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Polytocus focus: Uterine position effect is dependent upon horn size

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ABSTRACT

Understanding the variability caused by uterine position effects in polytocus species, such as rats, may enhance prenatal animal models for the study of drug and environmental agents. The primiparous litters of 42 intact female Sprague-Dawley rats were studied. Uterine position, fetal body weight, and fetal brain (wet) weight were recorded on gestation day (GD) 20 (GD 0 = sperm positive). Uterine position effect for brain and body weight varied depending upon horn size. Furthermore, an inverse relationship between horn size (and, to a lesser extent, litter size) and fetal weight applied to both body and brain weight measures. There were no statistical differences in brain and body weights between the left and right uterine horns. The position of the uterine horn (left vs. right) and litter size did not influence the uterine position effect in the rat. Collectively, the present data suggest the presence of a significant uterine position effect. Prenatal differences based on uterine position provide an untapped opportunity to increase our understanding of developmental neurotoxicological and teratological studies that employ a polytocus species as an animal model.

1. Introduction

The prevalence of substance abuse disorder is widespread across the United States, currently affecting approximately 23.9 million people (National Institute of Drug Abuse, 2012). Approximately 90% of drug-abusing women are of reproductive age, increasing the risk of substance abuse during pregnancy (Kuczkowski, 2007). Substance abuse during pregnancy increases premature birth rates, decreases birth weight, and is associated with changes in neurobehavior, such as high arousal or depression (Lester et al., 2002). Animal models can be used to replicate the conditions of substance abuse disorder, studying various aspects of substance abuse that cannot be ethically studied in humans.

Approximately 95% of the animals used for preclinical research are rodents, including mice and rats (Foundation for Biomedical Research, 2014). Understanding the uterine position effect in polytocus species, such as the rat, may enhance prenatal animal models for drug and environmental studies. If uterine position has an effect on fetal body or brain weight, it is important to understand these effects so they can be considered when making inferences concerning the potential adverse effects of perinatal exposure to drugs or environmental agents.

Fetal growth may be affected by the relative intrauterine position of male and female fetuses in polytocus species. The variability caused by intrauterine position may explain hormonal, morphological and behavioral differences between two fetuses (Ryan and Vandenbergh, 2002). A significant amount of research has been conducted in rats (Bell and Hallenbeck, 2002; Nagao et al., 2004), mice (Hurd et al., 2008; Morley-Fletcher et al., 2003), rabbits (Argente et al., 2008; Banszegi et al., 2009), and sheep (Lang et al., 2003) to confirm the existence of an intrauterine position effect. Previous research has demonstrated a uterine position effect for fetal weight in the mouse and the rabbit specifically. These results suggest that the nature of the uterine position effect may be species dependent. In the mouse, the uterine position effect for fetal body weight is typically reported as the heaviest male and female fetuses being surrounded by two male fetuses (Kinsley et al., 1986). Furthermore, the heaviest fetuses typically occupy the ovarian and cervical ends of the uterine horn and the lightest fetuses occupy the middle horn position (Louton et al., 1988; McLaren and Michie, 1960). A contrasting uterine position effect is present in rabbits and pigs. Specifically, a linear relationship between fetal body weight and uterine position exists, such that the heaviest fetuses are located at the ovarian (tubal) end of the uterine horn and the lightest fetuses are located at the cervical end (Stuckhardt et al., 1981; Wise et al., 1997).

The intrauterine environment of the fetal rat has been shown, through hormonal (Hernández-Tristán et al., 2006) and uterine blood and nutrient supply (McLaren and Michie, 1960; Wentzel et al., 1995) to have an effect on growth pattern and fetal survival

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(Chahoud and Paumgartten, 2009). Such factors, and likely currently unknown differences in the intrauterine environment in rats, could cause significant variation in fetal body or brain weight as a function of uterine position of the fetus.

The uterine position effect for fetal body weight in the rat, contrary to the data for mice, rabbits and lightweight pigs, initially described the heaviest fetuses being found at the mid-horn position whereas the lightest fetuses were located at the extreme cervical and ovarian ends (Barr et al., 1969). This inverted U-shaped curve of fetal body weight as a function of intrauterine position has since been replicated in numerous studies (Barr et al., 1970; Barr and Brent, 1970; Jensh et al., 1970; Padmanabhan and Singh, 1981). Furthermore, previous data has suggested that there is a tendency for the right horn to carry more fetuses (Brent, 1965), although other researchers did not find the horns to be statistically different (Norman and Bruce, 1979; Zamenhof and Van Marthens, 1986). In contrast to this classic inverted U-shaped effect of uterine position on fetal body weight, other data suggests that the uterine position effect in the rat is more linear than curvilinear in nature (Norman and Bruce, 1979). Fetuses at the ovarian end of the horn were significantly lighter than fetuses in the middle or cervical end of the horn (Norman and Bruce, 1979).

Fetal body weight, in both rats and mice, is also affected, at least indirectly, by the number of fetuses occupying the individual horn, and to a somewhat lesser extent, by the overall litter size. Specifically, as the number of fetuses in both the horn and litter increases, the average fetal body weight decreases (Barr et al., 1970; Chahoud and Paumgartten, 2005, 2009; Ishikawa et al., 2006).

The possible existence of a uterine position effect for rat fetal brain weight has, in contrast, received very little attention, with one notable exception (Zamenhof and Van Marthens, 1986). Specifically, fetal brain weight, brain DNA (cell number), and brain protein was collected when the fetuses were at gestation day (GD) 21. The uterine location of the maximal fetal brain weight was not found to be significantly greater than other possible uterine locations using a Students *t*-test. Unfortunately, no direct within-litter intrauterine comparisons were available (i.e., only the maximal fetal brain and body weights were recorded).

Due to the disparity of findings on the nature of the uterine position effect as it relates to fetal body weight in the laboratory rat, the present study attempted to determine the nature of this position effect without some of the limitations dictated by in the methodology used in prior research. The studies that have reported uterine position effects for fetal body weight that were markedly different from the classic inverted U-shaped function have based their findings on data obtained from relatively few litters (as indicated above). These findings could, therefore, represent a sampling bias rather than a true uterine position effect. The seminal series of papers reporting the U-shaped function for mice subjected the pregnant animals to fertility enhancing drugs in order to produce very large litter sizes (McLaren and Michie, 1959). Accordingly, the current study sought to avoid the aforementioned limitations, by utilizing data from over 40 rats and avoiding fertility enhancing treatments, in the attempt to find any naturally occurring uterine position effect.

Thus the aims of the current study were threefold. First, to determine if a significant uterine position effect exists for fetal body weight, or fetal brain weight, in the Sprague-Dawley rat. Second, to determine if the relationship between horn size (or potentially litter size) and fetal weight applied to both body and brain weight measures. Third, to determine if other factors, such as litter size, horn size (i.e., number of pups per horn) or uterine horn position (left vs. right) influence the nature or expression of the uterine position effect in the rat. Prenatal differences based on uterine position provide an untapped opportunity to increase our understanding of developmental neurotoxicological and teratological studies that employ a polytocus species as an animal model.

2. Methods

2.1. Animals

Litters of 42 intact, female Sprague-Dawley rats were studied. Female and male animals were obtained from Harlan Laboratories, Inc. (Indianapolis, IN). Upon arrival at the animal care facilities, rats were placed in quarantine for 7 days, then transferred to the colony. Animals were paired-housed with members of the same sex until their use in the experiment. Rodent food (Pro-Lab Rat, Mouse, Hamster Chow #3000) and water were provided ad libitum. The colony was maintained at 21 ± 2 °C, 50 ± 10% relative humidity and a 12 h light: 12 h dark cycle with lights on at 7:00 AM (EST). The animal protocol for this research was approved by the institutional IACUC.

2.2. Procedure

Each female rat was placed with a male rat in a cage overnight, across consecutive days, until pregnancy was confirmed by presence of sperm with a vaginal lavage. The female rats were euthanized (by overdose with pentobarbital) on gestational day (GD) 20 and the maternal weight, the total number of pups in the litter, the total number of pups in each horn, and position of the fetuses in each uterine horn were recorded. The fetuses were subsequently removed from the uterus, the placenta and membranes removed, and then they were blotted dry and weighed to the nearest hundredth of a gram. Prior to weighing, the umbilical cords were clamped in order to prevent excessive blood loss during placental removal, and the fetuses were stored in plastic boxes containing moist sponges in order to prevent desiccation. Fetal position was identified and recorded as in the schematic shown in Fig. 1. After the fetal body weight was recorded, the fetal brain was removed, blotted dry, and weighed (wet weight) to the nearest thousandth of a gram.

2.3. Statistical analyses

For statistical analyses, the individual fetal body and brain weights were arranged as a function of their uterine position (see Fig. 1).

For example, in a uterine horn with 10 fetuses, the data from those occupying the mid position in the horn was placed at positions 5 and 6 (5 if there were an odd number of pups in the horn, or 5 and 6 if there were an even number). The data from the fetuses occupying the extreme cervical or ovarian ends were placed at positions 1 and 10, respectively. Data from subsequent pups were placed at positions 2 and 9, than at positions 3 and 8, and finally at positions 4 and 7, if there were the maximum of 10 pups in the horn. The mean fetal weights and the standard deviations were then computed collapsing across all litters and all horns.

Two data sets of these standard scores were created for comparison purposes. The deviation of each fetal weight from the appropriate mean (litter or horn) was then divided by the standard deviation of that mean, according to the formula: ((mean fetal weight) − (individual fetal weight))/(standard deviation). The unmodified data set included all the data except for the data from horns containing less than three pups or from litters containing less than six pups. A trimmed data set was also computed consisting of all the data in the untrimmed set, but it excluded individual fetal brain and body weights from fetuses that did not fall into the range of the mean

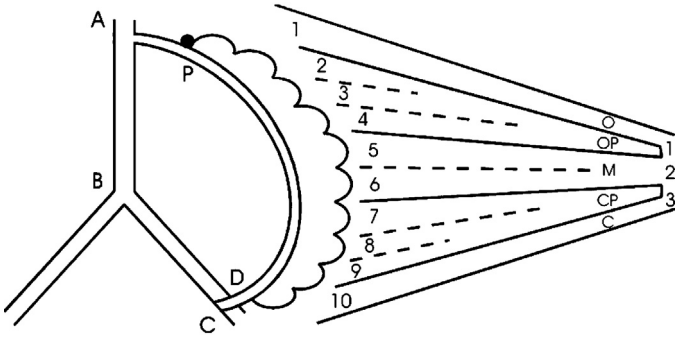

Fig. 1. Schematic representation of blood supply of uterine horn as well as identification and labeling of uterine position. *Abbreviations*: Point **A** represents the bifurcation of the ovarian artery from the abdominal aorta, point **B** represents the bifurcation of the right and left common iliac arteries from the abdominal aorta, and point **C** represents the bifurcation of the cervical artery from the iliac artery. Points **D** and **P** are the distal and proximal ends of the parametrial artery. ● Left ovary, blood flow is bi-directional in the parametrial artery, from **D** ↔ **P**. Identification of uterine position is as depicted, position 1 as the most proximal to the ovary. Adapted from Gorodeski et al. (1995) and Barr et al. (1970).