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Blastocyst recovery and multifactorial gene expression analysis in the wild guinea pig (*Cavia aperea*)

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ABSTRACT

The expression of specific developmentally important genes in preimplantation embryos is an accepted marker for unraveling the influence of single factors in studies that are mostly related to artificial reproduction techniques. Such studies, however, often reveal high levels of heterogeneity between single embryos, independently of the influence of factors of interest. A possible explanation for this variation could be the large variety of physiological and environmental factors to which early embryos are exposed and their ability to react to them. Here, we investigated several potentially important parameters of development at the same time, in blastocysts of the wild guinea pig (*Cavia aperea*) generated *in vivo* after natural mating. The optimal time for flushing fully developed blastocysts was between 123 and 126 hours after mating. The abundance of *POU5F1* ($P = 0.042$), *BAX* ($P < 0.001$), *SLC2A1* ($P = 0.017$), and *DNMT3A* ($P < 0.001$) mRNA changed significantly over time after mating. The number of sibling embryos present influenced *STAT3* levels significantly ($P = 0.02$). Levels of *BAX* and *POU5F1* were significantly affected by season ($P = 0.03$ and 0.04). The temporal pattern of *SLC2A1* levels was significantly altered both after feeding a protein-deficient diet ($P = 0.04$) and temperature treatment ($P = 0.04$) of the sire. In addition, the identity of the father had a significant influence on *POU5F1* ($P = 0.049$) and *STAT3* ($P < 0.001$) mRNA abundances. These data report that the expression of specific genes in early embryos reflects the entire heterogeneity of their surroundings and that it is a plastic reaction toward a multifactorial environment.

1. Introduction

Embryonic gene expression is an accepted marker for assessing the quality of an individual embryo, reflecting the conditions under which it is generated [1–5]. Furthermore, not only the direct milieu of the embryo itself but also the maternal and paternal environment during gamete maturation and growth, influence the subsequent embryo and its gene expression. Maternal nutrition, specifically, nutritional restriction during superovulation [6] and particularly obesity- or diet-induced hyperlipidemia, either in the mothers [7] or simulated by IVC conditions [8,9], was found to alter gene expression levels in blastocysts in different animal models. Another environmental factor that might modulate gene expression in early embryos is heat exposure: a single, mild short-term heat stress has already been shown to have negative consequences on fertility and embryo development in mice [10].

Male factors correlating with embryonic gene expression have, to our knowledge, not yet been investigated, but the influence of the status of the father, especially nutrition-related, is no longer neglected [11]. Obesity in fathers is known to impair embryo quality and developmental competence [12]. Epigenetic modulations are responsible for alterations in gene expression by the offspring, thereby adjusting the fetus to variations in

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environmental conditions [13–15]. Variable environmental conditions might be related directly to the parent's stress situation (e.g., nutritional stress) or might reflect ecologically relevant information. A variety of ecological and social factors such as food availability or seasonal changes in temperature and/or drought have been shown to affect performance, physiology, and reproduction in many species.

To investigate multiple environmental effects on embryonic gene expression, we chose to study the wild guinea pig (*Cavia aperea*). Unlike in many other small mammal species, offspring of *C. aperea* are likely to encounter the same environmental conditions as the mother and the father because males and females live in stable harem groups [16]. Therefore, paternal epigenetic programming is likely to occur and has indeed been proven recently [17]. Furthermore, the domesticated congener, the guinea pig (*Cavia porcellus*), has been a model species for physiology and reproduction studies for decades [18] and information about embryonic developmental speed and transport timing through the oviduct to the uterus is available [19]. Factors that commonly influence reproduction and later development of offspring are litter size and the season in which offspring are sired [20,21].

Within the present study, we aimed to investigate whether two different paternal factors, a change of nutrition toward protein deficiency and a constant elevated ambient temperature, cause alterations in gene expression levels by individual blastocysts sired by treated males.Furthermore, the study design allowed us to examine simultaneously the effect of additional potentially important parameters such as the time after mating (corresponding to the age of blastocysts after fertilization), the season, the identity of the father and the number of sibling embryos.

2. Materials and methods

All husbandry and experimental procedures were approved by the German committee of Animal Welfare in Research (permit no. V3-2347–35–2011).

2.1. Animal housing

Animals were kept as described previously [21]. All animals were fed guinea-pig pellets (Altromin GmbH, Lage, Germany), water and hay *ad libitum*, and apples, bell pepper, cucumber or carrots were given daily. Vitamin C was added to their drinking water once a week. They were kept in enclosures that constantly allowed them to choose indoor or outdoor locations. The enclosures had woodchips for flooring, a feeding trough, a water bottle, and shelters for hiding. The indoor enclosures were $0.75 \times 0.75 \times 1$ m and the contiguous outdoor enclosures were $0.75 \times 1 \times 1$ m (length $\times$ width $\times$ height). The animals experienced natural seasonal variations in photoperiod, temperature, and rainfall, except that during frost periods, a radiator prevented indoor freezing of the drinking water. These conditions induce predictable differences in reproductive performance including changes in the numbers of *Corpora lutea* and litter sizes [21]. From controlled laboratory experiments investigating seasonal effects, it is known that changes in photoperiod (mainly in the duration of daylight) are the main factor responsible for inducing these biological differences [20,22]. Daylight photoperiod changes at our animal facilities in Germany are as pronounced as the photoperiod changes observed in Uruguay, from where the animals originated. Thus, the seasonal variations we observe in our captive population mirrors the natural environmental conditions for this species.

During nonbreeding periods, males were kept individually away from one another, whereas females were kept in groups consisting of two adults together with their female offspring from previous breeding events.

2.2. Stress exposure and breeding

Animals were bred during three different mating periods: in July and August 2012 (period 1), then in November and December 2012 (period 2), and finally in April–June 2013 (period 3). Males were divided into two experimental groups, each consisting of five individuals. These two groups were exposed to two different stressors: a protein-deficient diet or constant elevated temperature of 30 °C. The animals were exposed to one or the other of the stressors before the subsequent breeding and embryo retrieval period. Males in group one were exposed to the dietary stressor for 60 consecutive days before mating period 1, whereas those in group two were exposed to the temperature stressor. Before mating period 2, the groups were subjected to the alternate stressor for 60 days, so that both groups received the same two stresses but in a different order. For the protein deficiency stress, normal pellets were replaced by pellets with 75% lower crude protein content (Altromin GmbH, Lage, Germany) and no vegetables, but hay *ad libitum* was given. The constant elevated temperature stress was induced with a heating mat (Candor GmbH, Leipzig, Germany) at the bottom of the cage. For the controls, the same 10 males were kept without stressors, under natural temperatures and with normal food, for 60 days before mating period 3. In this way, the two groups of males subjected to the two stressors subsequently became their own controls. During all breeding periods, males were kept under the normal conditions described previously together with 1–6 primiparous females (corresponding to the female offspring of another male from the previous breeding interval).

2.3. Animal observation and embryo collection

The aim was to obtain embryos at the blastocyst stage because this is the most appropriate stage for single embryo gene expression analysis because of the relatively high number of blastomeres. For this, knowing the specific time of mating was essential. Because the animals were very shy and furthermore, mating occurred predominantly during the night hours, we observed them with a camera system equipped with infrared diodes (Carlights.de, Tschernitz, Germany). The cameras were connected to EverFocus EDR 410Hs recorders, which were equipped with Seagate 500 GB Pipeline HD drives (EverFocus Electronics GmbH, Emmerich am Rhein, Germany). These drives were read by EPR 100C Harddisc Readers and