FERTILIZATION RATE AND EARLY EMBRYO DEVELOPMENT IN TWO RABBIT LINES SELECTED ON UTERINE EFFICIENCY

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Abstract - A number of 128 unilaterally ovariectomized females ULO [all of them belonging to two lines of a divergent selection experiment on uterine efficiency (EU+ and EU-)] and a number of 30 intact females were used in the experiment. The criteria of selection for ULO females was litter size. Fifteen does (ULO and intact females) were mated with vasectomized males. All does were slaughtered 30 h. post-coitus, the oviduct was flushed and the total number of embryos (EMB) and recovered ova (Ov) were assessed. The ovulation rate (OR) was estimated by accounting the number of haemorrhaghic corpora on the ovary. The recovery rate (RR=(Ov+EMB)/OR) and the fertilization rate (FR=EMB/(Ov+EMB) were calculated. RR (88%) was similar to the recovery rate of ova from the does mated with vasectomized males (92%). FR was close to 100%, in both intact and ULO does. A similar result was also obtained from the two selected lines on uterine efficiency, EU+ and EU-. No differences were found between EU+ and EU- in the average number of embryo cells per litter or in the standard deviation of the number of embryo cells within litter.

INTRODUCTION

Uterine efficiency has been defined as the maximum number of embryos that a female can gestate. The technique employed in most experiments to estimate uterine efficiency is the unilateral ovariectomy (ULO) for rabbits and mice, and the unilateral ovarian-histerectomy for pigs. The remaining ovary nearly duplicates its ovulation rate (BLASCO *et al.*, 1994 and BOLET *et al.*, 1994 for rabbits; LAMBERSON *et al.*, 1989, for mice; and CHRISTENSON *et al.*, 1987, for pigs). Given that there is no embryo transmigration in rabbits, it is possible to establish a large number of embryos in half the uterine space. Thus litter size in ULO females estimates uterine efficiency. In the studies previously cited, the ULO females showed a smaller litter size that intact does did. This was apparently due to differences in number of implanted embryos (BLASCO *et al.*, 1994; LAMBERSON *et al.*, 1989). In a divergent selection experiment for litter size in ULO does, SANTACREU *et al.* (1994) showed that the differences in litter size obtained were due to differences in number of implanted embryos.

Embryo losses before implantation might be due to characteristics inherent to the uterine efficiency --i.e., quality and quantity of uterine secretions (ULBERG and RAMPANCEK, 1974). They can also be due to other causes such as a low fecundity rate resulting from immature ova, which either presented some chromosomic anomaly or aged during a very long period of ovulation (WILMUT *et al.*, 1986). The objective of this paper is to study fertilization rate and embryo development in intact does and in ULO does selected in two divergent lines for litter size.

MATERIALS AND METHODS

Animals

A total of 158 females from three lines were used in the experiment. Two of these lines (EU+ and EU-) are currently selected for uterine efficiency in a divergent selection experiment described by BOLET *et al.* (1994), and the third line (V) is a synthetic line selected for litter size at weaning. Seventy-nine does were ULO from the high line of on uterine efficiency (EU+), and forty-nine were ULO from the low line (EU-). The remaining thirty females were intact does from the three lines. Uterine efficiency was estimated in lines EU+ and EU- as litter size of ULO females. Unilateral ovariectomy was performed at 14 to 16 weeks of age. Eight ULO females and seven intact females from the EU+ line were mated with vasectomized males in order to estimate the recovery rate of the ova.

Traits

All the females were slaughtered 30 hours post-coitus. Oviduct was flushed with 5ml. of phosphate buffer (PBS). All ova (Ov) and embryos (EMB) were counted and observed under a microscope. The number of cells of the

embryos was estimated by observing them with a phase contrast microscope. Corpora lutea on each ovary were counted to estimate the ovulation rate (OR). Besides, all the haemorrhagic follicles on the ovary were counted. The recovery rate RR=100x(EMB+Ov)/OR, and the fertilization rate FR=100xEMB/(EMB+Ov) were calculated. The average number of embryo cells per litter (M) as well as the standard deviation of the number of embryo cell per litter (SD) were also calculated. Data from females mated with infertile males were used only for the RR analysis.

Statistics Analysis

Least square means were calculated on a model with effects of parity (7 levels), generation (4 levels), type of doe (2 levels: intact and ULO does) and line nested to type of doe (5 levels: ULO EU+, ULO EU-, intact EU+, intact EU-, and intact does of line V). A second analysis was performed including the effect of the presence of haemorrhagic follicles (3 levels: no haemorrhagic follicles, less than 5, and more than 4 haemorrhagic follicles) was introduced. Doe weight was included as a covariate in the analysis of OR GLM procedure of the SAS statistics package (1989) was used.

RESULTS AND DISCUSSION

The OR of ULO females is slightly lower than that of intact females (tables 1 and 3). These results agree with the OR data obtained from animals of the same lines (ARGENTE *et al.*, 1996). Other authors have also found a complete ovarian compensation (LAMBERSON *et al.*, 1989, in mice; CHRISTENSON *et al.*, 1987, in pigs; BOLET *et al.*, 1994, in rabbits) or an almost complete one (GION *et al.*, 1990, in mice). Nevertheless OR duplication of the remaining ovary is not necessary as far as overcrowding has been achieved.

Table 1. Least square means and standard deviation (between brackets) in intact and ULO females of recovery rate (RR), ovulation rate (OR), recovered embryos (EMB) and fertilization rate (FR).

	ULO		INTACT	
RR(%)	88	(9.8)	88.8	(7.1)
OR	12.7	(2.2)	14.5	(2.4)
EMB	10.9	(3.1)	12.0	(3.0)
FR(%)	99.4	(21)	97.9	(20.9)

The recovery rate is high (88%) and similar to that obtained from other experiments –TORRES *et al.*, 1987, BOLET and THEAU-CLEMENT, 1994– (table 1). A small difference between EU+ and EU- ($P<0.10$) was found for RR (table 2), which disappeared when the haemorrhagic follicles factor was included in the model. No differences were detected for RR between intact and ULO females (table 2).

The fertilization rate is very high, nearly 100%, similar to the FR of Adams (1960) and higher than the values given by BOLET and THEAU-Clement (1994) (85% and 95%). FR can be estimated either as EMB/(Ov+EMB) or as EMB/OR, depending on whether ova and embryos are lost in the same proportion or only ova are lost. The ova recovery rate in a group of females mated with vasectomized males did not significantly differ from the recovery rate of does mated with entire males (table 2), thus the first definition was used. These results agree with those obtained by VILLALON *et al.* (1982) in rats.

Table 2. Orthogonal contrasts, standard error (S.E.) and significance levels of recovery rate (%).

	Contrast	S.E	Sig. (p-tail)
Fertile - Vasec	- 5.3	2.5	N.S.
Intact- Ulo	0.8	2.3	N.S.
(EU+) - (EU-)	4.5	2.4	+
CFH<5 - CFH>4	3.5	2.2	N.S.

N.S. non significant, +: $P<0.10$. Vasec: females mated with vasectomized males. Fertile: females mated with fertile males. Intact: intact females. ULO: unilaterally ovariectomized females. EU+: ULO high line. EU-: ULO low line. CFH<5: ovary with less than 5 haemorrhagic follicles. CFH>4: ovary with more than 4 haemorrhagic follicles.

No differences were found for FR between ULO and intact does. After unilateral ovariectomy, the compensatory effect of the functional ovary is due to an accelerated growth of pre-ovulatory follicles (MARIANA and DERVIN, 1992). This does not seem to produce the effect of increasing the number of immature ova, as LAMBERSON *et al.* (1989) suggested for mice. As chromosomic abnormalities seem to be rare in rabbit (less than a 5%, FECHNEIMER and BEATTY, 1974), the number of implanted embryos in ULO females should be a result of the competition between embryos for uterine factors before implantation, and could be a way to estimate the preimplantation uterine efficiency. FR also seems to be the same for EU+ and EU-, therefore differences in uterine efficiency could explain the

Table 3. Orthogonal contrasts, standard errors and significance levels of ovulation rate (OR), recovered embryos (EMB), fertilization rate (FR), average number of embryo cells per litter (M) and standard deviation of the number of embryo cell per litter (SD)

	Intact - ULO	S.E.	Sig.	EU+ EU-	S.E.	Sig.
OR	1.83	0.67	0.01	0.47	0.56	N.S
EMB	1.04	0.77	N.S	0.47	0.61	N.S.
FR	-1.43	1.89	N.S.	-3.63	1.49	*
M	0.21	0.20	N.S.	-0.02	0.11	N.S.
SD	-0.14	0.21	N.S.	-0.16	0.11	N.S.

N.S.: non significant. *:P<0.05. Intact: intact females.ULO: unilaterally ovariectomized females. EU+: ULO high line. EU-: ULO low line.

differences of number of implanted embryos between EU+ and EU- found by SANTACREU *et al.* (1994).

For both selected lines (EU+ and EU-) and for the intact females group, the average number of cells at 30 hours post-coitus is around four, which agrees with what other authors have previously found (see BRACKETT *et al.*, 1972, for a review). Pope (1988) suggested that a higher uniformity in embryo development can be an important factor for embryo survival. However, no differences in SD between EU+ and EU- were found in our experiment, which stress the importance of the doe uterus environment in the preimplantation process.

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