Reciprocal expression of 17α-hydroxylase-C17,20-lyase and aromatase cytochrome P450 during bovine trophoblast differentiation: a two-cell system drives placental oestrogen synthesis

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Abstract

No definitive information is yet available on the steroidogenic capacity of the two morphologically distinct cell types forming the bovine trophoblast, the uninucleated trophoblast cells (UTCs) and the trophoblast giant cells (TGCs). Hence, in order to localise 17α-hydroxylase-C17,20-lyase (P450c17) on a cellular level and to monitor its expression as a function of gestational age, placentomes from pregnant (days 80–284; n = 19), prepartal (days 273–282; 24–36 h prior to the onset of labour; n = 3) and parturient cows (n = 5) were immunostained for P450c17 using an antiserum against the recombinant bovine enzyme. At all stages investigated, P450c17 was exclusively found in the UTCs of chorionic villi (CV), where staining was ubiquitous between days 80 and 160, but was largely restricted to primary CV and the branching sites of secondary CV between days 160 and 240. Thereafter, a distinct ubiquitous staining reoccurred in the UTCs of all CV in late pregnant, prepartal and parturient animals. Using an antiserum against human aromatase cytochrome P450 (P450arom), specific cytoplasmic staining was observed in TGCs. In placentomes from pregnant cows, staining intensity was higher in mature compared with immature TGCs and was more pronounced in the trophoblast covering big stem villi compared with the trophoblast at other sites of the villous tree. In placentomes of a parturient cow, specific staining was only found in mature TGCs that survived the normal, but substantial, prepartal decline in TGC numbers. These results clearly showed that bovine UTCs and TGCs exhibit different steroidogenic capacities, constituting a ‘two-cell’ organisation for oestrogen synthesis. P450c17 expression appears to be quickly down-regulated and P450arom is up-regulated when UTCs enter the TGC differentiation pathway.


Introduction

The formation of progestagens and oestrogens occurs in the placenta of many mammalian species, with both classes of steroids obviously playing important roles in the establishment and maintenance of gestation (Pepe & Albrecht 1995). In cattle, our preceding studies suggest a particular role of placental progesterone and oestrone as local regulators of placental growth and differentiation (Hoffmann et al. 1997, Schuler et al. 1999, 2002, 2005, Hoffmann & Schuler 2002). Sites of placental steroid production are the fetal cotyledons (Hoffmann et al. 1979, Conley & Ford 1987, Schuler et al. 1994) which, together with the maternal caruncular tissue, form multiple discrete sites of placenta, the placenomes (Leiser & Kaufmann 1994).

The regulation of bovine placental steroidogenesis is still unclear, since specific tropic hormones have not yet been identified. Generally, steroid production is regulated by the relative levels and tissue-specific arrays of steroidogenic enzymes. As has been shown for dominant ovarian follicles or adrenal cortex, steroidogenic organs may exhibit functional zonation or compartmentalisation. Partitioning the expression of key steroidogenic enzymes into different, perhaps adjacent, cell types provides an important additional level of regulation of steroidogenesis in each cell compartment which facilitates or limits androgen and oestrogen synthesis in particular (Conley & Bird 1997). In the ovarian follicle, androgen synthesis catalysed by 17α-hydroxylase-C17,20-lyase cytochrome P450 (P450c17) and the conversion of androgens into oestogens.
by aromatase cytochrome P450 (P450arom) are partitioned into the theca and granulosa layers respectively (Richards & Hedin 1988, Gore-Langton & Armstrong 1994). Another notable example of compartmentalisation is found in human and equine pregnancy where, due to a lack of a placental P450c17 expression, oestrogen synthesis from placental P450arom expression relies on androgens synthesised by P450c17 expressed in the fetal adrenal (humans) or fetal gonads (equine), the two compartments then comprising the so-called ‘feto-placental unit’ (Diczfalusy 1964, Pashen & Allen 1979, Conley & Mason 1994, Kuss 1994). In contrast, the bovine trophoblast has been shown to express all enzyme activities necessary for the conversion of pregnenolone into oestrone (Schuler et al. 1989; P450arom: Hinshelwood et al. 1993, Fuerbass et al. 1994). However, no definitive information is available on the steroidogenic capacity of the two morphologically distinct cell types forming the bovine trophoblast, the uninucleated trophoblast cells (UTCs) and the generally binucleated trophoblast giant cells (TGCs) (Wooding & Wathes 1980, Klisch et al. 1999).

This current study focuses on the cell-specific and therefore potential compartmental expression of P450c17 and P450arom in bovine placenta. In cattle, both enzymes are well characterised at the molecular level (P450c17: Zuber et al. 1986a, 1986b, Estabrook et al. 1988, Bhasker et al. 1989; P450arom: Hinshelwood et al. 1993, Fuerbass et al. 1997). However, data on their spatio-temporal expression in the bovine placenta are sparse, and no definitive information is available on their expression on a cellular level. Using Western and Northern blots, high P450c17 expression levels have been found previously in bovine placentomes prior to months 4–5 of gestation, whereas they were markedly lower in mid-gestation (Conley et al. 1992). In vitro investigations using placental homogenates suggest that, as in the sheep (Mason et al. 1989), P450c17 activity in bovine trophoblast is significantly up-regulated at parturition (Schuler et al. 1994). Aromatisation in microsomes prepared from bovine cotyledons exhibits a distinct peak in the fifth month of gestation but is low thereafter until the last month of gestation, when it increases again around the time of parturition (Tsumagari et al. 1993). Only sporadic, and in part contradictory, information is available on the expression of P450arom on a cellular level in bovine placentomes. From studies with enriched fractions of UTCs and TGCs, Gross & Williams (1988) concluded that the TGCs were the primary site of bovine placental oestrogen production; in contrast, Matamoros et al. (1994) found the UTCs to be the major cellular source of oestrone which is the predominant oestrogen produced by the bovine placenta (Hoffmann et al. 1997).

Thus, the aims of this study were to localise P450c17 and P450arom in the bovine trophoblast at the cellular level and to characterise their expression with respect to the location within the chorionic villous tree, gestational age and the process of TGC differentiation. Materials and Methods

Sample collection and fixation

The study utilised placental specimens from 32 animals ranging from 80 days of gestation to parturition. Placentomes from healthy 80- to 270-day pregnant cows (n = 18) were collected at a local slaughterhouse. Gestational age was assessed according to fetal crown–rump length (Schnorr & Kressin 2001). In order to obtain placentomes immediately after spontaneous initiation of parturition, three cows (partum observational group) were subjected to Caesarean section after the maternal progesterone levels had dropped below 1.5 ng/ml (days 273–282; calculated from registered insemination dates) as detected by regular measurements at 8-h intervals (ACS:180 automated chemiluminescence system with kit PRGE; Bayer Vital GmbH, Fernwald, Germany) during the last 2 weeks of gestation. No signs of active labour were observed at the time of surgery. One additional cow was initially included in this group based on clinical signs of impending parturition on day 284. Retrospective measurements revealed that the prepartal decline in progesterone had not yet begun. Thus, this animal was included with the group of pregnant cows. Placentomes from parturient cows (term; n = 5) were obtained from animals undergoing routine Caesarean section for fetal/pelvic mismatch after spontaneous onset of labour. They were collected immediately after the delivery of the calves which were normally developed and vital in all cases.

Three to five placentomes were removed from the mid-region of the uterine horn that contained the fetus from each of these animals. Tissue samples were fixed overnight in 10% phosphate-buffered formalin and subsequently dehydrated in a graded ethanol series. They were finally embedded in paraffin.

A second set of samples collected from 90- and 180-day pregnant cows (n = 4 animals per age group), from a cow after glucocorticoid-induced parturition in late gestation and from a cow after normal parturition, was obtained from the Department of Animal Science, University of California, Davis. These samples were paraffin embedded after overnight immersion fixation in 4% paraformaldehyde (PFA) and subsequent dehydration in a graded ethanol series.

For the preparation of microsomal protein for Western blot analysis, small pieces of placentomes from pregnant cows were snap-frozen on dry ice and stored at −80 °C until analysis. Additionally, cotyledonal tissue was prepared from a placenta of a parturient cow immediately after timely spontaneous release, and conserved in the same manner.

Collection of samples from living animals was approved by the local competent authorities.

Immunohistochemical staining procedures

Indirect immunoperoxidase staining methods were employed using the streptavidin–biotin technique for signal enhancement following standard procedures. For the
detection of P450arom, 5 μm tissue sections were prepared from PFA-fixed samples and incubated in a steamer at 95°C for 20 min in antigen unmasking solution (Vector Laboratories, Burlingame, CA, USA) prior to immunohistochemistry. For the immunolocalisation of P450c17, 5 μm sections from formalin- or PFA-fixed samples were used without retrieval. Antiseras produced in rabbits against recombinant bovine P450c17 (Peterson et al. 2001) and recombinant human P450arom (Conley et al. 1996) served as primary antibodies. Additionally, a murine monoclonal antibody against a synthetic peptide corresponding to amino acids 376–390 of human P450arom (Turner et al. 2002) was tested (clone H4, purchased from Serotec GmbH, Düsseldorf, Germany); the peptide sequence used for immunisation is completely conserved in bovine P450arom. Serum from a non-immunised rabbit or dilution buffer were used as negative controls. A dominant bovine ovarian follicle served as positive control tissue.

**Semiquantitative assessment of immunostaining for P450c17**

Immunostaining was evaluated by one person blinded as to the animals at defined locations (see Fig. 1) and classified by comparison with pre-assigned photographic standards.

**Western analysis**

Microsomal preparations obtained by differential centrifugation (Moran et al. 2002) of placentomal or cotyledonary homogenates were electroblotted onto nitrocellulose membranes after PAGE and immunoblotted using the polyclonal antisera against P450c17 and P450arom and the monoclonal anti-P450arom antibody (clone H4) as primary antibodies to verify specificity. The preparation of microsomal protein from different steroidogenic organs of several species and of human recombinant aromatase included for comparison in the Western blot analysis has been described previously (Corbin et al. 2003). Dilution of primary antibodies was 1:5000 for the two polyclonal antisera and 1:200 for the monoclonal antibody. After incubation with the respective peroxidase-labelled secondary antibodies (PI-1000 and PI-2000; Vector Laboratories) a chemiluminescent detection system (Perkin-Elmer Life Sciences, Boston, MA, USA) was used as recommended by the supplier.

**Results**

**P450c17**

In Western blot using cotyledonary microsomal preparations, the polyclonal anti-P450c17 antiserum recognised a single band with an approximate molecular weight of 58 kDa (Fig. 2). With immunohistochemistry, in all placentomes investigated P450c17 was found exclusively in UTCs of chorionic villi (CV) (Fig. 3). No expression was detected in mature TGCs or trophoblast cells exhibiting signs of TGC differentiation such as increased cell volume and nuclear size, or the presence of more than one nucleus (Fig. 3A). The trophoblast of the chorionic plate remote from the insertions of stem villi and of the intercotyledonary chorion (not shown) exhibited no evidence of P450c17 expression. Between days 80 and 160, P450c17 in UTCs of villous trophoblast was highly and almost evenly expressed (Figs. 3B, 4 and 5). Afterwards, explicit staining was mostly restricted to primary CV and the branching sites of secondary CV, whereas staining in the remaining parts of the secondary CV and in tertiary CV was clearly reduced or completely absent. At day 270, distinct and more homogenous staining reappeared in the UTCs of all CV until parturition (Figs. 3C and D, 4 and 5).

**Figure 1** Schematic presentation of the bovine placental villous tree indicating the sites of semi-quantitative evaluation after immunostaining for P450c17. The dotted line indicates the arbitrary division of the interdigitation area in a basal and an apical zone of equal height. As with the exception of the tips of the villous tree (no. 10) no differences in staining intensity of tertiary villi was noticeable between basal and apical zone, they were evaluated as one category (no. 6).
compared with immature precursors (Fig. 7A and C). In TGCs with higher staining intensities in mature TGCs was absent in the respective negative controls (Fig. 7B). Occasionally, a weak to moderate staining was also observed only with the polyclonal anti-P450arom anti-
type (not shown). However, in the similarly PFA-fixed placentomes from bovine placentomes (Fig. 6). Virtually no additional bands occurred when using the monoclonal antibody (clone H4). When using the polyclonal antiserum a prominent band with a molecular weight of about 75 kDa was observed in microsomal preparations from placentomes which, to a lesser extent, was also found in a sample from bovine granulosa cells. In immunohistochemistry using tissue sections prepared from PFA-fixed, paraffin-embedded ovaries from cows at oestrus used as positive controls, both anti-P450arom antibodies yielded intense cytoplasmic staining in bovine granulosa cells of the dominant ovarian follicle; no signals were found in any other cell type (not shown). However, in the similarly PFA-fixed placentomal tissue samples, cell-specific staining was observed only with the polyclonal anti-P450arom antibody (Fig. 7). Specific cytoplasmic signals were observed in TGCs with higher staining intensities in mature TGCs compared with immature precursors (Fig. 7A and C). Occasionally, a weak to moderate staining was also observed in the caruncular epithelium (Fig. 7C) which was absent in the respective negative controls (Fig. 7B). In 90- and 180-day pregnant cows, staining was more pronounced in the trophoblast covering big stem villi compared with the trophoblast at other locations (Fig. 7D and E). Due to a highly variable signal-to-noise ratio in these samples no efforts were made at quantitative evaluation. In the placentome from a cow after induced parturition in late pregnancy, a homogenous and distinct staining was present in the cytoplasm of TGCs in all parts of the villous tree (Fig. 7A and C). In cotyledons of a cow at normal term, specific staining was only found in TGCs having escaped the substantial immediate prepartal decline in TGC numbers that normally occurs at the time of partu-
tion (Fig. 7F).

When using formalin-fixed placentomal tissue samples, no staining was obtained with the monoclonal antibody H4 to P450arom. With the polyclonal anti-human P450arom antiserum, a moderate cytoplasmic staining was observed in TGCs. However, because of the occurrence of a considerable diffuse unspecific background, no further evaluation was performed.

### Discussion

In Western blot using bovine cotyledonary microsomal protein preparations, the primary antiserum against bovine recombinant P450c17 specifically recognises a single band of 58 kDa which is consistent with the molecular weight previously determined in cattle (Lund et al. 1988, Conley et al. 1992) and in other species (Johnson 1992, Albrecht et al. 2001). These data corroborate the specificity of this antiserum also in the immunolocalisation of P450c17 in bovine placentomes. With immunohistochemistry, P450c17 was clearly and exclusively localised in the UTCs of CV whereas no signal was detected in mature TGCs or their precursors. Thus, bovine TGCs and UTCs clearly have different steroidogenic capacities. As it is generally accepted that bovine TGCs differentiate from a homogenous population of UTCs (Wooding & Wathes 1980, Klisch et al. 1999), this staining pattern implies that P450c17 expression is down-regulated at a very early stage of TGC differentiation. Whether the loss of P450c17 expression plays a functionally important role or is just a consequence of TGC differentiation needs further investigation. No signals were found in trophoblast cells that were not in intimate contact with maternal epithelium, that is the trophoblast covering the chorionic plates remote from the insertions of chorionic stem villi and the intercotyledonary regions. This observation suggests that cotyledonary P450c17 expression may also be controlled by factors secreted from the maternal tissues.

During the period under investigation, immunostaining for P450c17 in UTCs exhibited a triphasic pattern with a high, ubiquitous expression in the villous trophoblast between days 80 and 160. This was followed by a substantial reduction of expression and restriction of staining predominantly to major stem villi until late gestation, when a rapid up-regulation and spreading of the signal across the villous trophoblast occurred. No evaluation on the variability between the placentomes of one cow or between different sections of one placentome was performed. However, the high consistency of results obtained with placentomes from different animals at exactly defined stages of gestation (Fig. 5) provides evidence for the validity of the method used for the semi-quantitative assessment of immunostaining. The immunohistochemical results were consistent with data of earlier Western and Northern analyses showing a marked decrease in bovine placental P450c17 expression around mid-pregnancy after a high expression in early gestation (Conley et al. 1992). Moreover, bovine placental P450c17 expression was shown to follow a similar pattern found in bovine fetal adrenals (Conley et al. 1992). This corresponds with an early rise in bovine fetal adrenal cortisol concentration (Lund et al. 1988) and is reminiscent of the prepartum cortisol rise (Comline et al. 1974, Fairclough et al. 1975, Hunter et al. 1977) that is similarly associated with the increase in placental P450c17 expression shown here. Together, this suggests that there may be a functional
coupling of adrenal and placental P450c17 expression that can be activated at any stage of gestation once adrenal organogenesis is complete.

The expression of a number of other genes has been shown to increase in bovine placentomes in a cell-specific fashion at the onset of parturition. As for P450c17, a pre-partal up-regulation of expression starting from areas close to the chorionic plate towards the tips of CV located adjacent to the caruncular stalk has also been observed for the glucocorticoid receptor in TGCs and caruncular epithelial cells.

Figure 3 Immunostaining for P450c17 in bovine placentomes. (A) Specific cytoplasmic staining is exclusively present in the UTCs of CV. Mature TGCs (arrowheads) and their immature precursors (arrows) are negative (day 90). (B) Typical staining pattern in a 90-day pregnant cow. Distinct to intense signals occur in the villous trophoblast over the complete height of the interdigitation area. The trophoblast covering the chorionic plate (CP) is devoid of staining. (C) Typical staining pattern in a 180-day pregnant cow. Significant staining is virtually restricted to primary and secondary villi, whereas the signal in tertiary villi has widely disappeared. (D) Ubiquitous intense staining in the villous trophoblast of a prepartal cow. CS = caruncular stalk; pCV = primary chorionic villus; pMS = primary maternal septum. Black bars = 25 μm; white bars = 200 μm.
cells (Boos et al. 2000), cyclo-oxygenase-II (COX-II) in UTCs (Schuler et al. 2006) and steroid sulphatase in caruncular epithelial cells (Greven et al. 2005), possibly indicating a similar transcriptional control. In the sheep, a pre- and intrapartal increase of placental P450c17 has been shown to promote the increased production of oestrone, a crucial step in the initiation of parturition in this species (Mason et al. 1989, Gyomorey et al. 2000, Whittle et al. 2001). A similar process previously confirmed for the cow at the level of enzyme activity (Schuler et al. 1994) has now been characterised at a cellular level in this study. In the sheep, the up-regulation of COX-II in UTCs and a subsequent rise in placental prostaglandin E₂ levels have been suggested to stimulate the pre- and intrapartal rise of P450c17 expression (Whittle et al. 2000, 2001). A similar prepartal rise in COX-II expression of UTCs has recently also been confirmed for the cow (Schuler et al. 2006). However, during the initial period of high placental P450c17, expression between days 80 and 160 COX-II levels are basal. Thus P450c17 expression is obviously stimulated by other factors during this phase of gestation.

The success of immunohistochemistry for P450arom in bovine placentomes was dependent on the primary antibody used. Specific signals were only obtained with the polyclonal anti-human P450arom antiserum but not with the monoclonal antibody H4. This was true even though both primary antibodies yielded specific staining of granulosa cells in fixed sections of bovine preovulatory dominant follicles and each recognised a band of the expected size of 50 kDa in Western blot analysis of bovine placental microsomal preparations. As there is only one gene encoding aromatase in cattle (Fuerbass et al. 1997, Conley & Hinshelwood 2001, Vanselow et al. 2001),

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**Figure 4** Results of a semi-quantitative evaluation of immunostaining for P450c17 in UTCs of bovine placentomes at defined sites as specified in Fig. 1. Tissue sections were prepared from formalin-fixed samples. *Sampling was performed during the prepartal decline of progesterone which occurs 24–36 h prior to the onset of active labour. No staining (open boxes) through light grey and darker grey boxes to intense staining (solid boxes).

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**Figure 5** Results of a semi-quantitative evaluation of immunostaining for P450c17 in UTCs of placentomes from 90- and 180-day pregnant cows (four animals per group) at defined sited as specified in Fig. 1. Tissue sections were prepared from PFA-fixed samples. No staining (open boxes) through light grey and darker grey boxes to intense staining (solid boxes).
the diverging staining patterns of the two primary antibodies in bovine placentomes cannot be ascribed to the expression of tissue-specific isoforms which is the case in pigs (Conley & Hinselwood 2001). In fact, although two different translational start sites have been demonstrated for the bovine recombinant enzyme, the size of the protein detected by immunoblot in the current studies corresponded closely to those obtained previously for the purified protein and enzyme expressed in bovine granulosa cells (Corbin et al. 2003). It is notable that with the polyclonal antiserum a non-specific band of high molecular size similar to the one found in bovine placental microsomal preparations even appeared in Western blot analysis of purified protein (Corbin et al. 2003), which may therefore represent P450arom in aggregated or undernated form.

The failure of clone H4 to detect P450arom in bovine trophoblast by immunohistochemistry may possibly be explained by the relatively low level of expression in relation to tissue mass and a lower sensitivity of this monoclonal antibody for the fixed protein compared with the polyclonal antiserum used in parallel. The relatively low level of P450arom expression based on tissue mass is indicated by the high amount of protein load in Western blot necessary to produce a specific band equal to those obtained with clearly lower amounts of proteins prepared from other steroidogenic organs (Fig. 2). Alternatively, the epitope of clone H4 may have been masked in bovine placentomes by differential glycosylation or by a cell-specific interaction with microsomal membranes or binding of other molecules.

When using formalin-fixed samples evaluation of immunostaining was generally impaired by a low signal-to-noise ratio not allowing a further assessment. With PFA-fixed tissue fewer problems were encountered and specific staining was clearly identified in differentiating and mature TGCs. The results of positive control experiments using PFA-fixed ovaries from cows at oestrus and of negative placental controls established with non-immunised rabbit serum provide strong evidence for the specificity of the signals observed in TGCs. Staining in placentomes from animals at day 90 and 180 clearly showed higher signal intensity in the trophoblast covering primary villi compared with other parts of the villous tree. Observations in placentomes from a cow after induction of parturition and in a cow at normal term indicates that prepartal up-regulation of aromatase activity (Tsumagari et al. 1993) follows a pattern similar to the one identified for P450c17. The up-regulation of P450arom and oestrogen receptor β (Schuler et al. 2005) concomitant with TGC differentiation points to an autocrine regulatory role of placental oestrogens during this process in addition to their previously postulated functions as local caruncular growth factor (Schuler et al. 2002). Accordingly, a similar role of placental oestrogens has been suggested previously in the differentiation of syncytiotrophoblast from cytotrophoblast in the human placenta (Bukovsky et al. 2003, Rama et al. 2004).

In addition to the trophoblast, signals for P450arom were also found in the caruncular epithelium clearly associated with migrating TGCs or with the short-lived feto-maternal hybrid cells resulting from fusions of the weakly invasive TGCs with individual caruncular epithelial cells (Wooding & Wathes 1980, Klisch et al. 1999). Besides the distinct signals restricted to these large cells exhibiting more or less pronounced features of degeneration (Fig. 7C), occasionally homogenous weak to moderate cytoplasmic staining was also found in genuine caruncular epithelial cells (see also Fig. 7C). A significant uptake of P450arom released from disintegrating TGCs or hybrid cells by caruncular epithelial seems rather unlikely, as primarily the trophoblast is considered to phagocytose TGC remnants (Wooding & Wathes 1980, Klisch et al. 1999). Although not present in sections stained with non-immune rabbit serum, the question is open as to whether this signal is actually specific for P450arom, as generally the fetal part of the placentomes has been regarded as the primary site of production of pregnancy-associated oestrogens (Hoffmann et al. 1979, Conley & Ford 1987). However, considering observations in other species, an intrinsic oestrogen production in the endometrium (Tseng et al. 1982, Tseng 1984, Huang et al. 1991), albeit at a very low level, cannot be ruled out.
Figure 7 Immunostaining for P450arom in placentomes from a cow after induced parturition in late gestation (A–C), a 180-day pregnant cow (D and E) and in CV of a spontaneously released placenta at normal term (F). (A) Positive cytoplasmic staining in TGCs of cross-sectioned CV. (B) Specific staining is absent in the negative control in which the primary polyclonal antiserum was replaced by the serum of a non-immunised rabbit at the same dilution. (C) Distinct positive staining in mature TGCs of a chorionic villus and in degenerating invasive TGCs (*) situated in the maternal crypt epithelium (ME). Weak to moderate staining is found in immature TGCs (white arrows) and ME, whereas UTCs (black arrows) and maternal stromal cells (MS) are negative. (D) Distinct cytoplasmic staining in TGCs at the basal part of a primary chorionic villus. (E) Weak signals in TGCs at the tips of the chorionic villous tree. (F) In term placenta, specific staining is still present in degenerating TGCs having survived the dramatic prepartal decrease in TGC numbers. Black bars = 50 μm; white bars = 25 μm.
These data show for the first time that there are clear functional distinctions in terms of steroidogenic capacity of UTCs and TGCs of the bovine placenta. P450c17 expression in UTCs and P450arom expression in TGCs suggest partitioning of androgen and oestrogen synthesis between placental cell types. The results of the present study are of additional interest in advancing our current understanding of the differences in how placental oestrogen synthesis is achieved across species. As noted above, several species apparently require co-operation between placental and fetal or maternal tissues expressing different steroidogenic enzymes to promote normal levels of oestrogen synthesis during pregnancy. In contrast, the bovine placenta is considered to express the full complement of steroidogenic enzymes needed for oestrogen production, in particular P450c17 as well as P450arom, as supported by the present findings. However, the current data demonstrate that their expression is still very distinctly partitioned at the cellular level within the placenta. The ‘two-cell type’ theory of oestrogen secretion by ovarian follicles, suggested by the transplantation studies of Falck (1959) but first proposed by Short (1962), has been controversial (Lieberman 1996) if only relative to its relevance in different species. Nevertheless, in cattle the expression of P450c17 in UTCs and P450arom in TGCs appears similar to the partitioning of P450c17 into theca and P450arom in the granulosa of follicles in most species studied to date. Clearly, there are sites where both enzymes have been localised to the same oestrogen-synthesising cell; the Leydig cells of the testis and the reticularis cells of the adrenal cortex in pigs are notable examples (Conley et al. 1996). Thus, arguments for regulation by different trophic stimuli or particular cytokines or growth factors would be difficult to defend in sites other than the ovary. Why the expression of key enzymes in oestrogen synthesis are compartmentalised at the tissue, cell and sub-cellular levels remains a mystery, but the bovine placenta appears to conform to, rather than contradict, this general phenomenon.

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