Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development in utero

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The interacting influences of maternal size and fetal genotype on placental and fetal development in the mare were assessed by comparing conventional within-breed Thoroughbred (Tb-in-Tb, n = 7) and Pony (P-in-P, n = 7) control pregnancies established by artificial insemination (AI) with between-breed (Tb-in-P, n = 8; deprived in utero condition and P-in-Tb, n = 7; luxurious in utero condition) experimental pregnancies established by embryo transfer. All foals were born spontaneously and the mean (± SEM) duration of gestation in the two groups of control mares was significantly different (P < 0.001) at 325 ± 3.0 days for the P-in-P pregnancies and 339 ± 3.0 days for the Tb-in-Tb pregnancies, whereas the durations of gestation for the two experimental groups were very similar and midway between those of the control pregnancies at 332 ± 2.8 days for the Tb-in-P and 331 ± 2.7 days for the P-in-Tb. Mean (± SEM) foal birth weight and the mean (± SEM) values for the mass, gross area and volume of the allantochorion were all highest in the seven Tb-in-Tb pregnancies (53.1 ± 2.6 kg, 3.8 ± 0.3 kg, 12.9 ± 0.3 × 10⁴ cm², 3.5 ± 0.2 l, respectively) and lowest in the seven P-in-P control pregnancies (24.0 ± 1.3 kg, 1.7 ± 0.1 kg, 8.3 ± 0.3 × 10³ cm², 1.8 ± 0.1 l, respectively). These parameters were higher in the seven P-in-Tb pregnancies (37.9 ± 2.1 kg, 2.7 ± 0.1 kg, 10.1 ± 0.5 × 10³ cm², 2.5 ± 0.1 l, respectively) than in the eight Tb-in-P (33.0 ± 2.4 kg, 2.3 ± 0.2 kg, 9.0 ± 0.5 × 10³ cm², 2.1 ± 0.1 l) experimental pregnancies. Foal birth weight was positively correlated with the mass (r = 0.84, P < 0.001), gross area (r = 0.87, P < 0.001) and volume (r = 0.91, P < 0.001) of the allantochorion, and maternal weight was also positively correlated with both the mass and gross area of the allantochorion (r = 0.64 and 0.69, respectively; both P < 0.001). Application of stereology to multiple random biopsies recovered from each placenta produced mean values for the surface density of microcotyledons on the allantochorion (Sv). Values were higher in Thoroughbred than in Pony mares regardless of the breed of fetus being carried. Multiplication of Sv by the volume of the allantochorion to give the total microscopic area of fetomaternal contact at the placental interface was also positively correlated with foal birth weight (r = 0.84, P < 0.001).

Foal birth weight was determined by the microscopic area of fetomaternal contact of the placenta and there were no differences in foal weight per m² of placenta regardless of fetal or maternal genomes. Thus, the results indicate that in equids, maternal size interacts with both the maternal and fetal genotypes to control the rate and extent of fetal growth by influencing the gross area of the diffuse allantochorion, and the density, complexity and depth of the microcotyledons on its surface.

Introduction

Epidemiological studies in several human populations have shown that the pattern of fetal growth is an important risk factor for various adult onset degenerative diseases. In particular, impaired growth in utero or a small fetus relative to the size of the placenta increases the incidence of adult hypertension, ischaemic heart disease and a variety of metabolic disorders, such as dyslipaemia, glucose intolerance and Type II diabetes (Barker, 1995). The associations between low birth weight and specific adult diseases occur independently of current weight, obesity or degree of exercise and they have been linked to poor nutrition during pregnancy. These population-based observations have led to the hypothesis that adult disease may originate in fetal life as a consequence of abnormalities in nutrient provision at critical periods of development.

This hypothesis has been tested in a number of species by reducing maternal dietary intake during pregnancy (Robinson et al., 1999) or by altering the size and functional capacity of the placenta (Robinson et al., 1979), the main determinants of the fetal nutrient supply (Hoet and Hanson, 1999).

¹Deceased
The functional mass of the placenta has been reduced using a variety of techniques, including embolization, carunclectomy, multiple pregnancies and reduced uterine blood flow (Harding and Johnson, 1995; Clarke et al., 1998). All these methods retard fetal growth and, in several instances, alter cardiovascular and metabolic functions, both before and after birth (Ozanne et al., 1996; Hoet and Hanson, 1999). Similarly, in sheep and rats, restriction of maternal dietary intake, either in total or of protein, specifically impairs intrauterine growth and results in adult hypertension and glucose intolerance (Robinson et al., 2000). However, compared with humans, the lifespan of species studied to date, is short. In addition, few of the studies have considered the potential contribution made by changes in placental structure.

The domestic horse (Equus caballus, 2n = 64) could be a good model for investigating the fetal origin of adult disease. It has a long lifespan relative to other experimental animals and has a diffuse epitheliochorial placenta that attaches to the entire endometrium. As uterine size is directly related to the size of the mare, these parameters will, in turn, govern the area for placentation and, hence, fetal growth. Indeed, Walton and Hammond (1938) showed that the size of the mare affects not only the intrauterine development of her foal but also its postnatal growth rate. AI was used to cross large Shire horses with small Shetland ponies; a foal from a Shetland mare was produced that was half the size of its reciprocal half sibling born from a Shire mother; these differences in size persisted until adulthood (Hammond, 1940). Tischner (1985) produced three pairs of full sibling sex-matched Konik pony embryos and transferred one of each pair to a large draught-type mare, whereas the other embryo developed within its smaller genetic mother. The foals transferred to the larger mares were all taller and heavier at birth than their non-transferred siblings and they continued to grow faster after birth while nursing from their larger surrogate mothers. These earlier experiments demonstrated that in equids, fetal growth can be either enhanced above, or restricted below, the normal genetic potential for the breed by varying maternal size. However, neither the size nor the morphology of the placenta were investigated in the studies of Walton and Hammond (1938) and Tischner (1985).

In the present study, Thoroughbred embryos were transferred to the uteri of smaller Pony mares and Pony embryos were transferred to the uteri of Thoroughbred mares. The specific aim of this study was to establish whether differences in fetal growth due to variations in maternal size could be related to changes in the gross mass and microstructure of the placenta. The overall objective was to establish whether the horse might be a useful model for investigating the intrauterine programming of adult disease.

### Materials and Methods

#### Establishment of pregnancies

Thoroughbred-in-Thoroughbred (Tb-in-Tb) and Pony-in-Pony (P-in-P) pregnancies (controls) were established by artificial insemination (AI) using standard techniques (Table 1). Experimental pregnancies of Pony-in-Thoroughbred (P-in-Tb, luxurious in utero environment) and Thoroughbred-in-Pony (Tb-in-P, deprived in utero environment) were established by transferring Pony embryos into the uteri of Thoroughbred mares and Thoroughbred embryos into the uteri of Pony mares (Table 1), using standard embryo transfer techniques described by Allen (1982). One Thoroughbred sire was used for all Thoroughbred conceptuses, whereas the Pony conceptuses were conceived using semen from one Pony stallion.

#### Parturition and placental sampling

Each foal was weighed within 30 min after birth, before it had sucked. The allantochorionic was weighed and laid flat on a laminated board in the typical ‘F’ configuration with the fetal surface outermost (Fig. 1a). The allantochorionic was then cut open around its entire perimeter (Fig. 1b) so that its

<table>
<thead>
<tr>
<th>Genotype of the mare</th>
<th>Genotype of the fetus</th>
<th>Number of pregnancies established</th>
<th>Number of foals born alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb</td>
<td>Tb</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Tb</td>
<td>P</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>P</td>
<td>P</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>P</td>
<td>Tb</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

Tb: Thoroughbred; P: Pony.
gross area could be measured by suspending above it a 1 m² sheet of perspex marked with grid lines to every 10 cm with a small hole for every 100 cm². The surface area was then estimated by counting the number of holes positioned above the allantochorion; each point was equivalent to an area of 100 cm² (Mayhew, 1991). As there was a random positioning of the test grid and a linear magnification of 1, the total number of points (P) that fell above the allantochorion allowed an estimation of its total area (A) in cm². The area was calculated using the following equation: $A = P \times 100$.

The recovery of ten biopsies of the allantochorion for histological and stereological analyses was randomized by dropping a wooden cocktail stick through every nth hole above the allantochorion, where n equaled the number of holes above placental tissue divided by 10. Two pieces of tissue, each about 4 cm × 1 cm, were cut through the full depth of the allantochorion at each biopsy point. One piece of tissue was immersed in 10% (v/v) neutral buffered formol saline and another piece in Bouin's solution. Both tissue samples were processed for standard light microscopy. A third small piece (approximately 1 mm³) of tissue was collected and fixed in an equal mixture of 1% (v/v) glutaraldehyde and 3% (v/v) paraformaldehyde for electron microscopy.

When histological sampling was complete, the allantochorion was cut into four pieces, each of which was dropped into a 2 l measuring cylinder half filled with water. The volume of water displaced was recorded and the total volume of the allantochorion was calculated.

**Stereological analysis**

The total area of fetomaternal contact via the microcotyledons was estimated by first calculating the surface density of the microcotyledons ($S_v$; Fig. 2a). Isotrophic uniformly random (IUR) linear probes were generated with the aid of a computer program package (Digital Stereology 2.4; Confocal Technologies, Liverpool), a Lietz Laborlux microscope (Leica UK Ltd, Milton Keynes) and a colour video camera (JVC, TK-1085E; Leica UK Ltd). An image of a section of the chorionic microcotyledon viewed at × 100 magnification was digitized and projected on to a monitor via the video camera mounted on the microscope; sections were orientated by rotating the camera so that the vertical direction ran vertically on the monitor. The digital stereology package superimposed a uniform grid of points overlying the tissue image on the monitor to delineate the reference space (Fig. 2b); the number of points that fell inside the area of chorion was then entered into the computer. Subsequently, a multiplicity of linear probes or ‘needles’ was applied randomly to the image on the monitor (Fig. 2c) and the total number of times that the edges of chorionic villi were intersected by needles was entered into the computer. A figure for $S_v$ was then calculated automatically by the computer, using the equation $S_v = 2I/L$, where $I =$ number of intersections between probe and chorionic surface and $L =$ total length of the line falling within the object (Browne et al., 1995).

For each allantochorion under examination, the procedure was repeated at five random sites on each of the ten biopsies to give a total of 50 counts, each using a test system of five rows and five columns of test points and linear probes (that is, 25 ‘needles’). The computer program automatically calculated a running mean of the chorionic $S_v$ as each section was tested and then presented as an overall figure of $S_v$ in μm⁻¹ (μm²/μm³) for each placenta. A coefficient of error was also calculated for all the individual values of $S_v$. This value was consistently lower than 0.05, thereby indicating that 10 samples were sufficient to achieve a representative estimate of $S_v$ for the whole allantochorion.

The total microscopic area of fetomaternal contact was calculated by multiplying the mean $S_v$ value for each allantochorion by the volume of the chorionic portion of the organ. This value was calculated using a variation of Cavalieri’s Principle (Browne et al., 1995). Each section

![Fig. 1. The allantochorion from a Pony-in-Thoroughbred (P-in-Tb) pregnancy. (a) The allantochorion intact and arranged in the typical ‘F’ configuration with the allantoic (fetal) surface outermost and (b) opened at the periphery and spread out to reveal the chorionic (maternal) surface.](image-url)
from the ten biopsies was viewed under the microscope at \( \times 25 \) magnification to enable visualization of the full depth of the allantochorion. An eyepiece graticule (Taab Laboratories Equipment Ltd, Aldermaston) marked with a 10 × 10 grid of equidistant points allowed visualization of the allantochorion with the grid superimposed above. By counting the total number of points lying above the allantochorion, dividing this figure by the total number of points positioned above just the chorion and multiplying by 100, a value for the percentage of the total allantochorion that was just chorion \((P_c)\), was obtained. The procedure was carried out on each section at three separate locations to give a total of 30 estimations per placenta. Means were calculated for these values so that it was possible to calculate the volume of the chorion \((V_c)\) from the volume of the allantochorion \((V_a)\) using the equation \(V_c = V_a \times P_c / 100\).

Finally, the total microscopic area of fetomaternal contact at the placental interface \((S)\) in m\(^2\) was calculated using the formula \(S = S_v \times V_c\) (Baddeley et al., 1987). No corrections were made for any possible shrinkage of allantochorion that may have occurred during fixation in 10% (v/v) buffered formal saline, because Gerstenberg (1998) reported that this reduction was negligible.

**Statistical analysis**

Mean and standard errors (± SEM) have been presented throughout. Statistical analyses were performed using one-way or two-way ANOVA with Tukey’s post hoc test (SigmaStat v. 2.0 program; SPSS Inc. Chicago, IL). Data were also analysed by linear regression analysis using this program. Differences were considered significant at \( P < 0.05 \).

**Results**

**Pregnancies**

In the two breeding seasons of 1996 and 1997, a total of 11 Tb-in-P (deprived *in utero* existence), eight P-in-Tb (luxurious *in utero* existence), eight Tb-in-Tb (control) and seven P-in-P (control) pregnancies were established. One Tb-in-P aborted spontaneously at day 299 of gestation for no apparent reason. The fetus weighed 29 kg and appeared to be physically mature. One P-in-Tb also aborted (fetal...
body weight 13 kg) on day 275 of gestation. One Thoroughbred mare carrying a control Thoroughbred fetus was killed humanely on day 149 of gestation when it accidentally broke a hind leg. Two other Tb-in-P foals were stillborn at, respectively, day 328 and on day 335 of gestation, one of which had led a relatively luxurious existence in utero, and was not different between the groups of mares.

**Parturition**

The mean (± SEM) durations of gestation for the control mares were significantly different ($P < 0.001$) at 325 ± 3.0 days for the P-in-P and 339 ± 3.0 days for the Tb-in-Tb pregnancies. The durations of gestation of the two experimental groups were intermediate at 332 ± 2.8 days for the Tb-in-P and 331 ± 2.7 days for the P-in-Tb groups (Table 2). Two-way ANOVA of the data with maternal and fetal genome as factors showed significant effects of both the maternal ($P < 0.029$) and fetal ($P < 0.015$) genome on the duration of gestation, and a longer gestation was associated with a Thoroughbred genome irrespective of whether this was the mare or foal.

All the foals were healthy and reasonably vigorous at birth, but the Tb-in-P foals (deprived in utero existence) showed various degrees of muscle underdevelopment about the body and upper limbs (Fig. 3a). In addition, some Tb-in-P foals showed hyperextension of the fetlock joints and had a silky hair coat (Fig. 3a), characteristic signs of prematurity or dysmaturity as described by Rossdale and Silver (1982). These Tb-in-P foals required various degrees of assistance to stand and suck during the first 24 h after birth, but improved thereafter. In contrast, P-in-Tb foals, which had led a relatively luxurious existence in utero, were all healthy and physically mature (Fig. 3b). Overall, the Pony foals, regardless of the breed of their mothers, were all healthy and physically mature (Fig. 3b). Overall, the Pony foals, regardless of the breed of their mothers, were able to stand and suck earlier, and were generally more able and ‘worldly wise’, than the Thoroughbred foals. Delivery of the placentae occurred spontaneously between 19 min and 63 min after birth (mean ± SEM = 47 ± 6.3 min), and was not different between the groups of mares.

**Foal and mare body weights**

The mean (± SEM) body weights of the mares and their newborn foals are shown (Table 2). Tb-in-Tb control foals were significantly ($P < 0.001$) heavier (53.1 ± 2.6 kg, $n = 7$) than the P-in-P foals (24.0 ± 1.3 kg, $n = 7$), reflecting the significant difference in maternal body weights (Tb = 589.6 ± 12.7 kg, $n = 14$ versus Pony = 329.5 ± 17.0 kg, $n = 15$). The mean (± SEM) birth weight of the ‘luxurious’ P-in-Tb foals (37.9 ± 2.1 kg, $n = 7$) was significantly higher ($P < 0.001$) than that of the Tb-in-Tb foals. Two-way ANOVA showed significant effects of both maternal ($P < 0.001$) and fetal genome ($P < 0.01$) on foal birth weight and greater birth weights were associated with a Tb genome regardless of maternal or fetal origin. There was a significant linear relationship between the weight of the mother and the newborn foal ($r = 0.74$, $P < 0.01$; Fig. 4). Calculation of foal birth weight as a function of pre-pregnancy maternal metabolic weight (weight ($0.75$) showed that the fetal genome affected birth weight (Table 2) even when differences in

**Table 2. Mean (± SEM) maternal, fetal and placental parameters measured in the four types of equine pregnancy**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tb-in-Tb ($n = 7$)</th>
<th>P-in-Tb ($n = 7$)</th>
<th>Tb-in-P ($n = 7$)</th>
<th>P-in-P ($n = 7$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of gestation (days)</td>
<td>338.9 ± 3.0$^{a}$</td>
<td>331.1 ± 2.7$^{ab}$</td>
<td>331.6 ± 2.8$^{ab}$</td>
<td>324.6 ± 3.0$^{b}$</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>597.9 ± 18.2$^{a}$</td>
<td>581.3 ± 18.6$^{a}$</td>
<td>355.5 ± 30.8$^{b}$</td>
<td>300.1 ± 8.6$^{b}$</td>
</tr>
<tr>
<td>Foal birth weight (kg)</td>
<td>53.1 ± 2.6$^{a}$</td>
<td>37.9 ± 2.1$^{b}$</td>
<td>33.0 ± 2.4$^{b}$</td>
<td>24.0 ± 1.3$^{c}$</td>
</tr>
<tr>
<td>Foal weight/kg maternal weight $^{0.75}$</td>
<td>0.44 ± 0.02$^{a}$</td>
<td>0.32 ± 0.02$^{b}$</td>
<td>0.41 ± 0.04$^{b}$</td>
<td>0.34 ± 0.03$^{bc}$</td>
</tr>
<tr>
<td>Mass of allantochorion (kg)</td>
<td>3.8 ± 0.3$^{a}$</td>
<td>2.7 ± 0.1$^{b}$</td>
<td>2.3 ± 0.2$^{b}$</td>
<td>1.7 ± 0.1$^{c}$</td>
</tr>
<tr>
<td>Gross area of allantochorion (cm$^2$ × 10$^3$)</td>
<td>12.9 ± 0.3$^{a}$</td>
<td>10.1 ± 0.5$^{b}$</td>
<td>9.0 ± 0.5$^{b}$</td>
<td>8.3 ± 0.3$^{c}$</td>
</tr>
<tr>
<td>Volume of allantochorion (l)</td>
<td>3.5 ± 0.2$^{a}$</td>
<td>2.5 ± 0.1$^{b}$</td>
<td>2.1 ± 0.1$^{b}$</td>
<td>1.8 ± 0.1$^{c}$</td>
</tr>
<tr>
<td>Volume of chorion ($V_c$; l)</td>
<td>1.1 ± 0.09$^{b}$</td>
<td>0.8 ± 0.12$^{b}$</td>
<td>0.9 ± 0.07$^{ab}$</td>
<td>0.6 ± 0.04$^{b}$</td>
</tr>
<tr>
<td>Microcotyledon surface density ($S_{sc}$ μm$^{-1}$)</td>
<td>0.037 ± 0.001$^{a}$</td>
<td>0.036 ± 0.001$^{a}$</td>
<td>0.032 ± 0.001$^{b}$</td>
<td>0.030 ± 0.001$^{b}$</td>
</tr>
<tr>
<td>Total microscopic area of fetomaternal contact ($S_{vm} \times V_c$; m$^2$)</td>
<td>42.0 ± 4.4$^{a}$</td>
<td>34.2 ± 2.0$^{b}$</td>
<td>29.4 ± 2.6$^{bc}$</td>
<td>18.7 ± 1.3$^{c}$</td>
</tr>
<tr>
<td>$R_v$ ($S_{vm} \times V_c$/gross area) m$^2$</td>
<td>32.7 ± 3.6$^{ab}$</td>
<td>33.5 ± 2.3$^{a}$</td>
<td>35.3 ± 2.3$^{c}$</td>
<td>22.8 ± 1.8$^{b}$</td>
</tr>
<tr>
<td>Foal weight per m$^2$ placenta (kg (m$^2$))</td>
<td>1.30 ± 0.09</td>
<td>1.15 ± 0.08</td>
<td>1.15 ± 0.09</td>
<td>1.30 ± 0.09</td>
</tr>
</tbody>
</table>


$^{a,b,c}$Values within a horizontal row with different superscripts are significantly different (one-way ANOVA; $P < 0.05$).

Two-way ANOVA data are presented only in the text.
maternal genome are taken into account (two-way ANOVA; fetal genome, P < 0.01; maternal genome, not significant). Thoroughbred foals were larger per kg maternal metabolic weight irrespective of the genome of the mother (P < 0.05).

Placental characteristics

The mean (± SEM) values for the mass, gross area and volume of the allantochorion are shown (Table 2) together with the volume of the chorion, the surface density of the microcotyledons ($S_m$), the total microscopic area of fetomaternal contact at the placental interface and the ratio of total microscopic to gross area of the allantochorion ($R_v$).

Gross parameters. The mass, gross area and volume of the allantochorion were significantly greater in Tb-in-Tb than in P-in-P pregnancies, whereas intermediate values were obtained for P-in-Tb and Tb-in-P groups (Table 2).

Two-way ANOVA of the data showed that maternal and fetal genomes were significant factors in determining all three of these parameters (P < 0.001 in all cases). There were also interactions between the maternal and fetal genomes in determining the volume and gross area of allantochorion, with larger volumes and areas in Thoroughbred versus Pony foals that were gestated in Thoroughbred mares but not in Pony mares (P < 0.02, P > 0.05, respectively). There were significant positive correlations between maternal weight and the gross area ($r = 0.69$, P < 0.01, n = 29), mass ($r = 0.64$, P < 0.001, n = 29) and volume ($r = 0.68$, P < 0.01, n = 29) of the allantochorion.

Histological and stereological analyses. The size (that is, height, width and breadth) of the microcotyledons (Fig. 2a), and their density on the surface of the allantochorion (that is, the number of microcotyledons per length of allantochorion surface), varied greatly within each placenta. As described by Cottrill et al. (1991), the microcotyledons were considerably larger and more densely packed in the non-gravid uterine horn and the tip of the gravid uterine horn, and were smaller and more widely spaced in the base of the gravid uterine horn and in the body region of the placenta. This wide distribution pattern probably resulted from greater stretching of the uterine body and gravid uterine horn by the body of the growing fetus combined with the compressive effects of its gravitational weight on the undermost surface of the allantochorion–endometrium interface (Cottrill et al., 1991). Despite these within-placenta variations, significant differences in the microscopic structure of the placenta were observed between the four groups of animals. The volume of the chorion, microcotyledonalny surface density and the total microscopic area of fetomaternal contact were highest in the Tb-in-Tb pregnancies and lowest in the P-in-P pregnancies; intermediate values
were observed for the other two groups (Table 2). Two-way ANOVA for these three parameters showed that the total microscopic area and the volume of chorion were significantly affected by both the maternal and fetal genomes (P < 0.002 in all cases), whereas Sv was determined only by the maternal genome (P < 0.001). The ratio of total microscopic to gross area of the allantochorion (Rv) was significantly lower in the P-in-P mares than in the Th-in-P and P-in-Tb mares (Table 2). There was also a significant interaction between the maternal and fetal genomes in determining Rv, with a larger Rv in Thoroughbred versus Pony foals that were gestated in Pony but not in Thoroughbred mares (two-way ANOVA, P < 0.016). The total microscopic area of fetomaternal contact was positively correlated with maternal weight (r = 0.61, P < 0.001, n = 29).

Relationships between foal birth weight and placental parameters

There were significant positive correlations between foal birth weight and volume (r = 0.910, P < 0.01, n = 29; Fig. 5a), gross area (0.870, P < 0.001, n = 29; Fig. 5b) and mass (r = 0.843, P < 0.01, n = 29; Fig. 5c) of the allantochorion, and the total microscopic area of fetomaternal contact (r = 0.84, P < 0.001, n = 29; Fig. 5d). When fetal weight was expressed as a function of total microscopic area of the allantochorion, there was no significant difference in the weight of foal produced per m² of allantochorion between the four groups of animals (Table 2).

Discussion

The results of the present study confirm and extend the results of earlier studies (Walton and Hammond, 1938; Tischner, 1985, 1987) to show that foal birth weight is determined primarily by the total microscopic area of the allantochorion, independently of the maternal or fetal genotype. However, development of the total area of microscopic fetomaternal contact was itself dependent on both the maternal and fetal genotypes so that fetal growth was indirectly affected by both genotypes. Pony embryos transferred to Thoroughbred mares produced larger placentae.
and, hence, were larger foals at birth compared with their P-in-P counterparts. Nevertheless, the placenta mass and birth weights of these P-in-Tb foals were still lower than those of the Tb-in-Tb control animals. Conversely, transferring a Thoroughbred foal into a Pony uterus restricted placental, and hence fetal, growth compared with that in the Tb-in-Tb pregnancies, but it also gave rise to a larger total microscopic area of fetomaternal contact and larger birth weight than in the Pony foals born to similarly sized Pony mares. Therefore, genetic factors enhance placental growth in Tb-in-P pregnancies but restricted it in the P-in-Tb pregnancies. The cellular and molecular mechanisms whereby the fetal and maternal genotypes control placental development remain unknown.

As in laboratory rodents and other large mammalian species that have been studied (see Fisher and Lakshmanan, 1990), it is likely that growth factors secreted locally by both the endometrium and placenta synergize in stimulating the large degree of hyperplasia and tissue remodelling associated with placentation in mares, and may account for the differences in placental development observed between the groups. The mRNA for insulin-like growth factor II (IGF-II) is expressed strongly in equine trophoblast cells and many fetal organs from as early as day 20 of gestation (Lennard et al., 1995), and the mRNA for the potent mitogen, epidermal growth factor (EGF), is upregulated markedly in the epithelium lining the apical secretory portions of the endometrial glands in the uterus of the mare between day 35 and day 40 after ovulation (Stewart et al., 1994). This sudden increase in EGF production immediately precedes microvillus attachment of the outermost trophoblast of the allantochorion to the luminal epithelium of the endometrium and the commencing interdigitation of blunt villi of allantochorion with accommodating crypts in the surface of the endometrium (Samuel et al., 1975). Over the following 80–100 days, extensive branching of the allantochorionic villi and upward growth of the accommodating frond-like endometrial protruberances creates the mature microcotyledons which cover the entire surface of the allantochorion and maximizes the degree of haemotrophic exchange between mother and fetus during the second half of pregnancy (Steven, 1982; Bracher et al., 1996). EGF produced locally in large quantities by the endometrial glands is the most likely candidate to drive this whole process and there is preliminary evidence that the endometrium of the Thoroughbred mare contains a higher density of endometrial glands than that of the Pony (Lefranc, 2001), thereby providing the mechanism for the larger $S_V$ seen in the Thoroughbred mares.

Despite the increased growth of the placenta in the Tb-in-P relative to the P-in-P pregnancies, the Tb-in-P foals were severely growth retarded and had a mean birth weight that was only just over half that of the Tb-in-Tb foals. The intrauterine growth restriction of these foals acted to reduce their muscle and fat deposition and, thereby, facilitated safe delivery in most cases. The increased nutritional and spatial stress imposed on the Tb-in-P fetuses may have increased the output of pregnenolone by their adrenal glands and, thereby, initiated the hormone cascade that resulted in parturition. Indeed, these foals were delivered 6 days earlier than the Tb-in-Tb foals and these mares suffered a higher incidence of pregnancy failure than the other groups of mares. The finding that maternal progestagen concentrations were higher in Tb-in-P than in P-in-P pregnancies during the last 3 months of gestation (Allen et al., 2001) is consistent with the suggestion that the Tb-in-P foals were stressed in utero, as high maternal progestagen concentrations are common in clinical cases of fetal stress (Rossdale et al., 1991). In the P-in-Tb pregnancies, foals were born 6 days later than in the P-in-P controls, indicating that Pony foals were not stressed in the larger Thoroughbred uterus and may have delayed the normal signal for parturition in this situation.

The increase in the total microscopic area of the allantochorion in the Tb-in-P versus the P-in-P pregnancies occurred in the absence of any significant increase in its gross surface area. As $S_V$ was maternally determined, being lower in Pony than in Thoroughbred mares, the larger $R_V$ value in the Tb-in-P pregnancies was more likely to have been due to an increase in the length of the fetal villi than to an increase in their branching. Lengthening of the fetal chorionic villi, which is known to occur throughout gestation in normal P-in-P pregnancies (Macdonald et al., 2000), therefore appeared to be enhanced when the genetic potential for growth was constrained by transferring the Thoroughbred foal into a Pony uterus. The inherent drive of the Thoroughbred foal to produce a larger allantochorion may explain, in part, the greater mass of Thoroughbred foal produced per kg of maternal metabolic weight. However, the Thoroughbred foal may have also been more effective at adapting maternal metabolism in favour of its own nutrient supply. Certainly, maternal progestagen concentrations were higher when a Thoroughbred foal was being gestated, irrespective of maternal genotype (Allen et al., 2001), which indicates that factors other than placental growth were affected by the genotype of the fetus.

In summary, transfer of equine embryos between breeds led to either restricted (Tb-in-P) or enhanced (P-in-Tb) growth of the foal, which was directly determined by the growth of the allantochorion. Development of the total microscopic area of fetomaternal contact was affected by both the maternal and fetal genotypes; the maternal genotype controlled microcotyledonal surface density ($S_V$), whereas the fetal genotype appeared to affect gross placental area and the $R_V$ value, probably by influencing the length of the fetal villi. The growth retardation of the Tb-in-P foals and the overgrowth of the P-in-Tb foals indicate that the horse is likely to be a good model for investigating the intrauterine programming of adult disease. Indeed, physiological studies of the foals produced by transfer between breeds in this experiment showed that the pattern of growth in utero affected both cardiovascular and metabolic functions in the 7 days after birth (Forhead et al., 1999; Giussani et al., 1999). However, whether these
changes in growth and physiological function induced in utero persist into adult life remains to be determined.

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