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Embryologic changes in rabbit lines selected for litter size variability

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ABSTRACT

A divergent selection experiment on litter size variability was carried out. Correlated response in early embryo survival, embryonic development, size of embryos, and size of embryonic coats after four generations of selection was estimated. A total of 429 embryos from 51 high-line females and 648 embryos from 80 low-line females were used in the experiment. The traits studied were percentage of normal embryos, embryo diameter, zona pellucida thickness, and mucin coat thickness. Traits were measured at 24, 48, and 72 hours postcoitum (hpc); mucin coat thickness was only measured at 48 and 72 hpc. The embryos were classified as zygotes or two-cell embryos at 24 hpc; 16-cell embryos or early morulae at 48 hpc; and early morulae, compacted morulae, or blastocyst at 72 hpc. At 24 hpc, the percentage of normal embryos in the high line was lower than in the low line (−2.5%), and embryos in the high line showed 10% higher zona pellucida thickness than those of the low line. No differences in percentage of zygotes or two-cell embryos were found. At 48 hpc, the high-line embryos were less developed, with a higher percentage of 16-cell embryos (23.4%) and a lower percentage of early morulae (−23.4%). At 72 hpc, high-line embryos continued to be less developed, showing higher percentages of early morulae and compact morulae and lower percentages of blastocyst (−1.8%). No differences in embryo diameter or mucin coat thickness were found at any time. In conclusion, selection for litter size variability has consequences on early embryonic survival and development, with embryos presenting a lower state of development and a lower percentage of normal embryos in the line selected for higher variability.

1. Introduction

Selection for environmental variability has interest in animal production, evolutionary biology, and medicine [1]. In animal production, dams with lower resilience (more sensitive to stress or to diseases) can have a higher litter size variability. In a divergent selection experiment on litter size variability [2], we found the more variable line to be more sensitive to diseases and less able to withstand adverse environmental conditions [3]. We also found that the more variable line had lower litter size at birth and lower prenatal survival [2]. It seems relevant to examine the consequences on embryo development.

Early embryo losses are related to embryonic development [4] and embryo coat sizes [5]. During the first 3 days postcoitum, rabbit embryo acquires a glycoprotein layer, the mucin coat, which is accumulated during oviductal transport [6,7]. This secures timely, appropriate implantation and prevents the embryo from exposure to the pathogenic viruses [5,8]. This mucin coat is peculiar to rabbit and hare and is not common in other mammals. It would be interesting to know how selection for variability of litter size affects the evolution of the rabbit embryo mucin coat and zona pellucida (ZP).

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We know that embryonic development in early gestation is under genetic control in rabbits [4,9], and we also know that litter size variability has been modified by selection [2]; thus, we presume that genetic modifications have occurred in the embryos because of selection on litter size variability. This will affect embryo survival and will be related to the differences in litter size between the high and low lines selected for litter size variability, found by Argente et al. [2], explaining at least part of this difference. The aim of this study was to assess the effect of selection for litter size variability on early embryo development and survival.

2. Material and methods

All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee on June 21, 2011 (Reference 98 number DTA-MJA-001-11), in accordance with Council Directives 98/58/EC and 2010/63/EU.

2.1. Animals

Animals came from a divergent selection experiment for litter size variability [2]. Litter size variance of all parities of each female was calculated, and high and low lines were created by selecting the females with higher and lower variance of litter size, respectively. As a female can have higher litter variability for purely environmental reasons, e.g., for having a litter in one season and another litter in another season, litter size was precorrected by the effects of year-season and lactation status (nuliparous, lactating, and nonlactating females). Each divergent line had approximately 125 females and 25 males per generation. Selection pressure on females was approximately 30% in each line. Males were chosen within sire families to avoid inbreeding. The numbers of does and embryos used in the experiment are shown in Table 1.

All animals were bred at the farm of the University Miguel Hernández of Elche. They were kept under a constant photoperiod of 16 hours continuous lighting: 8 hours continuous darkness and controlled ventilation. Does were mated first at 18 weeks of age, and at Day 10 after parturition thereafter.

2.2. Traits

All does came from the fourth generation of selection. The average litter size was 7.70 and 8.34 for the high and low lines, respectively. The average number of parities was 5.0 and 4.7 for the high and low lines, respectively. A total of 51 and 80 nonlactating multiparous females from the high and low lines, respectively, were euthanized at 24, 48, or 72 hours postcoitum (hpc) by intravenous administration of sodium thiopental in a dose of 50 mg/kg of body weight (Thiobarbital, B. Braun Medical S.A., Barcelona, Spain). The entire reproductive tract was immediately removed. Total embryos (TEs) were recovered by perfusion of oviducts and uterine horns with 10 mL of Dulbecco's phosphate-buffered saline containing 0.2% of BSA. Embryos were classified as normal embryos (NEs) when they presented homogenous cellular mass and intact embryo coats [10], using a binocular stereoscopy microscope (Leica Mz 9.5; × 600). Percentage of normal embryos was calculated as ([NE/TE] × 100). At 24 hpc, normal embryos were classified as zygotes (Z) or 2-cell embryos (2-cells) so that NE = Z + 2-cells. At 48 hpc, normal embryos were classified as 16-cell embryos (16-cells) or early morulae (EM), thus NE = 16-cells + EM. At 72 hpc, normal embryos were classified as early morulae, compacted morulae (CM) or blastocysts (B), and NE = EM + CM + B. In all cases, zygotes, 2-cells, 16-cells, early morulae, compact moruale, and blastocysts were expressed as percentages of their respective NEs.

Embryo images were recorded using a color digital camera (LEICA DFC 420) mounted on the stereomicroscope. The setting for microscopic observations (magnification × 600) and bright field was kept constant throughout the study. Mucin coat thickness (MC, μm), ZP thickness (μm), and embryo diameter excluding ZP (ED, μm) were measured immediately after recovery of embryos. To minimize experimental distortion, the same technician performed all image recordings and measurements. The ZP thickness and the embryo diameter were measured at 24, 48, and 72 hpc, and the mucin coat thickness of embryos was measured at 48 and 72 hpc.

Table 1
Means (standard deviation) of types of embryo and embryo development traits at 24, 48, and 72 h postcoitum of two divergent lines selected for high and low litter size variability.

Number of data and traits	High line			Low line		
	24 h	48 h	72 h	24 h	48 h	72 h
Number of does	15	23	13	25	28	27
Number of embryos	98	190	141	166	245	237
Normal embryos (%)	97.1 (4.3)	96.9 (9.9)	95.7 (13.4)	99.6 (4.2)	97.7 (9.9)	97.5 (12.6)
Zygotes (%)	28.5 (32.7)			39.1 (32.2)		
2-cells (%)	71.5 (32.7)			60.9 (32.2)		
16-cells (%)		29.2 (28.1)			4.5 (28.1)	
Early morulae (%)		70.8 (28.1)	18.6 (26.7)		95.5 (28.1)	13.1 (30.0)
Compacted morulae (%)			73.9 (30.1)			66.0 (31.8)
Blastocyst (%)			7.5 (20.5)			20.8 (24.1)
Embryo diameter (μm)	127.0 (9.3)	123.3 (9.3)	119.3 (9.6)	123.5 (9.6)	124.0 (9.5)	118.2 (9.6)
Zona pellucida (μm)	17.5 (4.0)	16.9 (3.6)	17.9 (3.4)	15.8 (3.9)	16.9 (3.6)	17.8 (3.4)
Mucin coat (μm)		50.3 (14.7)	103.7 (25.0)		50.5 (14.8)	101.7 (25.4)