Physiology & Behavior 138 (2015) 101–106

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Intrauterine position as a predictor of postnatal growth and survival in the rabbit

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HIGHLIGHTS

- Intrauterine position is determined stochastically and non-genetically.
- It is an important determinant of fetal growth and resulting birth mass.
- Effects of implantation site on postnatal growth and survival were shown in rabbits.
- There were no such effects of the number of adjacent male fetuses.
- This has consequences for individual differences in phenotype and life histories.

ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form 21 October 2014
Accepted 24 October 2014
Available online 29 October 2014

Keywords:
Early development
Body mass
Individual differences
Sibling competition
Oryctolagus cuniculus

ABSTRACT

In mammals, body mass at birth is an important predictor of early postnatal growth and survival. Within litters, heavier young are more successful in competing for limited resources and show higher rates of growth and survival than their lighter sibs. In the present study, we investigated the contribution of two aspects of the intrauterine environment to within-litter differences in birth mass, growth and survival in the rabbit (*Oryctolagus cuniculus*): implantation site along the uterine horns and number of adjacent male fetuses. We used unilaterally ovariectomized mothers in order to infer relative sites of implantation from the birth order of pups from the single functional uterine horn. Pups from the extremities of the horn were significantly heavier at birth and weaning than their siblings from more central positions and had a higher probability of survival. The effect on body mass was still apparent 3 weeks after weaning in pups that had occupied positions at the ovarian end of the horn. The number of adjacent male fetuses did not affect individuals' growth or survival, and there were no differences between females and males. There were also no significant interactions between the different variables considered, indicating that the effects of implantation site on individuals' birth mass, growth and survival relative to littermates were independent of number of male neighbors, sex or litter size. Our study clearly demonstrates that in the rabbit, the site of implantation along the uterine horns is a major contributor to individual differences among littermates in early postnatal growth and survival.

1. Introduction

Interest has been growing in recent years in the developmental origins of individual differences in physiology and behavior and how such differences contribute to individual life histories and fitness [1,2]. In mammals, the early postnatal period is a critical life stage that a high percentage of young will not survive [3]. In litter-bearing species, an important factor influencing an individual's early growth and probability of survival is the presence of siblings [4,5]. There are often considerable individual differences in body mass among littermates at birth, and heavier young are typically more successful in competing for maternal and other resources than their lighter sibs [6–9,5]. This raises the question if and in what way features of the prenatal environment contribute to intra-litter differences in birth mass, an important predictor of postnatal development and life chances?

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http://dx.doi.org/10.1016/j.physbeh.2014.10.028
0031-9384/

In litter-bearing species, a notable feature of the prenatal environment is intrauterine position, that is, the site of implantation of a fetus relative to its littermates. This appears to be the result of a stochastic process, with no evidence to date for a genetic component [10]. In the rabbit (*Oryctolagus cuniculus*) and pig (*Sus scrofa*), for example, fetuses occupying end positions in the uterine horns (closest to the ovaries or to the cervix) are typically heavier than their more centrally located littermates [11–13]. In addition, the sex of neighboring fetuses can significantly affect the intrauterine environment of an individual. Testosterone produced by male fetuses can alter the development of litter siblings, in particular of those developing between two brothers [10]. This can have immediate and long-term effects on the morphological, physiological and behavioral development of littermates, including on postnatal body mass and growth [14,10]. In laboratory mice (*Mus musculus*), for example, young of both sexes located between two male fetuses (2M offspring) have greater postnatal body mass and long-term growth than fetuses with no male neighbors (0M offspring) [15,16]. It is presently unclear, however, whether this depends on a species' degree of sexual dimorphism. These two aspects of intrauterine position—the site of implantation along the uterine horns and sex of adjacent fetuses—may thus potentially interact to influence postnatal body mass, growth and survival in more complex ways than has usually been considered in the literature.

To our knowledge, there has been no longitudinal study investigating the relative contribution of these two aspects of intrauterine position to body mass at birth and so to differences among littermates from unmanipulated litters on postnatal growth and survival. It was therefore our aim to examine this in the domestic rabbit from birth until the post-weaning juvenile period. In domestic rabbit breeds, litters can comprise 14 or more altricial young, which can differ in body mass at birth by more than 100% [17,5]. Heavier pups are typically more successful in obtaining milk during the highly competitive once-daily nursing visits of the mother [6], and in occupying central, thermally advantageous positions in the litter huddle [18,7,19], of major importance given the rabbit's system of absentee mothering [20–22]. They are also more likely to survive the critical first postnatal week and to have greater body mass at weaning than their lighter sibs [23,17,9]. We hypothesize that in the rabbit the site of implantation along the uterine horns, with its known relation with fetal body mass, is a stronger predictor of postnatal growth and survival than the sex of neighboring fetuses.

2. Methods

2.1. Study animals

We used a total of 32 litters ($N = 220$ pups) of chinchilla-breed domestic rabbits from 20 different females each mated with one of 8 different stud males, bred and maintained at the Centro Tlaxcala de Biología de la Conducta. Females were kept in individual stainless steel cages 90 × 60 × 40 cm high and under fluorescent lights set to a 16:8 h light:dark cycle, which approximates conditions at the height of the summer breeding season for rabbits in Europe. Ambient air temperature was maintained between 17 °C and 24 °C, and water and food (Purina rabbit chow, Purina Mills, USA) were available ad libitum.

Experimental animals were kept and treated according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA, and the National Guide for the Production, Care and Use of Laboratory Animals, Mexico (Norma Oficial Mexicana NOM-062-200-1999).

2.2. Experimental procedure

2.2.1. Surgery

When the females for breeding were 4 months old, we removed the left ovary via a lateral ventral incision. Surgery was conducted by a qualified veterinarian after anesthetizing the females with 35 mg/kg (i.m.) of ketamine (Keminova, Mexico). Unilateral ovariectomy was done in order to establish pups' relative implantation sites (i.e. with respect to their siblings) along the right uterine horn as determined by birth order [24]. One month post-ovariectomy, we mated females by placing them individually with a stud male in a 1-m diameter arena, where copulation occurred within 5 min. After mating we returned females to their home cages.

2.2.2. Registration of birth order

At 10:00 h on gestational day 30, we transferred the females to a quiet room to register birth order. For this, each female was placed in a cage with a floor of 2.5 cm^2 wire mesh and mounted on the open frame of a table. A mirror was mounted below the table at an angle of 45° for continuous observation of the female's ventrum. At 09:00 h on gestational day 31, we induced parturition by the administration i.m. of 5 IU (1 ml) of oxytocin (Syntocinon, Basel, Switzerland). Approximately 8 min later (7.7 min ± 3.1 SD; measured in 6 litters) females started to give birth, adopting the typical crouching posture just before the expulsion of each pup. As each pup was born and fell through the mesh, it was caught by an observer who placed it in an open box divided into 10 sequentially numbered compartments. Meanwhile, a second observer took the pups in sequential order, cleaned them of birth fluids, and numbered them on the back (dark gray skin in this breed) with white correcting fluid for individual identification. This marking method is a standard procedure in our laboratory, where we have not found it to interfere with the behavior or health of the young [7,9,25,26]. The pups were then weighed on an electronic balance to the nearest 0.1 g and placed together in a nest-box in which a foster mother had built a nest and recently given birth (see below). The day of birth was taken as postnatal day 0. In total, birth order and birth mass was recorded for a total of $N = 220$ pups from 32 litters born to 20 mothers.

2.2.3. Postnatal procedures

Immediately after birth, 17 of the litters were assigned to another different experiment. The remaining 15 litters ($N = 110$ pups, born to 11 mothers) were brought to one of the 15 foster mothers according to whose nest box they occupied. The pups were allowed to suckle once before any human disturbance. To ensure the lactational readiness of the foster mothers, these had been mated one day before mating the mothers that gave birth to the experimental litters. We used foster mothers because in a pilot study we found that females treated with oxytocin to induce parturition did not always show appropriate nursing behavior.

2.2.4. Preweaning: day 1

At 10:00 h, after litters had been nursed once, each was removed in its nest box from the foster mother's home cage. The pups were individually weighed and their identification numbers were repainted; after day 7 when the pups had fur, the numbers were painted in their ears using a marker pen. They were then returned to their nest box, which was covered with flannel and kept separate from the foster mother but in the same room.

2.2.5. Days 2 to 25 pre-weaning

Each day at 11:30 h, pups were individually weighed and at 12:00 h the nest box containing the pups was placed in the foster mother's cage in a standardized way for the approximately 3-min once-daily nursing characteristic of rabbits [20,21]. As soon as the mother jumped away from the litter, the nest box was removed and the pups kept apart from the foster mother until nursing the following day. After weighing and nursing on day 25, we sexed the young, tattooed them in the ears for individual identification and placed them in individual stainless steel cages (34 cm × 34 cm × 30 cm height) with free access to water and standard pelleted food (Purina rabbit chow, Purina Mills, USA). Using the sex and birth order of each pup, we determined the number