Pregnancy detection in mice using ultrasound

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DIAGNOSTIC ultrasound has been used in almost all medical fields and is recognised as an increasingly important modality in a variety of clinical situations. The mouse is currently the most widely used animal in biomedical research (Marshall 2000). Diagnosis of pregnancy in experimental mice is possible at 12 to 14 days gestation, when abdominal distension is apparent. Mice with large litters may show distension slightly earlier (Kaufman 1989). Diagnosis of pregnancy at 7·5 days in experimental mice is currently performed by direct inspection of the uterine horns after laparotomy, with no routine utilisation of imaging techniques.

The embryonic mouse is a ubiquitous model of mammalian development, due to the obvious benefits of a short gestation cycle, and genetic homologies with developmental genes in other mammals. Such models should yield further insight into the mechanisms responsible for human developmental and disease processes. Most previous investigations of the gestation period in the mouse have been invasive in nature.

Video microscopy has been used to monitor heart dynamics in surgically exposed embryos, magnetic resonance microscopy has been successfully utilised to visualise three-dimensional anatomy and vasculature in fixed mouse embryos (Smith and others 1994), and servo-null pressure measurement and Doppler interrogation using implanted crystals have also been used (Keller and others 1996, MacLennan and Keller 1999). However, none of the previous methods used have provided real-time imaging of live embryos as early as 7·5 days. The lack of effective in utero imaging methods has been a significant limitation.

This short communication describes the capabilities of a conventional ultrasound system as a tool for the non-invasive investigation and diagnosis of early pregnancy in the mouse. All the animals in the present study were maintained according to protocols approved by the Institutional Animal Research and Care Committee, University of Naples.

Fifty-one pregnant CD1 mice, ranging in age from seven to nine weeks, were used in the study. The mice were divided into three groups of 17 mice at three different stages of pregnancy (7·5 days, 12·5 days and 16·5 days). In staging the embryos, gestational day 0·5 was defined as noon of the day a vaginal plug was found following overnight mating.

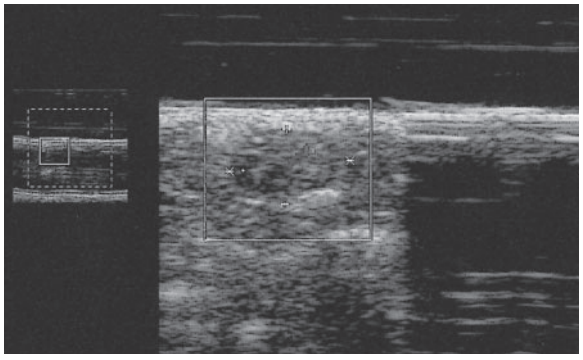

FIG 2: Mouse embryo at 12·5 days showing the gestational sac (arrows) with detectable cardiac activity; the global shape at this time is oblong

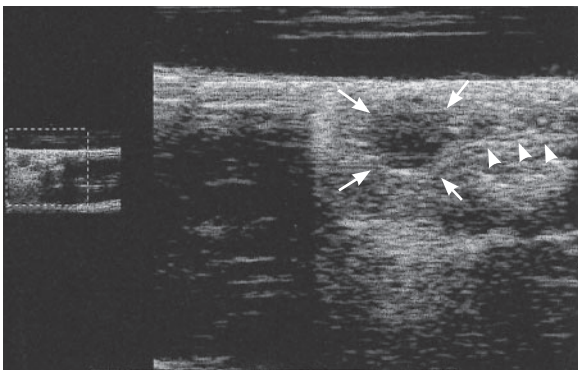

FIG 1: Longitudinal section of the gestational sac (arrows) and placenta (arrowheads) at 7·5 days. The image to the left shows the full urinary bladder

Ultrasonographic examination was performed with a GE ultrasound machine (Logiq MD 400) using a multifrequency linear transducer (7 MHz to 11 MHz). Before ultrasonographic evaluation, the mice were anaesthetised with 0·45 to 0·75 ml 2,2,2-tribromoethanol (Avertin solution T4, 840.2; Aldrich Chemical) or equivalent tertiary amyl alcohol, administered intraperitoneally.

Once each mouse had been anaesthetised, its lower abdomen and flanks were wet shaved to provide a clear window to the embryos, because hair and microbubbles of air trapped in the hair present an impenetrable barrier to ultrasound. The ultrasonographic examination was consistently performed with each mouse in dorsal recumbency. Imaging was started from one flank, and embryos were sequentially imaged across the lower abdomen to the opposite flank. An effort was made to obtain views approximating the transverse, frontal or sagittal planes. Characterisations and measurements of the size of the gestational sacs were obtained at 7·5 and 12·5 days of pregnancy. Head diameter (HD), body diameter (BD) and crown-rump length (CRL) were obtained at 16·5 days of pregnancy. Heart rate (HR) and heart peak blood flow velocities (HPBFV) were also obtained using colour flow Doppler ultrasound at 12·5 and 16·5 days.

All mice were found to be pregnant and were successfully diagnosed by ultrasound examination. The procedure took an average of 10 minutes in each case. In general, it was possible to differentiate those with large litters from those with small litters. Conceptuses were identified and measurements of the gestational sacs were then taken in longitudinal and transverse sections.

In the 7·5-day-old embryos, ultrasound permitted only the visualisation of gestational sacs that appeared relatively hypoechoic, approximately circular in shape (mean [sd] diameter 4·06 [0·6] mm) (Fig 1). In the 12·5-day-old embryos, the mean diameter of the gestational sac was almost doubled (7·9 [1·08] mm). The sac was oblong in shape, and heart activity was recognisable (Fig 2) (HR 172·29 [27·39] bpm, HPBFV 175 [64·55] m/s); in some gestational sacs, the CRL and HD could be assessed. In the 16·5-day-old embryos, fetal structures were easily visible, in particular it was possible to clearly differentiate the head from the trunk of the fetuses (Fig 3) (HD 4·45 [0·79] mm, BD 8·4 [0·76] mm,

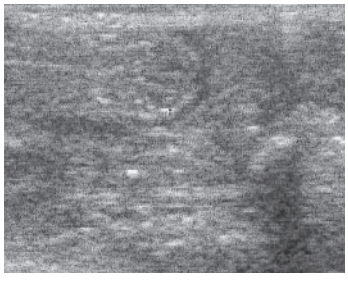

FIG 3: Mouse embryo at 16·5 days showing an easily distinguishable head (arrows) and cervical column (arrowheads)

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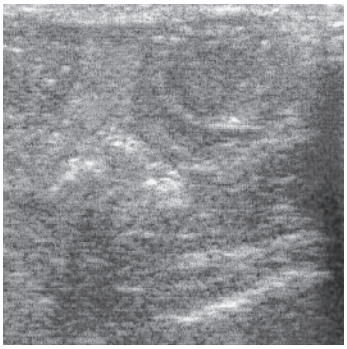

FIG 4: Longitudinal section through the uterus of a pregnant mouse, in which a disc-shaped placenta is visible (arrows)

CRL 17·8 [1·47] mm); the cardiac parameters were: HR 157·5 (43·5) bpm, HPBFV 175 (27·39) m/s. At this stage, it was possible to visualise the disc-shaped placenta that appeared 'quarter moon shaped' and hypoechoic compared with the embryos (Fig 4). All the results were based on a total of 204 fetuses examined.

The equipment used in the present study did not permit visualisation of fetal structures at 7·5 days or 12·5 days; however, at 16·5 days, some body structures such as the head, thorax and heart could be differentiated. Compared with other mammalian species, there is a limited amount of amniotic fluid in the mouse embryos (M. Russo, personal communication). In conclusion, conventional ultrasound is a valuable and non-invasive tool that can be successfully used in early pregnancy diagnosis in the mouse, as an alternative method to the surgical exposure of embryos.

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