Growth and in-vitro metabolism of placental tissues of cows from Day 100 to Day 250 of gestation

L. P. Reynolds, D. S. Millaway, J. D. Kirsch, J. E. Infeld and D. A. Redmer

Department of Animal and Range Sciences, North Dakota State University, Fargo, North Dakota 58105, USA

Summary. Weight of placental tissues of cows increased exponentially from Day 100 to Day 250 of gestation, but at much slower relative and absolute rates than fetal weight. In addition, growth rate of fetal placental tissues was less than that of maternal placental tissues. Concentrations of DNA, RNA and protein, however, increased in fetal placental but not in maternal placental tissues. Fetal placental tissues therefore exhibited hyperplasia, which probably contributes to increased functional capacity of the placenta during late gestation. The rate of O₂ uptake in vitro was greatest for maternal placental tissues, suggesting that the maternal portion of the placenta accounts for most of the large rate of placental O₂ utilization in vivo. Compared with other placental tissues, rate of secretion of macromolecules by intercaruncular endometrium was high, but decreased from Day 100 to 250, suggesting that uterine glandular secretory activity may decrease as gestation advances. Rate of secretion of macromolecules also was high for intercotyledonary tissues and increased with day of gestation, suggesting a role for secretory products of chorioallantois in gravid uterine function.

Keywords: placenta; growth; metabolism; cow; gestation

Introduction

The mammalian placenta consists of fetal and maternal tissues and is the site of exchange of respiratory gases, nutrients and waste substances between the fetal and maternal systems (Barcroft, 1946; Ramsey, 1982). Normal growth and development of the placenta are therefore vital to normal fetal growth and development. It is well known that treatments which reduce fetal growth (e.g. maternal genotype, reduced uterine surface area per fetus, maternal nutrient deprivation, environmental heat stress) are associated with decreased placental size (Wallace, 1948; McKeown & Record, 1953; Eckstein et al., 1955; Joubert & Hammond, 1958; Alexander, 1964a, b; Bell et al., 1987). In addition, in normal pregnancy placental and fetal weights are highly correlated (Eckstein et al., 1955; Alexander, 1964a; Anthony et al., 1986). Size of the placenta and its blood supply are primary determinants of the rate of physiological exchange between the fetal and maternal systems (Reynolds et al., 1985a, b, 1986; Bell et al., 1986, 1987; Reynolds & Ferrell, 1987). Conditions associated with reduced rate of fetal growth also are associated with reduced rates of placental blood flow and reduced fetal O₂ and nutrient uptakes (Wootton et al., 1977; Reynolds et al., 1985a, b; Bell et al., 1987; Ferrell & Reynolds, 1987). Therefore, factors which influence placental growth and development will probably have a substantial influence on fetal growth and development. Before the relationships between fetal and placental growth can be determined, however, an understanding of normal placental growth must be obtained.

The ruminant placenta is divided into placentomes comprising maternal caruncular endometrium and fetal cotyledon, and also into interplacentomal areas comprising intercaruncular endometrium

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and intercotyledony fetal membrane (Ramsey, 1982). Growth of these placental tissues, however, has not been well characterized in cows. Prior & Laster (1979) measured weights and surface areas for cotyledons and placenta (defined as fetal membranes with cotyledons removed) of cows, but did not report weights or surface areas of maternal caruncles. Ferrell et al. (1976a) reported weights of fetal membranes (presumably including fetal cotyledons) at several stages of gestation, but found growth rate of fetal membranes to be about half that observed by Prior & Laster (1979). Anthony et al. (1986) reported a 2-fold increase in total placental weight from Day 200 to 260 of gestation. Additionally, we are not aware of any reports which describe gestational changes in cellular composition (DNA, RNA and protein contents) of placental tissues in cows. An important consideration in describing the relationship between fetal and placental growth is the high metabolic rate exhibited by the placenta. Indeed, major portions (60–80% at midgestation) of O₂ and nutrients taken up by the gravid uterus are utilized by the placenta, which has a high rate of metabolism compared with other gravid uterine or maternal tissues (Meschia et al., 1966; Reynolds et al., 1986; Bell et al., 1986). Which of the placental tissues accounts for this high metabolic activity, however, has not been determined.

The objectives of the present study were to determine the relationship between fetal and placental growth, and to characterize cellular growth and metabolism of individual components of the placenta at several stages of gestation in cows.

Materials and Methods

Hereford and Hereford-cross cows were mated with Hereford bulls (Day 0 of gestation) and fed alfalfa/grass hay at a level calculated to maintain body weight, with free access to salt and mineral supplements. The 21 cows were assigned randomly to slaughter on approximately Days 100 (103 ± 0.7, N = 4), 150 (151 ± 0.5, N = 5), 200 (200 ± 1.2, N = 6) and 250 (250 ± 0.5, N = 6) of gestation. For each cow, the entire gravid uterus (from mid-cervix to both utero-tubal junctions) was obtained. For 9 cows (2 at Day 150, 4 at Day 200 and 3 at Day 250), a sample (≥2 g) of maternal liver was also obtained. Gravid uteri and maternal liver samples were immediately transported to the laboratory (within 10 min after collection and within 30 min after slaughter).

**Weights of gravid uterine tissues.** At the laboratory, the entire gravid uterus of each cow was weighed and fetus, fetal membranes and uterus were separated. Cotyledons were carefully dissected from fetal membranes. Caruncles were dissected from the uterine luminal surface, taking care not to include intercaruncular tissue. Any cotyledony villi remaining in caruncular crypts were removed with forceps and included with cotyledons. Weights of fetus, uterus (minus caruncles), total caruncles, total cotyledons and total fetal membranes (chorioallantois plus amnion) were determined. Weight of fetal fluids was calculated as gravid uterine weight minus total weights of gravid uterine tissues (fetus + uterus + caruncles + cotyledons + fetal membranes). Placental weight was calculated as the sum of caruncular and cotyledony weights. Weights of tissue samples obtained for evaluation of cellular growth, metabolic rate and [³H]leucine incorporation (see below) were included with the appropriate tissue weight. Curved crown–rump lengths of fetuses were determined as described by Ferrell et al. (1976a).

**DNA, RNA and protein concentrations.** To evaluate cellular growth of placental tissues, samples (≥2 g) of caruncular, intercaruncular, cotyledony and intercotyledony (chorioallantoic) tissues were obtained from the middle portion (near the fetus) of the gravid uterine horn of each cow and stored frozen at −80°C. Approximately 1 g of each tissue sample was homogenized in PBS-EDTA buffer (0.01 M-sodium phosphate, 0.14 M-NaCl, 3 mM-Na₂HPO₄, 1 mM-EDTA, pH 7.3) by using a Polytron (Brinkmann, Westbury, NY, USA). Tissue homogenates were analysed for concentrations of DNA and RNA by using diphenylamine and orcinol procedures, respectively, as described previously (Reynolds et al., 1985a). Standards were DNA Type I from calf thymus and RNA Type IV from calf liver (both from Sigma, St Louis, MO, USA). Concentrations of protein in tissue homogenates were determined by the method of Lowry et al. (1951) with bovine serum albumin (Fraction V, Sigma) as standard. Concentration and total content of DNA were used as indexes of hyperplasia, and ratios of RNA:DNA and protein:DNA were used as indexes of tissue hypertrophy (Enesco & Leblond, 1962; Rattray et al., 1975). For caruncular and cotyledony tissues, total content of DNA, RNA and protein were determined by multiplying concentrations in tissue samples by total tissue weight.

**Oxygen consumption.** To evaluate metabolic rate of placental tissues, samples (≥1 g) of caruncular, intercaruncular, cotyledony and intercotyledony (chorioallantoic) tissues were obtained from the middle portion (near the fetus) of the gravid uterine horn of each cow, and samples of maternal liver were obtained from 9 of the cows. Tissue samples were sliced by using a Stadie-Riggs microtome (Thomas, Philadelphia, PA, USA), and slices (0.5 mm thick, 66.6 ± 1.4 mg) were placed into 35-mm Petri dishes containing 3 ml Krebs’–Ringer buffer (37°C) of the following composition (mm): NaCl, 118.1; NaHCO₃, 25.0; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; glucose,
11-1 (Reynolds & Ford, 1984). Tissue slices were weighed on a torsion balance (Roller-Smith, Bethlehem, PA, USA), and placed into a stirred-cell O2 consumption monitor with a Clark-type polarographic electrode (model 5300, Yellow Springs Instruments, Yellow Springs, OH, USA). Oxygen consumption of tissue slices was monitored for 5 min in 1 ml of O2 consumption buffer (Krebs'-Ringer buffer containing 5.0 mM-sodium pyruvate, 5.0 mM-sodium glutamate, 4.5 mM-sodium acetate and 4.5 mM-malic acid; Ferrell et al., 1976b) at 37°C and recorded on a chart recorder. To minimize time effects, all tissues were analysed within 4 h after collection and in random order. Available O2 in solution was assumed to be 224 nmol/ml Krebs'-Ringer buffer at 760 mmHg and 37°C (Umbricht et al., 1972) and was adjusted daily for barometric pressure, which was determined in the laboratory by using a mercury barometer. Oxygen consumption was calculated as percentage utilization of available O2 and is reported as μmol·min⁻¹·g tissue⁻¹.

**Incorporation of [3H]leucine into macromolecules.** To evaluate rate of synthesis/secretion of macromolecules, samples (~1 g) of caruncular, intercaruncular, cotyledonary and intercotyledonary (chorioallantoic) tissues were obtained, as above, from the middle portion of the gravid uterine horn of each cow. Explants (175·7 ± 7·4 mg) of these tissues were incubated for 24 h in 10-ml low-leucine Eagle's MEM containing ∼20 μCi (17·6 ± 0·3 μCi; 293·7 ± 4·3 pmol) [3H]leucine (1-[4,5-3H(N)]leucine: New England Nuclear, Boston, MA, USA), as described previously (Bartol et al., 1985a, b; Millaway et al., 1989). After incubation, duplicate 0·25 ml aliquots of media were diluted with 1·75 ml distilled H2O and dialysed (SpectraPor 6, 1000 M, Spectrum, Los Angeles, CA, USA) against 1000 volumes phosphate-buffered saline (pH 7·4) at 4°C, as described by Millaway et al. (1989). Radioactivity remaining after dialysis was determined by liquid scintillation counting of 0·5 ml samples of retentates. To determine rate of synthesis/secretion of macromolecules, duplicate explants (185·7 ± 17·1 mg) of placental tissues were obtained from 2 additional cows (Days 197 and 202 of gestation) and incubated as described above. Samples (0·25 ml) of media from these explant incubations were obtained at 8, 16 and 24 h of incubation, diluted, dialysed and counted for radioactivity as described above. Data are reported as pmol [3H]leucine incorporated into non-dialysable macromolecules per unit time per gram of tissue.

**Statistical analysis.** To describe growth of gravid uterine tissues, an exponential growth model was used:

\[ W = W_0e^{b_1t + b_2} \]

where \( W \) = weight in grams, \( W_0 \) = initial weight in grams at Day 0 of gestation, \( b_1 \) = initial growth rate, \( b_2 \) = change in growth rate and \( t \) = day of gestation (Koong et al., 1975; Ferrell et al., 1976a; SAS, 1985). This exponential model also was used to describe total contents of DNA, RNA and protein in caruncular and cotyledonary tissues, except that content in milligrams was used instead of weight in grams. For curved crown–rump length, linear regression on day of gestation was used. Least-squares (General Linear Models) analysis of variance was used to determine differences in DNA, RNA and protein concentrations and ratios. O2 consumption rates, and [3H]leucine incorporation rates of placental tissues, with day of gestation, tissue and day × tissue interaction included in the model (SAS, 1985). When a main effect or interaction F-test was significant \((P < 0·05)\), differences between specific means were determined by using Bonferroni's \( t \) test (Kirk, 1968). Data are reported as means ± s.e.m.

**Results**

**Weights of gravid uterine tissues**

Weights of gravid uterine tissues and fetal fluids, and also curved crown–rump lengths, are given in Table 1. Given the weights of all the gravid uterine tissues increased exponentially \((P < 0·01)\) from Day 100 to Day 250 of gestation, and the model used to describe growth of these tissues explained a large proportion of the variation in weights \((R^2 = 0·80–0·98; \text{Table 2, Fig. 1a and 1b})\). Although weight of fetal fluids also increased exponentially \((P < 0·02)\), the model explained only 26% of variation in fetal fluid weight (Table 2). Curved crown–rump length increased as a linear function of day of gestation [curved crown–rump (cm) = −21·032 + 0·457 (day of gestation), \(R^2 = 0·96, N = 21, P < 0·01\)].

The initial rate of increase in fetal weight was 8·02% per day, and this rate decreased by 0·014% per day as gestation advanced (Fig. 1a). This model agrees with the data of Koong et al. (1975) for sheep and Ferrell et al. (1976a) for cattle, who also reported a decrease in relative rate (%/day) of fetal growth as gestation progressed. Although relative rate decreased, absolute rate (kg/day) of fetal growth continued to increase throughout gestation because of increasing fetal mass. Thus, in the present study, increase in fetal weight from Days 100 to 150, 150 to 200 and 200 to 250 of gestation was 816, 407 and 220% on a relative basis but 2·72, 9·53 and 15·12 kg on an absolute basis (Table 1). The initial rate of increase in placental (caruncular + cotyledonary) weight was 6·16% per day, which decreased by 0·012% per day thereafter (Fig. 1a). Because the relative rate of...
increase of fetal weight was greater than that of placentomal weight, fetal weight increased 73-fold whereas placental weight increased only 16-fold from Day 100 to 250 of gestation (Table 1; Fig. 1a). Thus, although fetal and placental weights were similar at Day 100 of gestation (0-38 and 0-29 kg, respectively), by Day 250 fetal weight was 6-fold greater than placental weight (Table 1, Fig. 1a). Similarly, because of a difference in relative rate of increase in weight of maternal caruncles and fetal cotyledons, these components of the placentome exhibited differential growth (Fig. 1b). Although their weights were similar at Day 100, caruncular weight was 2-fold greater than cotyledonary weight by Day 250 of gestation (Table 1, Fig. 1b). Although caruncular and cotyledonary tissues cannot be separated with 100% efficiency, our experience suggests that only a relatively small amount of fetal cotyledonary tissue remains in maternal caruncular crypts when these tissues are carefully separated, and we have substantiated this observation histologically (Reynolds & Redmer, 1988). It therefore seems unlikely that the 2-fold difference between caruncular and cotyledonary weights at Day 250 could be explained by poor efficiency of separation of these tissues.

### DNA, RNA and protein concentrations

Concentrations and ratios of DNA, RNA and protein in placental tissues are given in Table 3. A significant effect of day of gestation was found only for tissue protein concentrations, which...
increased \((P < 0.01)\) from Day 100 to 250. When averaged across all days of gestation, concentrations of DNA, RNA and protein \((\text{mg/g tissue})\) were greater \((P < 0.01)\) in caruncular \((4.31 \pm 0.22, 3.88 \pm 0.12 \text{ and } 51.1 \pm 1.4)\) than in intercaruncular \((2.70 \pm 0.22, 1.85 \pm 0.14 \text{ and } 37.2 \pm 2.2)\) or cotyledonary \((2.54 \pm 0.21, 1.79 \pm 0.19 \text{ and } 36.4 \pm 2.9)\) tissues, and were greater \((P < 0.01)\) in intercaruncular and cotyledonary than in intercotyledonary \((1.62 \pm 0.21, 1.24 \pm 0.16 \text{ and } 22.7 \pm 1.9)\) tissues (Table 3). Additionally, significant \((P < 0.01)\) day \times\ tissue interactions were observed for DNA, RNA and protein concentrations of placental tissues. Concentrations of DNA, RNA and protein remained constant across day of gestation in caruncular and intercaruncular tissues. For cotyledonary and intercotyledonary tissues, however, concentrations of DNA, RNA and protein increased \((P < 0.01)\) from Day 100 to 250 of gestation. As mentioned above, rate of increase in cotyledonary weight was less than that of caruncular weight. However, since concentrations of DNA increased 2-fold in cotyledonary tissues while remaining constant in caruncular tissues, the rate of increase of total DNA was similar for cotyledonary and caruncular tissues (Fig. 1c). The ratio of cotyledonary to caruncular DNA therefore remained constant across day of gestation, averaging 0.39 \pm 0.02 (Fig. 1c). Similarly, because concentrations of RNA and protein in cotyledonary tissues increased 3-fold across gestation, rates of increase of total tissue RNA and protein also were comparable for cotyledonary and caruncular tissues (data not shown). These differences in cellular growth of maternal and fetal placental tissues also were observed for interplacentomal tissues, with intercotyledonary tissues exhibiting 3-4-fold increases.

**Fig. 1.** Relationships of tissue weights (g) with day of gestation (t) in cows. Exponential regressions: (a) fetal weight = \(0.463e^{0.0802 - 0.0001452t}\), \(R^2 = 0.99\), \(N = 21\), C.V. = 1.6\%, \(P < 0.01\); and placental weight = \(1.757e^{0.0616 - 0.0001203t}\), \(R^2 = 0.97\), \(N = 21\), C.V. = 2.5\%, \(P < 0.01\). (b) caruncular weight = \(0.532e^{0.0682 - 0.0001343t}\), \(R^2 = 0.98\), \(N = 21\), C.V. = 2.6\%, \(P < 0.01\); and cotyledonary weight = \(3.680e^{0.0458 - 0.0000859t}\), \(R^2 = 0.91\), \(N = 21\), C.V. = 4.2\%, \(P < 0.01\). (c) caruncular DNA content = \(5.347e^{0.0589 - 0.0001115t}\), \(R^2 = 0.94\), \(N = 21\), C.V. = 3.4\%, \(P < 0.01\); and cotyledonary DNA content = \(3.422e^{0.0515 - 0.0000891t}\), \(R^2 = 0.89\), \(N = 21\), C.V. = 5.6\%, \(P < 0.01\).
in DNA, RNA and protein concentrations, whereas concentrations of DNA, RNA and protein in intercaruncular tissues remained constant from Day 100 to 250. Ratios of RNA:DNA and protein:DNA remained constant across day of gestation for all placental tissues, and no day \times tissue interaction was observed. When averaged across all days of gestation, ratio of RNA:DNA was greatest ($P < 0.05$) in caruncular (0.94 ± 0.05), intermediate in intercotyledonary (0.82 ± 0.08) and least ($P < 0.05$) in intercaruncular and cotyledonary (0.73 ± 0.05 and 0.74 ± 0.09) tissues (Table 3). Protein:DNA ratio was greater ($P < 0.05$) in intercotyledonary (16.7 ± 1.5) than in caruncular (12.4 ± 0.6) tissues, and was intermediate in intercaruncular and cotyledonary (15.3 ± 1.5 and 15.3 ± 1.4) tissues (Table 3).

**Table 3. Mean ± s.e.m. DNA, RNA and protein concentrations and ratios in placental tissues of cows**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day of gestation</th>
<th>DNA (mg/g)</th>
<th>RNA (mg/g)</th>
<th>Protein (mg/g)</th>
<th>RNA:DNA</th>
<th>Protein:DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caruncle</td>
<td>100</td>
<td>4.88 ± 0.35</td>
<td>4.16 ± 0.26</td>
<td>49.9 ± 6.2</td>
<td>0.86 ± 0.05</td>
<td>10.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>4.55 ± 0.52</td>
<td>4.09 ± 0.18</td>
<td>51.9 ± 2.3</td>
<td>0.94 ± 0.09</td>
<td>11.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>3.77 ± 0.37</td>
<td>3.46 ± 0.20</td>
<td>47.5 ± 2.2</td>
<td>0.97 ± 0.11</td>
<td>13.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>4.25 ± 0.44</td>
<td>3.92 ± 0.27</td>
<td>54.7 ± 0.8</td>
<td>0.96 ± 0.11</td>
<td>13.5 ± 1.2</td>
</tr>
<tr>
<td>Intercaruncular</td>
<td>100</td>
<td>2.66 ± 0.53</td>
<td>2.10 ± 0.46</td>
<td>31.6 ± 10.2</td>
<td>0.90 ± 0.14</td>
<td>11.7 ± 4.5</td>
</tr>
<tr>
<td>endometrium</td>
<td>150</td>
<td>2.23 ± 0.27</td>
<td>1.64 ± 0.34</td>
<td>36.7 ± 2.5</td>
<td>0.75 ± 0.14</td>
<td>17.9 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2.87 ± 0.34</td>
<td>1.90 ± 0.15</td>
<td>39.2 ± 3.1</td>
<td>0.70 ± 0.10</td>
<td>14.6 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2.94 ± 0.60</td>
<td>1.80 ± 0.29</td>
<td>39.4 ± 2.1</td>
<td>0.65 ± 0.08</td>
<td>16.1 ± 3.0</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>100</td>
<td>1.56 ± 0.32</td>
<td>0.76 ± 0.20</td>
<td>17.1 ± 2.0</td>
<td>0.49 ± 0.10</td>
<td>11.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>2.48 ± 0.24</td>
<td>1.30 ± 0.13</td>
<td>29.1 ± 1.0</td>
<td>0.53 ± 0.06</td>
<td>12.1 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2.39 ± 0.40</td>
<td>2.03 ± 0.29</td>
<td>40.4 ± 2.9</td>
<td>1.02 ± 0.29</td>
<td>19.7 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>3.39 ± 0.31</td>
<td>2.65 ± 0.26</td>
<td>51.3 ± 1.3</td>
<td>0.78 ± 0.04</td>
<td>16.0 ± 1.9</td>
</tr>
<tr>
<td>Intercotyledonary</td>
<td>100</td>
<td>0.66 ± 0.28</td>
<td>0.36 ± 0.10</td>
<td>7.8 ± 1.4</td>
<td>0.79 ± 0.26</td>
<td>18.4 ± 5.7</td>
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<tr>
<td>chorioallantois</td>
<td>150</td>
<td>1.40 ± 0.18</td>
<td>1.30 ± 0.30</td>
<td>23.2 ± 2.0</td>
<td>0.91 ± 0.17</td>
<td>18.3 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2.05 ± 0.51</td>
<td>1.47 ± 0.24</td>
<td>24.8 ± 1.6</td>
<td>0.82 ± 0.16</td>
<td>14.6 ± 2.1</td>
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<tr>
<td></td>
<td>250</td>
<td>2.03 ± 0.32</td>
<td>1.55 ± 0.28</td>
<td>30.0 ± 1.6</td>
<td>0.76 ± 0.08</td>
<td>16.4 ± 2.2</td>
</tr>
</tbody>
</table>

Numbers of observations were 4 on Day 100, 5 on Day 150, 6 on Day 200 and 6 on Day 250 for all tissues.

**Oxygen consumption**

Day of gestation had no significant effect on O2 consumption of placental tissues, and no day \times tissue interaction was found. Oxygen consumption differed, however, among placental tissues (Table 4). Across all days of gestation, O2 uptake (μmol·min\(^{-1}\)·g tissue\(^{-1}\)) of intercaruncular tissues (0.37 ± 0.02) was greater ($P < 0.01$) than that of other placental tissues. In addition, O2 uptake of caruncular tissues across gestation (0.30 ± 0.02) was greater than that of cotyledonary and intercotyledonary tissues (0.10 ± 0.01 and 0.15 ± 0.01) which were similar.

**Incorporation of [3H]leucine into macromolecules**

For all tissues, rate of incorporation of [3H]leucine into non-dialysable macromolecules was linear from 8 to 24 h of culture (regressions, where \(y = \text{pmol/g tissue and } x = \text{time in hours: caruncular incorporation } = -0.587 + 0.1312x, R^2 = 0.44, P < 0.05; \) intercaruncular incorporation = 1.682 + 1.8169x, \(R^2 = 0.34, P < 0.10; \) cotyledonary incorporation = -1.965 + 0.5936x, \(R^2 = 0.52, P < 0.01; \) and intercotyledonary incorporation = 0.756 + 0.3478x, \(R^2 = 0.29, P < 0.10.\) Incorporation of [3H]leucine by placental tissues did not vary across day of gestation. Significant differences ($P < 0.01$) in incorporation of [3H]leucine into secreted macromolecules were found, however, among placental tissues, and a day of gestation \times tissue interaction ($P < 0.01$) was also observed. When averaged across all days of gestation, rates of secretion...
Table 4. Mean ± s.e.m. oxygen consumption (µmol·min⁻¹·g tissue⁻¹) of placental tissues of cows

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Caruncle</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>Intercaruncular endometrium</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Intercotyledonary chorioallantois</td>
<td>0.14 ± 0.06</td>
</tr>
</tbody>
</table>

Maternal liver samples averaged 0.48 ± 0.06 µmol·min⁻¹·g tissue⁻¹ (n = 9). Number of observations is indicated in parentheses.

of macromolecules (µmol·24 h⁻¹·g tissue⁻¹) by intercaruncular and intercotyledonary tissues (31.3 ± 4.8 and 31.3 ± 3.0) were greater (P < 0.01) than that of cotyledonary tissues (21.0 ± 1.9; Table 5). In addition, secretory rate of cotyledonary tissues was greater (P < 0.01) than that of caruncular tissues (6.2 ± 0.8; Table 5). Rate of secretion of macromolecules by caruncules and cotyledons remained constant across day of gestation. Rate of secretion of macromolecules by intercaruncular endometrium, however, decreased (P < 0.01), whereas that of intercotyledonary chorioallantois increased (P < 0.05) from Day 100 to Day 250 of gestation (Table 5).

Table 5. Mean ± s.e.m. incorporation of [³H]leucine into macromolecules by placental tissues of cows*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Caruncle</td>
<td>7.2 ± 2.0</td>
</tr>
<tr>
<td>Intercaruncular endometrium</td>
<td>49.0 ± 22.8</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>18.8 ± 5.6</td>
</tr>
<tr>
<td>Intercotyledonary chorioallantois</td>
<td>16.5 ± 6.4</td>
</tr>
</tbody>
</table>

*Non-dialysable [³H]leucine in culture media expressed as µmol·24 h⁻¹·g tissue⁻¹. Numbers of observations were 4 on Day 100, 5 on Day 150, 6 on Day 200 and 6 on Day 250 for all tissues.

Discussion

Although placental weight increased exponentially throughout gestation, the absolute rate of increase was much less than that of fetal weight. In the ewe, placental weight ceases to increase or even decreases after Day 90 of gestation (Barcroft, 1946; Wallace, 1948; Alexander, 1964a). A similar pattern of continued growth of the fetus, but limited placental growth during the last half of gestation has been observed in several other mammalian species (Ibsen, 1928; Warwick, 1928; Hammond, 1935). In addition, a positive correlation between fetal and placental weight has been reported for several species (Ibsen, 1928; Warwick, 1928; Hammond, 1935; McKeown & Record,
intercaruncular growth, and of metabolic gestation endometrium, placental 0-32
First, Reynolds placental studies similar Redmer, tissue diffusion several later al, In gestation ewes to 02 than cows 1987). (Hammond, et al, 1985a). These observations led Huggett & Hammond (1952) to propose that “the size to which the fetal placenta grows during the early stages of pregnancy may determine, other things being equal, the amount of nutrition that is at the disposal of the fetus for growth during the later stages of pregnancy”. However, Huggett & Hammond (1952) also pointed out that this proposal may not be entirely valid since plancetal weight may not be indicative of placental function. Indeed, placental clearance of antipyrine, sodium, urea and H₂O increases with gestational age in several species (Flexner & Gellhorn, 1942; Meschia et al., 1966, 1967). In cows, transplacental diffusion of H₂O not only increases with gestational age, but remains constant when expressed as ml-min⁻¹.kg fetus⁻¹ (Reynolds & Ferrell, 1987). In the present study, DNA, RNA and protein concentrations of fetal placentomal tissues increased whereas those of maternal placentomal tissues remained constant from Day 100 to Day 250 of gestation. Because DNA, RNA and protein increased at about the same rates, growth of fetal placentomal tissues did not result from cellular or tissue hypertrophy, but rather from substantial hyperplasia. A portion of this hyperplasia may have resulted from increased density of vascular endothelial cells, which has been shown to occur primarily in fetal compared with maternal placentomal tissues of cows and ewes during late gestation (Hammond, 1927; Barcroft, 1946; Teasdale, 1976). In support of this proposal, umbilical blood flow and fetal O₂ and glucose uptakes increased 20-fold whereas uterine blood flow and uterine O₂ and glucose uptakes increased only 5-fold from mid- to late gestation in cows (Reynolds et al., 1986). Based upon these observations, it seems that, during the second half of gestation, fetal placental functional capacity increases at a greater rate than mass, due, in part, to hyperplasia of fetal placentomal tissues.

In the present study, the in-vitro rate of oxygen consumption by bovine placentomal tissues was similar to that reported by us and others for ovine placentomal tissues (Bell et al., 1987a; Reynolds & Redmer, 1987). The large proportion of O₂ taken up by the gravid uterus and utilized to support placental metabolism varies from ≈80% at mid-gestation to ≈40% during late gestation in cows and ewes (Battaglia & Meschia, 1981; Bell et al., 1986; Reynolds et al., 1986). In addition, in-vivo studies have shown a relatively high rate of O₂ consumption for the placenta compared with the fetus in cows (0.46 versus 0.25 μmol-min⁻¹·g⁻¹; Reynolds et al., 1986) and ewes (0.63 versus 0.32 μmol-min⁻¹·g⁻¹; Meschia et al., 1966). In the present study, in-vitro uptake of O₂ by maternal placentomal tissues was comparable to that reported for placental tissues in vivo and was 2-3-fold greater than O₂ uptake of fetal placentomal tissues. Similar observations have been reported for sheep placentomal tissues (Reynolds & Redmer, 1987). Therefore, maternal placentomal tissues probably account for a major portion of the high metabolic demand of the placenta. Intercaruncular endometrium, which is the site of uterine glands (Ramsey, 1982), exhibited a greater metabolic rate than did any of the other placentomal tissues. Uterine glands continue to develop throughout gestation in cows and ewes and secrete large amounts of histotrophe, thereby providing a portion of the nutritional demands of the fetus (Hammond, 1927; Bazer et al., 1979; Ramsey, 1982; L. P. Reynolds & D. A. Redmer, unpublished observations), which may account for the high metabolic rate of intercaruncular endometrium. Fetal placentomal tissues had relatively low growth and metabolic rates, yet, as discussed above, probably continue to increase their functional capacity throughout gestation. Thus fetal placentomal tissues appear to provide for the metabolic demands of fetal growth while their own metabolic demands are minimal.

As further indication of the importance of uterine glandular secretions in supporting fetal growth, intercaruncular tissues exhibited a relatively high rate of production of macromolecules. Secretory activity of intercaruncular endometrium, however, decreased from Day 100 to Day 250 of gestation. Assuming that all non-dialysable [³H]leucine was incorporated into macromolecules and that in-vitro secretory rate reflects secretory activity in vivo, decreased secretory activity of intercaruncular endometrium may indicate reduced importance of histotrophic secretion during late gestation. Uterine-specific proteins secreted by intercaruncular endometrium of cows throughout gestation may also have other functions in addition to providing for fetal nutrition (Bazer & First, 1983; Bartol et al., 1985a). Reynolds & Redmer (1988) found that, from Day 100 to 250 of
gestation, intercaruncular endometrium secreted a factor(s) which may regulate growth of blood vessels (angiogenesis) in the placenta. In contrast with intercaruncular tissues, intercotyledonary tissues increased their rate of secretion of macromolecules from Day 100 to 250. The role of secretory products of the chorioallantois in gravid uterine function is not known. Bartol et al. (1985b) found a glycoprotein of high molecular weight (735 000) secreted by bovine choriion up to Day 69 of gestation, but did not evaluate later stages and reported no function for this glycoprotein. Reynolds & Redmer (1988) reported that intercotyledonal tissues also secreted a factor(s) which may modulate placental angiogenesis. Additionally, products of intercotyledonary chorioallantois may regulate growth of non-vascular components of placenta. That intercotyledonal tissues can affect endometrial function is indicated by the observation that ‘adventitious placentomes’, which are smaller and more diffuse than normal placentomes but exhibit similar arrangement of fetal and maternal tissues, frequently form in interplacentomal areas during late pregnancy in cows (Hammond, 1927; L. P. Reynolds & D. A. Redmer, unpublished observations). Therefore, as stated by Hammond (1927), “it is apparent that the power, not only of developing the dormant caruncles of the uterus, but also of initiating the formation of new adventitious placental growths rests with the foetal membranes”.

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