UTEROGLOBIN LEVELS AT DAY 6 OF GESTATION IN TWO LINES OF RABBITS DIVERGENTLY SELECTED FOR UTERINE CAPACITY

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ABSTRACT

The aim of this work was to study the expression of uteroglobin in uterine fluid from two lines of rabbits divergently selected for uterine capacity during ten generations. In addition, the relation between embryo survival and uteroglobin expression and between progesterone levels in uterine flushings and uteroglobin expression were studied. Females from the 12th generation were used. Nineteen females from High line and fifteen females from Low line were slaughtered at day 6 of gestation. The genital tracts were removed and uterine flushings were obtained to measure uteroglobin expression and progesterone concentration. Number of recovered embryos (RE), ovulation rate (OR) and embryo survival (ES=RE/OR) were counted. The uteroglobin expression and progesterone concentration in uterine fluid was 2.44±1.29 and 15.48±5.88 ng/ml, respectively. Progesterone concentration and uteroglobin expression in uterine fluid were similar in both lines. Uteroglobin was slightly correlated with levels of progesterone in uterine fluid ($r=0.29$; $P<0.05$). However, uteroglobin expression was not correlated to the number of embryos ($r=-0.21$; $P>0.05$) or to embryo survival ($r=-0.13$; $P>0.05$). Thus, differences in embryo survival between lines do not seem to be caused by differences in uteroglobin expression at day 6 of gestation.

Key words: Uterine capacity, Uteroglobin, Divergent selection, Embryo survival.

INTRODUCTION

The effect of selection for litter size or its components on prenatal survival and ovulation rate has been studied in a large number of papers (reviewed by Blasco *et al.*, 1993). However, the number of papers dealing with the effect of selection for these traits on protein factors regulating litter size is quite small.

In rabbits, an experiment of divergent selection for uterine capacity has been conducted for 10 generations and an asymmetric correlated response on litter size was achieved. Divergence for litter size between High and Low lines was 2.35 kits and both lines differed in embryo and fetal survival (Santacreu *et al.*, 2005).

Embryo survival depends on uterine protein secretions. The pattern of uterine protein secretions is modified throughout gestation. In rabbits, Tucker *et al.* (1977) reported that uteroglobin was the main protein in the uterine secretions. Uteroglobin levels increase from day 1 to day 6 of gestation and then disappear around the 9 day of gestation (Beier, 1976). The peak of uteroglobin is detected around implantation time, thus it seems that this protein plays an important role during implantation although its physiological function still remains unclear. Progesterone is required to establish the secretion of uteroglobin during the preimplantation (reviewed by Beier, 2000).

The aim of this work is to study the expression of uteroglobin in uterine fluid from two lines of rabbits divergently selected for uterine capacity and to study the relationships of uteroglobin expression with embryo survival and progesterone levels in uterine flushings.

MATERIALS AND METHODS

Animals and experimental design

Animals came from the 12th generation of an experiment of divergent selection on uterine capacity: 19 multiparous females from the High line and 15 multiparous females from the Low line. All data were taken in the third, fourth and fifth parities. Animals were kept under controlled 16L:8D photoperiod and housed at the experimental farm of the Polytechnic University of Valencia in individual wired metal cages. Females were mated to males of the same line as the females. Does were slaughtered at day 6 of gestation (implantation takes place at day 7). The reproductive tract was removed and uteri were flushed once with 8 ml of saline solution. Number of recovered embryos per uterine horn (RE) was counted and ovulation rate (OR) was estimated as the number of corpora lutea. Embryo survival (ES) by uterine horn was estimated as number of recovered embryos divided by ovulation rate. Uterine flushings were centrifuged at 3000 r.p.m for ten minutes and the supernatant was stored at -20ºC until uteroglobin and progesterone assays were performed.

Chemical Analyses

Progesterone levels were measured in uterine flushings (UPr) by radioimmuno assay (RIA) (PROG-CTRIA, CIS bio international, Filiale de Schering S.A., B.P. 32-F91192 Gif-Sur-Yvette Cedex/France). RIA was carried out using 125I labelled progesterone. The sensitivity for the assay was 0.05 ng/ml. The intra- and inter-assay variation coefficients were 3.5% and 4.5% respectively. The cross-reactions were: 6.2% with deoxycorticosterone, 2.2% with 20-α-dihydroprogesterone, 2.1% with 6-β-dihydroprogesterone and less than 2% with other steroids.

For Western blot analysis we used uteri fluid. The protein content of all samples was determined as described by DC Protein Assay from Bio-Rad (Munich, Germany). The samples were subjected to SDS-PAGE using a 5% stacking gel and a 15% resolving gel under reducing conditions. Seventy µg protein was diluted in loading buffer (5% [v/v] mercaptoethanol, 30 mM Tris pH 6.8, 1.5 M urea, 7.5% [v/v] glycerol, 0.5% [w/v] SDS, and 0.05% [w/v] bromophenol blue) and heated at 100°C for 3 min. After electrophoresis, the gel was blotted onto a polyvinylidene difluoride membrane (S Millipore, Schwalbach, Germany) by semidry blotting for 40 min at 100 mA. Following blotting, the membrane was blocked with 5% (w/v) milk powder in PBS-Tween-20 (0.1% w/v) overnight at 4°C. The primary antibody (Anti-Urinprotin-1, DAKO, Hamburg, Germany) was diluted 1:2000 in 1% milk powder in PBS and incubated with the blot at room temperature overnight. After three washes in PBS/Tween, the second antibody was diluted 1:5000 (Goat anti-mouse HRP, DAKO, Hamburg, Germany) and added and left at room temperature for 1 h. After three washing, detection of the second antibody was performed using enhanced chemiluminescence reagents (ECL reagents (Amersham, Little Chalfont, UK) and exposure to X-ray film (X-Omat UV Plus, Kodak New York, USA) for 1 min.

Statistical Analysis

Embryo survival was transformed following Freeman and Tukey (1950) transformation for hypothesis testing, but results are reported in the original scale. Ovulation rate, number of embryos recovered, embryo survival, concentration of progesterone in uterine fluid and uteroglobin expression of each uterine horn were analyzed using a model with fixed effects of line (High and Low), uterine side (right and left), lactation status (lactating and non-lactating) and the random effect of doe. The Mixed procedure of SAS (SAS Inst., Inc., Cary, NC) was used.

RESULTS AND DISCUSSION

Raw means, standard deviation, coefficient of variation and minimum and maximum values for the traits measured are presented in Table 1. Results for ovulation rate, number of recovered embryos,

embryo survival and concentration of progesterone in uterine fluid agree with mean values published previously in the same lines (Mocé *et al.*, 2002). Uteroglobin was detected in all uterine flushings that were analyzed and its expression was highly variable (CV=0.53). This protein is predominant in rabbit uterine secretion during the phase of preimplantation (Beier, 1976).

Table 1: Raw means, standard deviation (SD), coefficient of variation (CV), minimum and maximum values and number of data (N) for ovulation rate (OR), number of recovered embryos (RE), embryo survival (ES=RE/OR), concentration of progesterone in uterine fluid (UPr) and expression of uteroglobin in uterine fluid (UG) at day 6 of gestation. Results are given per uterine horn

Trait	Mean	SD	CV	Minimum	Maximum	N
OR	7.46	2.30	0.31	3	14	65
RE	6.23	2.20	0.35	1	10	65
ES	0.85	0.19	0.22	0.17	1	65
UPr (ng/ml)	15.48	5.88	0.38	1.81	28.2	63
UG	2.44	1.29	0.53	0.48	6.81	65

Both lines presented similar ovulation rate (Table 2) in agreement with results published earlier (Santacreu *et al.*, 2005, Peiró *et al.*, 2007). No differences were detected between lines either for number of embryos recovered or for embryo survival. These results are unusual and do not corroborate the studies that have been published during the last years for the same lines (Mocé *et al.*, 2002, Santacreu *et al.*, 2005, Peiró *et al.*, 2007). The number of females used in this experiment was low compared to the number used in a study that was carried out by Mocé *et al.* (2002) in the same generation, in which significant differences between High and Low lines were obtained for number of embryos (12.8 vs. 11.0). The concentration of progesterone in uterine fluid was similar in both lines in agreement with results reported previously by Mocé *et al.* (2002) and both lines showed similar uteroglobin expression (Table 2). Furthermore, no correlations were found between uteroglobin and number of recovered embryos ($r=-0.21$; $P>0.05$) nor between uteroglobin and embryo survival ($r=-0.13$; $P>0.05$). Thus, differences reported in embryo survival between both lines (Mocé *et al.*, 2002, Santacreu *et al.*, 2005) do not seem to be related with differences in uteroglobin expression at day 6 of gestation.

The secretion of uteroglobin is regulated by progesterone (reviewed by Beier, 2000). Uteroglobin was slightly correlated with levels of progesterone in uterine fluid ($r=0.29$; $P<0.05$). Cowan *et al.* (1976) studied uterine fluid before implantation and observed that concentration of progesterone in uterine flushings increased from day 4 to day 6 and this rising was accompanied by an increasing in uteroglobin concentration. In rabbits, the only significant source of progesterone is the corpus luteum (Harrington and Rothermel, 1977), but no correlation was found between uteroglobin and ovulation rate ($r=-0.08$; $P>0.05$).

Table 2: Differences of least squares means between High and Low lines (H-L) for ovulation rate (OR), number of embryos recovered (RE), embryo survival (ES=RE/OR), concentration of progesterone in uterine fluid (UPr) and expression of uteroglobin in uterine fluid (UG) at day 6 of gestation. Results are given per uterine horn

Trait	(H-L) ± SE	Significance
OR	-0.48 ± 0.55	ns
RE	-0.61 ± 0.52	ns
ES	-0.01 ± 0.04	ns
UPr	2.19 ± 1.80	ns
UG	0.53 ± 0.34	ns

SE: standard error; ns: non significant

CONCLUSIONS

No differences between High and Low lines were found for the expression of uteroglobin at day 6 of gestation. Besides, neither number of embryos nor embryo survival was related to uteroglobin expression. Thus, uteroglobin does not seem to be responsible for differences in embryo survival between both lines at day 6 of gestation.

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