

## EFFECT OF DIVERGENT SELECTION FOR UTERINE CAPACITY ON EMBRYONIC SURVIVAL AND DEVELOPMENT AT 30 H POST-MATING IN UNILATERALLY OVARIECTOMIZED RABBIT FEMALES

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**Abstract:** Uterine capacity has been proposed as an indirect way to increase litter size. The aim of this work is to study the effect of a divergent selection for uterine capacity (UC) on reproductive traits at 30 h post mating in unilaterally ovariectomized (ULO) females. A total of 62 ULO females from the high line (selected to increase UC) and 39 ULO females from the low line (selected to decrease UC) were used. Ovulation rate was estimated as the number of corpora haemorrhagica and early embryonic survival was estimated as the ratio between number of embryos and ovulation rate. No differences in ovulation rate and early embryonic survival at 30 h post mating were found between high and low lines. Selection for UC did not change the embryonic stage of development either, the majority of embryos being at 4-cell stage. Additionally, the embryos were evaluated according to morphological criteria and more than 95% of the embryos were evaluated as good or fair quality. No differences in embryonic morphological criteria between high and low lines were found either. Thus, selection for UC did not modify the early embryonic survival and development in ULO females at 30 h post mating.

**Key Words:** rabbit, divergent selection, early embryonic survival, embryo development, uterine capacity.

### INTRODUCTION

Uterine capacity (UC) has been defined by Christenson *et al.* (1987) as the maximum number of foetuses that the dam is able to support at birth when ovulation rate is not a limiting factor. In rabbits, like mice but unlike pigs, there is no embryo migration between uterine horns, so unilateral ovariectomy (ULO) is enough to assess uterine capacity (Blasco *et al.*, 2005).

Uterine capacity has been proposed as an indirect way to improve litter size. Two different experiments have been performed in rabbits; Bolet *et al.* (1994) used the number of dead foetuses between implantation and birth in ULO females, whereas Argente *et al.* (1997) used litter size in ULO females. Using the latter criterion, not only the foetal survival could be modified, as in the first criterion, but also the embryonic survival. After 10 generations of divergent selection, ULO high line (selected to increase UC) had a higher number born alive (1.01 kits) than ULO low line (selected to decrease UC), mainly due to a higher number of implanted embryos (1.06 implanted embryos), as ovulation rate and foetal survival were similar (Mocé *et al.*, 2005). Differences in number of liveborn and number of implanted embryos were approximately twice (2.35 and 1.79, respectively) the difference reported for litter size using intact females. Difference between high and low lines for litter size in intact females was also due to embryonic survival until implantation (Santacreu *et al.*, 2005); differences in embryo development appeared at least from 48 h post-mating, and in embryonic survival at least from 62 post-mating (Peiró *et al.*, 2007). These differences, in intact

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females, were higher when gestation lasted (Mocé *et al.*, 2004). However, the correlated response on early embryonic survival in ULO females is unknown.

The aim of this work was to study the effect of divergent selection for UC on ovulation rate, fertilization rate and embryonic survival and development at 30 h post-mating in ULO females.

## MATERIALS AND METHODS

All experimental procedures were approved by the Committee of Ethics and Animal Welfare of the Universitat Politècnica de València. All the animals were handled according to the principles of animal care published by Spanish Royal Decree 1201/2005 (BOE, 2005).

### *Animals and environmental conditions*

A total of 101 unilaterally ovariectomized (ULO) females from the 4<sup>th</sup> and 5<sup>th</sup> generations of a divergent selection experiment for uterine capacity (UC) were slaughtered at 30 h post-mating. Sixty-two females belonged to the line selected to increase UC (ULO high line) and 39 females belonged to the line selected to decrease UC (ULO low line). The ULO females had the right functional ovary, as an ovariectomy was performed before puberty via mid-ventral incision at 14 to 16 wk of age (see details in Blasco *et al.*, 1994).

Animals were housed at the experimental farm of the Universitat Politècnica de València in individual cages and were fed a commercial diet with a photoperiod of 16-h light: 8-h dark. The females were first mated at 18 wk of age and at 10 d after parturition thereafter.

### *Traits analysed*

Body weight at slaughter time was recorded in all females. Ovulation rate was estimated as the number of corpora haemorrhagica in the ovary and the haemorrhagic follicles were counted. In order to recover embryos and oocytes, the oviduct was flushed with 5 mL of Dulbecco's Phosphate Buffered Saline (DPBS®, Sigma) supplemented with 0.132 g/L calcium chloride, 0.2% Bovine Serum Albumin (BSA®, Sigma) and 0.2 mL of antibiotic. Ova and embryos were recovered at room temperature and counted using a microscope at 6.3× magnification. Early embryonic survival was estimated as the ratio between embryos and ovulation rate and fertilization rate was estimated as the ratio between embryos and the total recovered, i.e. embryos plus ova.

Number of cells of the embryos was estimated by observing them with a phase-contrast microscope at 40× and 100× magnification. Average number of embryo cells per female (MNC) as well as standard deviation of them (SDNC) were calculated.

Additionally, embryos were graded following the Veeck and Maloney criterion (Veeck and Maloney, 1986) as: Grade 1: embryo with blastomeres of equal size and no cytoplasmic fragments; Grade 2: embryo with blastomeres of equal size and minor cytoplasmic fragments; Grade 3: embryo with blastomeres of distinctly unequal size and none or few cytoplasmic fragments; Grade 4: embryo with blastomeres of equal or unequal size and significant cytoplasmic fragmentation; Grade 5: embryo with few blastomeres of any size and severe or complete fragmentation. Finally, embryos were clustered in embryo-grade categories following the Shulman criterion (Shulman *et al.*, 1993); good quality clustered grade 1 and 2, fair quality clustered grade 3 and poor quality clustered grade 4 and 5. Embryo classification was always carried out by the same operator.

### *Statistical analyses.*

A least squares analysis using the GLM procedures of SAS (2008) was carried out on the following model:

$$y_{ijklm} = \mu + L_i + G_j + OP_k + FH_l + e_{ijklm}$$

where  $\mu$  is the general mean,  $L_i$  is the line effect (with 2 levels: ULO high and ULO low lines),  $G_j$  is the generation effect (with 2 levels);  $OP_k$  is the gestation order (with 5 levels; from 3<sup>th</sup> to 7<sup>th</sup>),  $FH_l$  is the haemorrhagic follicles effect (with 3 levels: 0, between 1 to 4 follicles, and 5 or more) and  $e_{ijklm}$  is the residual effect.

The effect of haemorrhagic follicles was included because several authors observed a negative effect on ovulation rate, fertilization rate and survival traits (García-Ximénez and Vicente, 1992) which may be due to an unbalanced hormonal profile (Hunter, 1982).

Body weight at slaughter time was considered as a covariate to analyse OR.

Chi-square analysis was performed to determine the difference on embryo-grade scores and categories between ULO females from the high and low lines.

### RESULTS AND DISCUSSION

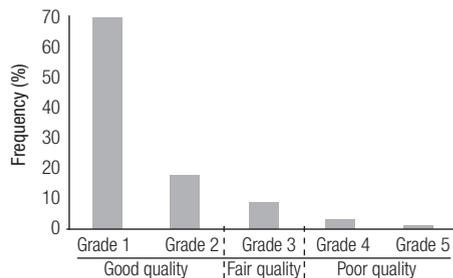
Raw mean, standard deviation and coefficient of variation for ovulation rate and fertilization rate are presented in Table 1. The mean value for ovulation rate was within the range of those obtained by other authors in multiparous females selected by reproductive traits (reviewed by Blasco *et al.*, 1993). Fertilization rate, estimated 30 h post-mating, was high (99.4%), similar to the results obtained in lines selected for reproductive traits (Torres *et al.*, 1987; García-Ximénez and Vicente, 1992; Bolet and Theau-Clément, 1994; Peiró *et al.*, 2007).

The majority of embryos catalogued in this study were in 4-cell stage (87.6%) at 30 h post-mating and the more advanced stage of development, 8-cell stage, were 5.0%; these values correspond to an average of 3.74 embryo cells per female. Moreover, the majority of embryos were catalogued as grade 1, which corresponds to good quality category (Figure 1). These results were in concordance with previous results. In intact females, the majority of embryos were in 2-cell stage and 8-16-cell stage at 25 and 48 h post-mating, respectively (Ménézo and Renard, 1991; García-Ximénez and Vicente, 1992; Peiró *et al.*, 2007).

No differences in ovulation rate were found comparing ULO high and ULO low line (Table 2), in agreement with previous results obtained in these lines in other generations using ULO females (Blasco *et al.*, 2005; Mocé *et al.*, 2005), as well as intact females (Mocé *et al.*, 2004; Santacreu *et al.*, 2005; Peiró *et al.*, 2007).

Unilaterally ovariectomized females from low line showed a higher fertilization rate than ULO females from high line ( $P < 0.05$ ) 30 h post-mating; however, this difference corresponds to around 0.2 embryos, which is considered an irrelevant difference.

Regarding early embryonic survival 30 h post-mating, ULO high line showed embryonic survival similar to ULO low line. Besides, neither different embryonic stages of development



**Figure 1:** Percentage of embryos within each of the 5 embryo-grade scores and embryo-grade categories (good, fair and poor quality) at 30 h post-mating.

**Table 1:** Number of data (N), raw mean (Mean), standard deviation (SD) and coefficient of variation (CV) for ovulation rate (OR), fertilization rate (FR, %), percentage of early embryonic survival (EES, %), percentage of 2-cell stage, percentage of 4-cell stage, percentage of 8-cell stage, number of embryo cells per female (MNC) and its standard deviation (SDNC) at 30 h post-mating.

	N	Mean	SD	CV
OR	101	12.4	2.2	0.18
FR, (%)	87	99.4	21.0	0.23
EES, (%)	85	88.0	9.0	0.11
2-cell, (%)	85	7.4	1.6	0.22
4-cell, (%)	85	87.6	15.4	0.18
8-cell, (%)	85	5.0	1.2	0.30
MNC	85	3.74	0.36	0.09
SDNC	85	0.66	0.42	0.73

**Table 2:** Difference between unilaterally ovariectomized females for the line selected to increase uterine capacity (ULO high) and to decrease uterine capacity (ULO low), standard error (SE) of the difference and significance level for ovulation rate (OR), fertilization rate (FR, %), percentage of early embryonic survival (EES, %), number of embryo cells per gestation (MNC) and its standard deviation (SDNC) at 30 h post-mating.

	ULO high-ULO low	SE	Significance level <sup>a</sup>
OR	0.45	0.54	NS
FR, (%)	-3.62	1.49	S
EES, (%)	4.52	2.36	NS
MNC	-0.02	0.11	NS
SDNC	-0.16	0.11	NS

<sup>a</sup>NS=not significant,  $P>0.05$ ; S= Significant,  $P\leq 0.05$ .

(measured as the average of embryo cells per female and the standard deviation of them; Table 2) nor embryo-grade scores and categories were found at this stage of gestation (Figure 1). A lower embryonic stage of development and uniformity are related to lower embryonic survival (Torres *et al.*, 1987). Thus, similar embryo development and uniformity found in both lines are in agreement with no difference on embryonic survival at 30 h post-mating. Previous results showed that ULO high line had a higher number of implanted embryos and embryonic survival until implantation than ULO low line (Mocé *et al.*, 2005). Our results indicated that these differences in ULO females would appear after 30 h post-mating, as similar embryonic survival and development at this stage of gestation were found. Peiró *et al.* (2007) obtained similar results at 25 h post-mating using intact females from high and low lines; difference in embryo development appeared at least from 48 h post-mating and in embryonic survival at least from 62 h post-mating. These differences were higher at 72-75 h post-mating in intact females (Mocé *et al.*, 2004).

## CONCLUSION

Selection for uterine capacity did not modify fertilization rate and early embryonic survival and development 30 h post-mating in ULO females, as in previous results found in intact females. All the results obtained until implantation in females divergently selected by UC suggest that mechanisms regulating embryonic survival and development are similar in ULO and intact females.

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