Altered arterial concentrations of placental hormones during maximal placental growth in a model of placental insufficiency

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Abstract

Pregnant ewes were exposed chronically to thermoneutral (TN; 20 ± 2 °C, 30% relative humidity; n = 8) or hyperthermic (HT; 40 ± 2 °C 12 h/day, 35 ± 2 °C 12 h/day, 30% relative humidity, n = 6) environments between days 37 and 93 of pregnancy. Ewes were killed following 56 days of exposure to either environment (days in treatment (dit)), corresponding to 93 ± 1 day post coitus (dpc). Maternal core body temperatures (CBT) in HT ewes were significantly elevated above the TN ewes (HT; 39.86 ± 0.1 °C vs TN; 39.20 ± 0.1 °C; P < 0.001). Both groups of animals displayed circadian CBT, though HT ewes had elevated amplitudes (HT; 0.18 ± 0.002 °C vs TN; 0.091 ± 0.002 °C; P < 0.001) and increased phase shift constants (HT; 2100 h vs TN; 1800 h; P < 0.001). Ewes exposed to chronic heat stress had significantly reduced progesterone and ovine placental lactogen (oPL) concentrations from 72 and 62 dpc respectively (P < 0.05), corresponding to approximately 30 dit. However, when compared with the TN ewes, HT cotyledonary tissue oPL mRNA and protein concentrations were not significantly different (P > 0.1). Prolactin concentrations rose immediately upon entry into the HT environment, reaching concentrations approximately four times that of TN ewes, a level maintained throughout the study (HT; 216.31 ± 32.82 vs TN; 54.40 ± 10.0; P < 0.0001). Despite similar feed intakes and euglycemia in both groups of ewes, HT fetal body weights were significantly reduced when compared with TN fetuses (HT; 514.6 ± 48.7 vs TN; 703.4 ± 44.8; P < 0.05), while placental weights (HT; 363.6 ± 63.3 vs TN; 571.2 ± 95.9) were not significantly affected by 56 days of heat exposure. Furthermore, the relationship between body weight and fetal length, the ponderal index, was significantly reduced in HT fetuses (HT; 3.01 ± 0.13 vs TN; 3.57 ± 0.18; P < 0.05). HT fetal liver weights were also significantly reduced (HT; 27.31 ± 4.73 vs TN; 45.16 ± 6.16; P < 0.05) and as a result, the brain/liver weight ratio was increased. This study demonstrates that chronic heat exposure lowers circulating placental hormone concentrations. The observation that PL mRNA and protein contents are similar across the two treatments, suggests that reduced hormone concentrations are the result of impaired trophoblast cell development, specifically trophoblast migration. Furthermore, the impact of heat exposure during maximal placental growth is great enough to restrict early fetal development, even before the fetal maximal growth phase (100 dpc-term). These data highlight that intrauterine growth retardation (IUGR) may result primarily from placental trophoblast cell dysfunction, and secondarily from later reduced placental size.

Journal of Endocrinology (1999) 162, 433–442

Introduction

In sheep, fetal membranes attach to the maternal uterine mucosa at 15–20 days post coitus (dpc) (Nephew et al. 1989), and by 35–40 dpc, trophoblastic interdigitation of developing placenta is well underway (Boshier 1969). The ruminant placenta develops through a non-invasive trophoblast attachment to specialized zones within the uterus, fusing with the maternal epithelium (Wooding 1982). Trophoblast cells are responsible for the synthesis and secretion of placental steroid and protein hormones (Strauss et al. 1996, Wooding et al. 1997). However, the interactions of the various hormones, angiogenic growth factors and receptors responsible for this active phase of placental growth and development are still to be fully documented. Placental growth proceeds rapidly from 40 dpc to an apex at 75–80 dpc, with maximal accretion rates for protein and dry matter contents at approximately 55 dpc (Ehrhardt & Bell 1995). Associated with this phase of rapid placental growth are rapid increases in DNA
Intrauterine growth retardation (IUGR) occurs in pregnant ewes exposed to high ambient temperature during the middle and last third of gestation (Bell et al. 1989, Dreiling et al. 1989, Thureen et al. 1992), after the period of maximal placental growth and development. The successful development and subsequent growth of the placenta is required for the desirable outcome of pregnancy, and modulators of placental development and growth include steroids and peptide hormones. Alterations in steroid and protein hormones of placental origin have been shown to occur in IUGR pregnancies, specifically progesterone and placental lactogen (PL) (Nisbet et al. 1982, Westergaard et al. 1984, Yamaihara et al. 1984, Laurin et al. 1987). Changes in hormonal profiles, placental functional capacities, and IUGR have been associated with changes in placental mass (Brown et al. 1977, Bell et al. 1987, 1989, Thureen et al. 1992). The underlying associations made from such studies have been that these hormonal and fetal alterations are a result of reduced placental growth. An alternative hypothesis is that the mechanisms involved in steroid and protein hormone synthesis and secretion may have been compromised earlier in gestation and it is this factor that results in reduced placental development and growth.

The relationship between placental and fetal growth implies that placental growth must precede fetal growth, and potential deficiencies in placental growth will interfere with subsequent fetal development. These observations highlight that the period of maximal placental development and growth, 40–80 dpc, would be an important time frame in which to investigate the effects of heat on placental hormone production and associated IUGR. We consider that the mechanisms responsible for IUGR following heat exposure during late gestation are different from those that occur following exposure during maximal placental growth. Heat exposure earlier in gestation potentially involves alterations in placental trophoblastic capacity. In addition to studying progesterone and ovine placental lactogen (oPL) concentrations, prolactin, a stress related hormone, was also measured. The objective of this study was to determine the effects of chronic heat stress through the period of maximal placental growth, 40–80 dpc, on placental endocrine function, specifically focusing on alterations in progesterone, oPL and prolactin concentrations, and consequences on placental and fetal growth.

Materials and Methods

Experimental protocol

This study was approved by the University of Colorado Health Sciences Center Animal Care and Use Commit-
glucose/lactate analyzer (YSI Model 2700 Select and Dual Standard). Ewe breaths per minute (bpm) were also recorded, consisting of four, 15-s observation periods.

Tissue collection

Ewes were killed at 93 ± 1 dpc, corresponding to approximately 56 days in treatment (d). The gravid uterus was removed and dissected into placental and fetal components. Placentomes were trimmed of endometrium and fetal membranes and weighed, placental weight being recorded as the sum of the placentomes. An average placentome weight was recorded as the total placentome weight divided by the total placentome number. Placentome cotyledonary tissue was manually separated from caruncular tissue, rinsed in 4°C physiologic saline, frozen in liquid N₂, and stored at −80°C until RNA isolation. Fetuses were towel dried, weighed, and crown-rump length (CRL) measured (Mellor & Matheson 1979). Fetal liver, heart and brain were removed and weighed. From these measurements fetal brain/liver index (brain weight (g)/liver weight (g)) and fetal ponderal index (fetal weight (g)/liver weight (g)) were calculated.

Hormone assays

Blood samples were analyzed for progesterone, PL and prolactin. Progesterone concentrations were determined using a validated (Bevers et al. 1989) Coat-A-Count progesterone RIA kit (DPC, Los Angeles, CA, USA), with interassay and intra-assay coefficients of variation (CV (%)) for the low quality control (QC) being 9·23 and 6·02% respectively, and for the high QC, 6·68 and 3·44% respectively.

oPL concentrations were determined as previously described (Kappes et al. 1992), with changes in sample volume to take into account for the early gestational age of the samples and advancing gestational age. Interassay and intra-assay CV values were 8·25 and 6·15%, respectively, for the low QC, 6·68 and 3·44% for the medium QC, and 3·67 and 3·45% for a high QC, over three assays.

Ovine prolactin concentrations were determined using a double antibody RIA with ovine prolactin standards (oPRL NHPP–AFP–10789B) and anti-ovine prolactin antiserum (anti-oPRL NHPP–AFP–C358106) following methods previously described (Nett et al. 1975). Interassay and intra-assay CV values were 8·64 and 8·13% respectively for a low QC, 3·07 and 3·07% for a medium QC, and 7·47 and 5·53% for a high QC, over three assays.

Cotyledonary mRNA extraction and Northern blot analysis

Total cellular RNA was isolated from cotyledonary tissue and Northern analysis performed as previously described (Warren et al. 1990a, Kappes et al. 1992). Cotyledonary DNA was determined in tissue samples extracted for RNA by the fluorometric assay as previously described (Labarca & Paigen 1980). A total of 20 μg total cellular RNA was analyzed using standard Northern blot and hybridization techniques (Kappes et al. 1992). A 866 bp oPRL cDNA fragment was isolated from EcoRI sites in its pBluescript SK(−) vector, as described previously (Kappes et al. 1992). The oPRL cDNA and a 250 bp oGAPDH cDNA were random prime labeled with [32P]deoxy-CTP to a specific activity of 1·5 × 10⁶ c.p.m./μg DNA. Autoradiographs were obtained by exposure to film. Densitograms were performed using a scanner and National Institutes of Health Image software.

Western blot analysis

Cotyledonary tissue was homogenized on ice in a tissue homogenate buffer (25 mM Tris(hydroxymethyl) aminomethane (Tris) HCl, pH 7·4, containing 1 mM EDTA, 1 mM EGTA, 0·1% (vol/vol) 2-mercaptoethanol, 1 mM PMSF, 2 μM leupeptin and 1 μM pepstatin A). The homogenate was centrifuged at 1500 g at 4°C for 10 min to remove cell debris. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed on 20 μg aliquots homogenate protein with a 15% (wt/vol) polyacrylamide gel. Prior to loading, samples were mixed with Laemmli sample buffer (1:2), heated at 95°C for 5 min and then centrifuged briefly. Proteins were transferred to nitrocellulose membrane using an electrophoretic transfer cell. The blot was blocked in TBS-T (50 mM Tris HCl pH 7·4, 150 mM NaCl, 5% (vol/vol) bovine serum albumin (BSA), and 0·1% (vol/vol) Tween-20) for 1 h at room temperature. The blot was then incubated with a polyclonal antibody directed against oPL (Warren et al. 1990b), diluted 1:15 000 in buffer overnight at 4°C. The blot was then washed three times, 10 min per wash, TBS-T at room temperature. The blot was then incubated for 1 h with anti-rabbit IgG antibody–horseradish peroxidase dase diluted in blocking buffer. The blot was then washed three times, 10 min per wash, with TBS-T at room temperature, and proteins were visualized by enhanced chemiluminescence (Le Cras et al. 1996). To take into account possible differences in gel loading, oPL values were normalized against the cell adhesion protein, Pecam-1 (M–20) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Following oPL immunodetection, antibodies were removed by incubation in 100 mM 2-mercaptoethanol, 62·5 mM Tris HCl, pH 6·8, 2% (wt/vol) SDS, at 50°C for 30 min with slight agitation, and then washed with TBS-T twice for 10 min at room temperature. The blot was then blocked in TBS-T, plus 5% (wt/vol) BSA, as described, and then incubated for 1 h with Pecam antibody (1:20 000). The same procedure as described for PL immunodetection followed. The intensity of the bands were determined by densitometry using a scanner and National Institutes of Health Image software.

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Apparent molecular weight for oPL and Pecam was confirmed by comparison with prestained molecular size markers.

Statistical analysis

CBT of the two treatment groups were modeled to take into account the repeated measures of the data for each ewe. SAS PROC SPECTRA and moving average methodology were used to detect the frequency of the cyclic fluctuations of the equally spaced time series. Following this, a best fit sinusoidal wave equation was determined for each group using circadian rhythm analysis (Zerbe & Jones 1998) in combination with mixed model methodology implemented with SAS PROC MIXED (Littell et al. 1996). These equations account for the variability between sheep and the variability between multiple measurements within sheep for each group. The fitting of a first order harmonic equation allowed modeling of the 24 h cyclic nature of the CBT. The model provided estimates of amplitude (peak height above the mean group CBT) and phase (the amount of time between time zero and the first peak of the cosine wave). Ten time periods were defined for the analysis, period 1 was 0–1 dit, period 2, 1–2 dit, period 3, 2–3 dit, period 4, 3–4 dit, period 5, 4–5 dit, period 6, 5–10 dit, period 7, 10–20 dit, period 8, 20–30 dit, period 9, 30–40 dit, and period 10, 40–56 dit.

Hormone data were analyzed using a general linear mixed model, which accounts for the variability within sheep as well as between sheep (Littell et al. 1996). For each treatment group, mixed models featuring polynomial group mean response curves were fitted. These were modeled with stochastic parameters in order to account for variability between sheep and between multiple measurements on the same sheep. Polynomial models took the form of a first or second degree, or intercept-only polynomial. The Scheffé method was used to adjust for comparisons of the mean response curves at multiple times as discussed previously (Young et al. 1997). Comparisons were made among the two groups at 1, 10, 20, 30, 40, 50 and 55 dit.

Three TN ewes and one HT ewe were not killed at 90 dpc in order to be available for later gestational studies. As a result, reduced animal numbers were analyzed for placental and fetal parameters using Student’s t-test. Placental and fetal data analysis was conducted on TN (5) and HT (5). oPL mRNA and protein concentrations were also analyzed using Student’s t-test, however a shortage of tissue reduced animal numbers to four in each group.

Results

Core body temperature and breaths per minute (bpm)

All animals obtained a resting CBT of 39·2 °C following surgery before being moved to either the TN or HT treatment. TN ewes had a mean CBT throughout the study of 39·19 ± 0·1 °C (Fig. 1). HT ewes had CBT significantly greater than TN ewes within 48 h of entry into the HT environment. From 2 dit onwards, CBT of HT ewes were significantly greater than TN ewes (HT 39·86 ± 0·1 °C vs TN; 39·19 ± 0·1 °C; *P* <0·001, Fig. 1). Ewes exposed to elevated ambient temperatures (HT), had significantly higher circadian amplitude and altered phase angles when compared with TN ewes. While TN ewes’ peaks occurred at 1800 h, HT ewes’ peaks occurred 3 h later at approximately 2100 h. TN ewes’ peak CBT occurred in 100% light, while HT peak CBT occurred approximately 2 h after entry into 0% light and the drop from daytime temperature of 40 °C, to the nighttime regime of 35 °C.

Exposure of ewes to elevated ambient temperature resulted in bpm increasing from a pre-exposure rate
of 50 ± 3 up to 134 ± 9 bpm for the HT group within 3 dit. The HT group appeared to acclimatize with bpm declining to 80 ± 5 at the studies conclusion.

**Feed and water intakes, and glucose concentrations**

Average feed intake of the HT ewes over the experimental period rose from 9·1 to 10·7 MJ/day at the end of the study period. TN ewe feed intake was that of the gestational age mated HT ewes. Ewes exposed to high ambient temperature had significantly greater water intake than TN ewes (HT; 4·48 ± 0·35 L vs TN; 3·42 ± 0·28 L; *P* < 0·05). Glucose concentrations were not significantly different between groups, though concentrations declined from an average for both treatments of 3·8 ± 0·08 mmol after entry into treatment, settling at 3·5 mmol at approximately 30 dit, corresponding to 70 ± 2 dpc.

**Hormone concentrations during hyperthermic exposure**

Pre-treatment concentrations of the hormones examined were not significantly different between the allocated groups (Table 1). Stochastic parameter models with second-degree polynomials, fitted for the progesterone, oPL and prolactin data for each of the treatments are provided in the associated figures. Arterial progesterone concentrations became significantly lower in HT ewes between 30 and 40 dit, corresponding to approximately 72 dpc (*P*<0·05; Fig. 2). HT ewes displayed a constant progesterone concentration throughout the study, whereas TN ewes displayed a pregnancy associated increase. Arterial oPL concentrations became significantly reduced in HT ewes, between 20 and 30 dit, corresponding to approximately 62 dpc (*P*<0·05; Fig. 3). However, concentrations did increase with advancing gestation, though concentrations remained significantly reduced when compared with TN ewes. Prolactin concentrations immediately rose in the HT ewes to concentrations approximately four times greater than in TN ewes, levels which were significantly different from 5 dit onwards (*P*<0·001; Fig. 4).

**Hyperthermic effects on cotyledonary PL mRNA and protein concentration**

Exposure to hyperthermia for 56 days had no effect on cotyledonary oPL mRNA expression, collected at approximately 90 dpc (Fig. 5). oPL mRNA expression, normalized for cellular DNA was similar in both groups (Data not shown). The cotyledonary PL protein was also found to be similar in both groups of animals (Fig. 6).

**Placental and fetal weights**

Placental weights at 93 ± 1 dpc, while not significantly different, were reduced in HT ewes (Table 2). Fetal weights were significantly reduced by 27% (*P*<0·05; Table 2). CRL were unaffected by heat, though calculated Ponderal index was significantly reduced in the HT fetuses (*P*<0·05; Table 2). Liver weights were significantly reduced in HT fetuses (*P*<0·05; Table 3), although when

<table>
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<th>TN</th>
<th>HT</th>
<th>S.E.M.</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone (ng/ml)</td>
<td>6·69</td>
<td>6·67</td>
<td>0·5</td>
<td>&gt;0·1</td>
</tr>
<tr>
<td>Placental lactogen (ng/ml)</td>
<td>6·0</td>
<td>7·75</td>
<td>1·95</td>
<td>&gt;0·1</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>97·8</td>
<td>125·5</td>
<td>11·21</td>
<td>&gt;0·1</td>
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</tbody>
</table>

**Figure 2** Gestational changes in arterial progesterone concentrations and regression lines for TN (▲, 6·958 - (0·014 × dit) + 0·002 × dit²) and HT (■, 7·025 - (0·011 × dit) + 0·00002 × dit²) ewes. Values are means ± S.E.M.

**Figure 3** Gestational changes in arterial placental lactogen concentrations and regression lines for TN (▲, 4·011 - (0·167 × dit) + 0·0127 × dit²) and HT (■, 10·090 - (0·646 × dit) + 0·0127 × dit²) ewes. Values are means ± S.E.M.
expressed relative to body weight, liver weight was not significantly different. Hyperthermia had no effect on brain and heart weights (Table 3).

Discussion

The results reported here show that following approximately 30 days of heat exposure, during active placental development, progesterone and oPL concentrations become significantly depressed. These data suggest that elevated CBT impair placental hormone synthesis and/or metabolism in pregnant ewes. Ovine progesterone concentrations during pregnancy are initially solely a measure of ovarian secretion, but placental secretion becomes the dominant source by approximately 50 dpc, increasing as gestation advances (Bassett et al. 1969). We now report that ewes exposed to heat, 20 days earlier than the previous reports (Bell et al. 1987), display a reduction in placental hormone concentration around the time of the onset of placental dominance of progesterone secretion. Progesterone concentrations from 35 to 70 dpc (35 dit), during the period of active interdigitation, were similar in TN and HT ewes. However, at the time when

Table 2 Placental and individual placentome weights, placentome numbers, fetal/placenta weight ratios and the fetal weights, CRL, Ponderal indexes of TN (5) and HT (5) fetuses following 56 dit. Values are means ± pooled standard error of treatment means

<table>
<thead>
<tr>
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<th>HT</th>
<th>S.E.M.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental weight (g)</td>
<td>571.2</td>
<td>363.6</td>
<td>64.3</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Individual placentome (g)</td>
<td>7.13</td>
<td>5.53</td>
<td>0.91</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Placentome number</td>
<td>81.0</td>
<td>72.0</td>
<td>4.42</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Fetal/placenta weight ratios</td>
<td>1.4</td>
<td>1.6</td>
<td>0.2</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Fetal weight (g)</td>
<td>703.4</td>
<td>514.6</td>
<td>44.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fetal CRL (cm)</td>
<td>27.0</td>
<td>25.7</td>
<td>0.53</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Ponderal index</td>
<td>3.57</td>
<td>3.01</td>
<td>0.13</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 4 Gestational changes in arterial prolactin concentrations and regression lines for TN (Δ, 54.402) and HT (■, 139.528 + (3.680 × dit) – 0.9405 × dit²) ewes. Values are means ± S.E.M.

Figure 5 Northern blot analysis of oPL mRNA levels of TN and HT cotyledons. The oPL mRNA was determined to be approximately 1 kb. Quantification of the oPL mRNA levels normalized to the signal from GAPDH mRNA is also shown. The results depict oPL mRNA expression in five TN and four HT ewes. Values are means ± S.E.M.

Figure 6 Western blot of cotyledonary oPL protein content of TN and HT ewes. The oPL antibody detected a single band in the cotyledonary homogenates of 22 kDa. Quantification of the oPL protein signal is also shown. The results depict oPL protein concentration in four TN and four HT ewes. Values are means ± S.E.M.
concentrations of progesterone would be expected to increase with the decline of ovarian progesterone secretion and increase in placental secretions (50–60 dpc), progesterone concentrations in HT ewes became depressed in comparison to rising TN concentrations.

Circulating progesterone concentrations became significantly lower just prior to term in ewes subjected to heat from 60 dpc, attributed to a reduced placental mass (Bell et al. 1987). While placental weight was not significantly different in our study (~90 dpc), HT placental weights were 36% less than TN placentae. This suggests that, late in gestation, reduced progesterone concentrations could be a result of a reduced placental mass, as postulated in other studies (Bell et al. 1987, Wallace et al. 1997). However, the observed reduced progesterone concentrations early in gestation in the present study implies heat adversely affects the development of placental cells responsible for steroid production and metabolism, before factors such as a reduced placental weight might affect circulating hormone concentrations. In contrast, in cattle, progesterone levels increase under HT conditions during the estrus cycle (Abilay et al. 1975). Exposure of cattle to elevated ambient temperature appears to promote ovarian synthesis and secretion of steroids during estrus (Abilay et al. 1975), and progesterin concentrations remain elevated throughout gestation (Collier et al. 1982). This elevation is attributed to stimulation of peripheral thermoreceptors, resulting in the activation of hypothalamic adrenocorticotropin releasing mechanisms (Abilay et al. 1975). Differences in the cellular source of progesterone during pregnancy between cattle and sheep may explain differences in responses to elevated temperatures. The corpus luteum is the primary source of progesterone throughout pregnancy in cattle, while in sheep, placental production of progesterone is the dominant source during the later two thirds of pregnancy (Bassett et al. 1969). Hyperthermia induced changes in steroid production by the placenta in cattle, could be masked by the dominance of ovarian progesterone secretion, which is stimulated by hyperthermia. Our data shows that there is a reduced concentration of placental steroids and hormones, following ovarian demise at approximately 50 dpc. This suggests that in sheep, heat may adversely affect placental trophoblast function and or development. Although the exact cause of reduced progesterone concentration in our study is unknown, two possibilities exist. Hyperthermia is affecting either placental steroid synthesis and secretion through interactions upon trophoblast function, or it is altering steroid clearance rate following secretion.

The oPL data may help answer this question. In HT ewes, PL concentrations decline around the same time as progesterone concentrations decline. The binucleated cells (BNC) of the fetal trophoblast are the only cells that produce PL (Kappes et al. 1992). PL is considered an important marker of placental integrity and function, with impaired secretion being implicated in altered maternal and fetal metabolism (Freemark & Comer 1989, Handwerger 1991). As gestation advances, PL plasma levels rise (Kelly et al. 1974, Oddy & Jenkin 1981, Gluckman & Barry 1988), as a function of total BNC within the placenta (Kappes et al. 1992). In our study, heat exposure from 37 dpc, was a critical determinant for circulating oPL concentrations, significantly reduced concentrations suggesting an altered BNC population. Examination of cotyledonary PL mRNA and protein concentration revealed a lack of difference between TN and HT ewes. This suggests that HT treatment does not effect the translation or transcription of oPL, and therefore differences in both maternal PL and progesterone concentrations could be attributable to an impaired trophoblast migration.

Trophoblast cells share the common feature to elaborate and metabolize steroid hormones (Strauss et al. 1996). In ruminant placentae, 15–20% of the chorionic epithelia consists of granulated BNC, the percentage of which remains constant over gestation (Wooding 1992). While these cells represent only a fifth of the placental cell population, they are the major source of placental protein and steroid hormones responsible for the establishment and maintenance of pregnancy. Binucleate cells migrate through the apical tight junction of the primary chorionic epithelium, across the microvillar junction of the fetomaternal interface, and fuse into the uterine syncytium (Wooding et al. 1986). The syncytium is closely associated with the maternal circulation, and following fusion, the BNC granules are released by exocytosis into the syncytium. The delivery of placental products throughout gestation that this initiates, may be critical to the development of a successful metabolic and/or immunologic dialogue between mother and fetus (Wooding 1992). The failure of human cytotrophoblast cells to differentiate into invasive cells, can result in abnormal placentation and potentially in IUGR and/or preeclampsia (Cross 1996). In human and sheep IUGR, reduced placental hormone and steroid concentrations have been associated with reduced

Table 3 Fetal organ and relative organ weights of TN (5) and HT (5) fetuses following 56 dit. Values are means ± pooled standard error of treatment mean

<table>
<thead>
<tr>
<th>Organ weight (g)</th>
<th>TN</th>
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<th>S.E.M.</th>
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<tbody>
<tr>
<td>Brain</td>
<td>16·11</td>
<td>13·38</td>
<td>0·93</td>
<td>&gt;0·1</td>
</tr>
<tr>
<td>Liver</td>
<td>45·16</td>
<td>27·31</td>
<td>4·60</td>
<td>&lt;0·05</td>
</tr>
<tr>
<td>Heart</td>
<td>5·18</td>
<td>4·65</td>
<td>0·25</td>
<td>&gt;0·1</td>
</tr>
<tr>
<td>Brain/liver</td>
<td>0·37</td>
<td>0·54</td>
<td>0·05</td>
<td>&gt;0·1</td>
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<tr>
<th>Realte organ weight (g/kg fetus)</th>
<th>TN</th>
<th>HT</th>
<th>S.E.M.</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Brain</td>
<td>2·29</td>
<td>2·65</td>
<td>0·14</td>
<td>&gt;0·1</td>
</tr>
<tr>
<td>Liver</td>
<td>6·35</td>
<td>5·20</td>
<td>0·40</td>
<td>&gt;0·1</td>
</tr>
<tr>
<td>Heart</td>
<td>0·73</td>
<td>0·94</td>
<td>0·06</td>
<td>&lt;0·1</td>
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trophoblast function and trophoblast mass (Westergaard et al. 1984, Maruyama et al. 1986, Newnham et al. 1986). In the sheep, the secretion pattern of protein hormones, such as oPL, is postulated to depend on the continual movement of these cells from the fetal to maternal component (Steven et al. 1978, Duello et al. 1986, Kappes et al. 1992). A failure of normal fetomaternal syncytium development could result in abnormal placental function.

Radiotelemetry has allowed detailed analysis of CBT in both TN and HT ewes during maximal placental growth. An immediate rise in HT ewes’ CBT was observed, reaching an asymptote within 2 days of HT treatment. During that period, HT animals maintained their circadian pattern, though with increasing amplitude around their mean CBT. Elevated CBT has been correlated with changes in circulating prolactin concentrations (Wettemann & Tucker 1974, 1976). Hyperthermia increases prolactin concentration in ovariecomized ewes (Schillo et al. 1978), similar to rises observed in cattle (Wettemann & Tucker 1974, 1976), mid to late gestation ewes (Bell et al. 1989), and those changes reported here. While prolactin is involved in the initiation of lactation (Forsyth 1986), a role during mid pregnancy has not been established in sheep. Circulating concentrations of prolactin do not change during normal gestation until just prior to the onset of lactation (Wettemann et al. 1973, Bell et al. 1989), and it seems to have no function in human osmoregulation (Baumann et al. 1977), suggesting that the observed elevated prolactin is a chronic response to a physiologically stressful situation.

To date, studies concerning the effects of heat stress upon pregnancy have concentrated on the metabolic adaptations by the IUGR fetus to placental failure, in late gestation (Bell et al. 1987, 1989, Thureen et al. 1992, Ross et al. 1996, Anderson et al. 1997). Elevated CBT from approximately 35 dpc results in a placental mass that, at term, is significantly reduced and associated with an IUGR fetus (Thureen et al. 1992). These fetuses are approximately 40% lighter than a normal fetus and display increased brain/liver ratios and altered fetal biometric ultrasonographic measurements associated with growth retardation (Thureen et al. 1992, Galan et al. 1999). Reduced birth weights and IUGR are associated with poor maternal nutrition in humans, however, heat induced IUGR in the sheep is considered independent of maternal nutrition (Dreiling et al. 1989). In the data reported here, the object of pair feeding the animals was to remove any effects of nutrition on conceptus development and growth. This regime allows developmental or growth impairment to be attributed to the effects of chronic heat exposure. When TN ewes are fed the same feed intake of gestational age matched heat stressed ewes, heat stressed lamb birth-weights are significantly reduced, while TN weights are unaffected (Cartwright & Thwaites 1976, Brown et al. 1977). Furthermore, both groups of ewes consumed approximately 10.7 MJ per day, an amount greater than the estimated 10.5 MJ required metabolizable energy intake of pregnant ewes during the first 105 days of gestation (Subcommittee on Sheep Nutrition 1998). In addition, all ewes displayed normal glycemia over the course of this study, concentrations similar to those previously reported to occur during mid gestation (Bell et al. 1989).

Under the environmental regimes used, HT fetal weights were significantly reduced, despite a non-significant reduction in placental weight. The fetus at this stage of gestation is not in a rapid growth stage, and reduced fetal weights suggest that what little nutrients the fetus requires, are not supplied adequately. A direct reduction of available nutrients from the maternal compartment can not be offered as the cause of this fetal weight reduction, since maternal glucose concentrations indicate HT animals are in a state of euglycemia. We postulate that fetal growth failure results from severe placental insufficiency, manifested by abnormal trophoblast development and function. In another report, following exposure to heat between 50 and 75 dpc, fetal weights are observed not to be affected (Vatnick et al. 1991). The differences between this report and our data highlight two important suggestions, first that the effect of heat exposure prior and during the maximal growth of the placenta is critical to later placental transfer capacities. Secondly, fetal development and growth occurring between 75 and 90 dpc, can be impaired in a heat stressed placenta. Liver weights were significantly reduced in HT fetuses, similar to changes reported in late gestation IUGR fetuses (Bell et al. 1987), which together with a maintained brain weight produced an increased brain/liver weight ratio (Thureen et al. 1992). The reduced liver weight in term fetuses has been attributed to a reduced capacity of the heat-stunned placenta to supply nutrients. If this is the case in 90 dpc HT fetuses, then a major placental transport malfunction must be occurring at this relatively early stage in fetal development. Furthermore, fetal CRLs are similar between the two treatment groups, while calculated ponderal index is decreased in HT fetuses. These observations are similar to fetuses subjected to heat following maximal placental development, indicating that the growth of soft tissues is more affected than bone (Bell et al. 1989). Of equal importance is the fact that alterations in fetal development and growth are present in 90 dpc fetuses, suggesting that the factors responsible for soft tissue growth are adversely affected during early fetal development by elevated CBT.

Previously it has been suggested that heat induced alterations in plasma progesterone and oPL, associated with IUGR, were consequences rather than causes of placental stunting (Bell et al. 1989). However, we report that pregnant ewes exposed to chronic heat stress during maximal placental development display significantly reduced progesterone and oPL concentrations. This occurs prior to a significant placental weight divergence, highlighting a major disruption of early placental function and
or metabolism in the heat stress induced model of placental insufficiency. We postulate that heat stress induced impaired trophoblast development could result in dysfunc-
tional cells that are essential to normal placental develop-
ment, adversely affecting further placental development and maternal/fetal metabolic interactions. The migration of trophoblast BNC, those cells responsible for steroid and protein synthesis and secretion in the ruminant placenta, plays a major role in the delivery of hormones to the circulatory systems of mother and fetus. Alterations in this system would potentially limit the conceptus’ capacity to produce the biochemical signals, such as steroids, hor-
mones and growth factors, necessary for normal placental development and later fetal development and growth.

The relationship between placental and fetal growth implies that placental growth must precede fetal growth, and that potential deficiencies in early placental growth will interfere with subsequent fetal develop-
ment more severely than heat exposure later in placental development. HT exposure during maximal placental development appears to reduce circulating hormone concentrations, which adversely affects placental and fetal development and growth. As a result of compromised placental development, fetal requirements are impaired earlier in gestation, further impairing fetal development leading to IUGR. This study highlights that IUGR may result primarily from trophoblast cell dysfunction, and secondarily from reduced placental size.

Acknowledgements

The authors thank Dr A F Parlow and the NIDDK’s National Hormone and Pituitary Program for pro-
lactin hormone preparations. We are grateful to Karen Trembler, I-Da and Yu-Ching Fan, Pete Orchard and Willie Jones for their technical support and excellent care of the animals. Dr Gary Zerbe, Janet Tooze, Bonnie LaFleur and Anne Esler kindly assisted in statistical analy-
sis. This work was supported through NIH–NICHD PO1 HD20 761.

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Received 26 October 1998
Revised manuscript received 22 March 1999
Accepted 26 April 1999