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Non-invasive ultrasonographic characterization of phenotypic changes during embryo development in non-anesthetized mice of different genotypes

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Abstract

This study aimed to determine phenotypic changes during embryo development in the mouse, and the influence of genetic background, by non-invasive real-time ultrasonography. Serial scannings were performed from Day 4.5 after the appearance of the vaginal plug in a total of 34 adult mice of different strains (9 BALB/c, 10 C57BL/6 and 15 CD1). Embryonic vesicle diameter was measurable from Day 4.5 of pregnancy. Images of embryos were obtainable from Day 5.5 of gestation onwards, and crown-rump length and trunk parameters (diameter and area) were measured. At more advanced stages, the positions of fetuses prevented accurate measurement of crown-rump length; therefore, head diameters (occipito-snout length and biparietal diameter) were used as an alternative index of fetal size. All measurements correlated highly with gestational age ($P < 0.0005$). No significant differences were observed between genotypes in early pregnancy, but during the last week of gestation trunk measurements were larger in CD1 embryos ($P < 0.05$) while head diameters were larger in C57BL/6 conceptuses ($P < 0.05$). There was a significant effect of genetic background on heart rate throughout pregnancy; although heart rate was similar in CD1 and C57BL/6 embryos (154.5 ± 2.8 and 147.8 ± 4.5 beats/min, respectively), it was significantly lower in BALB/c mice (127.0 ± 2.1; $P < 0.005$ vs. CD1 and C57BL/6).

Keywords: Conceptus; Developmental biology; Early development; Pregnancy; Ultrasonography

1. Introduction

The mouse is a widely used model in biological research [1,2], and one of the major fields of study is developmental biology. However, analysis of the development of specific phenotypes, which is required for the progress of knowledge, is hindered by the fact that most methodologies are invasive and impede the progression of pregnancy. The use of non-invasive methods has been previously described, for example magnetic resonance imaging [3–5] and ultrasound microscopy (probes between 40 and 70 MHz of frequency; [6–8]). However, these methods are technically complex, very expensive and not widely available. Moreover, they require that mice be anaesthetized and immobilized, which prevents serial scannings on successive days.

A reliable non-invasive method for screening conceptus development would thus be very beneficial

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for research in developmental biology, by allowing examination on successive days, minimizing animal management, and avoiding the use of anesthesia and invasive techniques. Furthermore, it would contribute to the improvement of animal welfare, in accordance with the philosophy of the 3Rs model of Russell and Burch (refinement, reduction and replacement; [9]). Non-invasive techniques would reduce the number of experimental animals used and make studies more precise, by ensuring that only pregnant animals of known gestational age are used. The ability to determine gestational age would also allow determination of the expected time of parturition, an advantage in breeding programs with mouse strains that have difficulties at delivery. The exact time of fertile mating in mice is often unknown, so the ability to monitor and measure fetal organs would allow accurate estimation of gestational age, as in other species.

A routine method for assessing pregnancy and fetal development in humans and other animal species is real-time ultrasound imaging. However, the use of ultrasonography in mice has been described only very recently [10]. This study used a transducer with a frequency of 15 MHz, whereas commercial probes usually have a maximum of 10–11 MHz, frequencies first used in a study published during the developmental of our work [11]. Both studies examined a single strain (CD1), which is the most corpulent and which is not exactly the most widely used in research.

The objectives of the current study were (a) to characterize, by ultrasonography, changes of phenotypic parameters in the developing mouse embryo throughout pregnancy, and (b) to determine the influence of genetic background on these parameters.

2. Material and methods

2.1. Animals and husbandry

A total of 34 adult mice of breeding age were used (9 BALB/c, 10 C57BL/6 and 15 CD1). All animals were maintained at the CNIC Animal Unit in Madrid, Spain, which meets the requirements of European Union regulations regarding the use of animals for experimental and other scientific purposes. The study was carried out under Project License 156/07 from the CNIC scientific ethics committee. Animal manipulations were performed in accordance with the Spanish policy for animal protection (RD1201/05), which meets the requirements of European Union directive 86/609 regarding the protection of animals used for experimentation.

Females used in the study were placed with males at a 1:1 ratio. Pregnant females were identified by the presence of a vaginal plug after overnight mating, and gestational stage was estimated by defining the morning of that day as Day 0.5.

2.2. Ultrasonographic measurements of conceptus development

Observations were made by two sonographers, both of whom had no previous experience in the assessment of pregnancy in the mouse: one had prior experience with other species and the other was a complete novice. Serial scannings were done from Day 3.5 (estimated as the mean day of implantation; [12]) to Day 18.5. But to avoid excessive consecutive observations, which might damage the mouse or its conceptus, the mice were randomly divided into observation groups, and a different group was scanned each day. In this way individual mice were examined every 3 days. For ultrasound scanning, each mouse was held and maintained in dorsal recumbency by hand. Mice were not anesthetized, so as to avoid alterations to fetal parameters during observations, principally heart rate [13]. To minimize animal distress, abdominal hair was not shaved: coupling between transducer and skin was improved by thorough wetting of the abdomen with carboxymethylcellulose gel.

Two ultrasound scanners were used: an Aloka 2500 equipped with a multifrequency (7.5–10 MHz) sectorial array probe with a footprint of 40 mm; and a Siemens Antares connected to a multifrequency (7.5–10 MHz) linear array probe with a footprint of 35 mm. Ultrasound scanning was done by placing the transducer on one flank and moving it to the opposite flank, to view the uterine horns and fetus in transverse, frontal or sagittal planes. The probe was manipulated until the largest section of each structure was obtained. Scans were recorded, using the machines' ''cine-loop'' option; this allowed animals to be released quickly, minimizing stress caused by keeping them restrained. Complete examination of each female took approximately 3–4 min. The sizes (diameters and areas) of structures of interest were measured with built-in electronic calipers on the cine-loop recordings. To avoid distortions resulting from the corpulence of an individual fetus, measurements were taken from a minimum of three conceptuses in each scan recording.

2.3. Validation of the technique

Ultrasound determination of gestational stage in very early pregnancy was validated by anatomical inspection