# Ovine placenta at high altitudes: Comparison of animals with different times of adaptation to hypoxic environment

Víctor H. Parraguez [a,b,c,*], Miljenko Atlagich [a], Rodrigo Díaz [a], Raquel Cepeda [a], Carlos González [a], Mónica De los Reyes [a], María E. Bruzzone [b,c], Claus Behn [b,c], Luis A. Raggi [a,b,c]

[a] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Chile*
[b] *Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Chile*
[c] *International Center for Andean Studies (INCAS), Universidad de Chile, Chile*

**Abstract**

Fetal growth and newborn weight from ovine gestations at high altitudes (HA) are greater in ewes that live at HA for several generations than in those native to low altitudes (LA) exposed to HA only during pregnancy. Because the placenta is a key regulator of fetal growth, the present study compared placental characteristics in term pregnancies among ewes native to HA and LA. Conception occurred at HA and ewes continued to reside at HA throughout pregnancy or conception occurred at LA and ewes were transported to HA or remained at LA (controls). Ewes native to LA were moved to HA shortly after mating (group LH) and joined with pregnant ewes native to HA (group HH). After parturition, placental cotyledons were counted and measured for total area and histological estimation of surface occupied by vasculature. The total surface of the cotyledons and surface occupied by vasculature were greater at HA, whereas the number of cotyledons was smaller at HA. These changes were more pronounced in ewes of the HH compared with the LH group. The present study showed that exposure to HA induces, in pregnant ewes, placental morphological changes that may improve maternal–fetal exchange. Moreover, because of accentuation of placental changes in ewes with long-term residence at HA, this appears to be an efficient mechanism of adaptation to hypobaric hypoxia.

*Keywords:* Sheep-placenta; High altitude

* Corresponding autor at: Facultad de Ciencias Veterinarias y Pecuarias, International Center for Andean Studies (INCAS), Universidad de Chile, Casilla 2, Correo 15, La Granja, Santiago, Chile. Tel.: +56 2 978 5548; fax: +56 2 978 5526.

*E-mail address:* vparragu@uchile.cl (V.H. Parraguez).

## 1. Introduction

Previous work in sheep has demonstrated placental adaptations when ewes gestate at high altitudes (HA). Responses include a slight reduction in placentome number (Penninga and Longo, 1998) and a denser placentome capillary bed (Krebs et al., 1997). Moreover, there is an increased placental weight in native ewes that have resided for several generations at HA (Parraguez et al., 2005). In the human placenta HA induces an increase in villous vascularization (Zamudio, 2003). Though these changes may improve maternal–fetal exchange at HA, changes are associated with intrauterine growth retardation (IUGR).

Sheep pregnancies at HA also result in IUGR. In addition, the effects of HA on fetal growth and newborn body weight were more accentuated in native ewes at low altitude (LA) as compared with HA (Parraguez et al., 2005). Considering the previous results and the role of the placenta as a regulator of fetal growth, we hypothesized that the time of residence at HA modifies the response of sheep placenta to exposure to chronic natural hypobaric hypoxia. Thus, the aim of the present study was to compare placental characteristics in term pregnancies among ewes native to HA and LA, where pregnancy occurred at HA, and ewes native to LA that carried their pregnancies at LA.

## 2. Materials and methods

The present study is the continuation of a previous study. A detailed description of the characteristics, location, and general management of the animals involved in these studies were reported previously (Parraguez et al., 2005). In brief, animals in the present study were singleton pregnant Creole ewes. Ten ewes were native to HA (group HH). Twenty LA native ewes were separated into two groups. One of them was kept at 500 m above sea level (m.a.s.l.; control group LL), and the other group was moved to HA (group LH) shortly after diagnosis of pregnancy. The latter group, as well as the HH, was maintained at 3589 m.a.s.l. during gestation (INCAS, University of Chile). The food supply was calculated to satisfy the daily requirements for ewes during late gestation (NRC, 1985). Arterial blood gases were measured using an IL Synthesis 25$^{\mathrm{TM}}$ (Instrumentation Laboratory, Lexington, MA, U.S.A.) at 106–120 days of gestation.

After parturition, the placenta was recovered, rinsed with buffered saline, drained, and fixed for 48 h (10% buffered formalin). Counting of the cotyledons, measurement of the cotyledon diameters, estimation of the surface area of cotyledon–caruncle contact, and estimation of cotyledon surface area occupied by vasculature were then performed. Cotyledon diameters were estimated by averaging the longest axis and the length of the transversal axis at the center of the structure. Cotyledon surface area was estimated assuming a circumferential surface, using the formula $A = \pi r^2$, where $A$ is the surface area and $r$ is the radius. This criterion has been previously applied by Kaulfub et al. (2000) and Vonnahme et al. (2003). The total placental surface area in each animal was obtained by adding individual cotyledon surfaces. After an additional fixation period, 16 cotyledons of each group of ewes were processed for light microscopy histology. Serial 6 μm cuts from the middle zone through the transversal plane were obtained and stained with hematoxylin–eosin. Measurement of the cotyledon area occupied by the vasculature was done in four sections (four microscopic fields per section) through an Eclipse E-600 microscope (Nikon, Japan). Images were captured with 100× augmentation using a digital video camera (Cool Snap-Pro, Media Cibernetic, U.S.A.) coupled to the microscope. The percentage of vascular lumina of the total area was then calculated using Image Pro-Plus morphometric analysis software (Media Cibernetic, U.S.A.).

Table 1
Arterial blood variables in pregnant ewes (106–120 days of gestation) at high and low altitudes

| Group | $P_aO_2$ (mmHg) | $P_aCO_2$ (mmHg) | Ht (%) | Hb (mg/dL) | SatHb (%) | pH |
|---|---|---|---|---|---|---|
| LL | 99.0 ± 4.6 a | 37.0 ± 3.0 a | 29.4 ± 1.4 a | 10.9 ± 0.5 a | 98.0 ± 3.8 a | 7.492 ± 0.003 |
| LH | 54.6 ± 5.0 b | 28.7 ± 2.9 b | 35.0 ± 2.6 b | 14.0 ± 0.9 b | 83.3 ± 8.2 b | 7.507 ± 0.025 |
| HH | 62.2 ± 7.1 c | 25.7 ± 2.9 b | 30.5 ± 1.8 a | 11.8 ± 0.5 c | 84.7 ± 1.8 b | 7.490 ± 0.031 |

Distinct letters indicates significant difference ($P \leq 0.05$) among groups. LL: control group; ewes native to low altitude in which gestation took place at low altitude. LH: ewes native to low altitude in which gestation took place at high altitude. HH: ewes native to high altitude in which gestation took place at high altitude.

Table 2
Placental and newborn characteristics after delivery in ewes at high and low altitude

| Group | Newborn weight (kg) | Placental weight (g) | P/NB weight ratio | Number of cotyledons | Cotyledon diameter (cm) |
|---|---|---|---|---|---|
| LL | 4.20 ± 0.28 a | 280 ± 40 a | 0.07 ± 0.01 a | 72.50 ± 13.50 a | 1.59 ± 0.68 a |
| LH | 2.99 ± 0.53 b | 303 ± 64 a | 0.10 ± 0.01 b | 69.90 ± 13.27 ab | 2.01 ± 0.86 ab |
| HH | 3.26 ± 0.79 b | 396 ± 80 b | 0.12 ± 0.01 c | 54.62 ± 19.36 b | 2.70 ± 1.16 b |

Distinct letters indicates significant difference ($P \leq 0.05$) among groups. LL: control group; ewes native to low altitude in which gestation took place at low altitude. LH: ewes native to low altitude in which gestation took place at high altitude. HH: ewes native to high altitude in which gestation took place at high altitude.

Comparison of different placental characteristics among the three groups of ewes was done by ANOVA followed by the Tukey–Kramer test when needed. The statistical model was $y_i = \mu + G_{i=1-3} + \varepsilon_i$, where $y_i$ is the value for each placental trait, $\mu$ the general average value for the trait, $G_i$ the effect of group (LL, LH, or HH), and $\varepsilon_i$ is the error. Analyses were conducted using the Statistical Analysis System (SAS).[1] A difference was considered significant when $P \leq 0.05$. The results of each characteristic are expressed as the average ± S.D.

## 3. Results

The ewes' arterial blood gases at HA were similar to those of a hypoxemic state (Table 1). However, the effect of hypoxia was less marked in HH animals. All the parturitions occurred between 7:00 and 16:00 h, and placenta was passed in the normal period after delivery (Bazer and First, 1983).

Data for group averages for the placental/newborn weight ratio (P/NB), number of cotyledons, and cotyledon diameter are shown in Table 2. To enhance assessment of the data, the data for placental weight and newborn body weight are also presented in Table 2, although these data have been reported elsewhere (Parraguez et al., 2005). The value for P/NB was least the LL, intermediate in LH, and greatest in the HH groups ($P < 0.05$). The number of cotyledons was smaller in HH compared with LL group ($P < 0.05$), without differences between LH and LL or LH and HH groups. In contrast, the cotyledon diameter was greater in the HH compared with LL group ($P < 0.05$). No differences were observed between LH and LL or HH and LH groups. The estimated contact surface for the entire placenta is shown in Fig. 1. The HH group had the greatest value ($261.9 \pm 20.6\ cm^2$), followed by LH ($155.6 \pm 11.1\ cm^2$), and LL ($114.7 \pm 11.7\ cm^2$) group

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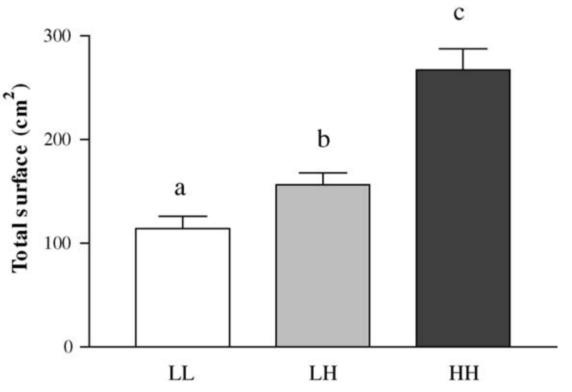


Fig. 1. Total surface area of cotyledon–caruncle contact in entire placentas from pregnancies at HA. LL: control group, ewes native to LA in which gestation took place at LA. LH: ewes native to LA in which gestation took place at HA. HH: ewes native to HA in which gestation took place at HA. Distinct letters indicate significant differences ($P \leq 0.05$) among groups.

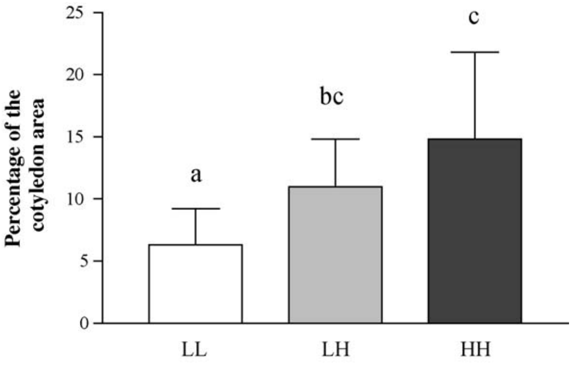


Fig. 2. Cotyledon area occupied by vascular lumina expressed as a percentage of the total cotyledon surface from pregnancies at HA. LL: control group, ewes native to LA in which gestation took place at LA. LH: ewes native to LA in which gestation took place at HA. HH: ewes native to HA in which gestation took place at HA. Distinct letters indicate significant differences ($P \leq 0.05$) among groups.

($P < 0.01$). The cotyledon area occupied by vascular lumina as a percentage of the total area surface is depicted in Fig. 2. This variable was greater in the HH (14.8 ± 7.0%) and LH (11.0 ± 3.8%) than in the LL group (6.3 ± 2.9%; $P < 0.05$). No difference was found between the HH and LH groups.

## 4. Discussion

To our knowledge, this is the first study comparing placental variables in pregnancies at HA involving ewes living for several generations at HA with those of ewes which were recently transported from LA to HA. The effects of HA hypobaric hypoxia on placental and newborn body weight, and differences between long- and short-term residence at HA, have been discussed previously (Parraguez et al., 2005). When analyzing the placenta and newborn weights together, the resulting P/NB ratios were greater at HA, being greatest in the HH group. The P/NB values were in the range for the species; however, no differences between HA and LA groups have been reported (Jacobs et al., 1988; Penninga and Longo, 1998). In humans, most of the studies find an increased P/NB ratio at HA (Zamudio, 2003). However, a lower ratio at HA has been reported in Arabian women (Zhang et al., 2002; Khalid et al., 1997). Whether this particular response to HA is associated with the ethnicity or was a coincidental result should be studied in future research.

The total number of cotyledons was less at HA. Compared with LL, the HH group had a 24.7% lesser total number of cotyledons. A result consistent with the general trend found in the present experiment was previously reported in ewes in which conception occurred at LA that were maintained at HA during gestation (Penninga and Longo, 1998). In contrast, no change in the number of cotyledons was observed in ewes under artificial hypoxia (Jacobs et al., 1988). Given that the times of exposure to natural or artificial hypoxia experienced by the ewes in the LH group in the present study and those in previous studies (Penninga and Longo, 1998; Jacobs et al., 1988) were similar, the observed differences were attributed to variations in susceptibility to hypoxia reported in different breeds of sheep (Moore et al., 2004). Moreover, specific constitutive differences are confirmed by the different numbers of cotyledons and placental weight in control animals in the three experiments considered.

Cotyledon diameter, total surface area covering the entire placenta, and cotyledon surface area occupied by vascular lumina were greater at HA. In the LA group where pregnancies were carried at HA, a trend of placentome weight increase was detected (Penninga and Longo, 1998), which could be associated with an increase in placentome diameter and cotyledon–caruncle contact surface area. However, in LA ewes subjected to artificial hypoxia from 30 to 135 days of gestation the opposite effect was observed (Jacobs et al., 1988). Krebs et al. (1997) reported an increase in the percentage of vessel lumina of the total area in ewes at LA exposed to HA between days 41–49 and 137–140 of gestation. This agrees with findings in the present study, although the magnitude of the increase was less than that observed in ewes of the LH group in the present study. Probably, a greater period of exposure to HA as occurred in the present experiment may result in a more profound effect on the placenta vasculature. Vascular density of maternal placental tissue exhibits a two-fold increase before day 30 of pregnancy, and in fetal cotyledons, the vascular density increases dramatically during the last month of gestation (Reynolds and Redmer, 2001). Studies in women support the relationship between HA and increased placental vascularization (Zamudio, 2003; Tissot van Patot et al., 2003; Zhang et al., 2002; Espinoza et al., 2001; Khalid et al., 1997). Placental angiogenesis/vasculogenesis is regulated by several growth factors (Reynolds and Redmer, 2001; Challier et al., 2001; Regnault et al., 2002). Early placentation occurs in a hypoxic environment, a condition that up-regulates VEGF gene expression, through a transcriptional process mediated by hypoxia-inducible factor-1 (HIF-1) (Anthony et al., 2001). Consistent with vascular development in the placenta, the expression of VEGF gene and its receptors also increase with advancing gestational age (Regnault et al., 2002). It is hypothesized that under a chronic hypoxemic environment the increased placental vascularization may be induced, at least partially, by a constant over-expression of this growth factor gene. No previous information was found about the effect of long-term hypoxia on placental expression of these growth factor genes in ewes. However, after 4 days under induced hypoxia, placental VEGF mRNA was significantly augmented in ewes near term (Matsumoto et al., 2002), which would support the present hypothesis.

It is noteworthy that all the observed placental characteristics at HA were accentuated more in the HH as compared with the LH group. This phenomenon may represent the difference between long- and short-term adaptation to a hypoxic environment. The longest-resident women at HA (Tibetans) have more efficient physiological attributes to prevent the effects of hypobaric hypoxia on reproductive success, when compared with women living less time at HA. This is considered as an adaptive mechanism developed over several generations at HA (Moore, 2003, 2001; Moore et al., 2004, 2001a,b; Monge and León-Velarde, 1991). Although the number of generations of a human population living at HA for ∼25,000–50,000 years is not comparable with that of the sheep herds present in the Andean highlands for ∼500 years, differences between HH and LH groups may also be attributed to an incipient long-term physiological adaptation phenomenon.

## 5. Conclusion

The results reported in the present study indicate natural HA hypobaric hypoxia impacts on the sheep placenta, both in macroscopic and microscopic characteristics. In addition, a different response to hypoxia is developed, depending on the time of residence at HA. In the present study, when pregnancy takes place at HA: (1) ewes with long-term residence at HA have increased cotyledon diameter, cotyledon–caruncle contact surface, and cotyledon area occupied by vascular lumina, but decreased total number of cotyledons and (2) ewes native to LA have increased cotyledon–caruncle contact surface and cotyledon area occupied by vascular lumina. In the latter group, however, a trend toward the other changes displayed by HA native ewes was also observed. All the placental modifications described may improve the maternal–fetal exchange, thus avoiding, at least partially, the effects of the lesser oxygen tension at HA.

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