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THE EFFECT OF UNILATERAL OVARIECTOMY ON EARLY EMBRYONIC SURVIVAL AND EMBRYO DEVELOPMENT IN RABBITS

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Abstract: Unilateral ovariectomy can be used to study uterine capacity in rabbits because an overcrowding of the functional uterine horn is produced. Due to the uterus duplex, the rabbit is the ideal model for such studies. However, this technique may affect embryo survival. The aim of this work is to study the effect of unilateral ovariectomy on early embryo survival and development in rabbit. A total of 101 unilateral ovariectomised females and 52 intact females were compared after slaughter at 30 h post-mating. Early embryo survival was estimated as the ratio between number of embryo recovered and ovulation rate. No differences were found between intact and unilaterally ovariectomised females in this trait. Unilateral ovariectomy did not change embryo development, measured as the number of embryo cells. Variability of embryo development was not affected either. At 30 h post-mating, the majority of embryos (86.2%) were 4-cell stage. Embryo quality was evaluated according to morphological criteria. No difference in embryo quality between intact and unilaterally ovariectomised females was found. Therefore, unilateral ovariectomy performed before puberty in rabbit does not modify early embryo survival and development.

Key Words: early embryo survival, embryo development, fertilisation rate, ovulation rate, unilateral ovariectomy.

INTRODUCTION

Litter size in prolific species depends mainly on uterine capacity. For prolific species, uterine capacity 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; i.e., when the uterus is overcrowded. This definition implies that fertilisation rate is high and a large number of embryos are produced.

Unilateral ovariectomy has been proposed as a technique to overcrowd the uterus in species having no embryo transuterine migration (Clutter *et al.*, 1990, in mice; Blasco *et al.*, 1994, in rabbits). Female rabbits have a uterus duplex, i.e. two separated functional uteri and cervices, with a vagina simplex. Thus, uterine anatomy in the rabbit does not allow embryo transit from one uterus to the other. Unilateral ovariectomy increases ovulation rate in the remaining ovary, producing a large number of embryos in the functional uterine horn (Blasco *et al.*, 1994; Argente *et al.*, 2008). However, this technique depends on two major assumptions to measure uterine capacity properly. The first assumption is that uterine capacity measured in one uterine horn will be highly correlated to uterine capacity when both uterine horns are functional. The second assumption is that unilateral ovariectomy will not increase embryo mortality. In this paper, we examine whether the second assumption holds. Unilateral ovariectomy can increase embryo mortality if the proportion of immature oocytes increases with ovulation rate, leading to a decrease in fertilisation rate and embryo survival. Moreover, higher ovulation rate may produce higher variation in embryo development and consequently an increase in embryo mortality (Torres *et al.*, 1987a).

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The aim of this work was to test the assumption that unilateral ovariectomy does not increase embryo mortality, in order to validate unilateral ovariectomy for measuring uterine capacity in rabbits.

MATERIALS AND METHODS

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

Animals and environmental conditions

A total of 160 females were slaughtered at 30 h post-mating; 101 of these females were unilateral ovariectomised (ULO) and the rest remained intact, i.e. with both functional ovaries.

Animals were housed at the experimental farm of the Universitat Politècnica de València in individual cages and 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.

Unilateral ovariectomy technique

Unilateral ovariectomy was performed before puberty by midventral incision at 14 to 16 wk of age. Females were anaesthetised with an IM administration of xylazine (Rompun 2%; Bayer AG, Leverkusen, Germany) at a rate of 4 mg/kg body weight; 5 min later, an IV dose of ketamine HCl and chlorbutol (Imalgene 500; Merial S.A., Lyon, France) at a rate of 15 to 30 mg/kg body weight was administrated in the marginal ear vein. The abdominal wall was shaved and washed using 4% chlorhexidine gluconate (Pharmaniaga, Bangi, Malaysia), scrubbed with povidone-iodine solution and draped with the aperture of the fenestrated drape at the intended operation site. A long 8 cm midventral skin and abdominal wall incision was made. After grasping the left ovary with haemostatic forceps, a ligation was placed around the oviduct and blood vessels and the ovary was removed. The abdominal wall was closed with 00 silk suture, and the skin with 000 silk suture. After surgery, an IV dose of 1 mL penicillin and streptomycin (Penivet 1; Divasa Farmavic, Barcelona, Spain) was administrated.

Traits analysed

All females were slaughtered at 30 h post-mating and body weight at slaughter time was recorded. Ovulation rate (OR) was estimated as the number of *corpora hemorrhagica*, i.e. follicles with ovulation stigmas, in the ovaries. Additionally, all the hemorrhagic follicles were counted. To recover embryos and oocytes, the oviduct was flushed with 5 mL of Dulbecco's Phosphate Buffered Saline (®DPBS, Sigma-Aldrich Química S.A., Alcobendas, Madrid, Spain) supplemented with 0.132 g/L calcium chloride, 0.2% Bovine Serum Albumin (®BSA, Sigma-Aldrich Química S.A., Alcobendas, Madrid, Spain) and 0.2 mL of antibiotic (penicillin G sodium 300000 IU, penicillin G procaine 700000 IU and dihydrostreptomycin sulfate 1250 mg; ®Penivet 1, Divasa Farmavic, Barcelona, Spain). The ova and embryos (EMB) were recovered at room temperature and counted using a microscope at 6.3× magnification. Early embryo survival was estimated as the ratio between embryos and ovulation rate (EES%=100×EMB/OR) and fertilisation rate was estimated as the ratio between embryos and the total recovered (FR%=100×EMB/[EMB+ova]).

The number of cells of the embryos was estimated by counting with a phase-contrast microscope at 40× and 100× magnification. The average number of embryo cells per female (AEC) and their standard deviations (SDEC) were calculated. The embryos were scored by the same operator, following the criteria outlined by Veeck and Maloney (1986). The score system is as follows: Grade 1 corresponds to embryos with blastomeres of equal size and no cytoplasmic fragments and represents the best morphological condition. Grade 2 corresponds to embryos with blastomeres of equal size and minor cytoplasmic fragments or blebs. Grade 3 corresponds to embryos with blastomeres of distinctly unequal size and negligible or few cytoplasmic fragments. Grade 4 corresponds to embryos with blastomeres of equal or unequal size and significant cytoplasmic fragmentation. Grade 5 corresponds to embryos with few blastomeres of any size and severe or complete fragmentation. The embryos were grouped in three embryo-grade categories: good quality (grade 1 and 2), fair quality (grade 3) and poor quality (grade 4 and 5) following the Shulman criterion (Shulman *et al.*, 1993).

Statistical analyses

A least squares analysis using the GLM procedures of Statistical Analyse System (SAS Inst. Inc., Cary, NC) was carried out on the following model: $y_{ijklm}=\mu+S_i+OP_j+SE_k+FH_l+e_{ijklm}$ where μ is the general mean, S_i is the status of the female (with 2 levels: intact and unilaterally ovariectomised females), OP_j is the order of gestation (with 5 levels from 1st to 5th gestation), SE_k is the season effect (with 4 levels), FH_l is the hemorrhagic follicles effect (with 3 levels: zero, between one to four follicles, and five or more) and e_{ijklm} is the residual effect (with 4 levels: every 3 mo).

The effect of hemorrhagic follicles (follicles without ovulation stigmas with a presence of blood in the antral cavity) was included because several authors observed a decrease on ovulation rate, fertilisation 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 ovulation rate.

Differences among embryo-grade scores and categories between unilaterally ovariectomised and intact females were analysed using χ^2 analysis.

RESULTS

The raw mean for ovulation rate was around 14 ova and the fertilisation rate was high, near 100% (Table 1). An increase of 6 ova was achieved in the only functional ovary in unilaterally ovariectomised (ULO) females, compared to one ovary in intact females. However, OR in ULO females, with only one functional ovary, was lower than in intact females ($P<0.05$). No difference between ULO and intact females was found for FR.

Early embryo survival at 30 h post-mating was high, around 90%. Rabbit embryos of 2 to 8-cell stage were recovered at this time of gestation, with the majority of embryos in 4-cell stage (86.2%; data not shown). No differences between ULO and intact females were found for early embryo survival, the average number of embryo cells per female and the standard deviation.

Early embryo quality was evaluated on the basis of blastomere shape and size and blastomere fragmentation. Percentages of embryos within each of the five embryo-grade scores and three embryo-grade categories are shown in Figures 1 and 2, respectively. Morphological evaluation of embryos showed that approximately 70% of them were grade 1 and approximately 20% were grade 2; both grades outline the good quality category. Only a small proportion, less than 5%, of rabbit embryos were catalogued as grade 4 and 5, which corresponds to the poor quality category. No differences in the percentage of each embryo-grade scores or categories between embryos from ULO and intact females were found. Therefore, early embryo quality does not seem to be affected by unilateral ovariectomy, as occurred for most of the other traits analysed in the present study.

DISCUSSION

Ovulation rate obtained in the present study was within the range of those obtained by other authors in females selected by reproductive traits (Blasco *et al.*, 1993). A quasi-complete ovarian compensation was obtained, since the remaining

Table 1: Number of data, raw mean (Mean), standard deviation (SD), difference between unilaterally ovariectomised (ULO) females and intact females and standard error of the difference (SED) for ovulation rate (OR), fertilisation rate (FR, %), percentage of early embryo survival (EES, %), average of embryo cells per female (AEC) and its standard deviation (SDEC) at 30 h post-mating.

	n	Mean	SD	ULO-Intact	SED	Significance level
OR	160	13.9	2.3	–1.8	0.7	<0.05
FR, %	131	98.5	21.0	1.4	1.9	NS
EES, %	134	86.9	10.4	–0.8	0.5	NS
AEC	130	3.82	0.42	–0.2	0.2	NS
SDEC	130	0.61	0.61	0.1	0.2	NS

NS: not significant, $P>0.05$.

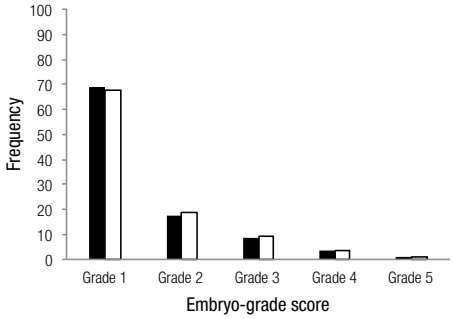

Figure 1: Percentage of embryos within each embryo-grade scores from unilaterally ovariectomised females (■) and intact females (□) at 30 h post-mating.

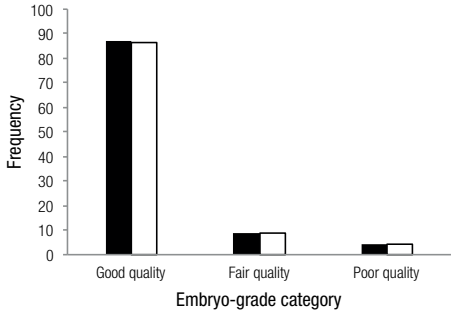

Figure 2: Percentage of embryos within each embryo-grade categories from unilaterally ovariectomised females (■) and intact females (□) at 30 h post-mating.

ovary almost doubled the number of ova, around 6 per female as average. Unilaterally ovariectomised females and intact does showed similar OR in other experiments using the same lines (Blasco *et al.*, 1994; Argente *et al.*, 2008). Little is known about the effect of increasing ovulation rate on survival rates without hormonal-induced superovulation. An increase in ovulation rate increases ovulatory timing (Fujimoto *et al.*, 1974), which could increase embryo development variability, and is related to lower embryo survival (Torres *et al.*, 1987a). Moreover, increasing OR could lead to a decrease in oocyte quality (Koenig *et al.*, 1986), and consequently in fertilisation rate and embryo survival.

Fertilisation rate was high, agreeing with results obtained by other authors in rabbits (Torres *et al.*, 1987b; García-Ximénez and Vicente, 1992; Bolet and Theau-Clement, 1994). There is no information on the effect of unilateral ovariectomy on fertilisation rate in rabbits. Previous results showed a negative effect of ovariectomy on the ability of the fallopian tube to accomplish rabbit spermatozoa capacitation (Bedford, 1970). On the other hand, a lesser amount of sperm in the reproductive tract of ULO females 10 h after insemination was found (Fateh-El-Bab *et al.*, 1983). According to our results, these phenomena are not relevant enough to affect FR in rabbits. In mice, in line with our results, a similar fertilisation rate was also found in ULO and intact females (Lamberson *et al.*, 1989).

Early embryo survival at 30 h post-mating was similar to those obtained by Torres *et al.* (1987b), García-Ximénez and Vicente (1992), Bolet and Theau-Clement (1994) and Peiró *et al.* (2007) in the first stages of gestation in maternal rabbit lines. Similar EES were found between ULO and intact females, suggesting unilateral ovariectomy did not produce an important hormonal imbalance leading to increased embryo losses, as occurs when hormone-induced superovulation is used (Tsiligianni *et al.*, 2004; Mehaisen *et al.*, 2006). Moreover, unilateral ovariectomy does not seem to change the early embryonic stage of development either. Similar results were obtained in ULO mice females (Lamberson *et al.*, 1989) and unilaterally hysterectomised-ovariectomised sows (Knight *et al.*, 1977).

Embryonic stage of development obtained at 30 h of gestation was in accordance with those obtained in maternal lines by Ménézo and Renard (1991), García-Ximénez and Vicente (1992) and Peiró *et al.* (2007). Similar embryonic stage of development between ULO and intact females was in concordance with no difference in EES. Positive relationships between embryo survival and embryo development as well as between embryo survival and uniformity have been documented in several mammals, such as rabbits, mice and pigs (Pope *et al.*, 1990; Al-Shorepy *et al.*, 1992; Duc-Goiran *et al.*, 1999; Herrler *et al.*, 2003; Fleming *et al.*, 2004).

To sum up, unilateral ovariectomised rabbit females achieved the ovulation rate without modifying early embryo survival at 30 h post-mating. Similar early embryo survival between ULO and intact females could be explained by similar fertilisation rates and embryonic features. Unilateral ovariectomy can therefore be used in rabbits to overcrowd one of the uterine horns with good quality embryos in order to assess uterine capacity.

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