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Polytocus Focus: Uterine Position Effect is Dependent Upon Horn Size

Kristen A. McLaurin and Charles F. Mactutus

Program in Behavioral Neuroscience, Department of Psychology, University of South Carolina, Columbia, SC 29208

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.

Keywords

Uterine position; Polytocus; Rats; Prenatal growth; Drug abuse; Environmental agents

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 (Kuczkoski 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

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Address proofs and correspondence to: Charles F. Mactutus, Ph.D., Department of Psychology, 1512 Pendleton Street, University of South Carolina, Columbia, SC 29208, PH: +1 (803) 777-4137, FAX: +1 (803) 777-9558, mactutus@mailbox.sc.edu.

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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 Vandenberg 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 (Chahoud and Paumgarten 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. 1970b; Barr and Brent 1970a; 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; Zamenhoff 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. 1970b; Chahoud and Paumgarten 2005; Chahoud and Paumgarten 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 Student's *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.

Methods

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°C +/- 2°C, 50% +/- 10% relative humidity and a 12h light: 12h dark cycle with lights on at 7:00 AM (EST). The animal protocol for this research was approved by the institutional IACUC.

Procedure

Each female was placed with a male in a cage overnight, across consecutive days, until pregnancy was confirmed by presence of sperm with a vaginal lavage. The females were euthanized (by overdose with pentobarbital) on gestational day (GD) 20 and the maternal rat 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 Figure 1. After the fetal body weight was recorded, the fetal brain was removed, blotted dry, and also weighed (wet weight) to the nearest thousandth of a gram.

Statistical Analyses

For statistical analyses, the individual fetal body and brain weights were arranged as a function of their uterine position (See Figure 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, then 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: $((\text{mean fetal weight}) - (\text{individual fetal weight})) / (\text{standard deviation})$. 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 fetal weight collapsed across all litters $\pm$ one standard deviation of that mean. This range was defined at 2.96-4.58 g. After both the unmodified and trimmed standard scores were obtained, the mean standard scores for each position (1-10) were then computed, along with the n and standard deviation for each position. In order to obtain a mean standard score for fetuses occupying only the ovarian (position 1), ovarian penultimate (positions 2-4), mid position (positions 5-6), cervical penultimate (positions 7-9), and cervical (position 10) positions in each horn, the standard score means were averaged together. After obtaining mean standard scores for each of these five positions, an index of curvature for each individual horn's data was then determined utilizing the formula: (mean of fetal weight at ovarian and cervical positions) – (fetal weight at the mid position).

The data were also analyzed to determine if any other litter variable (i.e., horn size) had an effect on the uterine position effect. A two-way ANOVA (litter or horn size $\times$ uterine position) with repeated measures on uterine position was employed to analyze the fetal body and brain weight data (2V, BMDP Statistical Software, Release 7, Los Angeles, CA, 2009). In all cases the Greenhouse-Geisser df correction procedure was applied to repeated-measure terms for violations of compound symmetry (Greenhouse and Geisser 1959; Winer 1971). The uterine position effect as a function of body and brain weight was then analyzed graphically using GraphPad Prism 5.0 (GraphPad, San Diego, CA). Where appropriate, power (P) of the statistical analysis was computed via the SOLO power analysis module of the BMDP Statistical Package. Regression analyses were employed to determine the equations which best fit the uterine position data from the litters with most linear and most curvilinear indices of curvature. An α level of $p = 0.05$ was the significance level set for rejection of the null hypothesis.

Results

The fetal body and brain weights were obtained from 488 fetuses from 42 litters. The average litter size was 11.62 ($SD=2.75$) pups. The mean maternal rat weight was determined to be 311.7 ± 39.9 g. No significant differences in the number of pups per horn in each litter were found between the right and left horns, $F(1,76)=1.27$, $p = 0.264$. There were 243 fetuses in the left horn with a mean of 5.78 ± 2.33 pups per horn for each litter, and there were 245 fetuses in the right horn with a mean of 5.83 ± 1.91 g pups per horn for each litter. There were no significant differences in the mean fetal body weight, or brain weight between the left and right horns. The mean fetal body weight for pups located in the left horn was 3.64 ± 0.90 g, and the mean fetal body weight for pups located in the right horn was 3.86 ± 0.84 g. The mean fetal brain weight for pups was 0.17 ± 0.02 g in both the left and right horn.

As litter size and horn size increased, mean fetal body and brain weight decreased (Tables 1 and 2, Figures 2a and 2b). Linear regression analyses were performed on both the mean fetal body and brain weight data (Data from three litters with sizes of 4, 15, and 17 were excluded as these were the only representation of those litter sizes). Significant linear regressions were found for fetal body weight as a function of litter size, $(1,37)=4.65$, $r=0.334$, $p = 0.05$, and as a function of horn size, $F(1,75)=5.24$, $r=0.255$, $p = 0.05$. Linear regression for fetal

brain weight as a function of litter size did not reach statistical significance, $F(1,37)=2.77$, $r=0.264$, $p = 0.10$, but a significant linear regression was noted for fetal brain weight as a function of horn size, $F(1,75)=4.53$, $r=0.239$, $p = 0.05$.

A significant intrauterine position effect for fetal body weight was found $F(4,292)=8.17$, $p = 0.001$. As illustrated in Figure 3a, this uterine position effect was determined to vary according to horn size, $F(16, 292)=2.39$, $p = 0.005$. Horn sizes were divided at fewer than four pups and greater than eight pups to provide estimates of the upper and lower quartiles. Sprague-Dawley rats with more than eight pups showed a U-shaped curve of fetal body weight as a function of intrauterine position and horn size ($R^2=0.6219$). In contrast, Sprague Dawley rats with fewer than four pups showed a linear relationship of fetal body weight as a function of intrauterine position and horn size ($R^2=0.9943$). As previously stated, this uterine position effect was not influenced by the position of the horn (left vs. right). The uterine position effect was also not significantly influenced by litter size, $F(36,272)=1.03$, $p = 0.426$. Thus, the horn size, but neither the position of the horn (left vs. right), nor the size of the litter, influenced the nature or expression of the uterine position effect for fetal body weight.

A significant intrauterine position effect was also found for fetal brain weight, $F(4,288)=3.44$, $p < 0.01$. As illustrated in Figure 3b, this uterine position effect also varied according to horn size, $F(16, 288)=1.93$, $p < 0.05$. For Sprague-Dawley rats with more than eight pups, brain weight is the least at the mid position ($R^2=0.8720$). In contrast, for Sprague Dawley rats with less than four pups, brain weight is the greatest at the mid position ($R^2=0.6745$). As with the uterine position effect for fetal body weight, the uterine position effect for fetal brain weight was not significantly influenced by horn position, $F(4,300)=1.05$, $p = 0.382$ or by litter size, $F(36, 268)=1.16$, $p = 0.253$. Therefore, the horn size, but neither the position of the horn (left vs. right) nor the size of the litter influenced the nature or expression of the uterine position effect for brain weight.

A significant intrauterine position effect was also found for the brain weight (mg): body weight (g) ratio. As illustrated in Figure 4, this uterine position effect varied according to horn position (left vs. right) when the horn size was small, $F(1,8)=12.885$, $p < 0.001$. Sprague-Dawley rats in the left horn with four or fewer pups had a greater brain weight (mg): body weight (g) ratio than rats in the right horn with four or fewer pups. In contrast, the uterine position effect was not significantly influenced when the horn size was large, $F(1,13)=0.19$, $p = 0.892$. Thus, the position of the horn (left vs. right) influenced the nature or expression of the uterine position effect for the brain weight (mg): body weight (g) ratio only when horn size is small.

An index of curvature was determined for each set of standard scores. The index of curvature was determined by the equation: index of curvature = (mean of weights at position 1 and position 10) – (weight at mid position, mean of weights at position 5 and 6). Utilizing this index of curvature, the six most curvilinear and six most linear sets of standard scores for fetal body weight were determined and analyzed. The data from the six most curvilinear data sets for fetal body weight (Figure 5a) were determined to fit to a second-order polynomial equation, $F(1,5)=627.88$, $p = 0.0001$ rather than a first-order polynomial

equation, $F(1,5)=9.71$, $p=0.0264$. The data from the six most linear data sets for fetal body weight (Figure 5b) were determined to fit to a first-order polynomial equation, $F(1,5)=963.22$, $p=0.0001$ rather than a second-order polynomial equation, $F(1,5)=3.15$, $p=0.1359$.

Discussion

Variability in body and brain weight between fetuses in the polytocus rat may be explained by intrauterine position. Intrauterine position effects for brain and body weight in the Sprague-Dawley rat are dependent upon horn size. Furthermore, an inverse relationship between horn size (and, to a lesser extent, litter size) and fetal weight, applies to both body and brain weight measures. There was no statistical difference between the number of pups in the left and right horns. In addition, there was no statistical difference between the left and right uterine horns in regards to producing pups with similar brain and body weights. Other factors, such as the position of the uterine horn (left vs. right) and litter size, did not influence the uterine position effect in the rat.

Some previous research has indicated that the right uterine horn contains more fetuses on average than the left uterine horn in rats (i.e., right vs. left, 5.2 vs. 4.5, Barr et al. 1970b; Brent 1965), while other reports have concluded that there are no statistical differences between horns (i.e., right vs. left, 4.6 vs. 4.5, Zamenhoff and van Marthens 1986; Norman and Bruce 1979). The current study found not only no significant differences in the mean number of fetuses (right vs. left, 5.8 vs. 5.8), but also found no significant difference in mean fetal body weight, mean fetal brain weight, or any intrauterine position effect between right and left uterine horns in the rat.

A negative correlation was confirmed between horn size (and, to a lesser extent, litter size) and mean fetal body and brain weight. As the horn or litter size increased, both the average fetal body weight and average fetal brain weight decreased. These findings are consistent with the results of research conducted across a variety of polytocus species, including mice (Healy et al. 1960; Ishikawa et al. 2006; McLaren and Michie 1960; McLaren 1965), rabbits (Hammond and Marshall 1952), guinea pigs (Peaker and Taylor 1996) and other strains of rats (Barr et al 1970b; Chahoud and Paumgartten 2005).

Despite the fact that this negative correlation between litter/horn size and fetal weight has been found across a large variety of animal species, the predominant factor (litter size or horn size) for the correlation varies among species. The variations due to increases in litter size have been termed “general” effects, while variations due to increases in horn size have been termed “local” effects. Previous research has concluded that the general effect of variation in litter size was the predominant factor on fetal weight in rabbits (Hammond and Marshall 1952). In research conducted on guinea pigs, the general effect (litter size) was predominant in small litters, but the local effect (horn size) predominated in larger litters (Peaker and Taylor 1996). In mice, some research suggests that the local effect of horn size is the predominating factor (McLaren and Michie 1960; McLaren 1965), while more recent research suggests that the general effect of litter size is the predominating factor (Ishikawa et al 2006). Recent research on the rat has found that the general effect was the predominant

factor on fetal weight in rats (Chahoud and Paumgartten 2005). In contrast, the current study found that the local effect of variations in horn size was more important than the general effect of variations in litter size for both fetal body weight and fetal brain weight in the rat, which is consistent with research conducted by Barr, Jensh and Brent (1970b).

A significant intrauterine position effect was obtained for both fetal body weight and fetal brain weight. Overall litter size and position of the horn (left vs. right) did not influence the nature of the intrauterine position effect. However, for both fetal body and brain weight, the nature or expression of this intrauterine effect was influenced by horn size. Specifically for fetal body weight, a larger horn size (fetuses = 8) shows the classically observed inverted U-shaped curve with the heaviest fetuses occupying the mid position in the horn and the lightest fetuses at the extreme ovarian and cervical positions. However, for small horn sizes (fetuses = 4), the uterine position effect is linear in nature with the heaviest fetuses occupying the extreme cervical position and the lightest fetuses occupying the extreme ovarian position. Thus, the nature of the uterine position effect on fetal body weight varies as a function of horn size.

Similarly, the uterine position effect for fetal brain weight varies as a function of the number of horn size. For small horns (fetuses = 4), a curvilinear relationship was found with the heaviest brain weights at the mid position and the lightest brain weights at the extreme cervical and ovarian positions. However, for large horns (fetuses = 8), a contrasting inverted curvilinear relationship was found with the lightest brain weights near the middle of the horn and the heaviest brain weights at the extreme ovarian and cervical positions of the horn.

Previous research regarding the effect of intrauterine position on fetal brain development in the rat concluded that there was no statistical difference between the left and right horns with regard to fetal brain weight. Furthermore, there was no intrauterine position effect with respect to fetal brain weight found within the horn (Zamenhof and van Marthens 1986). The current findings are likely different because of procedural changes. Conclusions from previous research were drawn from only the heaviest fetus of each litter ('maximal fetus') while the current findings are based on all fetuses within one standard deviation of the mean fetal body weight.

A significant intrauterine position effect was obtained for the brain weight (mg): body weight (g) ratio when the litter size was small (fetuses = 4). For small horns (fetuses = 4), the left horn had a significantly greater brain weight (mg): body weight (g) ratio than the right horn. However, when the litter size was large (fetuses = 8), there was no significant intrauterine position effect for brain weight (mg): body weight (g) ratio. Therefore, the nature of the intrauterine position effect varies, once again, as a function of horn size. Understanding the intrauterine position effect for brain weight (mg): body weight (g) ratio provides additional resources to assess the effects of prenatal toxicology studies (Bailey 2004).

Although the current study clarified the nature or expression of a uterine position effect as it relates to fetal body and brain weight; the implications for animal models of exposures are of concern. Prior research has demonstrated conflicting evidence concerning the distribution

of both drugs and environmental agents varies across fetuses due to their position in the uterine environment (Lipton et al. 2002; Mactutus et al. 1998; Withey and Karpinski 1985; Withey et al. 1992; Withey et al. 1993; Wood et al. 1999). Across a total of 40 litters, styrene seems to be differentially distributed to rat fetuses based on their intrauterine position following maternal exposure to the substance (Withey and Karpinski 1985). The fetuses at the ovarian and cervical ends of the uterine horn had the lowest fetal body weight and the highest concentration of styrene following maternal exposure. In contrast, across a total of 298 litters, differential distribution to fetuses based on uterine position was not observed for diethylstilbestrol, zeranol, 3,4,3',4'-tetrachlorobiphenyl, or cadmium (Cornwall et al. 1984). Levels of both pyrene and benzo[a]pyrene in fetuses following maternal exposure to these substances showed no relationship to the relative position of the fetus in the uterine horn in a total of 40 litters (Withey et al. 1993; Withey et al. 1992). It is possible that the differential distribution effects to fetuses across exposure to pyrene and benzo[a]pyrene versus styrene has some physiochemical basis. Pyrene and benzo[a]pyrene have similar physiochemical properties that may determine their similar actions in fetal tissues. However, styrene may possess some unique physiochemical properties that cause this agent to be differentially distributed to fetuses based on uterine position. For example, physiochemical properties of styrene may influence physiological degradation and, therefore, lipophilic and hydrophilic metabolites of the agent may be retained within fetal tissues.

With respect to more commonly abused drugs, again, evidence for differential drug distribution as a function of uterine position has been reported. The levels of fetal cocaine may vary as a function of uterine position when the pregnant rat was subcutaneously administered 30 mg/kg (Lipton et al. 2002). In contrast, when using a clinically relevant route of exposure, the intravenous route, no differential distribution of cocaine was detectable as a function of uterine position (Mactutus et al. 1998; Wood et al. 1999). It is possible that the differential uterine position effects for cocaine may be dependent upon the route of exposure (i.e. subcutaneously vs. intravenously) and that other drugs, due to unique physiochemical properties, may be differentially distributed to the fetus based on uterine position.

Differential effects of environmental or drug exposure as a function of uterine position in rats does not appear to have received much investigation. Further, the lack of consistent research may be due to small sample sizes (Lipton et al. 2002), possible methodological limitations (i.e. subcutaneous injection, oral administration, Lipton et al. 2002; Cornwall et al. 1984) and unequal treatment sizes (Withey et al. 1993; Withey et al. 1992). Beck (1981) suggests that, when studying teratogens, numerous variables must be considered before the results can be extrapolated to the human population. Thus, it is vital to further understand the variability found in intrauterine position in rats so that experimental findings of environmental and drug abuse research may be more confidently extrapolated to the human population.

Collectively, the current study suggests that a significant uterine position effect exists in the Sprague-Dawley rat for both fetal body and brain weight measures and that this uterine position effect interacts with horn size: a different intrauterine position effect is noted for

large and small horn sizes. No other maternal or litter variables (i.e., horn position, litter size, etc.) were found to influence the uterine position effect. In future studies, the variability caused by intrauterine position should be controlled to provide more reliable and reproducible results. 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.

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Highlight Statements

- Intrauterine position within polytocus species may affect fetal growth.
- Uterine position effect for brain and body weight was dependent upon horn size.
- An inverse relationship exists between horn size and fetal brain and body weight.

Figure 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., 1970b.

(Single Column Image)

Figure 2.

a: Regression of fetal body weight as a function of number of pups in the litter or number of pups in the uterine horn. **Figure 2b:** Regression of fetal brain weight as a function of number of pups in the litter or number of pups in the uterine horn.

(2-Column Fitting Image)

Figure 3.

a: Fetal body weight as a function of uterine position and horn size. **Figure 3b:** Fetal brain weight as a function of uterine position and horn size. Standard scores for both fetal body weight and fetal brain weight were computed by calculating the horn mean, subtracting the individual fetal weight, and then dividing by the standard deviation collapsed across the horn. Therefore, a lower standard score indicates a heavier weight.

(2-Column Fitting Image)

Figure 4.

a: Fetal brain weight (mg) to body weight (g) ratio with 4 pups as a function of uterine position and horn. **Figure 4b:** Fetal brain weight (mg) to body weight (g) ratio with 8 pups as a function of uterine position and horn. Standard scores were computed by calculating the horn mean, subtracting the individual fetal weight, and then dividing by the standard deviation collapsed across the horn. Therefore, a lower standard score indicates a heavier weight.

(2-Column Fitting Image)

Figure 5.

a: Fetal body weight as a function of uterine position and horn size for the six litters with the smallest index of curvature. **Figure 5b:** Fetal body weight as a function of uterine position and horn size for the six litters with the greatest index of curvature. Standard scores were computed by calculating the horn mean, subtracting the individual fetal weight, and then dividing by the standard deviation collapsed across the horn. Therefore, a lower standard score indicates a heavier weight.

(2-Column Fitting Image)

Table 1
Mean fetal body weight (gm) by litter size and horn size

Size	<i>Litters</i>			<i>Horns</i>		
	Number of Fetuses	Number	Weight $\pm$ S D	Number of Fetuses	Number	Weight $\pm$ S D
0	0	---	---	1	0	---
1	0	---	---	2	2	4.550 $\pm$ 0.933
2	0	---	---	3	6	3.210 $\pm$ 0.973
3	0	---	---	7	21	3.963 $\pm$ 0.421
4	1	4	2.590 $\pm$ 0.186	7	28	4.430 $\pm$ 0.574
5	0	---	---	12	60	3.370 $\pm$ 0.657
6	2	12	4.397 $\pm$ 0.304	19	114	3.910 $\pm$ 0.881
7	0	---	---	17	119	3.660 $\pm$ 0.907
8	0	---	---	10	80	3.454 $\pm$ 0.756
9	6	54	3.895 $\pm$ 0.360	2	18	3.599 $\pm$ 1.004
10	6	60	4.111 $\pm$ 0.986	4	40	4.214 $\pm$ 0.980
11	4	44	3.737 $\pm$ 0.578	0	---	---
12	3	36	4.060 $\pm$ 1.166	0	---	---
13	10	130	3.405 $\pm$ 0.704	0	---	---
14	5	70	3.524 $\pm$ 0.704	0	---	---
15	3	45	3.680 $\pm$ 0.275	0	---	---
16	1	16	2.662 $\pm$ 0.183	0	---	---
17	1	17	5.921 $\pm$ 0.450	0	---	---

Table 2
Mean fetal brain weight (gm) by litter size and horn size

Size	<i>Litters</i>			<i>Horns</i>		
	Number of Fetuses	Number	Weight $\pm$ S D	Number of Fetuses	Number	Weight $\pm$ S D
0	0	---	---	1	0	---
1	0	---	---	2	2	0.182 $\pm$ 0.006
2	0	---	---	3	6	0.147 $\pm$ 0.024
3	0	---	---	7	21	0.170 $\pm$ 0.007
4	1	4	0.133 $\pm$ 0.008	7	28	0.181 $\pm$ 0.017
5	0	---	---	12	60	0.162 $\pm$ 0.011
6	2	12	0.174 $\pm$ 0.008	19	114	0.172 $\pm$ 0.018
7	0	---	---	17	119	0.166 $\pm$ 0.022
8	0	---	---	10	80	0.161 $\pm$ 0.013
9	6	54	0.171 $\pm$ 0.010	2	18	0.152 $\pm$ 0.020
10	6	60	0.174 $\pm$ 0.022	4	40	0.189 $\pm$ 0.028
11	4	44	0.169 $\pm$ 0.009	0	---	---
12	3	36	0.175 $\pm$ 0.020	0	---	---
13	10	130	0.161 $\pm$ 0.012	0	---	---
14	5	70	0.160 $\pm$ 0.011	0	---	---
15	3	45	0.173 $\pm$ 0.008	0	---	---
16	1	16	0.137 $\pm$ 0.005	0	---	---
17	1	17	0.235 $\pm$ 0.012	0	---	---