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A new rat model of prenatal bowel obstruction: development and early assessment

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Abstract

Purpose: Although intestinal motility disorders often complicate the postoperative surgical management of newborns with congenital intestinal atresia, their pathogenesis remains unclear. Animal models of prenatal intestinal obstruction have been mainly developed in the lamb and the chicken. Despite new insights brought by these models, they have one or more limitations, such as high fetal mortality rates, high costs, long gestation periods, and an insufficient number of fetuses per litter. Moreover, some species are phylogenetically distant from mammals.

Methods: We developed a reproducible model of prenatal intestinal obstruction in the rat to study the histologic changes induced by the obstruction. We report, the technical devices and the first assessment of this atresia model in a didactic way to allow other researchers to easily reproduce the model.

Results: Prenatal intestinal obstructions in this study fulfilled all the macroscopic and histologic criteria usually listed by other models of prenatal intestinal obstruction that have been developed in other species. Furthermore with our model, we obtained a high success rate at a low cost.

Conclusions: We presented in this study a reproducible model of prenatal intestinal obstruction in the rat with the macroscopical and histologic features of prenatal intestinal obstruction.

In about 20% of newborns operated on for intestinal atresia, small bowel function remains poor, making it impossible to feed these children orally [1]. Disturbances in absorption and motility of the operated bowel have been suspected in humans and in animal models. Studies have been performed on large animal models, such as ewes or dogs, or on small animals, such as chickens. These models cannot provide prognostic factors to predict which of these patients are going to be severely affected by these postoperative disorders [2]. Indeed, most of them have one or more significant limitations, such as high fetal

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mortality rates, high costs, long gestation, or an insufficient number of fetuses per litter. Moreover, models based on species phylogenetically distant from mammals may be open to question and may not be sufficiently related to contribute to the understanding of these conditions [3]. Furthermore, they are restricted by an absence of modern tools to investigate them (immunohistochemical reagents, species-specific DNA sequences). A valuable animal model of prenatal intestinal obstruction will be able to generate reproducible results at 2 levels. First, this model will allow numerous technicians to obtain obstructed fetuses, and second, the features of the obstructed animals would be standardized to assess valuable analyses [4]. Moreover, it should be inexpensive, and there should be an availability of molecular probes and reagents for analyzing the pathophysiology of postoperative disorders encountered after surgical treatment of intestinal atresia. The aim of this study was to design an experimental model of intestinal obstruction in rat fetuses, which allows for reproducibility and an acceptable rate of success. All of the technical devices are illustrated by step-by-step pictures guiding the reader to reproduce the model.

1. Materials and methods

1.1. Animals

Animals were reared under French national guidelines (French law N°87-848). Timed and dated pregnant Wistar rats (Elevage Depré, France) were identified by analyzing vaginal smears twice a day to obtain accurate timings. Visualization of spermatozoa defined embryonic day 0 (day E0). The animals were transferred to the animal care facility 5 days before start of experimental work, to ensure optimal acclimatization. Animals were housed in groups of 2 per cage and were allowed food and water, ad libitum. Dams were housed in standard laboratory conditions, with constant temperature (21°C) and 12-hour day and night cycles.

1.2. Preoperative care and anesthesia

Food and water were not restricted before the anesthesia. Pregnant rats were anesthetized by a solution (0.7-0.9 mL per 200 g of body weight) composed of 7.5 mg of chlorpromazine (Rhône-Poulenc, Vitry sur Seine, France) and 250 mg of ketamine (Parke-Davis, Courbevoie, France) administered intramuscularly. Fetuses were anesthetized with 1% lidocaine (Roger-Bellon, Montrouge, France) injected into the amniotic cavity. Abdominal hair was removed with clippers, and the surgical site was prepared using a povidone-iodine solution (10% Betadine, Laboratoires Meda Pharma SAS, Paris, France). The procedure was carried out under a dissecting microscope (OPM1; Carl Zeiss, Le Pecq, France; magnification, ×8 to ×50) with microsurgical instruments. Intestinal obstruction was performed in fetuses of pregnant Wistar rats, between day E16 and day E20 of gestation. A midline laparotomy was performed on the dam rat, followed by exteriorization of one horn of the bicornuate uterus (Fig. 1A).

1.3. Fetus selection and positioning

Before performing surgery, fetuses have to be carefully selected (Fig. 1B-E). Anatomical preliminary studies on rat fetuses showed that the abdominal incision in the fetus should be performed on the fetal lower left abdominal quadrant to avoid damage to the liver. To reach this point without damaging the umbilical vessels, the fetus had to be selected in an amniotic cavity with the placenta on its right-hand side (Fig. 1B and D). Thus, the umbilical vessels run from the fetus through to the placenta on the right-hand side, ensuring free access to the operative target. This was determined by gentle palpation and visualization of the placenta through the transparent uterus (Fig. 1D and E).

1.4. Suture of the small bowel

After fetal selection, the upper and lower left limbs were then attached to the uterus using an 8/0 polypropylene (Prolene) stitch to facilitate exposure (Fig. 2A). A hysterotomy was performed with an 8/0 polypropylene purse-string suture incorporating amniotic membranes followed by a 3-mm incision within the purse-string suture overlying the fetal lower left abdominal quadrant. A full-thickness incision was initiated on the fetal lower left abdominal quadrant, with a 25-gauge needle, which then allowed the use of microsurgical scissors (Fig. 2A). The exteriorization of a single bowel loop was achieved by a gentle instillation of prewarmed sterile saline solution (37°C) into the fetal peritoneal cavity. A 10/0 polypropylene ligature was performed around the intestinal loop, which was reintegrated later into the abdominal cavity (Fig. 2B). The fetal abdominal wall was closed with a single 9/0 polypropylene ligature (Fig. 2C).

1.5. Abdominal closure and postoperative care

After the uterine cavity had been filled with a prewarmed saline solution, the purse string was tied (Fig. 2D). The stitches encircling the limbs were removed, and the uterus was returned into the maternal abdomen. The maternal laparotomy was closed in 2 layers with a continuous 4-0 silk suture. This surgical procedure was used for fetuses undergoing operation between days E17 and E20 of gestation. On day E16, the procedure was modified because midgut loop is located outside the abdominal cavity and only covered by a fine layer of tissue. The ligature was performed directly through the amniotic membrane (Fig. 2E). Control groups consisted of fetuses from pregnant rats time-dated