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An ecologically relevant guinea pig model of fetal behavior

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HIGHLIGHTS

- IAF hairless guinea pigs were acclimated to ultrasound procedures without restraint.
- Behavior of fetal offspring was observed weekly across gestation.
- No elevation of cortisol was observed during procedures with pregnant females.
- Fetal development of movement coordination was similar to other species studied.
- Fetal behavior may be useful in the study of prenatal origins of disability.

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ABSTRACT

The laboratory guinea pig, *Cavia porcellus*, shares with humans many similarities during pregnancy and prenatal development, including precocial offspring and social dependence. These similarities suggest the guinea pig as a promising model of fetal behavioral development as well. Using innovative methods of behavioral acclimation, fetal offspring of female IAF hairless guinea pigs time mated to NIH multicolored Hartley males were observed longitudinally without restraint using noninvasive ultrasound at weekly intervals across the 10 week gestation. To ensure that the ultrasound procedure did not cause significant stress, salivary cortisol was collected both before and after each observation. Measures of fetal spontaneous movement and behavioral state were quantified from video recordings from week 3 through the last week before birth. Results from prenatal quantification of Interlimb Movement Synchrony and state organization reveal guinea pig fetal development to be strikingly similar to that previously reported for other rodents and preterm human infants. Salivary cortisol readings taken before and after sonography did not differ at any observation time point. These results suggest this model holds translational promise for studying the prenatal mechanisms of neurobehavioral development, including those that may result from adverse events. Because the guinea pig is a highly social mammal with a wide range of socially oriented vocalizations, this model may also have utility for studying the prenatal origins and trajectories of developmental disabilities with social-emotional components, such as autism.

1. Introduction

Recent interest in delayed effects from early insults, known as Prenatal Programming [1], Developmental Origins [2], or Fetal Basis of Adult Disease, suggests a need for longitudinal models that include prenatal observations of behavior. These models would not only link early changes in behavioral development with postnatal outcomes, but could also provide important information about mechanisms that underlie the early etiology of these phenomena. More importantly, models that systematically explore the ontogeny of prenatal behavior after insult would also provide translational information for the development of prenatal diagnostics [3]. These diagnostics could lead to early identification and treatment of developmental disabilities, which has been demonstrated to increase quality of life dramatically for affected individuals [4].

Despite possible benefits, few longitudinal animal models include prenatal observations of behavior. Recent advances in ultrasound technology, however, have made possible the addition of longitudinal study with smaller mammals such as guinea pigs [5–7]. Historically, the laboratory guinea pig (*Cavia procellus*) has proved an excellent model for human development due

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to numerous commonalities. For example, guinea pigs need folic acid and vitamin C during prenatal development because, like humans, guinea pigs cannot manufacture vitamin C [8]. Guinea pigs have a placental system similar to humans, which has been more extensively studied than that of any other non-human species [9]. Guinea pigs also have a high dependence on social structure [10,11]. Although not nearly as complex as human language, they exhibit a wide range and type of vocalizations for communication [12–17]. Similar to human attachment, developing guinea pig offspring demonstrate a crucial need for mother/infant bonding, in that pups not only exhibit signs of distress such as vocalization when separated from the mother, but develop symptoms of depression and even physical illness after only a few hours of separation [18,19].

As a result of this sensitive nature, guinea pigs have been an instrumental model in the study of stress, particularly during prenatal development and infancy [19–24]. This sensitivity requires special consideration during potential stress-inducing procedures. For example, the use of ultrasound technology as a non-invasive means to observe behavioral development longitudinally in the fetus often requires both restraint of the pregnant female, and removal of abdominal fur [6,7,25]. Restraint is a known stressor for rodents, and in fact is often used as a reliable method to induce stress [26]. Use of such methods with pregnant animals is doubly problematic, because elevated stress can significantly alter both behavior and hormonal development in the fetus [27].

In order to create a longitudinal model of prenatal development that is free from restraint and/or stress invoking procedures, we combine three features: (a) pregnant IAF hairless guinea pigs, (b) handling and acclimation techniques, and (c) non-invasive ultrasound to visualize fetal behavior. We hypothesize that the use of these techniques with the IAF hairless strain of guinea pig will result in data reflecting clear patterns of behavioral development in the fetal guinea pig, without raising levels of stress in the pregnant female, as evidenced by quantification of salivary cortisol. Based on prior studies with fetal rats and mice [28,29], specific patterns of behavior hypothesized include emergence of interlimb coordination as evidenced by increasing rates of synchronous limb movements across development.

2. Material and methods

2.1. Subjects

Subjects ($n = 5$) were heterozygous offspring of Institute Armand Frappier (IAF) hairless female guinea pigs (Crl:HA-Hr^{hr}, Charles River Laboratories, Kingston, NY) mated to an NIH multicolored Hartley male obtained from Elm Hill Labs (Chelmsford, MA). The hairless guinea pig was chosen to avoid the stress associated with shaving and/or depilatories during preparation of fur-bearing animals for fetal sonography. First identified from a spontaneous mutation in a colony of Hartley guinea pigs, the IAF hairless strain has been utilized in research for over 40 years. Unlike nude rats and mice, the hairless guinea pig is euthymic and possesses a functional immune system [30,31]. Developmental and breeding parameters are all nearly identical to the Hartley strain from which they are derived. Preliminary analyses of behavior in adults also reveal few differences between the IAF and Hartley strains [32]. When mated to Hartley males, the heterozygous IAF offspring are indistinguishable from the Hartley background animals. Offspring from this pairing are born with multicolored markings on their fur. These natural patterns of coloration facilitate easy identification after birth without tattoos or tagging [33,34].

IAF females were housed in pairs throughout mating, pregnancy, and lactation. Housing was standard polycarbonate (76 cm × 56 cm) caging lined with recycled shredded office paper. Guinea pigs were provided food (Teklad-7006 guinea pig pellets) and water ad libitum. Cages were maintained on a 12:12-h light/dark cycle in a temperature (23 °C) and humidity-controlled room. Each IAF female was also provided a Polar Tek fleece blanket (Monsanto) sewn into the shape of a tunnel. The fleece tunnel blankets provided the hairless animals an additional source of warmth, a place to hide, and an opportunity for socialization when shared with a cagemate. All females were also provided enrichment as a herd for 1 h each weekday [32].

Table 1
Pregnancy data.

A. Female IAF weight (g) with [95% confidence intervals][a]

Pre-pregnancy	**Week 1**	**Week 2**	**Week 3**
765.00	775.80	790.40	806.60
[697.91–832.09]	[700.54–851.06]	[699.92–880.88]	[734.78–878.42]
Week 4	**Week 5**	**Week 6**	**Week 7**
805.60	831.80	873.60	932.00
[728.03–883.18]	[747.80–915.80]	[799.10–948.10]	[846.93–1017.07]
Week 8	**Week 9**	**Birth**	
958.60	1009.60	1057.40	
[814.25–1102.95]	[846.44–1172.76]	[892.12–1222.68]	

B. Uterine position

Fetal Subject	Uterine horn	Number in horn	Total number of offspring	GA at birth (days)
1	Right	1	2	70
2	Left	2	3	68
3	Left	1	1	71
4	Right	2	2	68
5	Right	2	2	67

C. Postnatal growth (g) with [95% confidence intervals][a]

P1	P3	P5	P7
92.50	104.33	125.42	148.67
[86.55–98.45]	[90.68–117.99]	[110.94–139.89]	[133.31–164.02]

[a] Weight data presented are for group means.

During mating, a single male was placed in the cage with the two females until both females had cycled through a period of estrus. The timing of conception was determined by keeping a daily record of vaginal membrane readings of estrus. In female guinea pigs, the vaginal membrane is typically closed, except during estrus [35]. Prior records charting IAF estrus and delivery in our laboratory were used to determine that the day of conception (Gestational Age 0, GA 0) occurred on the first day of a fully open membrane. Using these metrics, and depending on the size of the litter, the hairless guinea pig generally delivers 2–3 offspring approximately 70 days after conception (Table 1).

At all times, guinea pigs and their offspring were cared for in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [36]. All procedures, husbandry, and care for the subjects in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Wright State University.

2.2. Salivary cortisol

Salivary cortisol samples were collected both prior to and after the ultrasound observations to determine if the ultrasound procedures resulted in elevated stress in the pregnant females. A period of at least 30 min elapsed between pre- and post-sampling. Saliva was collected using 2–3 cotton swabs per subject (Fisher Scientific), extracted from the swabs by centrifuge, and stored at −80 °C until processing. Cortisol levels were determined with an ELISA salivary