EFFECTS OF INTRAUTERINE CROWDING ON UTERINE AVAILABLE SPACE PER FETUS IN RABBITS

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ABSTRACT

The aim of this study is to examine the effects of uterine crowding on uterine available space per fetus and fetal development at 18 days of gestation in unilaterally ovariectomized and intact does from a divergent selection experiment on uterine capacity. Uterine capacity was estimated as litter size in unilaterally ovariectomized (ULO) does. Records were available from 37 ULO and 26 intact does. All the 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 a 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 uterine available space by fetus (4.36 cm *vs.* 4.96 cm, $P < 0.01$). The uterine available space by implantation site showed a negative quadratic regression coefficient with the number of implanted embryos ($P < 0.001$), and a negative linear regression coefficient with the number of dead fetuses (-0.16 $\pm$ 0.06 in ULO does and –0.19 $\pm$ 0.10 in intact does). The uterine available space had a positive quadratic relationships with the length of maternal placenta ($P < 0.001$), and it was linearly associated to the development of fetal placenta and fetus ($P < 0.01$). These results would suggest that each embryo requires a certain minimum space of uterus to attach, survive and develop. The fetal position within the uterus did not affect the proportion of dead embryos. However, the fetuses with placentas receiving less than three blood vessels showed a higher probability of death ($P < 0.01$) than those receiving more than three blood vessels (35 % *vs.* 4 %). The implantation sites receiving less than three blood vessels had lighter placentas (1.31 g *vs.* 1.41 g, $P < 0.05$) and fetuses (2.03 g *vs.* 2.12 g, $P < 0.05$) that those receiving more than three blood vessels.

Key words: blood vessels, fetal development, placenta, intrauterine crowding, uterine horn.

INTRODUCTION

Litter size depends on ovulation rate and prenatal survival. Prenatal survival ranges from 60 to 80 % (reviewed by BLASCO *et al*., 1993). Superovulation (ADAMS, 1960 in rabbits; WEBEL and DZIUK, 1974 in pigs) or transferring a large number of fertilized ova (HAFEZ, 1966 in rabbits; DZIUK, 1968 in pigs) increase number of embryos at implantation; however, litter size at term of gestation is not increased in the same proportion because many embryos are reabsorbed from implantation to birth. VALLET *et al*. (2002) have suggested that losses resulting from intrauterine crowding are due to limitations in uterine capacity. Uterine capacity is defined as the maximum number of fetuses that the dam is able to support at birth when ovulation rate is not a limiting factor (CHRISTENSON *et al*., 1987). Therefore, uterine capacity depends on number of implanted embryos and subsequent fetal survival. Fetal survival in rabbits seems to be associated with fetal development which is related to development of placenta (ARGENTE *et al*., 2003). Development of placenta seems to be primarily influenced by availability of space as VALLET and CHRISTENSON (1993) reported in pigs and by vascular supply within the uterus as DUNCAN (1969) 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 is to examine the effects of uterine crowding on uterine available space per fetus and fetal development to d 18 of gestation in unilaterally ovariectomized and intact does from a divergent selection experiment on uterine capacity.

MATERIALS AND METHODOS

Animals

Records were available from 37 unilaterally ovariectomized (ULO) does and 26 intact does. These females came from the sixth generation of a divergent selection experiment on uterine capacity. Uterine capacity was estimated as litter size in ULO does (BLASCO *et al*., 1994). More details on this divergent selection experiment can be found in ARGENTE *et al*. (1997). All does were slaughtered on d 18 of their last gestation (fifth or sixth). The ovaries and the uterine tract were collected. The number of blood vessels arriving at implantation sites was counted to estimate the vascular supply to each fetus as proposed by WIRTH-DZIECIOLOWSKA (1987) in mice. Each uterine horn was opened lengthwise and the position (oviductal, middle or cervical) and status (live or dead) of each fetus were recorded by starting on the ovarian end.

Traits

The variables measured on does were ovulation rate estimated as the number of corpora lutea, number of implanted embryos estimated as the number of implantation sites, number of live fetuses at 18 days of gestation, embryo survival (implanted embryos/ovulation rate), fetal survival (live fetuses at 18 days of gestation/implanted embryos), prenatal survival (live fetuses at 18 days of gestation/ovulation rate), length

and weight of the each full and empty uterine horn, distance between the oviduct and the first maternal placenta, and distance between the last maternal placenta and the cervix. The traits measured on fetus were length of maternal placenta, weight of fetal placenta and fetus, and available uterine space for each fetus (measured as figure 1 shows).

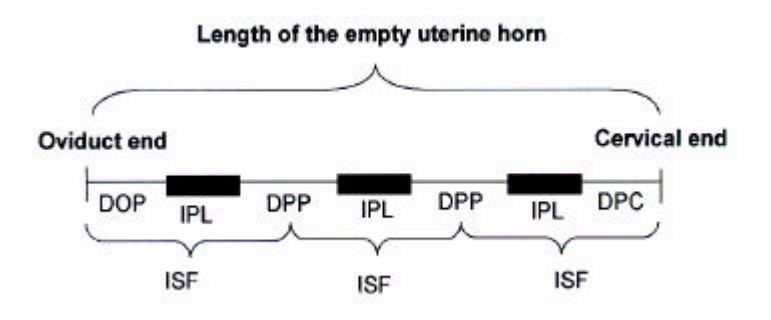

Figure 1. Measurement of individual uterine available space by fetus (ISF). DOP: distance between oviduct end and maternal placenta. IPL: length of maternal placenta. DPP: distance between two adjacent maternal placentas. DPC: distance between maternal placenta and cervical end.

Statistical Analyses

The doe traits were analyzed using the following model:

$$y_{ijklm} = \mu + T_i + L_{j(i)} + OP_k + SU_{l(i)} + m_{ijklm} + e_{ijklmn}$$

Where μ is the general mean, T_i the treatment effect (intact or ULO females), $L_{j(i)}$ the selection effect (High or Low uterine capacity line) nested within treatment, OP_k the order of parity effect (fifth or sixth), $SU_{l(i)}$ the side effect (right or left) nested within treatment, m_{jklm} the random effect of female, and e_{ijklm} the random residual term. For uterine length and weight and distance between the oviduct and the first maternal placenta, the number of implanted embryos was added to the model as a covariate. The individual traits of live fetus were analyzed with a mixed model that included the uterine position effect (P_m: oviductal, middle or cervical). For uterine available space by fetus, the number of implanted embryos was included in the model as a covariate. In order to examine the effect of vascular supply, the fixed effect of the number of blood vessels reaching the implantation site of each fetus (less than three vessels, three vessels, or more than three vessels) was added to the model. In order to asses the relationships between two fetal traits, the followed model was used:

$$y_{ijklmn} = \mu + T_i + L_{j(i)} + OP_k + SU_l + P_m + m_{ijklmn} + b\, x_{ijklmnp} + (b*T)_i\, x_{ijklmnp} + e_{ijklmnnp}$$

Where $x_{ijklmnp}$ was the covariate, b was the overall regression coefficient, and $(b*T)_i$ was the interaction between the regression coefficient and the treatment. A quadratic term was included in the former model to study the quadratic relationships between traits. The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) was used for these analyses.

RESULTS AND DISCUSSION

Differences per side between ULO and intact females for doe traits are shown in Table 1. 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 the other one. This agrees with the results of BLASCO *et al*. (1994) in rabbits, CLUTTER *et al*. (1990) in mice, and CHRISTENSON *et al*. (1987) in pigs. The number of implantation sites per uterine horn was a 75% higher in ULO does than intact does. ULO does showed a lower prenatal survival than intact does (0.63 vs. 0.73), due to a lower embryo and fetal survival. Embryo survival is related to the embryo quality and the amount and composition of uterine secretions (reviewed by POPE, 1992). After implantation, there is a key 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 for fetus begins to be control by the placenta. The placenta would require an adequate surface area for its development and for nutrient exchange from the maternal to fetal streams. ADAMS (1960) found that a 66 % of the total fetal losses occur in this period.

Full and empty uterine horn in ULO does were 45% and 40% longer and 38% and 51% heavier than in intact does, due to a larger number of implanted embryos (Table 1). ARGENTE *et al*. (2003) observed an increment for uterus in the length (7%) and weight (9%) with each additional fetus in rabbit at d 25 of gestation. Distance between ovarian end and first implantation site was 20% lower in the uterine horn of ULO does.

Table 1. Least square means per side for doe traits in ULO and intact females.

Item.	ULO	Intact	Sig
Ovulation rate	12.41 + 0.39	6.47 + 0.30	***
Number of implanted embryos	9.36 ± 0.39	5.31 ± 0.30	***
Number of live fetuses	7.68 ± 0.34	4.73 ± 0.27	***
Embryo survival	0.76 ± 0.03	0.81 ± 0.04	ns
Fetal survival	0.84 ± 0.02	0.90 ± 0.02	*
Prenatal survival	0.63 ± 0.03	0.73 ± 0.03	*
Length of the each full uterine horn, cm	23.49 ± 0.68	17.58 ± 0.54	***
Length of the each full uterine horn[A], cm	21.06 ± 0.60	19.52 ± 0.58	†
Length of the each empty uterine horn, cm	31.42 ± 0.93	22.22 ± 0.79	***
Length of the each empty uterine horn[A], cm	28.05 ± 0.78	24.99 ± 0.79	*
Weight of the each full uterine horn, g	93.01 ± 3.86	57.83 ± 3.15	***
Weight of the each full uterine horn[A], g	77.35 ± 2.40	72.07 ± 2.01	ns
Weight of the each empty uterine horn, g	38.21 ± 1.33	25.38 ± 1.04	***
Weight of the each empty uterine horn[A], g	32.52 ± 0.99	29.99 ± 0.93	†
Distance between ovary and first maternal placenta, cm	1.42 ± 0.15	1.77 ± 0.13	*
Distance between ovary and first maternal placenta[A], cm	1.75 ± 0.18	1.51 ± 0.14	ns
Distance between cervix and last maternal placenta, cm	2.94 ± 0.22	2.95 ± 0.18	ns

[A]Analysis fitting number of implanted embryos as a covariate. ***= P < 0.001, *P* < 0.05, † = *P* < 0.10, ns = non-significant.

The higher crowding in uterine horn of ULO does was associated with a lower uterine available space per fetus (12%, see Table 2). There were no differences between ULO

and intact does on the development of placentas and fetus on d 18 of gestation. However, ARGENTE *et al*. (2003) found a reduction of placental and fetal development in rabbit with each additional implanted embryo at 25 days of gestation, due to fetal development would be more limited by uterine available space at the end of gestation.

Table 2. Least square means for individual weight of the live fetus (IWF), individual weight of fetal placenta (IWFP), individual length of maternal placenta (IPL), and individual uterine available space by fetus (ISF) in ULO and intact does.

Item.	N[A]	ULO	N[A]	Intact	Sig
IWF, g	290	2.17 ± 0.09	235	2.00 ± 0.10	ns
IWFP, g	290	1.41 ± 0.04	235	1.36 ± 0.04	ns
IPL, cm	290	1.75 ± 0.04	235	1.80 ± 0.04	ns
ISF, cm	290	4.36 ± 0.16	235	4.96 ± 0.16	**
ISF[B], cm	290	4.59 ± 0.14	235	4.15 ± 0.16	†

[A] Number of observations for fetuses. [B] Analysis fitting number of implanted embryos with a quadratic regression. ** = P< 0.01, † = P < 0.10, ns = non-significant.

The uterine available space by implanted embryo decreased quadratically when the number of implantation sites per uterine horn increased (b_1 ± SE = -2.46 ± 0.18 and b_2 ± SE = 0.13 ± 0.01). A reduction in the uterine available space, even after adjusting by number of implanted embryos with a quadratic regression, was associated with an increase in number of dead fetus (-0.16 ± 0.06 in ULO does and –0.19 ± 0.10 in intact does). This result suggests that each embryo requires a certain minimum space of uterus to attach, survive, and develop. Besides, CHEN and DZIUK (1993) found an increase on fetal mortalty with a decrease on uterine available space in pigs. Table 3 shows as the uterine available space seems to affect especially the development of maternal placenta, and to a lesser extent the development of fetus and its fetal placenta. These relationships seem to be higher in ULO does, due to a larger intrauterine crowding in these females (9.36 embryos and 4.36 cm on uterine available space in ULO does vs. 5.31 embryos and 4.96 cm on uterine available space in intact does).

Table 3. Linear (b_1) and quadratic (b_2) regression coefficients with their standard error (SE).

		ULO		Intact	
Y	X	b_1 ± SE	b_2 ± SE	b_1 ± SE	b_2 ± SE
IPL, cm	ISF, g	0.49 ± 0.04***	-0.033 ± 0.003***	0.34 ± 0.03***	-0.019 ± 0.002***
IWFP, g	ISF, g	0.14 ± 0.02***		0.04 ± 0.01***	
IWF, g	ISF, g	0.04 ± 0.01***		0.02 ± 0.01**	

IPL:individual length of maternal placenta; IWFP:individual weight of fetal placenta; IWF:individual weight of live fetus. ISF:individual uterine available space by live fetus. ***= P<0.001, **= P< 0.01.

The results of contingency chi-square test indicate that the fetal position within uterine horn did not affect the proportion of dead fetuses. However, the fetuses with placenta receiving less than 3 blood vessels showed a higher probability of death (P<0.01) than those receiving more than 3 blood vessels (35% vs. 4%). The implantation sites receiving less than 3 blood vessels showed lighter placentas (1.31 g vs. 1.41 g, P<0.05) and fetuses (2.03 g vs. 2.12 g, P<0.05) that those receiving more than 3 blood vessels.

CONCLUSION

An increase in the number of implanted embryos by uterine horn decreases the uterine available space by fetus. The relationship between both traits is quadratic at 18 days of gestation. A reduction of the uterine available space seems to be linearly associated with a reduction of the fetal survival, possibly by means of a limitation in the development of the maternal placenta.

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