells observed in the maternal tissue very likely originated from the foetal trophectoderm and have migrated into the maternal tissue.

The 5 to 10% non-stained foetal binucleate cells are either binucleate cells that elaborated some other secretory products (Watkins & Reddy 1980) or bCS binucleate cells that have already released their bCS content to the foetal connective tissue.

Beckers et al. (1982) have found by RIA 4 to 5 times more bCS in foetal cotyledons than in maternal ones. Moreover the ratio of the foetal plasma bCS to maternal plasma bCS was about 20 to 30. We think that the bCS assayed in the maternal cotyledons might well originate mostly from foetal fragments included in the maternal placenta, since the close interdigitation of placenta villi makes it impossible to separate clearly the foetal and maternal parts. We would suggest that, as in the sheep, the granules of the migrating binucleate cells synthesised in the foetal trophoectoderm are, after migration, released into the maternal connective tissue. However, a great number of the binucleate cells probably releases their granules within the foetal trophectoderm epithelium. This would explain the higher concentration of bCS in the foetal circulation.

Beckers et al. (1982) also showed that ratio of foetal to maternal plasma bCS concentration decreases during the pregnancy. In this immunostaining study, we did not see any corresponding change in topography and intensity of the immunostaining during gestation. The RIA results indicate that in the bovine the granule release needs to be mostly in the foetal compartment. In human and ovine species, near birth the maternal plasmatic concentration of placental lactogen is always greater than in foetus (Kaplan & Grumbach 1965; Chan et al. 1978). In the bovine, it is the other way round. Our results, with those of others (Levasseur 1983), suggest that in this species the foeto-placental unit is much more independent than in other species.

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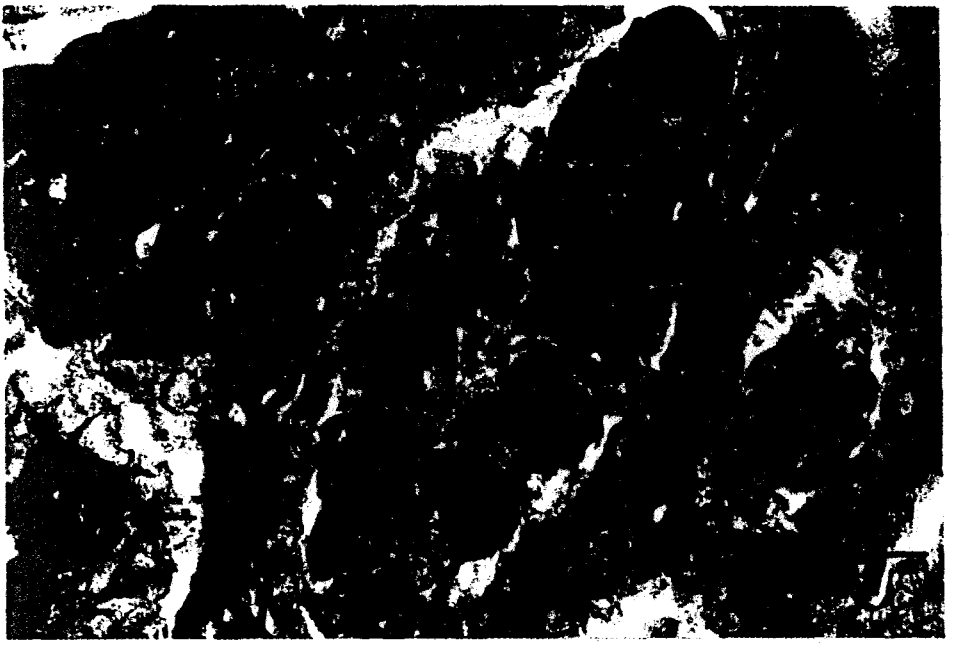

*Fig. 5.*

Section stained for bCS. Black arrows show binucleate cells in foetal trophectoderm. Red arrows: cells stained in maternal part. Dashed arrows: maternal epithelium, most cells stained. Green arrows: a binucleate cell probably beginning its migration from foetal villi to maternal epithelium. The connective tissue is stained with toluidine blue. Animal No. 1894; 7th month of pregnancy.

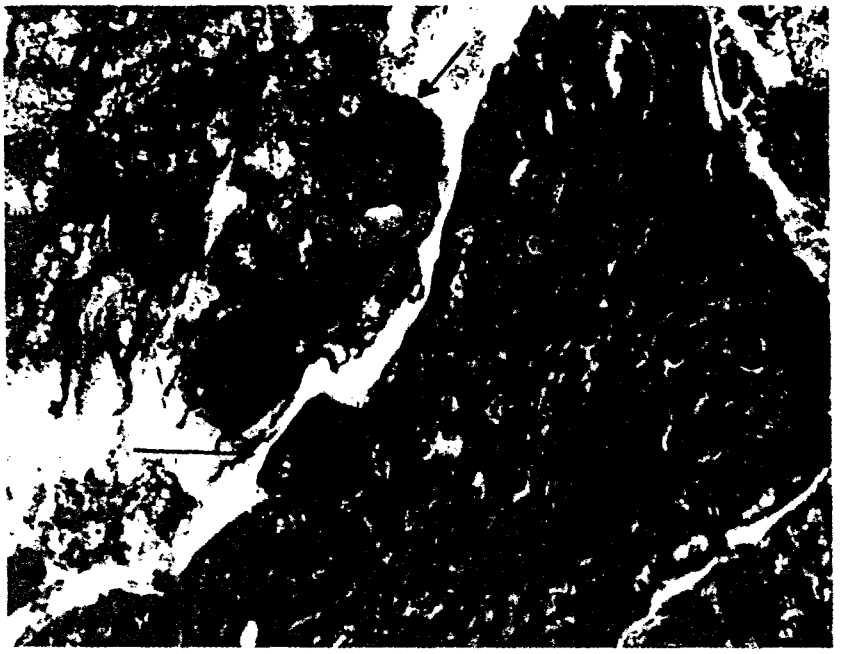

*Fig. 6.*

Showing stained with anti-bCS in foetal villi. Black arrows show stained cells in foetal trophectoderm. Red arrow shows one trinucleate cell in maternal part, probably due to the fusion between a binucleate migrating cell and an epithelial cell. Connective tissue is stained with toluidine blue. Animal No. 1848; 4th month of pregnancy.

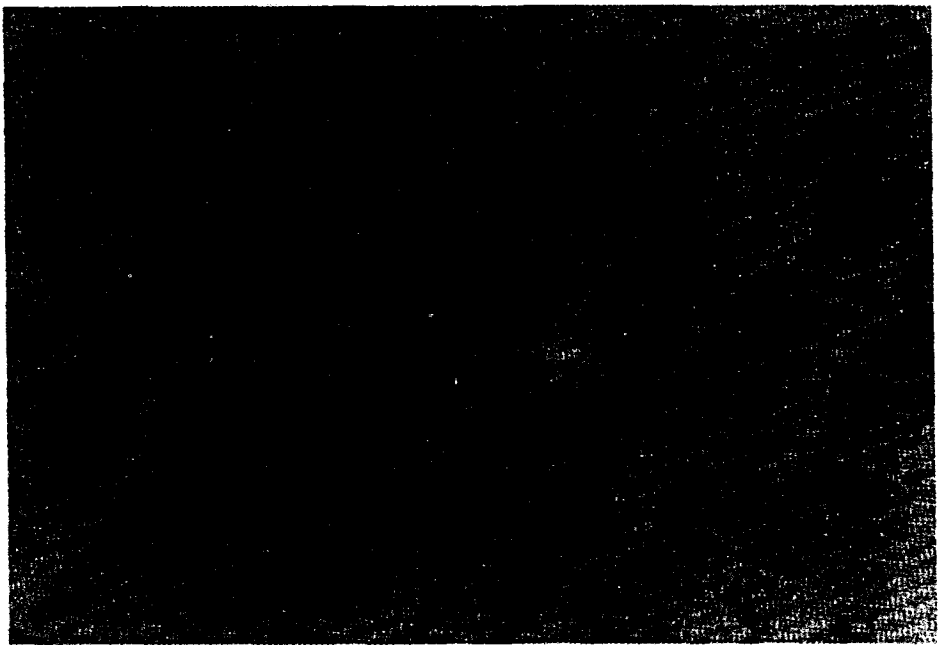

*Fig. 7.*
Section incubated with NSS instead of anti-bCS. No staining is observed. The nuclei were stained with Mayer's Carmalun. No filter used for microphotography. Animal No. 1843; 5th month of pregnancy.

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