PERGAMON

Neuroscience and Biobehavioral Reviews 26 (2002) 665–678

NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS

www.elsevier.com/locate/neubiorev

Review

Intrauterine position effects

Bryce C. Ryan*, John G. Vandenbergh

Department of Zoology, North Carolina State University, Raleigh, NC 27695, USA

Received 6 April 2002; revised 7 July 2002; accepted 30 July 2002

Abstract

A review of the literature suggests that individual variability in sex-related traits may be influenced by variations in hormonal exposure during fetal development. In litter-bearing mammals, fetuses develop in utero and may be subjected to differing hormonal environments based upon the sex of neighboring fetuses. Female fetuses developing between two males tend to show masculinized anatomical, physiological and behavioral traits as adults. Female fetuses developing without adjacent males, on the other hand, tend to show more feminized traits as adults. These traits include permanently altered hormone levels, reproductive organs, aggressive behaviors, secondary sex ratios and susceptibility to endocrine disruption. This intrauterine effect is due to the transfer of testosterone from male fetuses to adjacent fetuses. While these effects have been most clearly demonstrated in mice, other rodents and swine also show intrauterine position (IUP) effects. Some of these effects are similar to the influence of prenatal stress on adult phenotypes. A few reports on human twins suggest that variability in some masculine and feminine traits may be due to intrauterine hormonal signals. IUP effects may impact a number of scientific fields of research such as endocrine disruption, toxicology, population biology, animal production and health.
Published by Elsevier Science Ltd.

Keywords: Anogenital distance; Behavior; Endocrine disruption; Human; Intrauterine position; Rodent; Stress; Testosterone; Toxicology

Contents

* Corresponding author. Tel.: +1-919-515-3883; fax: +1-919-515-5327.
E-mail address: bcryan@unity.ncsu.edu (B.C. Ryan).

0149-7634/02/$ - see front matter Published by Elsevier Science Ltd.
PII: S0149-7634(02)00038-6

1. Introduction

> It has been disputed at what period of life the causes of variability, whatever they may be, generally act; whether during the early or late period of development of the embryo, or at the instant of conception
>
> –Charles Darwin 'On the Origin of Species'[31]

Explaining variability lies at the heart of biology, yet at the same time this variability must be experimentally controlled. Variability is typically controlled by standardizing the genetic background of the subjects and the environment in which they are maintained after birth. Here we review another source of variability not often recognized. This variability is not genetic or environmental in origin, but rather hormonal.

Intrauterine development in eutherian mammals allows for precise control of the fetal environment and allows the fetus to directly interact with the mother. In litter bearing mammals, pups from the same litter must share space in their mother's uterus. This space sharing results in pups from large litters developing in slightly different environments from each other. For example, a mouse or swine fetus that occupies a position at either end of a uterine horn receives more nutrient rich blood and is subsequently heavier at birth than other fetuses [6,117,125]. A rat fetuses located at the cervical end of the uterus receives maternal blood flow prior to fetuses in other uterine positions [42,67]. Any fetus not located at an end of a uterus will be located between two males (2M), two females (0M), or one male and one female (1M) (Fig. 1). This intrauterine position (IUP) has significant and wide-ranging effects on the development of the fetus.

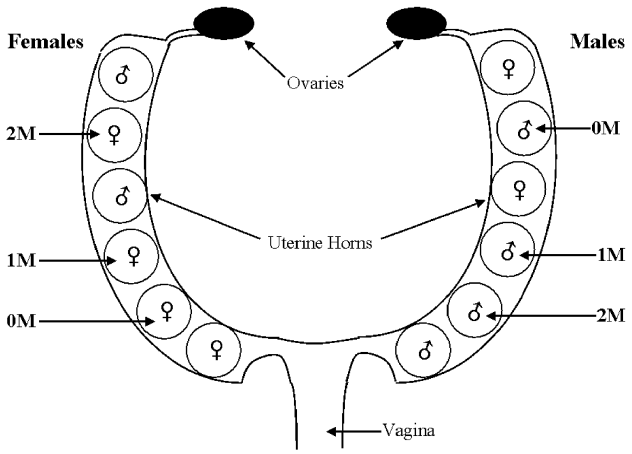

Fig. 1. Schematic illustrating fetus positions in a womb. The 0M–2M classification refers to the number of males flanking the fetus. This classification is the most common categorization system used in the literature. However, this system does not appear to be the most appropriate classification for rats or swine. In these species, the number of fetuses in the entire uterine horn must be taken into consideration.

In most mammals, male fetuses produce testosterone earlier and in higher amounts than females. Females, on the other hand, produce higher amounts of estradiol later in development. Being steroids, these hormones can diffuse through the amniotic fluid between fetuses. As a result, both male and female 2M mouse fetuses (flanked by males) have higher blood concentrations of testosterone and lower blood concentrations of estradiol than 0M fetuses (flanked by females) [122]. This mechanism of hormone transfer among mouse fetuses has become fairly well understood and accepted.

In addition to diffusion through the amniotic fluid, hormones may also travel among fetuses through the bloodstream of the mother. In pregnant rats, uterine blood flows predominantly in the caudal to distal direction (i.e. from the cervix to the ovaries) [42]. Fetuses located distally to male pups will thus be subjected to higher levels of testosterone and fetuses located caudally to male pups will not be subjected to high testosterone levels. Interestingly, this uterine blood flow also results in fetuses from the same litter receiving differing levels of cocaine (and presumably other drugs) administered to their mother [67,124]. Pregnant mice, in contrast to rats, show bi-directional blood flow through the uterus [117]. This makes a one-way movement of hormones among mouse fetuses unlikely. It is not known if the differences in circulation play some predictable role in developmental differences or IUP differences between species. However, this physiological difference between rat and mouse circulation has resulted in most investigators classifying rat fetuses by the number of males located upstream (caudal male hypothesis), whereas mice and gerbil researchers have maintained the classical 0M–2M classification (contiguous male hypothesis).

Regardless of the exact mechanism, it is clear that same-sex fetuses from the same litter will be subjected to different levels of steroid hormones. Many mammals seem to rely on this variable uterine environment to develop normally. Both gerbils and mice that do not have any littermates mature abnormally and reproduce poorly [23,40]. IUP, therefore, serves as a source of non-genomic variability in these animals.

Sexual differentiation in mammals is largely mediated by androgens early in development. Variable levels of androgens will therefore alter this process of differentiation. 2M pups subjected to high levels of androgens will show increased masculinization and 0M pups subjected to low levels of androgens will show increased feminization. Steroid hormone transfer among fetuses not only alters development, but it increases variability among individuals from the same litter. From an experimental perspective, variability is unwanted and may mask potentially significant results. For example, IUP alters sensitivity to certain endocrine disruptors [56,57] and therefore serves as an additional source of variation. Laboratory rodents are commonly used experimentally because of their genetic homogeneity and low variability. It is important to

understand the potential implications of this IUP effect in order to control and minimize this endogenous, non-genetic, variability.

The first report of an IUP effect came at the 68th Annual Meeting of the American Society of Zoologists, in 1971. At this meeting, Clemens and Coniglio [26] reported a "positive relation between the probability of female mounting and the number of males which were present during intrauterine development. This relation may suggest that the behavior of the female is influenced by fetal testicular secretions of male sibs". The initial work on rats was expanded in a subsequent publication [25]. It was followed up more extensively in mice [114], giving rise to a field of research that has remained active for 30 years and produced over 100 publications.

The purpose of this review is to identify areas of research where knowledge of IUP effects can reduce variability and increase the chance of detecting reproducible results. IUP has the potential to influence results in a wide variety of mammalian studies. A number of important fields that seem particularly susceptible to IUP effects will be discussed. This review will hopefully stimulate more efficient research design in these fields that may not be intuitively linked to IUP.

The recent public and scientific concern over the effect of hormone mimics in the environment has simulated a vast amount of work in the relatively new field of endocrine disruption. Similar concerns regarding low dose and mixture effects of certain toxicants have surfaced in recent years as well [52]. IUP, by altering prenatal endogenous hormone exposure, has the potential to interact with experimental manipulations of endocrine disruptors.

IUP has the ability to influence aggression, dispersal and mating in wild populations and could therefore affect studies dealing with the population biology of mammals as well as allow commercial breeders to have greater control over their animal production. IUP can also influence immunological responses as well as enzyme levels in the body and could therefore become important for general animal health and human health.

This paper reviews the literature generated in the past 30 years and is organized by specific IUP effects on physiology, morphology and behavior. Interactions between prenatal stress and IUP are also discussed.

2. Physiology

2.1. Hormone levels

A 2M female mouse fetus has a higher level of testosterone in both her blood and her amniotic fluid than does a 0M female [115,122] (Table 1). A female rat fetus located distally to males also has a high level of plasma testosterone [53], although this has not been shown in every case [47]. Both 2M male and female gerbil fetuses show high testosterone levels [13,22] (Table 2). These differences appear to originate from the male pups surrounding a fetus and not the mother's circulation. In mice, the difference in hormone levels drops off by adulthood [115]. Adult 2M male gerbils, however, retain a high level of plasma testosterone throughout life [22]. Swine do not show this difference in fetal testosterone levels [125]. The influence of IUP on estradiol levels is unclear. In one study, 0M female mice had higher fetal concentrations of estradiol than 2M mice [119]. Another study found no significant differences [115]. Differing levels of prenatal testosterone will alter the organizational function of this hormone and have effects that persist through adulthood. In addition, differing levels of adult steroid levels will obviously influence any activational steroid function as well.

Table 1
Selected IUP effects demonstrated in mice affecting physiology, morphology and behavior. It is also apparent that female mice are more sensitive to these effects than are male mice

Sex	0M	2M	Source
Physiology			
♀	Lower fetal testosterone levels	Higher fetal testosterone levels	[114,122]
♀	Earlier vaginal opening	Later vaginal opening	[71,113]
♀	Less male offspring	More male offspring	[105]
♀	Mate and impregnated earlier	Mate and impregnated later	[113]
♀	More sensitive to bisphenol-A	Less sensitive to bisphenol-A	[57]
♂ & ♀	Less sensitive to testosterone	More sensitive to testosterone	[41,119]
Morphology			
♀	Shorter AGD	Longer AGD	[41,105,114]
♂	Lower 5α-reductase levels	Higher 5α-reductase levels	[80]
Behavior			
♀	Less likely to mount other females	More likely to mount other females	[84,89]
♂	Less parental behavior	More parental behavior	[110,112]
♂ & ♀	Smaller home range	Larger home range	[32,129]
♂ & ♀	Less aggressive	More aggressive	[32,41,114]

Table 2
Selected IUP effects demonstrated in gerbils affecting physiology, morphology and behavior. In contrast to other rodents, male gerbils appear to be more sensitive to these effects than are female gerbils

Sex	0M	2M	Source
Physiology			
♀	Earlier first estrus	Later first estrus	[14,18]
♀	Less male offspring	More male offspring	[18,23]
♂	Lower adult testosterone levels	Higher adult testosterone levels	[22]
♂	Less attractive to females	More attractive to females	[21]
♂	Lower impregnation rate	Higher impregnation rate	[17]
♂ & ♀	Less fetal testosterone, more estrogen	More fetal testosterone, less estrogen	[13,22]
Morphology			
♂	Shorter AGD	Longer AGD	[19]
♂	Smaller bulbocavernosus and levator ani	Larger bulbocavernosus and levator ani	[38]
Behavior			
♂	Scent mark less often	Scent mark more often	[19,23]
♂	More parental behavior	Less parental behavior	[17,24]
♂ & ♀	Receive less parental attention	Receive more parental attention	[12]

2.2. Sensitivity to testosterone

Adult 2M female mice are more sensitive to testosterone than 0M females. This is shown by higher concentrations of epidermal growth factor in the submandibular glands of 2M mice, a response to increased testosterone bioactivity [5]. 2M female mice also require a shorter duration of testosterone treatment to induce aggression [41]. Female rats located distally to male rats in utero possess an increased sensitivity to testosterone as adults, showing an increase in mounting behavior, a known response to androgen treatments [54,97]. 2M female rats also show increased mounting behavior [27] and these females became sterile faster and anovulatory sooner than 0M females when injected with testosterone, again showing an increased sensitivity to testosterone [102]. Sensitivity to estrogen does not appear to be correlated with IUP, as measured by uterine weight and luteinizing hormone levels in mice [114]. However, more 0M males are mounted after treatment with estradiol than are 2M males, indicating that 2M males are more defeminized than are 0M males [110]. A pup's prior IUP can clearly influence sensitivity to androgens when adult. This sensitivity difference could theoretically alter any and all functions of the steroid hormones in adult animals.

2.3. Enzyme levels

IUP alters enzyme levels in a variety of different tissues. 0M female swine have higher levels of thymosin β4 than 2M females. This could be due to differing thymic function between 0Ms and 2Ms and may lead to differing immunological function between these animals [125] (Table 3). It is therefore possible that animals from different IUPs may have different susceptibility to diseases or stress.

0M female rats show an increased testosterone 5α-reductase activity [107]. 0M male mice have low 5α-reductase levels in the seminal vesicles and prostate. This could be due to high estrogen levels interrupting the normal organizing effect of testosterone in these animals [80]. 5α-Reductase converts testosterone to dihydrotestosterone, an androgen with high activity in many tissues. Altering 5α-reductase levels will alter dihydrotestosterone levels, which in turn will influence many areas of androgen activity, especially in the development of secondary sex characteristics. Aromatase, like 5α-reductase, shows sexual dimorphism in its activity. Aromatase, however, does not appear to vary as a result of IUP [103], nor do other testicular steroidogenic enzymes [80].

2M female gerbils show higher cytochrome oxidase reactivity, a measure of metabolism, in certain areas of the anterior hypothalamus [61]. 2M male rats show an increased level of NADPH cytochrome c reductase in their hepatic microsomes. In comparison to 0M females, 2M female rats show increased levels of cytochrome p450 in their hepatic microsomes. They also show an increased aniline hydroxase and 7-ethyoxycoumarin *O*-deethylase activity [107]. These results show that IUP has the ability to alter the metabolic functioning of multiple tissues in adult animals and suggests that IUP may alter the metabolism of selected toxins. Certain toxins show a sexual dimorphism in their metabolism [51]. It follows that IUP may influence the ability of an animal to metabolize selected toxins as well. 2M male rat pups show a decreased preference for ethanol when their mothers were given alcohol, possibly because 2M and 0M pups metabolize this alcohol with different efficiencies [70]. Similar trends may be seen with other toxins as well.

2.4. Reproduction

IUP can influence the onset of puberty and reproductive capacity. 0M female mice and gerbils show an earlier age at

Table 3
Selected IUP effects noted in several species demonstrating a similarity between these results and the results noted in mice and gerbils, indicating that IUP effects can impact a number of different species

Ferrets			
Sex	Located distally from <2 ♀s	Located distally from 2 + ♀s	Source
♀	Lower fetal androgen level	Higher fetal androgen level	[66]
Hamsters			
Sex	Located distally from 2 + ♂s	Located distally from <2♂s	Source
♂	Higher estrogen, lower testosterone levels	Lower estrogen, higher testosterone levels	[108]
Meadow voles			
Sex	Female biased litter	Male biased litter	Source
♂ & ♀	Better performance in a water maze	Worse performance in a water maze	[39]
Swine			
Sex	0M or female biased litter	2M or Male biased litter	Source
♀	Higher thymosin β4 levels	Lower thymosin β4 levels	[125]
♀	Inseminated more often	Inseminated less often	[33]
♀	Shorter AGD	Longer AGD	[33]
♀	More teats	Less teats	[35]
♂ & ♀	Less aggressive	More aggressive	[90]
Humans			
Sex	Same sex dizygotic twins	Opposite sex dizygotic twins	Source
♀	More acoustic emissions	Less acoustic emissions	[72,73]
♀	Lower levels of sensation seeking	Higher levels of sensation seeking	[87]

vaginal opening and earlier age at first estrus than do 2M females [13,14,18,71,105,113]. Anogenital distance (AGD) is a morphological measurement sensitive to prenatal testosterone levels (see Section 3.1). A female rat with a short AGD (more likely to be 0M) experiences vaginal opening and first estrus at an early age [127]. 0M female mice also produce more potent estrous delaying pheromones than do 2M mice, but do not appear to differ in sensitivity to males' estrous promoting cues [105,113,114,121].

A female mouse with a short AGD is more likely to become pregnant [32] and 2M females produce fewer viable litters overall and cease giving birth at younger ages than do 0M females [120]. 2M females tend to produce larger litters, however, especially later in life [105,118], whereas 0M mice produce more young in their first litter [63].

Female swine from female biased litters (thus more likely to be 0M) are inseminated more often and have fewer pregnancy failures than females from male biased litters (more likely to be 2M) [33]. 0M female swine are also more receptive to male advances [90]. Consequently, females exposed to few males in utero should have increased reproductive capabilities as adults.

2M male gerbils impregnate more females [17], are more attractive to females and sire more young per litter than do 0M males or isolate males [21,23]. In gerbils, a unique subset of 0M males exists. A proportion of 0M males has circulating testosterone levels similar to those seen in females and much lower than seen in other males. These 0M males do not show normal sexual behavior and are incapable of impregnating females. These same males also show much higher parental behavior than is normally seen in other 0M males [17]. It has been hypothesized that this subset of asexual, parental males may help increase a dam's fitness more than having all reproductive, non-parental males.

2.5. Secondary sex ratio

IUP alters secondary sex ratios (i.e. the ratio of male pups to female pups at birth). As adults, 2M female gerbils give birth to almost 60% male pups. Conversely, 0M females, and singleton females, give birth to about 40% male pups. 1M mothers produce the traditional 50% sex ratio [11,15,16,23]. Similar reproductive outputs are also seen in mice [50,104,105]. These effects do not seem to be caused by selective cannibalism because the litter sizes of 0Ms and 2Ms are similar, and the ratios remain if the pups are delivered via a cesarean section. The mechanism behind this phenomenon is unknown, however, the timing of insemination may cause these shifts in the sex ratio. Female rats inseminated close to the time of ovulation produce more females than males, while females inseminated a few hours before or after ovulation produce more males than females [46]. Interestingly, a similar 'U' shaped insemination curve has been reported in humans as well (for a review, see [60]).

This phenomenon has never been reported in mice or gerbils, but 0M female mice (and rats) have shorter estrous cycles and are more sensitive to the presence of pheromones that may alter the length of the estrous cycle [113,114,116,121,122,127].

This phenomenon of altered secondary sex ratio may serve as a form of non-genetic inheritance from mother to daughter. 0M females give birth to a high number of females and therefore, these females will be more likely to be surrounded by females in utero. In other words, a 0M female is likely to produce high numbers of 0M female. Similarly, a 2M female will be more likely to produce 2M offspring than will other mothers.

2.6. Asynchronous development

Gerbils show sexual dimorphism in the eye that opens first in pups. Females are more likely to have their right eye open first, and males are more likely to have their left eye open first. 0M males have a high proportion of right eyes opening first, and 2M females have a low proportion of right eyes opening first. A similar trend is seen in paw preference, with 0M gerbils holding the right paw aloft more than 2M gerbils [20]. These findings suggest that IUP may alter the development of laterality in the nervous system in gerbils.

2.7. Environmental estrogens

0M female mice are more sensitive to the environmental estrogen bisphenol-A than are 2M females. When exposed to this chemical prenatally, 0M females show an abnormally short interval between vaginal opening and first estrus. In addition, both male and female 0M and 1M mice are heavier at weaning than 2M mice [56,57]. These findings suggest that pups developing in the 0M position are more sensitive to bisphenol-A than other pups. These results are interesting due to the recent concern surrounding the harmful effects of environmental endocrine disruptors and reveal an important connection between IUP and endocrine disruption.

3. Morphology

3.1. Anogenital distance

Anatomically, a 0M female mouse has a shorter distance between the anus and the genital papilla than does a 2M female. This AGD difference is not surprising, as male rodents generally have longer AGDs than females. 2M female mice, subjected to high levels of testosterone, have a more masculine AGD. The AGD is simple to measure and, in mice, has become widely used as a marker for a female pup's prior IUP [41,62,71,82,105,114,117,122,128].

In addition to mice, female rats located downstream from males have longer AGDs than other females [25,55,88] as do 2M females [25,102]. This increase in AGD is most likely due to increased testosterone levels in utero because treatment with the anti-androgen flutamide abolishes the effect [25,27]. In contrast to the findings in mice and rats, 2M male gerbils possess longer AGDs than 0M male gerbils, with females showing no difference [19]. It is unclear why male gerbils appear to be more sensitive to fetal steroids than female gerbils, while in other rodents females show a higher sensitivity.

IUP effects on AGD in swine are unclear. Drickame *et al.* [33] found a significant effect of litter composition on females' AGDs when looking at the total number of males in utero. Rohde Parfet *et al.* [91] found no effect when using a 0M–2M classification system. A fetal pig, like a rat, may be sensitive to the hormonal contribution of every fetus, not just those directly surrounding it.

In mice and rats, some of the variability present in the AGD can be explained by the weight of the animal being measured. Heavier animals tend to have longer AGDs than lighter animals. Therefore, a more accurate measurement can be obtained by dividing the AGD by weight, yielding an AGD index (AGDI). The AGDI can, in some cases, serve as an accurate marker for a mouse pup's prior IUP [43,104,105] and for a rat pup's prior IUP [75]. A number of studies, however, have found that variations in weight do not account for a significant portion of the variability in AGD measurements [81,117]. A sensible policy would be to use an analysis of covariance to evaluate the importance of weight to the variability seen in AGD measurements before calculating an AGDI.

The AGD-IUP correlation allows for the identification of an adult animal's prior fetal position and in some cases eliminates the need for cesarean sections. Using the AGD as a biomarker for intrauterine hormone exposure is simple and non-invasive can be used on wild caught animals. This technique makes true field research on IUP effects a possibility.

3.2. Reproductive organs

2M male mice have larger seminal vesicles and smaller prostate glands than do their 0M brothers. These 2M mice also show lower androgen binding levels in their prostates but no difference in estrogen binding levels [80]. Similarly, 2M male rats [107] and gerbils [11,19] possess heavier testes than do 0M males. In contrast, male testis weights in swine do not vary with IUP [91]. The number of teats on female pigs, however, is partially correlated with the number of males in her birth litter. Females from male biased litters have fewer teats than females from female biased litters [35]. Nipple number in rats is sensitive to prenatal exposure to androgens or anti-androgens [44,79,126], however, nipple number has never been shown to correlate with IUP in the rat.

3.3. Other morphology

IUP influences body weight, with 2M mice of either sex weighing more than 0M mice at all ages tested [64,81]. This could be caused by differing metabolism, differing responses to stress or differing levels of aggression among the individuals in a population.

2M male gerbils possess heavier ventral scent glands than do 0M males [11,19,21,22]. 2M male gerbils also have a large bulbocavernosus and levator ani musculature, a sexually dimorphic muscle that is responsible for controlling penile reflexes. 2M females have an increased number of motoneurons in the spinal nucleus of the bulbocavernosus as well [38]. Differences in sexual morphology may explain some of the differences in reproductive efficiency seen in male and female gerbils from different IUPs.

Compared to females, male rats have a significantly larger sexually dimorphic nucleus of the preoptic area of the hypothalamus (SDN-POA). As expected, females with a large AGD also possess a significantly larger SDN-POA than females with a small AGD [37]. IUP does not appear to influence the size of the sexually dimorphic hippocampus in gerbils, however [93], nor does it influence corpus callosum size in mice [6]. It is clear, however, that IUP can influence the development of some areas of the nervous system and may subtly influence a variety of different behaviors in this fashion.

4. Behavior

4.1. Aggression

Adult 2M female mice injected with testosterone show greater frequencies of chasing and biting, and initiate fights more often than do 0M females receiving the same treatment. 2M females also begin displaying aggression after a shorter duration of testosterone treatment than do 0M females [41]. This again shows that 2M females are more sensitive to testosterone as adults than are 0M females. IUP not only alters sensitivity to testosterone, but it also influences endogenous levels of aggression. 2M female swine participate in and win more fights than 0M females [90]. Like swine, 2M female mice initiate more fights than do 0M mice, 2M mothers fight for a longer duration than do 0M mothers and pregnant 2M mice show more intense aggression than do 0M mice [63,84,114]. One study contradicts this, however [50]. These differences in aggression may be related to lower anxiety levels, as masculinized 2M female mice show less shock avoidance than 0M females [45].

Similarly to females, castrated 2M male mice require a shorter duration of testosterone treatment to induce aggressive behavior than do 0M males. This suggests that 2M male mice are more sensitive to testosterone treatment than are 0M males despite the fact that 2M male fetuses have never been shown to be exposed to higher levels of testosterone in utero [119]. Conversely, castrated 2M males show greater parental behaviors than castrated 0M males [110,112]. This result seems confusing, but may simply indicate that testosterone stimulates both aggressive behavior and parental behavior. In contrast, IUP does not appear to influence maternal behavior in female mice [63].

Even though IUP has never been correlated with AGDs in male mice, males with large AGDs are more aggressive than males with small AGDs [32]. 2M male swine gain more weight when placed on restricted feed and group housed with pigs from all IUPs, indicating that 2M male swine are more aggressive than other swine and therefore take more food for themselves [91]. High aggression in swine does not always correlate with social dominance, however [34].

4.2. Territoriality

When mice from known IUPs are placed in natural field settings, 2M individuals of both sexes maintain larger home ranges than 0M individuals. Both sexes also avoid odors of same sex individuals with long AGDs. AGD is also positively correlated with dispersal rate in male mice [32,36,81,129]. In addition, 2M males show a higher rate of novelty seeking than 0M males. These differences are not due simply to activity level, as 0M male mice are more active than 2M mice [81].

Urine marking is another testosterone dependent behavior in mice. A male urine marks more frequently when exposed to a 2M female as opposed to a 0M female [82], and 2M females themselves urine mark at a higher rate than 0M females [115]. 2M male gerbils scent mark more frequently than do their 0M or isolate brothers [19,21,22,23]. This greater frequency in scent marking persists even if the males are castrated and testosterone levels are controlled [11], showing that this effect is due to an organizational difference between animals as opposed to an activational effect of adult hormone levels. These differences in territorial behavior, especially home-range size, could have important effects on population dynamics of wild rodents.

4.3. Diet

0M male mice prefer greater amounts of saccharin than do 2M males [7], and 0M females are more likely to steal food from another animal [84]. This seems to conflict with other work that shows 2M mice to be heavier and more aggressive than their 0M siblings [64,119]. Differing metabolism in the 0M and 2M mice, however, could explain these discrepancies. 0M animals may have an increased motivation for food that drives them to be aggressive in certain situations.

4.4. Sexual behaviors

When treated with testosterone, 2M female mice [84,89] and rats [27,75] are more likely to exhibit mounting behavior than are 0M females, as are female rats located distally to male rats [25,26,54]. A few studies, however [96,106], have found no correlation between mounting behavior and IUP. These studies, however, used crude classifications of IUP and a lengthy period of hormone treatment, factors which may have obscured any potential differences.

0M female mice show a higher lordosis quotient and are more likely to receive a male's first ejaculation than are 2M mice [89]. Similarly, female rats located downstream from at least two males show a decreased lordosis quotient [54]. In contrast, however, Zehr *et al.* [127] found that female rats with a short AGD had less intense lordosis responses. This discrepancy in results may arise from the method of inducing lordosis (male-induced vs. manually palpated) or by the method of classifying the rats (downstream from males vs. short AGD). Yet another study found no effect of IUP on lordosis [106]. This study used an imprecise method of calculating IUP, however.

0M female mice are more likely to be chosen by a male, mounted by a male and are more likely to be attacked when rejecting a male's advance [114,115]. Male mice are also more attracted to the odors of females with small AGDs [36]. 0M female mice mate at a younger age than do 2M females [113], but do not differ in their ultrasonic mating vocalizations [62]. These results suggest that 0M females are more attractive to males than their 2M sisters.

2M male gerbils are more attractive to females [21] and show a decreased latency to mount and to ejaculate than 0M or isolate males [19,23]. Similarly, female mice prefer the odor of male mice with long AGDs to the odor of male mice with short AGDs [36]. 2M mice also show significantly less mounts and intromissions [119]. 0M male rats sniff females less than do 2M males [47]. 0M male gerbils are more likely to spend time with pups than are 2M males [24], and 'asexual' 0M males show an even greater frequency of parenting behaviors [17]. Both 2M males and females receive more attention (i.e. anogenital licking) from their parents relative to their 0M brothers and sisters [12]. High levels of anogenital licking may explain the increased number of motor neurons observed in the spinal nucleus of the bulbocavernosus of 2M female gerbils [38], as increased stimulation increases these motor neurons in rats [78]. Interestingly, anogenital stimulation also promotes hippocampal synaptogenesis and mRNA expression important for brain functioning in rats [68]. This difference in attractiveness between 0M and 2M individuals could alter mate selection and potentially influence the number of offspring an individual produces, as well as higher level brain functioning.

5. IUP effects on other species

The vast majority of work on IUP effects has been conducted on laboratory mammals. This poses a potential problem. Laboratory animals are generally inbred and therefore have low genetic diversity. The reported IUP effects could be construed purely as a laboratory phenomenon. The genetic diversity of a wild population could theoretically mask the IUP effects that are seen in lab colonies. Fortunately, a number of studies have been conducted on non-laboratory species, including humans. The positive results of these studies show that IUP effects can influence wild populations. We will review the limited work completed to date in this field. More studies must be completed, however, before the true importance of IUP effects in wild populations can be determined.

Female wild house mice (*Mus musculus*) from a 2M position have a longer AGD than mice from a 0M position [82,128]. Females with long AGDs also elicited more urine marking in male mice [82] and females with small AGDs were more likely to be pregnant and more likely to be in estrus [32]. In addition, males and females with long AGDs were more aggressive than mice with short AGDs [32,82]. Males with long AGDs are more likely to disperse than males with short AGDs [32] and 2M males and females maintain larger home ranges than 0M mice [129]. These results suggest that both male and female mice with long AGDs are masculinized in a similar fashion to laboratory mice. The parallel between these results and the results seen in laboratory colonies indicate that IUP effects are not masked by the genetic diversity of a wild population.

Large litters of four pups (average litter size is 2) in the California mouse (*Peromyscus californicus*) have a high proportion of males. This could be due to selective resorption or cannibalism in large litters to favor males, which are slightly smaller at birth and may be less costly to raise [9]. This is similar to the phenomenon seen in many animals, including swine, where one sex of offspring is favored in times of stress [10]. In addition, the California mouse shows a similar trend in anogenital morphology as other rodents. The AGDs of females from male biased litters were larger than the AGDs of females from female biased litters [9].

Female ferrets (presumably *Mustela furo*) located downstream from at least two males contain higher levels of androgens than other females [66]. With differing steroid levels, it is entirely possible that female ferrets show some of the IUP effects seen in other mammals. To date, however, no studies have demonstrated these potential effects.

A study on laboratory-bred hamsters (presumably *Mesocricetus auratus*) showed elevated estrogen levels and depressed androgen levels in males located downstream from two or more females. Unlike many of the other species studied, however, androgen levels in female hamsters did not correlate with the number of males located upstream [108]. It is unclear why androgen levels do not correlate

with the number of males in utero. It is possible that male hamster fetuses produce abnormally low levels of testosterone throughout development or produce high testosterone at a different time than was measured (gestational day 14).

Female grey-sided voles (*Clethrionomys rufocanus*) from male biased litters show a greater tendency to disperse than females from female biased litters [58,59]. This result shows a possible masculinization effect, as dispersal is more common in male voles. Meadow voles (*Microtus pennsylvanicus*) from male biased litters performed better on a water-maze task than voles from female biased litters [39]. This suggests that IUP alters nervous system development in voles, as seen in other rodent species.

Sheep (presumably *Ovis aries*) normally give birth to one offspring, however, litters of two or three are not uncommon. Avdi and Driancourt [2] investigated a number of reproductive parameters with these twin and triplet offspring. They found that a lamb born with one or two male siblings had abnormally high embryonic mortality when pregnant as adult in comparison to lambs born with other females.

A small number of studies conducted on human twins has produced interesting results. Humans show dental asymmetry, with males generally possessing larger teeth in the right jaw. In opposite sex dizygotic twins, however, this sexual dimorphism disappears. In addition, females who had an opposite sex twin show high levels of sensation seeking [87]. Other minor differences have been found as well (for a review, see Miller [76]). These studies should be interpreted cautiously because they are based more on human judgement than scientific quantification, or use non-randomly chosen participants.

Loehlin and Martin [69] completed an extensive survey of characteristics from a large pool of Australian twins. They found some minor differences between opposite sex twins and same sex twins, such as a higher rate of premature babies in opposite sex twins. Resnick et al. [87] also found that female, opposite sex dizygotic twins showed higher levels of sensation seeking than did female, same sex dizygotic twins (Table 3). Researchers have also investigated spontaneous otoacoustic emissions (SOAEs) and click-evoked otoacoustic emissions (CEOAEs) in opposite sex twins. SOAEs are continuous, tonal sounds produced naturally in the cochlea whereas CEOAEs are sounds produced in the cochlea in response to a click stimulus. Females generally exhibit a higher frequency of SOAEs and CEOAEs than males. McFadden [72] found that females with a male twin demonstrated significantly less SOAEs than other females. Females with a male twin also demonstrated less CEOAEs, although the difference did not reach significance [73]. Taken as a whole, the research conducted to date on humans suggests that twins may be subjected to some minor hormonal influences in utero. These hormonal influences do not appear to cause the same level of modifications in humans as they do in the other mammals with larger litters.

6. Stress

Prenatal stress, much like IUP, can permanently alter physiology, morphology, and behavior, with many effects persisting through adulthood. Any review of intrauterine effects would not be complete without a brief discussion on stress effects, although a comprehensive review is beyond the scope of this paper.

Pregnant mice, subjected to intense light and heat, show an increase in corticosterone [77]. This stress alters the development of the fetuses in a number of ways. Prenatally stressed mouse fetuses show increased plasma testosterone and are lighter at birth than control fetuses [110,122]. In female rodents, prenatal stress has been shown to lower aggression [118], lengthen AGD and estrous cycles, delay vaginal opening, increase receptivity to males [48,49,83] and causes some learning deficits [74,99]. In female rodents, stress appears to interact with IUP, and in many cases, the development of 0M females seems to be more drastically altered than the development of 2M females.

Male mice are not immune to the effects of stress. Prenatally stressed male mice demonstrate a higher level of parental behavior than control males, again with 0M males showing the greatest change in behavior [110]. Prenatally stressed male mice are also more attractive to females [83]. For a review of prenatal stress on reproductive behaviors, see Ref. [123].

Stress effects are seen in wild populations as well. Lab-reared descendants of wild house mice subjected to high population density (and therefore high stress) give birth to females that are lighter and have longer AGDs than did non-stressed females [128]. This suggests that a rodent population under stress might become disproportionately filled with individuals with 2M characteristics.

Stress and IUP interactions are not restricted to rodents. A piglet developing in a crowded uterus is more likely to die before birth or earlier in life than other piglets. In addition, the fetuses that die in utero are more likely to be male, showing that swine give birth to more female fetuses when resources are limited. There is some evidence that this regulation of litter size in swine is partially controlled by placental estrogen levels [10,65,100,101].

Human health is also influenced by prenatal stress. Maternal nutrition during pregnancy has been linked to development of fetal pancreatic beta cells. This suggests that the mother's diet can predispose her child to type II diabetes [94]. Maternal diet has also been linked to the offspring's adult blood pressure [3,8]. Children born to mothers who were stressed during pregnancy show high levels of blood glucose [85], hypertension [92], and obesity [86] in adulthood. These initial findings indicate a need for further study in the field of human stress and its possible effects on developing children.

7. Discussion

IUP accounts for a large amount of variability that is not genetic in origin. IUP causes an individual mammal to become more masculine or feminine physiologically, morphologically and behaviorally. IUP potentially may have profound effects at the population level of a species. Individuals have altered reproductive capabilities, propensities to show aggression, production of young or dispersal. These factors provide a population with phenotypic variability that allows it to adapt to changes rapidly even if individuals in a population are genetically homogenous.

As with any large body of research, some variable and contrasting findings have been reported. Multiple studies [95,106] have found no influence of IUP on traits strongly correlated with IUP in other studies, such as AGD, sexual behaviors and aggression. Prenatal stress or strain effects could account for these results. Strain differences in the effects of IUP on AGD have been reported in mice [62] and rats [55]. The strain used by Simon and Cologer-Clifford [95] (CF-1 mice) was shown to be one of the strains less sensitive to IUP effects [62]. Strain differences in sensitivity to hormones have also been described elsewhere [98] and may account for some of the variability present in the body of literature reviewed here.

Variability in endocrine studies may arise from a number of other unexpected sources as well. Rodent diets vary in their level of phytoestrogens and have been shown to differentially influence vaginal opening and onset of first estrus, two measures commonly seen in IUP studies. In addition, certain tissues, or assays, may have endogenous variability that could skew results. Both prostate and testis weights have been shown to vary in weight over time for no explained reason (for a review, see Ref. [1]). Lastly, differing laboratory environments may contribute to variability. Crabbe *et al.* [29] showed that different laboratories could easily obtain contrasting results while running the same behavioral tests on the same strain of mice.

Despite the variability, the vast majority of studies reviewed here show that IUP has predictable and often strong effects. These IUP effects have important implications for a number of other scientific disciplines.

It has been hypothesized that IUP may influence the population cycles seen in many small mammals [28,109, 111]. Females exposed to high population densities and high stress will show higher circulating levels of testosterone and will subsequently give birth to female pups that are all similar to 2M females. In general, these masculinized females are more aggressive, more likely to disperse, have lower fecundity and give birth to fewer females. This combination of factors can interact to lower a population. At low density, the more attractive 0M females will have more litters. These 0M females will have large litters with a high proportion of 0M female pups that show a low propensity for dispersal. These factors can result in a denser population. In this fashion, IUP may contribute to rodent population cycles.

IUP influences aggression, attractiveness, and mating efficiency among individuals in a predictable manner. These traits can also influence population dynamics. With the discovery of a strong correlation between testosterone exposure and AGD, recording IUP data becomes non-invasive and relatively simple. Researchers in the field of mammalian population biology should consider recording IUP data in order to explain some of the variability inherent in their studies.

Recent medical advances in human reproduction have led to a dramatic increase in the number of multi-child births. In 1995, approximately 37% of all fertility clinic live births resulted in multiple children, as compared to 2% in the general population. Of the multiple childbirths, approximately 1 in 7 resulted in triplets or greater [30]. Human fetuses sharing womb space with multiple siblings could have an increased exposure to steroid hormones. No data has yet been published on IUP effects in human children of these multi-child births. IUP has the potential to influence functioning of the immune system, reproduction and adult endogenous hormone levels. It would be interesting, and medically important, to conduct studies investigating possible IUP effects in these children.

For similar reasons, it is important to consider IUP in animal health as well. Commercial breeders depend on their animals being healthy and reproductively viable. Research conducted on swine indicates that IUP can influence the immune system as well as reproduction and aggression. Research on other commercially important animals is scarce. Both cats and dogs give birth to large litters. Some work has been completed that suggests that IUP may influence behavior in these species [4]. Sheep also appear to be susceptible to IUP effects [2]. Carefully controlled experiments exploring IUP effects have not been conducted in these species or in many other species that may be of commercial value. However, based on existing research, any mammal that gives birth to multiple offspring is susceptible to IUP effects that may influence reproductive capabilities and health.

IUP effects also have the potential to influence toxicological studies. Sexual dimorphism exists in mammals' susceptibility to certain chemicals. For example, female rats are more sensitive to the effects of warfarin, strychnine, hexobarbital and parathion whereas male rats are more sensitive to lead, aldrin and epinephrine [51]. It is possible that IUP may influence an individual's susceptibility to these and other compounds. Because recent trends in toxicology have placed a focus onto low doses of toxicants and mixtures of toxicants causing chronic effects, it becomes even more important to reduce variability as much as possible. This is especially true

considering that rodents are commonly used as animal models for human exposure. Future rodent studies on the effects of toxins and toxicants should consider incorporating IUP in order to lower variability and produce more standard results.

Endocrine disruption is another field that is receiving a great deal of attention. By its very nature, this is a body of research that focuses on low doses of fairly weak chemicals that cause chronic toxic effects. Not only can IUP be used to lower variability in these studies, but IUP can also directly influence sensitivity to certain endocrine disruptors [56,57]. It is also possible that endocrine disruptors and IUP may act via similar mechanisms to create additive or synergistic effects, not only altering sensitivity to an endocrine disruptor, but also enhancing their effects.

IUP may serve as a tool allowing researchers to study the effects of exogenous chemicals or toxicants on mammals. In addition, studying IUP effects allows researchers to observe hormonal actions with naturally differing groups within the same species or even within the same litter. This provides the ability to understand more fully how different hormones contribute to development and how the function of these hormones differs among species and strains.

IUP allows researchers to study the natural organizing effects of hormones using individuals from the same litter that are exposed to slightly different levels of steroid hormones. In this way, the uterus can act as a natural laboratory for determining prenatal organizational effects of steroid hormones. The body of research also clearly demonstrates important species differences in the organizational effects of these hormones. Even though they are all rodents, mice, rats and gerbils show tremendous variability in their IUP effects. Future research in this field should continue to focus on this variability in order to advance the current knowledge of species and strain differences.

Most importantly, IUP is a source of variability that is not often recognized. Genetic variation and environmental variation are commonly controlled. It is vital that future research involving mammals at least recognize this third, hormonal, source of variation.

Acknowledgements

The authors would like to thank Roger A. Powell, S. Miles Dean and Andrew K. Hotchkiss for their input and critique of this manuscript. The authors would also like to recognize the W.M. Keck Center for Behavioral Biology at North Carolina State University for support during the writing of this manuscript.

References

[1] Ashby JA. Testing for endocrine disruption post-EDSTAC: extrapolation of low dose rodent effects to humans. Toxicol Lett 2001;120:233–42.
[2] Avdi M, Driancourt MA. Influence of sex ratio during multiple pregnancies on productive and reproductive parameters of lambs and ewes. Reprod Nutr Dev 1997;37:21–7.
[3] Barker DJP, Shiell AW, Barker ME, Law CM. Growth in utero and blood pressure levels in the next generation. J Hypertension 2000;18: 843–6.
[4] Bateson P, Young M. The influence of male kittens on the object play of their female siblings. Behav Neural Biol 1979;27:374–8.
[5] Brown MJ, Schultz GS, Hilton FH. Intrauterine proximity to male fetuses predetermines level of epidermal growth factor in submandibular glands of adult female mice. Endocrinology 1984;115: 2318–23.
[6] Bulman-Fleming B, Wahlsten D. The effects of intrauterine position on the degree of corpus callosum deficiency in two substrains of BALB/c mice. Dev Psychobiol 1991;24:395–412.
[7] Bushong ME, Mann MA. Gender and intrauterine position influence saccharin preference in mice. Horm Behav 1994;28:207–18.
[8] Campbell DM, Hall MH, Barker DJP, Cross J, Shiell AW, Godfrey KM. Diet in pregnancy and the offspring's blood pressure 40 years later. Br J Obstet Gynaecol 1996;103:273–80.
[9] Cantoni D, Glaizot O, Brown RE. Effects of sex composition of the litter on anogenital distance in California mice (*Peromyscus californicus*). Can J Zool 1999;77:124–31.
[10] Chen Z-Y, Dzuik PJ. Influence of initial length of uterus per embryo and gestation stage on prenatal survival, development, and sex ratio in the pig. J Anim Sci 1993;71:1895–901.
[11] Clark MM, Bishop AM, vom Saal FS, Galef Jr. BG. Responsiveness to testosterone of male gerbils from known intrauterine positions. Physiol Behav 1993;53:1183–7.
[12] Clark MM, Bone S, Galef Jr. BG. Uterine positions and schedules of urination: correlates of different maternal anogenital stimulation. Dev Psychobiol 1989;22:389–400.
[13] Clark MM, Crews D, Galef Jr. BG. Concentration of sex steroid hormones in pregnant and fetal Mongolian gerbils. Physiol Behav 1991;49:239–43.
[14] Clark MM, Galef Jr. BG. Effects of uterine position on rate of sexual development in female Mongolian gerbils. Physiol Behav 1988;42: 15–18.
[15] Clark MM, Galef Jr. BG. Sexual segregation in the left and right horns of the gerbil uterus: the male embryo is usually on the right, the female on the left (Hippocrates). Dev Psychobiol 1990; 23:29–37.
[16] Clark MM, Galef Jr. BG. A gerbil dam's fetal intrauterine position affects the sex ratio of litters she gestates. Physiol Behav 1995;57: 297–9.
[17] Clark MM, Galef Jr. BG. Why some male Mongolian gerbils may help at the nest: testosterone, asexuality and alloparenting. Anim Behav 2000;59:801–6.
[18] Clark MM, Karpuik P, Galef Jr. BG. Hormonally mediated inheritance of acquired characteristics in Mongolian gerbils. Nature 1993;367:712.
[19] Clark MM, Malenfant SA, Winter DA, Galef Jr. BG. Fetal uterine position affects copulation and scent marking by adult male gerbils. Physiol Behav 1990;47:301–5.
[20] Clark MM, Robertson RK, Galef Jr. BG. Intrauterine position effects on sexually dimorphic asymmetries of Mongolian gerbils: testosterone, eye opening, and paw preference. Dev Psychobiol 1993;26: 185–94.
[21] Clark MM, Tucker L, Galef Jr. BG. Stud males and dud males: intrauterine position effects on the reproductive success of male gerbils. Anim Behav 1992;43:215–21.

[22] Clark MM, vom Saal FS, Galef Jr. BG. Intrauterine positions and testosterone levels of adult male gerbils are correlated. Physiol Behav 1992;51:957–60.
[23] Clark MM, Vonk JM, Galef Jr. BG. Reproductive profiles of adult Mongolian gerbils gestated as the sole fetus in a uterine horn. Physiol Behav 1997;61:77–81.
[24] Clark MM, Vonk JM, Galef Jr. BG. Intrauterine position, parenting, and nest-site attachment in male Mongolian gerbils. Dev Psychobiol 1998;32:177–81.
[25] Clemens LG. Neurohormonal control of male sexual behavior. In: Montagna W, Sadler WA, editors. Advances in behavioral biology, vol. 11. New York: Plenum Press; 1974. p. 23–53.
[26] Clemens LG, Coniglio L. Influences of prenatal composition on mounting behavior of female rats. Am Zool 1971;11:617–8.
[27] Clemens LG, Gladue BA, Coniglio LP. Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. Horm Behav 1978;10: 40–53.
[28] Cowell LG, Crowder LB, Kepler TB. Density-dependent prenatal androgen exposure as an endogenous mechanism for the generation of cycles in small mammal population. J Theor Biol 1998;190: 93–106.
[29] Crabbe JC, Wahlsten D, Dudek BC. Genetics of mouse behavior: interactions with laboratory environment. Science 1999;284: 1670–2.
[30] Danel IA, Green YT, Walter G. 1995 assisted reproductive technology success rates: national summary and fertility clinic report. J Women Health 1998;7:301–3.
[31] Darwin C. Variation under domestication. On the origin of the species; a Facsimile of the first edition (1859), Cambridge, MA: Harvard University Press; 1964.
[32] Drickamer LC. Intra-uterine position and anogenital distance in house mice: consequences under field conditions. Anim Behav 1996; 51:925–34.
[33] Drickamer LC, Arthur RD, Rosenthal TL. Conception failure in swine: importance of the sex ratio of a female's birth litter and tests of other factors. J Anim Sci 1997;75:2192–6.
[34] Drickamer LC, Arthur RD, Rosenthal TL. Predictors of social dominance and aggression in gilts. Appl Anim Behav Sci 1999;63: 121–9.
[35] Drickamer LC, Rosenthal TL, Arthur RD. Factors affecting the number of teats in pigs. J Reprod Fertil 1999;115:97–100.
[36] Drickamer LC, Robinson AS, Mossman CA. Differential responses to same and opposite sex odors by adult house mice are associated with anogenital distance. Ethology 2001;107:1–11.
[37] Faber KA, Hughes Jr. CL. Anogenital distance at birth as a predictor of volume of the sexually dimorphic nucleus of the preoptic area of the hypothalamus and pituitary responsiveness in castrated adult rats. Biol Reprod 1992;46:101–4.
[38] Forger NG, Galef Jr. BG, Clark MM. Intrauterine position affects motoneuron number and muscle size in a sexually dimorphic neuromuscular system. Brain Res 1996;735:119–24.
[39] Galea LAM, Ossenkopp K-P, Kavaliers M. Performance (re-acquisition) of a water maze task by adult meadow voles: effects of age of initial task, acquisition, and in utero environment (litter sex-ratio). Behav Brain Res 1994;63:177–85.
[40] Gandelman R, Graham S. Singleton female mouse fetuses are subsequently unresponsive to the aggression-activating property of testosterone. Physiol Behav 1986;37:465–7.
[41] Gandelman R, vom Saal FS, Reinisch JM. Contiguity to male foetuses affects morphology and behaviour of female mice. Nature 1977;266:722–4.
[42] Gorodeski GI, Sheean LA, Utian WH. Sex hormone modulation of flow velocity in the parametrial artery of the pregnant rat. Am J Physiol 1995;268:R614–24.
[43] Graham S, Gandelman R. The expression of ano-genital distance data in the mouse. Physiol Behav 1986;36:103–4.
[44] Gray Jr. LE, Ostby J, Monosson E, Kelce WR. Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. Toxicol Ind Health 1999;15: 48–64.
[45] Hauser H, Gandelman R. Contiguity to males in utero affects avoidance responding in adult female mice. Science 1983;220:437–8.
[46] Hendricks C, McClintock MK. Timing of insemination is correlated with the secondary sex ratio of Norway rats. Physiol Behav 1990;48: 625–32.
[47] Hernandez-Tristan R, Arevalo C, Canals S. Effects of prenatal uterine position on male and female rats' sexual behavior. Physiol Behav 1999;67:401–8.
[48] Herrenkohl LR. Prenatal stress reduces fertility and fecundity in female offspring. Science 1979;206:1097–9.
[49] Herrenkohl LR, Politch JA. Effects of prenatal stress on the estrous cycle of female offspring as adults. Experientia 1978;34:1240–1.
[50] Hirlemann S, Brain PF, Parmigiani S. Prior intra-uterine position of lactating mice: effects on attackability by adult resident females. Boll Zool 1990;57:67–71.
[51] Hodgson E. Modifications of xenobiotic metabolism. In: Hodgson E, Levi PE, editors. A textbook of modern toxicology. Stamford, CT: Appleton and Lange; 1997. p. 119–70.
[52] Hotchkiss AK. The effect if androgens and antiandrogens on sexual differentiation in male and female rats. Doctoral Dissertation, North Carolina State University; 2001.
[53] Houtsmuller EJ, de Jong FH, Rowland DL, Slob AK. Plasma testosterone in fetal rats and their mothers on day 19 of gestation. Physiol Behav 1995;57:495–9.
[54] Houtsmuller EJ, Slob AK. Masculinization and defeminization of female rats by males located caudally in the uterus. Physiol Behav 1990;48:555–60.
[55] Houtsmuller EJ, Thornton JA, Rowland DL. Using a regression approach to study the influence of male fetuses on the genital morphology of neonatal female rats. Multivar Behav Res 1997;32: 77–94.
[56] Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS. Exposure to bisphenol A advances puberty. Nature 1999; 401:763–4.
[57] Howdeshell KL, vom Saal FS. Developmental exposure to bisphenol A: interaction with endogenous estradiol during pregnancy in mice. Am Zool 2000;40:429–37.
[58] Ims RA. Kinship and origin effects on dispersal and space sharing in *Clethrionomys rufocanus*. Ecology 1989;70:607–16.
[59] Ims RA. Determinants of natal dispersal and space use in grey-sided voles, *Clethrionomys rufocanus*: a combined field and laboratory experiment. OIKOS 1990;57:106–13.
[60] James WH. The human sex ratio. Part 1: a review of the literature. Hum Biol 1987;59:721–52.
[61] Jones D, Gonzalez-Lima F, Crews D, Galef Jr. BG, Clark MM. Effects of intrauterine position on the metabolic capacity of the hypothalamus of female gerbils. Physiol Behav 1997;61:513–9.
[62] Jubilan BM, Nyby JG. The intrauterine position phenomenon and precopulatory behaviors in house mice. Physiol Behav 1992;51: 857–72.
[63] Kinsley CH, Konen CM, Miele JL, Ghiraldi L, Svare B. Intrauterine position modulates maternal behaviors in female mice. Physiol Behav 1986;36:793–9.
[64] Kinsley C, Miele J, Wagner CK, Ghiraldi L, Broida J, Svare B. Prior intrauterine position influences body weight in male and female mice. Horm Behav 1986;20:201–11.
[65] Knight JW. Aspects of placental estrogen synthesis in the pig. Exp Clin Endocrinol 1994;102:175–84.
[66] Krohmer RW, Baum MJ. Effect of sex, intrauterine position and androgen manipulation on the development of brain aromatase activity in fetal ferrets. J Neuroendocrinol 1989;1:265–71.
[67] Lipton JW, Robie HC, Ling Z, Weese-Mayer DE, Carvey PM. The magnitude of brain dopamine depletion from prenatal cocaine

exposure is a function of uterine position. Neurotoxicol Teratol 1998;20:373–82.
[68] Liu DJ, Dorio JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 2000;3:799–806.
[69] Loehlin JC, Martin NG. A comparison of adult female twins from opposite-sex and same-sex pairs on variables related to reproduction. Behav Genet 1998;28:21–7.
[70] Mankes RF, LeFevre R, Calvano C. Prenatal ethanol exposure significantly effects alcohol preference in masculinized male offspring of LE rats. Alcoholism 1992;16:379.
[71] McDermott NJ, Gandelman R, Reinisch JM. Contiguity to male fetuses influences ano-genital distance and time of vaginal opening in mice. Physiol Behav 1978;20:661–3.
[72] McFadden D. A masculinizing effect on the auditory systems of human females having male co-twins. Proc Natl Acad Sci 1993;90: 11900–4.
[73] McFadden D, Loehlin JC, Pasanen EG. Additional finding on heritability and prenatal masculinization of cochlear mechanisms: click-evoked otoacoustic emissions. Hear Res 1996;97:102–19.
[74] Meek LR, Burda KM, Paster E. Effects of prenatal stress on development in mice: maturation and learning. Physiol Behav 2000; 71:543–9.
[75] Meisel RL, Ward IL. Fetal female rats are masculinized by male littermates located caudally in the uterus. Science 1981;213:239–42.
[76] Miller EM. Prenatal sex hormone transfer: a reason to study opposite-sex twins. Pers Individ Diff 1994;17:511–29.
[77] Montano MM, Wang M-H, Even MD, vom Saal FS. Serum corticosterone in fetal mice: sex differences, circadian changes, and effect of maternal stress. Physiol Behav 1991;50:323–9.
[78] Moore CL, Dou H, Juraska JM. Maternal stimulation affects the number of motor neurons in a sexually dimorphic nucleus of the lumbar spinal cord. Brain Res 1992;572:52–6.
[79] Mylchreest E, Wallace DG, Cattley RC, Foster PM. Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to Di(*n*-butyl) phthalate during late gestation. Toxicol Sci 2000;55:143–51.
[80] Nonneman DJ, Ganjam VK, Welshons WV, vom Saal FS. Intrauterine position effects on steroid metabolism and steroid receptors of reproductive organs in male mice. Biol Reprod 1992;47: 723–9.
[81] Palanza P, Morley-Fletcher S, Laviola G. Novelty seeking in periadolescent mice: sex differences and influence of intrauterine position. Physiol Behav 2001;72:255–62.
[82] Palanza P, Parmigiani S, vom Saal FS. Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth. Physiol Behav 1995;58:827–35.
[83] Politch JA, Herrenkohl LR. Effects of prenatal stress on reproduction in male and female mice. Physiol Behav 1984;32:95–9.
[84] Quadagno DM, McQuitty C, McKee J, Koelliker L, Wolfe G, Johnson DC. The effects of intrauterine position on competition and behavior in the mouse. Physiol Behav 1987;41:639–42.
[85] Ravelli AC, van der Meulen JHP, Michels RPJ, Osmond C, Barker DJP, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. Lancet 1998;351:173–7.
[86] Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. New Engl J Med 1976; 295:349–53.
[87] Resnick SM, Gottesman II, McGue M. Sensation seeking in opposite-sex twins: an effect of prenatal hormones? Behav Genet 1993;23:323–9.
[88] Richmond G, Sachs BD. Furthur evidence for masculinization of female rats by males located caudally in utero. Horm Behav 1984;18: 484–90.
[89] Rines JP, vom Saal FS. Fetal effects on sexual behavior and aggression in young and old female mice treated with estrogen and testosterone. Horm Behav 1984;18:117–29.
[90] Rohde Parfet KA, Ganjam VK, Lamberson WR, Rieke AR, vom Saal FS, Day BN. Intrauterine position effects in female swine: subsequent reproductive performance, and social and sexual behavior. Appl Anim Behav Sci 1990;26:349–62.
[91] Rohde Parfet KA, Lamberson WR, Rieke AR, Cantley TC, Ganjam VK, vom Saal FS, Day BN. Intrauterine position effects in male and female swine: subsequent survivability, growth rate, morphology and semen characteristics. J Anim Sci 1990;68:179–85.
[92] Roseboom TJ, van der Meulen JHP, van Montfrans GA, Ravelli ACJ, Osmond C, Barker DJP, Bleker OP. Maternal nutrition during gestation and blood pressure in later life. J Hypertension 2001;19: 29–34.
[93] Sherry DF, Galef Jr. BG, Clark MM. Sex and intrauterine position influences the size of the gerbil hippocampus. Physiol Behav 1996; 60:1491–4.
[94] Shiell AW, Campbell DM, Hall MH, Barker DJP. Diet in late pregnancy and glucose-insulin metabolism of the offspring 40 years later. Br J Obstet Gynaecol 2000;107:890–5.
[95] Simon NG, Cologer-Clifford A. In utero contiguity does not influence morphology, behavioral sensitivity to testosterone, or hypothalamic androgen binding in CF-1 female mice. Horm Behav 1991;25:518–30.
[96] Slob AK, van der Schoot P. Testosterone induced mounting behaviors in adult female rats born in litters of different female to male ratios. Physiol Behav 1982;28:1007–10.
[97] Slob AK, Vreeburg JTM. Prenatal androgens in female rats and adult mounting behaviour. In: Gilles R, Balthazart J, editors. Neurobiology. Berlin: Springer; 1985. p. 165–79.
[98] Spearow JL, Doemeny P, Sera R, Leffler R, Barkley M. Genetic variation in susceptibility to endocrine disruption by estrogen in mice. Science 1999;285:1259–61.
[99] Szuran TF, Pliska V, Pokorny J, Welzl H. Prenatal stress in rats: effects on plasma corticosterone, hippocampal glucocorticoid receptors, and maze performance. Physiol Behav 2000;71: 353–62.
[100] Tarraf CG, Knight JW. Effect of uterine space and fetal sex on conceptus development and in vitro release of progesterone and estrone from regions of the porcine placenta throughout gestation. Domest Anim Endocrinol 1995;12:63–71.
[101] Tarraf CG, Knight JW. Effect of intrauterine position on conceptus development, placental and endometrial release of progesterone and estrone in vitro, and concentration of steroid hormones in fetal fluids throughout gestation in swine. Domest Anim Endocrinol 1995;12: 179–87.
[102] Tobet SA, Dunlap JL, Gerall AA. Influence of fetal position on neonatal androgen-induced sterility and sexual behavior in female rats. Horm Behav 1982;16:251–8.
[103] Tobet SA, Baum MJ, Tang HB, Shim JH, Canick JA. Aromatase activity in the perinatal rat forebrain: effects of age, sex and intrauterine position. Dev Brain Res 1985;25:171–8.
[104] Vandenbergh JG, Huggett CL. Mother's prior intrauterine position affects the sex ratio of her offspring in house mice. Proc Natl Acad Sci 1994;91:11055–9.
[105] Vandenbergh JG, Huggett CL. The anogenital distance index, a predictor of the intrauterine position effects on reproduction in female house mice. Lab Anim Sci 1995;45:567–73.
[106] van de Poll NE, van der Zwan SM, van Oyen HG, Pater JH. Sexual behaviors in female rats born in all-female litters. Behav Brain Res 1982;4:103–9.
[107] van der Hoeven T, LeFevre R, Mankes R. Effects of intrauterine position on the hepatic microsomal polysubstrate monooxygenase and cytosolic glutathione S-transferase activity, plasma sex steroids and relative organ weights in adult male and female Long-Evans rats. J Pharmacol Exp Theor 1992;263:32–9.
[108] Vomachka AJ, Lisk RD. Androgen and estradiol levels in plasma and amniotic fluid of late gestational male and female hamsters: uterine position effects. Horm Behav 1986;20:181–93.

[109] vom Saal FS. Variation in phenotype due to random intrauterine positioning of male and female fetuses in rodents. J Reprod Fertil 1981;62:633–50.
[110] vom Saal FS. Variation in infanticide and parental behavior in male mice due to prior intrauterine proximity to female fetuses: elimination by prenatal stress. Physiol Behav 1983;30:675–81.
[111] vom Saal FS. The intrauterine position phenomenon: effects on physiology, aggressive behavior and population dynamics in house mice. In: Flannelly K, Blanchard R, Blanchard D, editors. Biological perspectives on aggression. New York: Alan R. Liss, Inc; 1984. p. 135–79.
[112] vom Saal FS. Perinatal testosterone exposure has opposite effects on adult intermale aggression and infanticide in mice. In: Brain P, Mainardi D, Parmigiani S, editors. House mouse aggression. New York: Harwood Academic Publishers; 1989. p. 179–204.
[113] vom Saal FS. The production of and sensitivity to cues that delay puberty and prolong subsequent oestrous cycles in female mice are influenced by prior intrauterine position. J Reprod Fertil 1989;86: 457–71.
[114] vom Saal FS, Bronson FH. In utero proximity of female mouse fetuses to males: effects on reproductive performance during later life. Biol Reprod 1978;19:842–53.
[115] vom Saal FS, Bronson FH. Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. Science 1980;208:597–9.
[116] vom Saal FS, Bronson FH. Variation in length of the estrous cycle in mice due to former intrauterine proximity to male fetuses. Biol Reprod 1980;22:777–80.
[117] vom Saal FS, Dhar MG. Blood flow in the uterine loop artery and loop vein is bidirectional in the mouse: implications for transport of steroids between fetuses. Physiol Behav 1992;52:163–71.
[118] vom Saal FS, Even MD, Quadagno DM. Effects of maternal stress on puberty, fertility and aggressive behavior of female mice from different intrauterine positions. Physiol Behav 1991;49:1073–8.
[119] vom Saal FS, Grant WM, McMullen CW, Laves KS. High fetal estrogen concentrations: correlation with increased adult sexual activity and decreased aggression in male mice. Science 1983;220: 1306–9.
[120] vom Saal FS, Moyer CL. Parental effects on reproductive capacity during aging in female mice. Biol Reprod 1985;32:1116–26.
[121] vom Saal FS, Pryor S, Bronson FH. Effects of prior intrauterine position and housing on oestrous cycle length in adolescent mice. J Reprod Fertil 1981;62:33–7.
[122] vom Saal FS, Quadagno DM, Even MD, Keisler LW, Keisler DH, Khan S. Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. Biol Reprod 1990;43:751–61.
[123] Ward IL. Sexual behavior: the product of perinatal hormonal and prepubertal social factors. In: Gerall AA, Moltz H, Ward IL, editors. Handbook of behavioral neurobiology. Sexual differentation, vol. 11. New York: Plenum Press; 1992. p. 157–79.
[124] Welch MA, Dowell RT, Booze RM, MacTutus CF. Maternal/fetal cardiovascular effects of intravenous cocaine in unanesthitized, in utero, rats. Neurotoxicol Teratol Abstr 1996;18:340.
[125] Wise TH, Christenson RK. Relationship of fetal position within the uterus to fetal weight, placental weight, testosterone, estrogens, and thymosin β4 concentrations at 70 and 104 days of gestation in swine. J Anim Sci 1992;70:2787–93.
[126] Wolf CJ, Ostby J, Hotchkiss A, Gray Jr. LE. Effects of prenatal testosterone propionate on the sexual development of male and female rats. Biol Reprod 2000;62:247.
[127] Zehr JL, Gans SE, McClintock MK. Variation in reproductive traits is associated with short anogenital distance in female rats. Dev Psychobiol 2001;38:229–38.
[128] Zielinski WJ, Vandenbergh JG, Montano MM. Effects of social stress and intrauterine position on sexual phenotype in wild-type house mice (*Mus musculus*). Physiol Behav 1991;49:117–23.
[129] Zielinski WJ, vom Saal FS, Vandenbergh JG. The effect of intrauterine position on the survival, reproduction and home range size of female house mice (*Mus musculus*). Behav Ecol Sociobiol 1992;30:185–91.