Effects of intrauterine crowding on available uterine space per fetus in rabbits

M.J. Argente a,⁎, M.A. Santacreu b, A. Climent b, A. Blasco b

a Universidad Miguel Hernández de Elche, Departamento de Tecnología Agroalimentaria, División de Producción Animal, Carretera de Beniel Km 3.2, 03312 Orihuela, Spain
b Universidad Politécnica de Valencia, Departamento de Ciencia Animal, P.O. Box 22012, 46071 Valencia, Spain

Received 19 October 2006; received in revised form 3 May 2007; accepted 7 May 2007

Abstract

The aim of this study was to examine the effects of uterine crowding on available uterine space per fetus and fetal development at 18 days of gestation in unilaterally ovariectomized and intact does from the sixth generation of a divergent selection experiment on uterine capacity. Uterine capacity was estimated as litter size in unilaterally ovariectomized (ULO) does. Records from 37 ULO and 26 intact does were used. All does were slaughtered on d 18 of gestation. Ovulation rate per side in ULO does was almost twice as much as intact does (12.41 ova vs. 6.47 ova, \( P < 0.001 \)). ULO does showed higher intrauterine crowding at implantation than intact does (9.36 implanted embryos/uterine horn vs. 5.31 implanted embryos/uterine horn, \( P < 0.001 \)) and a lower available uterine space per live fetus (3.60 cm vs. 4.44 cm, \( P < 0.001 \)). The available uterine space per embryo decreased quadratically with the number of implanted embryos \( (b_1 = -2.46 \pm 0.18, b_2 = 0.13 \pm 0.01) \), and showed a negative linear regression coefficient with number of dead fetuses \( (−0.18 \pm 0.08) \). The available uterine space affects quadratically the development of the maternal placenta, and to a lesser extent is linearly related to the development of the fetus and its fetal placenta. The coefficients of these regressions were higher in ULO does than intact does, due to the higher degree of uterine overcrowding in these females.

Although the fetal position within the uterus did not affect the proportion of dead embryos, the uterine position could affect the survival of fetuses with a lower available uterine space. A poor blood supply had a negative effect on survival of the fetus and its development. Probability of death for fetuses with placenta receiving less than 3 blood vessels was higher than those receiving more than 3 blood vessels in both ULO and intact does (75.61% vs. 7.32%). Probability of survival asymptotically increases with available uterine space, as a result of the greater availability of uterine space which allows more blood vessels to reach each implantation site. The uterine overcrowding of ULO does was therefore associated with less uterine space and blood supply available at each implantation site, which could be related to higher fetal mortality in these females. Blood supply also affects fetal development. The implantation sites receiving less than 3 blood vessels showed lighter placentas (1.31 g vs. 1.41 g, \( P < 0.05 \)) and fetuses (2.02 g vs. 2.12 g, \( P < 0.05 \)) than those receiving more than 3 blood vessels in both ULO and intact does. These results suggest that available uterine space is a limitation component of fetal survival, which is related to an adequate vascular supply for nutrient exchange from the maternal to fetal blood streams and an adequate surface area for development of the placenta.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Blood vessels; Fetal development; Placenta; Rabbit; Uterine horn

⁎ Corresponding author. Tel.: +34 96 6749708; fax: +34 96 6749677.
E-mail address: mj.argente@umh.es (M.J. Argente).

Acknowledgments: This study was supported by CICYT (AGL2001-3068-C03-02 and AGL2005-07624-C03-03).

1871-1413/S - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.livsci.2007.05.008
1. Introduction

Litter size is determined by either ovulation rate and/or prenatal survival (Blasco et al., 1993a, in rabbits; Bennett and Leymaster, 1989, in pigs; Clutter et al., 1990, in mice). Uterine capacity is an important component in prenatal survival (see review by Ford, 1997). This trait measures the ability of the uterus to support development of embryos through gestation when ovulation rate is not a limiting factor (Christenson et al., 1987). Approximately 20% to 40% of the ova ovulated do not arrive at term of gestation in polytocous species such as the rabbit, pig and mouse (see review by Blasco et al., 1987). Development of placenta might be primarily influenced by availability of space as Vallet and Christenson (1993) reported in pigs and by vascular supply in the uterus as Mocé et al. (2004) found in rabbits. The number of blood vessels arriving at each implantation site has been used to estimate the vascular supply to each fetus in mice (Wirth-Dzieciolowska, 1987) and in rabbits (Argente et al., 2003). The aim of this study was to examine the effects of uterine crowding on available uterine space per fetus and fetal development to 18 days of gestation in unilaterally ovariectomized and intact does from a divergent selection experiment on uterine capacity.

2. Material and methods

2.1. Animals

Records from 37 unilaterally ovariectomized (ULO) does and 26 intact does were used in this experiment. These females came from the sixth generation of a divergent selection experiment on uterine capacity. Details on this experiment can be found in Argente et al. (1997). Uterine capacity was estimated as litter size in ULO does (Blasco et al., 1994). All does were first mated at 18 weeks of age and thereafter 10 days after parturition. They were slaughtered at 18 days of their fifth or sixth gestation. The ovariates and uterine tract were collected. The ovaries were weighed, and the number of corpora lutea was recorded. An external examination of each uterine horn was made to locate the implantation site of each fetus. The number of blood vessels arriving at the implantation sites was counted, as in Argente et al. (2003). The weight and the length of each gravid uterine horn were recorded. The functional uterine horn of ULO does and both uterine horns of intact does were then opened lengthwise. The position and the status of each fetus were recorded starting at the oviduct. Three uterine positions were considered: oviduct (the first fetus nearest the ovarian end), middle (fetuses in middle of the uterine horn) and cervix (the last fetus in the uterine horn from the ovarian end). Fetuses were classified according to their status as live or dead fetus; the presence in the implantation site of an atrophic fetus with maternal placenta or only atrophic maternal placenta was also considered as a dead fetus. All fetuses with their fetal placentas were removed from the uterine horn. The empty uterine horn was weighed and its length was measured. Fetuses and their fetal placentas were individually weighed, and fetal crown-rump length (CRL) was measured. The length of each maternal placenta and the distance between adjacent maternal placentas or to end of uterine horn were measured. In total, there were 290 and 235 live fetuses in ULO and intact does, and 62 and 38 dead fetuses in ULO and intact does, respectively.

2.2. Traits

2.2.1. Traits measured on each doe

Variables measured were ovulation rate (OR) estimated as the number of corpora lutea, number of implanted embryos (IE) estimated as the number of implantation sites, number of live fetuses at 18 days of gestation (LF), number of dead fetuses (ND = IE − LF), embryonic survival (ES = IE/OR), fetal survival (FS = LF/IE), prenatal survival (PS = LF/OR), ovarian weight (WO), length of each full and empty uterine horn (LFU and LEU), weight of each full and empty uterine horn (WFU and WEU), distance between the oviduct and the first maternal placenta (DOP), and distance between the last maternal placenta and the cervix (DPC).

2.2.2. Traits measured on each fetus

Variables measured were individual weight of the fetus (IWF) and its fetal placenta (IWFp), fetal crown-rump length (CRL), length of the maternal placenta (IPL), and available uterine space per fetus (ISF). ISF was measured as the length of its maternal placenta plus one-half the total distance to their two adjacent maternal placentas. For extreme fetuses, ISF was the distance from the tip of the uterine horn to the maternal placenta plus length of its maternal placenta and one-half the distance to adjacent maternal placenta (Fig. 1).
2.3. Statistical analyses

2.3.1. Differences between treatments for traits of the doe

Does traits were analysed with the following mixed model:

\[ y_{ijklmn} = \mu + T_i + L_j + (T \times L)_{ij} + OP_k + S_{ij} + m_{ijklm} + e_{ijklmn}, \]

where \( \mu \) is the general mean, \( T_i \) is the treatment effect (ULO or intact females), \( L_j \) is the line effect (High or Low uterine capacity line), \((T \times L)_{ij}\) is the interaction treatment per line, \( OP_k \) is the order of parity effect (fifth or sixth), \( S_{ij} \) is the side effect (right or left) nested within treatment, \( m_{ijklm} \) is the random effect of female and \( e_{ijklmn} \) is the random residual term. The model for uterine length and weight, and distance between oviduct and the first maternal placenta included the number of implanted embryos per uterine horn as a covariate.

2.3.2. Differences between treatments for individual traits of the fetus

Individual traits of the fetus were analysed using the following mixed model:

\[ y_{ijklmno} = \mu + T_i + L_j + (T \times L)_{ij} + OP_k + S_{ij} + S_{Um} + m_{ijklmn} + e_{ijklmno}, \]

where \( SU_{Um} \) is the status of the fetus effect (live or dead). The model for available uterine space per embryo included a linear and a quadratic regression coefficient on number of implanted embryos.

2.3.3. Relationship between ISF and ND

Average available uterine space per embryo (ISF) depends on the number of IE. To avoid the effect of IE on ISF, residuals of ISF were estimated using the following model:

\[ y_{ijklm} = \mu + T_i + L_j + (T \times L)_{ij} + OP_k + S_{ij} + b_1 IE_{ijklm} + b_2 IE_{ijklm}^2 + e_{ijklm}, \]

A regression of these residuals on the number of dead fetuses (ND) was then performed.

2.3.4. Relationships between individual traits of the fetus and ISF

The following model was used:

\[ y_{ijklmn} = \mu + T_i + L_j + (T \times L)_{ij} + OP_k + S_{ij} + b_1 x_{ijklmn} + (b_1 \times T)_{i} x_{ijklmn} + b_2 x_{ijklmn}^2 + (b_2 \times T)_{i} x_{ijklmn}^2 + e_{ijklmn}, \]

where \( x_{ijklmn} \) is the covariate, \( b_1 \) is the overall linear regression coefficient, and \((b_1 \times T)_{i}\) is the interaction between the linear regression coefficient and the treatment. If the interaction \((b_1 \times T)_{i}\) is significantly different from zero, the linear regression coefficients of ULO and intact does are different. \( b_2 \) is the overall quadratic regression coefficient, and \((b_2 \times T)_{i}\) is the interaction between the quadratic regression coefficient and the treatment. If \( b_2 \) was not different from zero, only the linear regression was considered.

2.3.5. Blood supply, uterine position and fetal development

The distribution of the percentage of live and dead fetuses in relation to the number of blood vessels reaching each implantation site and locations of the fetuses were analysed using a contingency \( \chi^2 \) test.
Weight of the live fetus and its fetal placenta, the length of its maternal placenta, and fetal crown-rump length were also analysed with a mixed model that included the random effect of mother of the fetus and number of implanted embryos (IE) as covariate. The model to study the fixed effect of position of the fetus in the uterine horn was

\[ y_{ijklmno} = \mu + T_i + L_j + (T^*L)_{ij} + \Omega_P + S_{il} + P_m + m_{ijklmno} + b_1 I_{Eijklmno} + e_{ijklmno}, \]

in which \( P_m \) is the fixed effect of position of the fetus in the uterine horn (oviduct, middle or cervix). The model to examine the effect of the vascular supply to the implantation site was

\[ y_{ijklmno} = \mu + T_i + L_j + (T^*L)_{ij} + \Omega_P + S_{il} + V_i + m_{ijklmno} + b_1 I_{Eijklmno} + e_{ijklmno}, \]

in which \( V_i \) is the fixed effect of the number of blood vessels reaching the implantation site of the fetus (less than three, three, or more than three vessels). Available uterine space per fetus was adjusted by a quadratic regression of number of implanted embryos per uterine horn.

The MIXED procedure of SAS statistical package (SAS Inst. Inc., Cary, NC) was used for all these analyses. The probability of the survival of kits was analysed by logistic regression, using the LOGISTIC procedure of SAS. A logistic regression model was used to study the probability of kits survival in relation to their available uterine space, their position within the litter and their blood supply. The model was

\[ y_{ijklmno} = \mu + T_i + L_j + (T^*L)_{ij} + \Omega_P + S_{il} + P_m + V_i + m_{ijklmno} + b_1 I_{Eijklmno} + e_{ijklmno}, \]

in which ISF is the available uterine space per fetus.

### 3. Results and discussion

#### 3.1. Differences between ULO and intact females

Differences per side between ULO and intact females for doe traits at 18 days of gestation are shown in Table 2. Ovulation rate per side in ULO does was almost twice as much as intact does because the remaining ovary compensates for the lack of ovulation in the other one. This agrees with the results of Blasco et al. (1994) in rabbits, Clutter et al. (1990) in mice, and Père et al. (1997) in pigs. Because transuterine migration is not found in rabbits, unilateral ovariectomy and consequent ovarian hypertrophy leads to double the number of fertilized ova in the uterine horn adjacent to the functional ovary. As a result of this uterine overcrowding, the functional uterine horn of ULO does had 76% more embryos at implantation and 62% more fetuses on day 18 of gestation (when gestation is 60% complete) than each uterine horn of intact does. At birth, Blasco et al. (1994) found 54% more kits per uterine horn in ULO does than in intact does (7.8 vs. 5.05 kits). In pigs, Fenton et al. (1970), Weibel and Dziuk (1974) and Knight et al. (1977) reported double the number of embryos per uterine horn at an earlier stage of gestation, up to day 30 of pregnancy, in unilaterally hysterectomized–ovariectomized (ULO) gilts than intact gilts (10.3 vs. 5.4, embryos, respectively). Litter size per uterine horn at birth was similar in both UHO and intact gilts (4.76 vs. 4.69 young pigs, respectively) (Père et al., 1997). These results show the higher capacity of the uterus of ULO rabbit does than the uterus of UHO gilts to maintain the gestation of a larger number of fetuses until the end of gestation when it is challenged with a high number of embryos.

As a consequence of the higher density of fetuses found in the uterus of ULO does, prenatal survival was lower \((P<0.05)\) in ULO does than intact does \((0.63\text{ vs. } 0.73, \text{ Table 2})\). This difference was due to a lower embryonic and fetal survival in these females \((0.76 \text{ ULO does vs. } 0.81 \text{ intact does for embryonic survival, and } 0.84 \text{ ULO does vs. } 0.90 \text{ \((P<0.05)\) intact does for fetal survival})\). In pigs, there is no difference for embryonic survival up to day 30 of gestation between UHO and intact gilts (Fenton et al., 1970; Weibel and Dziuk, 1974; Knight et al., 1977). A higher percentage

<table>
<thead>
<tr>
<th>Item</th>
<th>(N_{ULO})</th>
<th>(N_{Intact})</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>IE</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>LF</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>ES</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>FS</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>PS</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>WO, g</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>LFU, cm</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>LEU, cm</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>LFU corrected, cm</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>LEU corrected, cm</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>WFU, g</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>WFU corrected, g</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>WEU, g</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>WEU corrected, g</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>DOP, cm</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>DOP corrected, cm</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
<tr>
<td>DPC, cm</td>
<td>37</td>
<td>37</td>
<td>(*)</td>
</tr>
</tbody>
</table>

\(N\): number of data. OR: ovulation rate. IE: number of implanted embryos. LF: number of live fetuses at 18 days of gestation. ES: embryonic survival. FS: fetal survival. PS: prenatal survival. WO: ovarian weight. LFU: length of each full uterine horn. LEU: length of each empty uterine horn. WFU: weight of each full uterine horn. WEU: weight of each empty uterine horn. DOP: distance between the oviduct and the first maternal placenta. DPC: distance between the last maternal placenta and the cervix. \# Corrected by number of implanted embryos in this uterine horn. **\(*\): \(P<0.001\), \(*\): \(P<0.05\), \(+\): \(P<0.10\), ns=not significant.
of fetal death after day 30 of gestation in UHO gilts is responsible for similar litter size per uterine horn at birth in both groups. These results suggest that uterine capacity begins to affect litter size before implantation in rabbits (Mocé et al., 2004), nevertheless, the effects of uterine capacity are almost insignificant prior to implantation in pigs (Ford et al., 2002). Embryonic survival depends on the fertilization rate, embryo quality, oviductal and uterine environment. Although there is variability among genotypes, fertilization rate is generally high, almost 95% (Adams, 1960; Bolet and Theau-Clement, 1994). This agrees with the high fertilization rate (100 to 98%) reported by Santacreu et al. (1996) in ULO and intact does of this experiment. Therefore, embryonic survival depends mainly on embryo viability and oviducal and uterine environment. Several glycoproteins and proteins of oviduct and uterine fluid, such as oviductin or uterinoglobin, have an important role in embryonic survival because they are related to sperm capacitation, fertilization, rate of cleavage, blastocyst development, and implantation of embryo (Beier, 2000; Merchán et al., 2006 in rabbits). After implantation, there is a critical moment for fetal survival in rabbits between the day 8 and 17 of gestation, when the hemochorial placenta of rabbit has finished its development and the nutrition of the fetus begins to be controlled by the placenta (Adams, 1960). A secondary peak of mortality occurs between days 17 and 24 post coitus, which takes place at the same time as the period of uterine enlargement when the tension on the spherical conceptus is at a maximum and blood flow through the maternal vessels of the uterus decreases (Hafez and Tsutsumi, 1966). Therefore, in this period the placenta would require an adequate surface area for its development and an adequate vascular supply for nutrient exchange from the maternal to fetal blood streams. It has been reported that 52% of the fetal losses of ULO does in this population (3.2 fetuses) takes place between implantation and day 18 of gestation (Blasco et al., 2005; Mocé et al., 2005). In previous studies on this population, Blasco et al. (1994) and Argente et al. (2003) reported that 29% of the fetal losses in ULO does take place from day 19 to 25 of gestation and 19% of total fetal losses happen from day 26 to 30 of gestation. There is a similar pattern in fetal losses from implantation to birth for intact does (Blasco et al., 1994; Argente et al., 2006). Adams (1960) found two peaks of mortality after implantation in intact does, 66% and 27% of the total fetal losses occur between implantation and day 17 of gestation and between day 18 and 24 of gestation, respectively, in agreement with our results.

The weight of the ovaries of ULO does was 0.47% greater \( (*P<0.001) \) than of intact does, as a result of hypertrophy (Table 2). Full and empty uterine horns were 34% and 41% longer and 61% and 51% heavier \( (P<0.001) \) in ULO does than intact does due to larger growth of the conceptus, as was indicated by the ISF in the same horn. The ISF in the ULO horn was 13% greater \( (P<0.001) \) than intact does, and so was the length \( (13\%, P<0.001) \) and weight \( (14\%, P<0.001) \) of the live fetus. The height of the uterine horns in intact does was 38% longer \( (P<0.001) \) and 51% heavier \( (P<0.001) \) than in the ULO does, as was the ISF \( (25\%, P<0.001) \) and the length \( (20\%, P<0.001) \) and weight \( (23\%, P<0.001) \) of the live fetus.
numbers of implanted embryos (Table 2). These differences diminished when the number of implanted embryos per uterine horn was added to the model as a covariate. No difference was found in the weights of full uterine horns corrected for the number of embryos. Argente et al. (2003) found an increment in the length (7%) and weight (9%) of the uterus with each additional fetus on d 25 of gestation in rabbits. These observations show that the uterine horn has the capacity to continue expanding until the end of gestation to accommodate the large number of embryos entering. In pigs, several reports have indicated a relationship between the length of uterine horn and the number of embryos (Wu et al., 1987; Irgang et al., 1993). Ford et al. (2002) suggested that uterine length in pigs may be an important component of uterine capacity, which would limit litter size from an earlier stage of gestation around d 30.

Distance between the ovarian end and the first implantation site was 20% lower \( (P < 0.05) \) in the uterine horn of ULO does than in intact does, this difference disappeared when the number of implanted embryos per uterine horn was included as a covariate in the model. There was no difference between ULO and intact does for distance between the cervix and the last maternal placenta.

The greater crowding in the uterine horn of ULO does was associated with the lower available uterine space per fetus \( (P < 0.001) \) (Table 3). In spite of the smaller available uterine space per fetus found in ULO does, no differences were found for placental and fetal development on d 18 of gestation between ULO and intact does. At a later stage of gestation, Argente et al. (2003) found a reduction of placental and fetal development in rabbits with each additional implanted embryo at 25 days of gestation, due to fetal development being more limited by available uterine space at the end of gestation.

Lower availability of uterine space (ISF) was found for dead fetuses both in ULO and in intact does as compared to live fetuses (25% and 22%, respectively see Table 3). The available uterine space was lower \( (P < 0.05) \) for dead fetuses in ULO does than in intact does (22%, Table 3), as consequence of the higher degree of uterine overcrowding in these females. Higher fetal mortality in ULO does could be due to lower uterine space per fetus (Table 2). Fetal placetas of dead fetuses weighed nearly twice as much in intact does as in ULO does \( (P < 0.001) \). Because the dead fetuses of ULO does had a lower placenta weight, they may have died earlier (Table 3).

### 3.2. Relationships between traits of doe and live fetuses

The available uterine space per implanted embryo decreased quadratically in both ULO and intact does when the number of implantation sites per uterine horn increased \( (b_1 = -2.46 ± 0.18 \) and \( b_2 = 0.13 ± 0.01 \). A reduction in the available uterine space, even after adjusting for the number of implanted embryos with a quadratic regression, led to an increase in number of dead fetuses \( -0.18 ± 0.08 \). Figs. 2 and 3 show that the probability of survival of fetuses is asymptotically related to availability of uterine space, and increases to more than 90% from 4.5 cm of available uterine space onwards. This result suggests that each embryo requires a certain minimum space of uterus to attach, survive, and develop. Chen and Dziuk (1993) also found a reduction in prenatal survival with a decrease in available uterine space in pigs. Table 4 shows that available uterine space affects the

### Table 4

<table>
<thead>
<tr>
<th>Y</th>
<th>X</th>
<th>ULO</th>
<th>Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPL, cm</td>
<td>ISF, g</td>
<td>( b_1 = 0.455 ± 0.046^{***} )</td>
<td>( b_1 = 0.248 ± 0.027^{***} )</td>
</tr>
<tr>
<td></td>
<td>ISF, g</td>
<td>( b_2 = -0.034 ± 0.005^{***} )</td>
<td>( b_2 = -0.014 ± 0.002^{***} )</td>
</tr>
<tr>
<td>IWFP, g</td>
<td>ISF, g</td>
<td>( b_1 = 0.088 ± 0.015^{**} )</td>
<td>( b_1 = 0.006 ± 0.011^{ns} )</td>
</tr>
</tbody>
</table>
| IWF, g  | ISF, g  | \( b_2 = 0.023 ± 0.089^{*} \) | \( b_2 = 0.009 ± 0.006^{ns} \)

IPL: individual length of the maternal placenta; IWFP: individual weight of the fetal placenta; IWF: individual weight of the live fetus. ISF: individual available uterine space per live fetus. \(*^{***} = P < 0.001, *^{**} = P < 0.01, *^{*} = P < 0.01, \text{ns} = \text{not significant.}

Lower availability of uterine space (ISF) was found for dead fetuses both in ULO and in intact does as compared to live fetuses (25% and 22%, respectively see Table 3). The available uterine space was lower \( (P < 0.05) \) for dead fetuses in ULO does than in intact does (22%, Table 3), as consequence of the higher degree of uterine overcrowding in these females. Higher fetal mortality in ULO does could be due to lower uterine space per fetus (Table 2). Fetal placetas of dead fetuses weighed nearly twice as much in intact does as in ULO does \( (P < 0.001) \). Because the dead fetuses of ULO does had a lower placenta weight, they may have died earlier (Table 3).

### Table 5

<table>
<thead>
<tr>
<th>Status</th>
<th>Number of blood vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Less than 3</td>
</tr>
<tr>
<td>Live fetuses</td>
<td>25.66</td>
</tr>
<tr>
<td>Dead fetuses</td>
<td>75.61</td>
</tr>
<tr>
<td>( \chi^2 = 39.24 )</td>
<td>( P = 0.001 )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of blood vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULO</td>
<td>47.01</td>
</tr>
<tr>
<td>Intact</td>
<td>22.66</td>
</tr>
<tr>
<td>( \chi^2 = 24.22 )</td>
<td>( P = 0.001 )</td>
</tr>
</tbody>
</table>
development of the maternal placenta, and to a lesser extent the development of the fetus and its fetal placenta. The length of the maternal placenta (IPL) increases quadratically with the available uterine space per fetus. The linear and quadratic regression coefficients of IPL on available uterine space per fetus were higher in ULO does than intact does (Table 4), due to greater intrauterine crowding in ULO females (9.36 embryos and 3.60 cm available uterine space per implanted embryo in ULO does vs. 5.31 embryos and 4.44 cm available uterine space per implanted embryo in intact does). Knight et al. (1977) also found in pigs that intrauterine crowding of UHO gilts was associated with a lower endometrial surface area than in intact gilts, which resulted in an inhibition of placental development at an earlier stage of gestation (after d 30) and, in turn, increased fetal mortality and inhibited development of those fetuses which survived.

3.3. Blood supply, uterine position and fetal development

The results of the contingency $\chi^2$ test indicate that a poor blood supply had a negative effect on fetal survival (Table 5). Percentage of death for fetuses with placentas receiving less than three vessels was higher than for fetuses with placentas receiving more than three blood vessels in both ULO and intact does (75.61% vs. 7.32%). Argente et al. (2003) found a similar result at 25 days of gestation in ULO does. The estimated odds ratios by logistic regression for fetus survival were 2.79 for fetuses in oviduct position vs. cervical position, and 11.39 for fetuses in mid-uterine position vs. cervical position. These results suggest that there is a higher risk of mortality for fetuses in cervical position vs. oviduct and mid-uterine position. Moreover, Fig. 3 shows that position in the uterine horn affects individual survival. Fetuses with a lower available uterine space were more sensitive to this factor. Fetuses in the mid-uterine and oviduct position had a higher probability of survival if their available uterine space,

<table>
<thead>
<tr>
<th>Status</th>
<th>Position in the uterine horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live fetuses</td>
<td>Oviduct</td>
</tr>
<tr>
<td>Dead fetuses</td>
<td>Oviduct</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Status</th>
<th>Position in the uterine horn</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live fetuses</td>
<td>12.45</td>
<td>74.15</td>
<td>13.40</td>
</tr>
<tr>
<td>Dead fetuses</td>
<td>20.00</td>
<td>66.00</td>
<td>14.10</td>
</tr>
</tbody>
</table>

The implantation sites receiving less than three blood vessels showed lighter placentas and fetuses ($P<0.05$) than those receiving more than three blood vessels in both ULO and intact does (1.31 g vs. 1.41 g for fetal placental weight and 2.02 g vs. 2.12 g for fetal weight, see Table 6). Table 5 shows that ULO does have fetuses with worse blood supplies than intact does, which could be associated with a greater number of fetal losses in these females. There were no differences in the percentages of dead fetuses among the different positions in the uterine horn (Table 7). Argente et al. (2003) reported in ULO does on d 25 of gestation that fetal position within the uterus did not affect the proportion of dead embryos. However, the estimated odds ratios by logistic regression for fetus survival were 2.79 for fetuses in oviduct position vs. cervical position, and 11.39 for fetuses in mid-uterine position vs. cervical position. These results suggest that there is a higher risk of mortality for fetuses in cervical position vs. oviduct and mid-uterine position. Moreover, Fig. 3 shows that position in the uterine horn affects individual survival. Fetuses with a lower available uterine space were more sensitive to this factor. Fetuses in the mid-uterine and oviduct position had a higher probability of survival if their available uterine space...
was lower than fetuses in the cervical position. Table 8 shows that available uterine space for live fetuses in the cervical uterine position was 50% higher than in oviduct and 68% higher than in mid-uterine position ($P<0.05$). A higher availability of space seems to be linked to a better development of the maternal placenta and the fetus in both ULO and intact does. The smaller maternal placentas and lighter fetuses were located near the oviduct. This pattern seems to change in the later stages of gestation, and the heaviest fetuses were localized in the position nearest the oviduct and the lightest fetuses developed in the intermediate positions as Duncan (1969), Bruce and Abdul-Karim (1973) and Poigner et al. (2000) found. Argente et al. (2003) observed that, at 25 days of gestation in ULO does, fetuses at both ends of the uterus were heavier than fetuses in the middle. Similar results have been reported in pigs at the end of gestation (Waldorf et al., 1957; Perry and Rowell, 1969; Wise et al., 1997). Duncan (1969) and Bruce and Abdul-Karim (1973) indicate in rabbits that the fetuses located near the oviduct seem to have less competition for available uterine space and a better vascular supply at the end of gestation than at d 18 of gestation.

### 4. Conclusion

An increase in the number of implanted embryos in the uterine horn quadratically decreases the uterine space available per fetus at 18 days of gestation. A reduction of the uterine space available is linearly associated with a reduction of the fetal survival, possibly by means of a limitation in the development of the maternal placenta and a reduction in the number of blood vessels arriving at each implantation site. The greater crowding in the uterine horn of ULO does was associated with less uterine space and blood supply available at each implantation site, which is related to higher fetal mortality in these females.

The uterine position and blood supply available seems to affect fetus survival when available uterine space is low. However, the probability of survival asymptotically increases with available uterine space, as a result of the higher availability of uterine space allowing more blood vessels to reach each implantation site.

These results suggest that available uterine space is a limiting component of fetal survival, which is related to an adequate vascular supply for nutrient exchange from the maternal to fetal blood streams and an adequate surface area for development of the placenta.

### Acknowledgements

This experiment has been supported by the Spanish Research Projects AGL2001-3068-C03-02 and AGL2005-07624-C03-03.

### References


