EMBRYOLOGICAL CHANGES IN RABBITS LINES SELECTED ON LITTER SIZE VARIABILITY

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Abstract

A divergent selection experiment on litter size variability was carried out. Correlated response on early embryo survival, embryonic development, size of embryos and size of embryonic coats after 4 generations selection was estimated. A total of 429 embryos from 51 females of the High line and 648 embryos from 80 females of the Low line 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 h of gestation; mucin coat thickness was only measured at 48 and 72 h of gestation. The embryos were classified as zygotes or 2-cells embryos at 24 h of gestation; 16-cells embryos or early morulae at 48 h of gestation, and early morulae, compacted morulae or blastocyst at 72 h of gestation. At 24 h of gestation, percentage of normal embryos in the High line was lower than in the Low line (-2.5%), and the embryos in the High line showed 10 % higher zona pellucida thickness than those of the Low line. No differences in percentage of zygotes or 2-cells embryos were found. At 48 h of gestation, embryos of the High line were less developed, having higher percentage of 16-cells embryos (23.4%) and lower percentage of early morulae (-23.4%). At 72 h of gestation, embryos from High line continued being less developed, showing higher percentage of early morulae and compact morulae and lower percentage 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, having embryos lower state of development and a lower percentage of normal embryos in the line selected for higher variability

Keywords: Embryonic development; embryonic coats; litter size variability; rabbit; selection.

1. Introduction

A divergent selection experiment on litter size variability has been carried out in order to produce two lines with high and low litter size variability respectively [1]. No selection for this trait has been performed hitherto in prolific species. An important question is to examine the consequences of this selection on litter size, embryological changes and embryo survival. Effects on litter size have been studied by Argente et al. [1, 13], but no studies have been done on embryo development and survival.

Early embryo losses are related to embryonic development [2] and embryo coat sizes [3]. During the first 3 d postcoitum, rabbit embryo acquires a glycoprotein layer, the mucin coat, which is accumulated during oviductal transport [4,5]. This secures timely appropriated implantation, and prevents the embryo from exposure to the pathogenic viruses [3,6]. This mucin coat is peculiar to rabbit embryos and it 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.

We know that embryonic development in early gestation is under genetic control in rabbits [2,7], and we also know that litter size variability has been modified by selection [1, 13], thus we presume that genetic modifications have been produced in the embryos due to selection for litter size variability. This will affect embryo survival and it 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. [13], explaining at least part of this difference. The aim of this study is 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 University Miguel Hernández of Elche Research Ethics Committee on 21 June 2011 (Reference 98 number DTA-MJA-001-11), according to Council Directives 98/58/EC and 2010/63/EU.

2.1. Animals

Animals came from a divergent selection experiment for litter size variability [13]. Litter size variance of all parities of each female was calculated, and High (H) and Low (L) lines were created by selecting the females having a higher and lower variance of litter size respectively. As a female can have a higher litter variability for pure environmental reasons; for example, 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 non-lactating females). The number 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 h continuous lights: 8 h continuous darkness and controlled ventilation. Does were mated first at 18 wk of age, and at d 10 after parturition thereafter.

2.2. Traits

All does came from the fourth generation of selection. A total of 51 and 80 non-lactating multiparous females of the High and the Low lines respectively were euthanized at 24, 48 or 72 h postcoitum 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 (TE) were recovered by perfusion of oviducts and uterine horns with 10 mL of Dubelcco´s phospate buffered saline containing 0.2% of bovine serum albumin. Embryos were classified as normal embryos (NE) when they presented homogenous cellular mass and intact embryo coats [8], using a binocular stereoscopy microscope (Leica Mz 9.5-600x). Percentage of normal embryos was calculated as [(NE ⁄ TE) x 100]. At 24 h of gestation, normal embryos were classified as zygotes (Z) or 2-cells embryos (2-cells) so that NE = Z + 2-cells. At 48 h of gestation, normal embryos were classified as 16-cells embryos (16-cells) or early morulaes (EM), thus NE = 16-cells + EM. At 72 h of gestation, 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 percentage of their respective Normal Embryos.

Embryo images were recorded using a colour digital camera (LEICA DFC 420) mounted on the stereomicroscope. The setting for microscopic observations (magnification X 600) and bright field was kept constant throughout the study. Mucin coat thickness (MC, µm), zona pellucida thickness (ZP, 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 zona pellucida thickness and the embryo diameter were measured at 24, 48 and 72 h of gestation and the mucin coat thickness of embryos was measured at 48 and 72 h of gestation.

2.4 Statistical analyses

All traits were analysed with a model including the effects of line (High and Low lines) and season. The embryo diameter, zona pellucida thickness and mucin coat thickness included a random female effect.

The traits were analysed using Bayesian methodology. Bounded flat priors were used for all unknowns, with the exception of the female effect, which was considered normally distributed with mean **0** and variance $\mathbf{I}\sigma_f^2$, where **I** is a unity matrix, and σ_f^2 is the variance of the female effect. Residuals were normally distributed with mean **0** and variance $\mathbf{I}\sigma_e^2$. The priors for the variances were also bounded uniform. Features of the marginal posterior distribution of differences between lines were estimated using Gibbs sampling. The Rabbit program developed by the Institute for Animal Science and Technology was used for all procedures. Inferences were made from the estimated marginal posterior distributions of the differences between the High and the Low lines [9]. Probability of the difference being larger than zero ($P > 0$) and $HPD_{95\%}$ (shortest Bayesian confidence intervals with a 95% of probability) were calculated.

3. Results

Mean of all the traits are presented in Table 2 for the High and Low lines. Rabbits have ovulation induced by coitus, thus euthanizing the does at 24, 48 and 72h after coitus estimates the stage of gestation accurately in this species. The percentage of normal embryos was high at all stages of gestation in both lines. Most embryos were classified as two-cells at 24h of gestation, early morulae at 48 h, and compact morulae at 72h. Embryo diameter was remarkably constant during the three stages of development. The zona pellucida remained also constant along the embryo development, but mucin coat thickness was doubled from 48h to 72h of gestation.

Differences between the High and Low lines at 24h of gestation are shown in Table 3. The line selected for high litter size variability showed lower percentage of normal embryos than the line selected for low variability (-2.5%), with probability 0.05 of this difference being positive; i.e. probability 0.95 that the High line had a lower percentage of normal embryos than the Low line. Embryo development (2-cells embryos with respect to Zygotes) was similar in both lines. Embryo diameter was also similar in both lines, but the zona pellucida thickness was 10% greater in the High line.

Percentage of normal embryos was similar in both lines at 48h and 72h of gestation (tables 4 and 5). The line selected for higher litter size variability showed lower embryo development at 48h (less early morulae, table 4) and at 72h of gestation (less blastocysts, table 5). No evidence of differences for the embryo diameter, zona pellucida thickness and mucin coat thickness were found either at 48h or 72h of gestation.

4. Discussion

Selection for environmental variability have interest in animal production, evolutionary biology and medicine [Genetic Control of Environmental Variation of Two Quantitative Traits of Drosophila melanogaster Revealed by Whole-Genome Sequencing. Peter Sørensen,* Gustavo de los Campos,† Fabio Morgante,‡ Trudy F. C. Mackay,‡ and Daniel Sorensen*,1 Genetics, Vol. 201, 487–497 October 2015]. No selection experiments on litter size variability have been carried out hitherto. As variability is estimated within female, and the same genes control litter size in all parities [Genetics of litter size in three maternal lines of rabbits: Repeatability versus multiple-trait models M. Piles,*1 M. L. Garcı´a,† O. Rafel,* J. Ramon,* and M. Baselga‡, J. Anim. Sci. 2006. 84:2309–2315], only the environment explains having different litter size in different parities. Does with lower resilience can have a higher litter size variability; for example, does having higher sensitivity to stress of lower disease resistance. We have also found in former studies that the line selected for higher litter size variability is less robust; i.e., more sensitive to diseases and less able to withstand adverse environmental conditions [14]. We also found that the High line had lower litter size at birth and lower prenatal survival [13]. It seems relevant to examine the consequences of selection for litter size variability on embryo development.

We have seen that the percentage of normal embryos is similar in both lines, only being slightly lower in the High line at 24h of gestation. However, embryo development was clearly affected by selection, having the line selected for higher variability lower embryo development, which would agree with the lower prenatal survival found in this line [13]. Other authors have found that lower embryo development produced lower embryo survival in rabbits [3,7].

Differences in embryonic stage of development are principally due to timing of ovulation and oviductal and uterine fluid compositions [15, 19]. Torres et al. [16] showed that a high ovulation rate increases ovulatory timing and later ovulating follicles would be fertilized later [17]; however we have found that our lines have the same ovulation rate [13], so differences in timing of ovulation should be similar. Time spent in the oviduct should also be similar, since the mucin coat thickness was similar in both lines, and according to Murakami and Imai [3], the mucin coat thickness depends on the time spent in the oviduct. Therefore, the advanced embryonic development in the L line should be due to different oviductal and uterine fluid compositions, which influence in rabbit embryo development is well known [19].

A peculiarity of rabbit embryos is that they are surrounded not only by the zona pellucida but also by a mucin coat. Mucin coat thickness was not measured at 24 h of gestation because 90% of the embryos did not show it. At 24 h of gestation most of the embryos are still in the infundibulum at the start of the isthmus [12], but the secretion of mucopolysaccharides and some proteins forming the mucin coat occurs in both ampulla and isthmus and less so in the infundibulum [5]. The importance of the mucin coat lies in its relation to embryo mortality. Greenwald [27] and Murakami and Imai [3] found that low mucin coat thickness is the primary factor in embryonic loss in rabbit. However, we have not found differences between lines in mucin coat thickness. We have found differences in the zona pellucida thickness at 24h of gestation, being higher for the line selected for higher variability. Higher thickness of the zona pellucida, is related to lower fertilization [25], which would explain the lower percetange of normal embryos in the High line at 24h. No differences were found in embryo diameter, which remains approximately constant along the three stages of gestations investigated, which agrees with the results of other authors [22].

In summary, selection on litter size variability modifies early embryo development in rabbits, leading to a lower state of development and a lower percentage of normal embryos in the line selected for higher variability.

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Table 1. Number of does and embryos from the High and Low lines at different stages of gestation used in the experiment.

	24h		48h		72h	
	High	Low	High	Low	High	Low
Does	15	25	23	28	13	27
Embryos	98	166	190	245	141	237

274 Table 2. Means of types of embryo and embryo development traits at 24, 48 and 72
275 hours of gestation of two lines selected for high and low litter size variability.

	High Line			Low Line		
	24h	48h	72h	24h	48h	72h
Normal Embryos (%)	97.1	96.9	95.7	99.6	97.7	97.5
Zygotes (%)	28.5			39.1		
2-cells (%)	71.5			60.9		
16-cells (%)		29.2			4.5	
Early Morulae (%)		70.8	18.6		95.5	13.1
Commpacted Morulae (%)			73.9			66.0
Blastocyst (%)			7.5			20.8
Embryo Diameter (µm)	127.0	123.3	119.3	123.5	124.0	118.2
Zona Pellucida (µm)	17.5	16.9	17.9	15.8	16.9	17.8
Mucin Coat (µm)		50.3	103.7		50.5	101.7

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277 Table 3. Differences between the High and Low lines for types of embryo and embryo
278 development traits at 24 hours of gestation.

	High – Low	$HPD_{95\%}$	P
Normal Embryos (%)	-2.5	-0.2 , 5.4	0.05
Zygotes (%)	-9.5	-31.3 , 12.7	0.20
2-cells (%)	9.5	-12.1 , 32.0	0.80
Embryo Diameter (µm)	2.3	-4.7 , 8.5	0.76
Zona Pellucida (µm)	1.6	-0.2 , 3.7	0.95

279 HPD95%: shortest confidence interval at 95% probability. P: Probability of the
280 difference between High and Low lines being larger than zero.

281 Table 4. Differences between the High and Low lines for types of embryo and embryo
282 development traits at 48 hours of gestation.

	High – Low	$HPD_{95\%}$	P
Normal Embryos (%)	-0.9	-4.6 , 6.9	0.38
16-cells (%)	23.4	8.9 , 38.3	1.0
Early Morulae (%)	-23.4	-37.7 , -8.4	0.0
Embryo Diameter (µm)	-1.0	-4.4 , 2.5	0.30
Zona Pellucida (µm)	0.01	-1.3 , 1.2	0.51
Mucin Coat (µm)	0.2	-7.1 , 8.0	0.52

283 HPD95%: shortest confidence interval at 95% probability. P: Probability of the
284 difference between High and Low lines being larger than zero.

285 Table 5. Differences between the High and Low lines for types of embryo and embryo
286 development traits at 72 hours of gestation.

	High – Low	$HPD_{95\%}$	P
Normal Embryos (%)	-2.01	-11.1 , 6.9	0.33
Early Morulae (%)	5.3	-13.1 , 23.7	0.73
Compacted Morulae (%)	6.5	-15.6 , 27.9	0.72
Blastocysts (%)	-11.8	-27.8 , 3.6	0.07
Embryo Diameter (µm)	0.8	-3.1 , 4.6	0.66
Zona Pellucida (µm)	0.004	-1.3 , 1.3	0.51
Mucin Coat (µm)	1.4	-12.3 , 15.7	0.58

287 HPD95%: shortest confidence interval at 95% probability. P: Probability of the
288 difference between High and Low lines being larger than zero.