INFLUENCE OF PARITY ORDER ON AVAILABLE UTERINE SPACE PER FETUS, PLACENTAL AND FETAL DEVELOPMENT IN RABBITS

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ABSTRACT

Our objective was to study, in the rabbit of local population, the effect of parity, on fetal and placental development and available uterine space per fetus. Twenty rabbit does (10 nulliparous and 10 primiparous) were mated and sacrificed at 24th of pregnancy. The uterine horns were examined to estimate the vascularization of implantation sites, the intra-uterine position (oviductal, middle and cervical), available uterine space. The fetuses in oviduct position presented maternal placenta weight significantly different from those in middle (+12%) and cervical position (+8.6%). The vascularization influenced the weight, the crown-rump length and available uterine space. The fetuses receiving ≥5 blood vessels presented the best performances ($p<0.05$). The number of fetus per horn affects significantly the weight and available uterine space. At 24th of pregnancy, the vascularization influenced the fetal development and available uterine space but; the position influenced only the weight of maternal placenta.

Key words: Rabbits, parity order, placenta, fetus, available uterine space.

INTRODUCTION

The weight of rabbit kits at birth is influenced by several factors during the pregnancy period (litter size, kindling number, physiological status, nutrition, intra uterine position and vascularization of the fetuses). Indeed, a decrease in litter size is accompanied by an increase in average individual birth weight (Garcia-Ximenez and Vicente, 1993; Bolet *et al.,* 1996) and the progeny of nulliparous does is substantially lower than that of the offspring of multiparous does (Szendro *et al.,* 1998). Otherwise, the production of milk in the nursing does reduce the weight of the fetuses (-20%) (Fortun *et al.,* 1993).The weight of fetuses is related also to their position in the uterine horn. Indeed, the heaviest fetuses are localized in the position nearest the oviduct and the lightest fetuses developed in the intermediate positions (Poigner *et al.,* 2000). Finally, uterine vascular supply reaching each implantation site seems to have an important effect on development of fetuses and their placentae, as well as on fetal survival (Mocé *et al.,* 2004; Argente *et al.,* 2003). Poor blood supply is associated with lower fetal and placentae weight and higher probability of death (Argente *et al.,* 2003). These factors were studied generally for the same parity. The aim of that experiment was to study on nulliparous and primiparous rabbit does the available uterine space per fetus, the placental and fetal development in the local Algerian rabbits.

MATERIALS AND METHODS

Animals and experimental design

Rabbits used in this study came from an experimental farm of the High National Veterinary School (Alger's). Twenty rabbit females of local Algerian population were used in this work (10 nulliparous and 10 not-lactating primiparous). The criteria of selection were the age (4.5 months for nulliparous and 7 months for primiparous), the average live weight at mating (2576 ± 59 and 3188 ± 155 g respectively for nulliparous and primiparous does). All the primiparous nursed during their first

lactation the same number of kits (6-7), in order to avoid variability of this parameter (we used non lactating primiparous after weaning). After mating, the twenty pregnant rabbit does were slaughtered on d24, and immediately after their genital tract was removed and dissected. To locate the implantation site of each fetus, an external examination of the uterine horn was made. The number of blood vessels arriving at each implantation site was used to estimate the vascular supply to each fetus, as in Argente *et al.* (2003). Live fetuses were classified as receiving a number of blood vessels less-than or equal to 4 (≤4) or greater-than or equal to 5 (≥5). Afterward, the *mesometrium* was trimmed from both uterine horns. For each gravid uterine horn, fetuses were classified according to their status as alive or dead. In total, there were 55 and 84 alive fetuses in nulliparous and primiparous respectively. By horn, the number of dead fetuses was lower (3 fetuses in primiparous does). The position of each fetus was recorded by starting on the ovarian end. Three uterine positions were considered: oviduct (the first fetus nearest ovary), middle (fetuses in middle of uterine horn) and cervix (the last fetus in the uterine horn near cervix). For each uterine horn, fetuses and their fetal placentas were separated and individually weighted and fetal crown-rump was measured. Available uterine space per fetus was measured as in Argente *et al.* (2008): the length of maternal placenta plus half of the distance from its right and left boundaries to the nearest placenta. For embryos located at the beginning of the cervix, distance was calculated as the length of maternal placenta plus the distance from the boundary of the placenta to the beginning of the cervix, plus one-half of the distance from the other boundary to the nearest placenta. The same procedure was used for embryos located at the beginning of the oviduct.

Statistical analyses

The data were analyzed using an analysis of variance (ANOVA 3) taking into account the fixed effect of the parity (2 levels), vascular supply (2 levels) and intra uterine position (3 levels) as well as their interactions. The effect of the number of fetus per horn among parity order of the dam on different parameters measured was analyzed using analysis of variance (ANOVA 2) taking into account the fixed effect of parity (2 levels) and the number of foetus per horn (4 levels) as well as their interactions. Analyses were performed using the program Statview (Abacus Concepts 1996, Inc.., Berkeley, CA94704-1014, USA).

RESULTS AND DISCUSSION

The weight and the crown-rump of the fetus were not significantly influenced by the parity order of the dam and the intra uterine position of the fetus (Table 2). However, these traits were significantly influenced by the number of the blood vessels reaching each implantation site ($p<0.001$). Indeed, the individual weight and the crown-rump of the fetus were 24.4 and 12.6% lower respectively in the group of the fetuses with placentas receiving poor blood supply (≤4 blood vessels) compared to those receiving higher number of blood vessels (Table 1). Similar results were obtained in rabbit by Argente *et al.* (2008) at 18 d of pregnancy and Bruce and Abdul-Karim (1973) and Mocé *et al.* (2004) at 28 d. In mice, Wirth-Dzieciolowska (1987) obtains the same result in the late stages of gestation. The increase in the number of blood vessels reaching the implantation site is associated with a better nutrient supply and consequently, a better fetal development (Argente *et al.,* 2003). Table 2 shows that the intra uterine position affects significantly the individual weight of the maternal placenta ($p<0.05$). The heavier maternal placenta was located in the oviduct uterine position, weighting 12% and 8.6% higher than those in middle of uterine position and cervix position respectively. On the one hand, previous studies showed in the rabbits and in the pigs, that the fetuses in the mid-uterine position seem to have higher competition for availability of uterine space than fetuses near the oviduct, this smaller available uterine space could limit placenta development. (Bruce et Abdul-Karin, 1973; Wu *et al.,* 1987; Chen and Dziuk, 1993). On the other hand, the fetuses near the oviduct had a greater blood flow therefore better development of placentae (Duncan, 1969). At 28 days of pregnancy, Mocé *et al.* (2004) reported that the number of blood vessels received at each implantation site significantly affects the weight of fetal placenta, and the higher number of blood vessels is related to a better nutrient supply and consequently to a better development of placenta. Our data show that availability of uterine space was higher in the nulliparous compared to the primiparous does. The larger implanted

Table 1: Effect of parity, intra uterine position and vascularization on individual weight of fetus and its placentas, fetal crown-rump length and available uterine space per fetus (mean ± SD)

Parity	**Nulliparous**				**Primiparous**					
Position	O	M	C		O		M		C	
Blood vessels	≥5	≤4	≤4	≥5	≤4	≥5	≤4	≥5	≤4	≥5
Fetus weight (g)	15.5^{b} ± 1.91	12.2^{a} ± 1.07	12^{a} ± 1.08	16.1^{b} ± 1.64	12.9^{ac} ± 1.09	16.4^{b} ± 2.79	11.2^{a} ± 2.31	16.5^{b} ± 2.63	12.2^{a} ± 1.24	15.5^{bc} ± 2.43
Fetal crown-rump length (cm)	7.6^{b} ± 0.54	6.8^{c} ± 0.56	6.8^{c} ± 0.56	7.8^{ab} ± 0.63	7.3^{abc} ± 0.58	8.1^{a} ± 0.51	6.7^{c} ± 0.82	8.1^{a} ± 0.67	6.9^{c} ± 0.66	7.9^{ab} ± 0.69
Maternal placenta weight (g)	1.6a^{b} ± 0.29	1.53^{ab} ± 0.32	1.39^{cd} ± 0.18	1.74^{a} ± 0.22	1.73^{ab} ± 0.44	1.4^{cd} ± 0.32	1.41^{c} ± 0.33	1.25^{d} ± 0.22	1.42^{bc} ± 0.36	1.29^{d} ± 0.3
Weight of fetal placenta (g)	3.5a^{b} ± 0.47	3.1b^{c} ± 0.38	2.9^{cd} ± 0.43	3^{abc} ± 0.48	3.7^{ab} ± 1.01	3.8^{a} ± 0.45	2.6^{d} ± 0.8	3.6^{ab} ± 0.41	3.2^{bc} ± 0.72	3.5^{ab} ± 0.53
Uterine space(cm)	8.8^{a} ± 2.86	4.9^{cdf} ± 1.52	7.4^{b} ± 2.3	10.2^{a} ± 2.86	6.7^{bc} ± 1.44	5.4^{cd} ± 1.29	3.8^{ef} ± 1.04	4.1^{f} ± 0.75	4.6^{cdef} ± 0.63	4.4^{def} ± 0.63

a, b, c .Means within a row with different superscript differ (P<0.05). C: Cervix; M: Middle; N: Number; O: Oviduct; BV: Blood vessels

Table 2: Statistical analyses for different parameters measured (mean ± SD)

	Parity	Position	Vascularization	Parity*Position	Parity*Vascularization
Individual weight of fetus (g)	NS	NS	***	NS	NS
Fetal crown-rump length (cm)	NS	NS	***	NS	NS
Individual weight of maternal placenta (g)	NS	*	NS	NS	NS
Weight of fetal placenta (g)	NS	NS	NS	NS	*
Available uterine space per fetus (cm)	***	**	*	*	*

*p<0.05; **p<0.01; ***p<0.001; NS: non significant (p>0.05).

Table 3: Effect of crowding of the uterine horn, on individual number of blood vessels, weight of fetus, fetal crown-rump length and available uterine space per fetus (mean ± SD).

	Number of fetuses per horn								Statistical effects		
Fetus/horn	3		4		5		6		Parity	Fetus/horn	Parity*fetus/horn
Parity	N	P	N	P	N	P	N	P			
Number blood vessels	5.53^{ab} ± 1.95	4.9^{bcd} ± 1.64	3.81^{d} ± 1.51	5.0^{bcd} ± 1.71	3.80^{d} ± 1.31	5.32^{ab} ± 1.62	3.4^{a} ± 1.64	4.27^{cd} ± 1.87	NS	NS	***
Fetus weight (g)	15.04^{ab} ± 2.40	14.76^{ab} ± 3.33	12.7^{c} ± 1.34	12.94^{c} ± 1.98	12.1^{c} ± 1.51	16.01^{a} ± 2.87	11.4a^{b} ± 1.81	13.9^{bc} ± 4.15	NS	*	**
Fetal crown-rump length (cm)	7.41^{ab} ± 0.67	7.61^{ab} ± 0.83	7.23^{a} ± 0.58	7.35^{ab} ± 0.63	7.11^{b} ± 0.63	7.81^{a} ± 0.65	7.49^{ab} ± 0.69	7.63^{ab} ± 1.37	*	NS	NS
Uterine space/fetus (cm)	8.52^{a} ± 3.37	5.44^{bc} ± 1.12	5.74^{b} ± 2.42	4.32^{ce} ± 2.18	6.20^{b} ± 0.95	4.03^{e} ± 0.52	5.40^{d} ± 2.14	4.38^{ce} ± 1.29	***	***	***

N: Nulliparous; P: Primiparous, Means within a row with different superscript differ (P<0.05).

embryos observed in primiparous does (8.4 *vs* 5.5; $p<0.05$) seems to be responsible of this reduction in availability of uterine space. Similarly, Argente *et al.* (2008) have shown that available uterine space per implanted embryo, decreased when the number of implantation sites per uterine horn increased. Present data show that the fetuses near the oviduct had higher available uterine space than those in middle of uterine horn or near the cervix. This result is probably related to the less competition for available uterine space and a better vascular supply (Duncan, 1969; Bruce et Abdul-Karim, 1973; Argente *et al.,* 2003). Argente *et al.* (2008) reported that a higher availability of uterine space would allow more blood vessels to reach each implantation site. The number of blood vessels was not significantly influenced by either parity or the number of fetus per horn, but the interaction between those two factors was significant ($p<0.001$, Table 4). In primiparous, the horns with 5 or 6 fetuses present a higher number of blood vessels than those with the same number in nulliparous (+25%). The fetal weight decreased when the number of fetus per horn increased ($p<0.05$). The fetal crown-rump was affected by the parity order of the dam and when the horn contains 5 fetuses, the fetal crown-rump was reduced in nulliparous compared to primiparous does (- 9%). Available uterine space per foetus was higher in nulliparous than primiparous and increased when the number of implanted embryos per horn decreased. This result is probably related to the decrease of implanted embryos in primiparous does. Indeed, Argente *et al*. (2008) found that the crowding of the uterine horn was associated with the lower available uterine space per fetus.

CONCLUSION

The results of this study suggest that at 24th day of pregnancy, available uterine space is related to the parity of the dam, to the vascularization of the implantation site and to the intra uterine position. The weight of maternal placenta was influenced only by the intra uterine position of fetus.

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