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Minireview

Maternal Diet and Other Factors Affecting Offspring Sex Ratio: A Review

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ABSTRACT

Mammals usually produce approximately equal numbers of sons and daughters, but there are exceptions to this general rule, as has been observed in ruminant ungulate species, where the sex-allocation hypothesis of Trivers and Willard has provided a rational evolutionary underpinning to adaptive changes in sex ratio. Here, we review circumstances whereby ruminants and other mammalian species, especially rodents and primates, appear able to skew the sex ratio of their offspring. We also discuss some of the factors, both nutritional and nonnutritional, that potentially promote such skewing. Work from our laboratory, performed on mice, suggests that age of the mother and maternal diet, rather than the maternal body condition per se, play directive roles in controlling sex ratio. In particular, a diet high in saturated fats but low in carbohydrate leads to the birth of significantly more male than female offspring in mature laboratory mice, whereas when calories are supplied mainly in the form of carbohydrate rather than fat, daughters predominate. As the diets fed to the mice in these experiments were nutritionally complete and because litter sizes did not differ between treatments, dietary inadequacy seems not to be the cause for sex-ratio distortion. A number of mechanisms, all of which are testable, are discussed to provide an explanation for the phenomenon. We conclude the review by discussing potential implications of these observations to human medicine and agriculture.

early development, embryo, embryonic development, fertilization, nutrition and pregnancy, sex allocation, sex ratio, trophoblast, uterus

INTRODUCTION

Darwin surmised that some animal species can exhibit statistically significant shifts in the proportion of sons and daughters that are born, although the conditions and underlying mechanisms that prompt these changes were and still are, for the most part, unclear. In insects, reptiles, and birds, sex-ratio adjustments in response to food availability and other environmental factors, e.g., extreme sex-ratio skewing due to male-selective killing by *Wolbachia* infection in the Samoan butterfly *Hypolimnas polina* have long been characterized [1–5]. This work has contributed greatly to evolutionary theory, even to the extent that the experimental data can be fitted accurately to mathematical predictions [6–9]. It is now clear that the male-to-female sex ratio at the time of conception (primary sex ratio) and the secondary sex ratio at birth can be strikingly skewed from the theoretical 1:1 expected ratio [reviewed in 10, 11]. In the sections that follow, we first review the evidence that adaptive adjustments in sex ratio of offspring occurs in mammals in response to diet and report on some of our own experimental findings in the mouse. We conclude by discussing some of the mechanisms that might be responsible for skewing sex ratios.

Significance of Gender Differences at Birth and the Trivers and Willard Sex-Allocation Hypothesis

Trivers and Willard [12] pointed out that, in polygynous species, a small proportion of males, usually ones that are larger and more aggressive, share most of the lifetime reproductive success, while lower ranking males often father no offspring at all. By contrast, the majority of females, irrespective of their social rank and body condition, become pregnant through mating to this select group of males. In such species, fathers often play little part in rearing the young. The sex-allocation hypothesis of Trivers and Willard predicted that females in the best body condition would tend to produce offspring the gender of which favors the sex of greater variance, namely males. Their sons would benefit from greater parental investment and most likely, as adults, join the elite group of breeding males. As a consequence, such females are likely to pass on their genes to more of their grandchildren. Conversely, females lower in the social structure or in poorer body condition would be anticipated to invest more in female progeny because their daughters, rather than their sons, are likely to have greater lifetime reproductive success. The greater variance of males in polygynous species, both in terms of early mortality and reproductive success, is well established in wild populations. Males born to high ranking/better fed females may, in turn, have greater reproductive success than their contemporaries [13, 14]. Such a correlation seems also to hold true for mice, where larger males are more attractive to females than small males [15], and males born to food-deprived mothers are generally smaller as adults than males born to females fed ad libitum, even if such variance is not evident when they are born [16]. Moreover, males born to food-deprived female mice are more likely to lose agonistic encounters than sons born to control-fed females [17]. De-

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TABLE 1. Relative energy content (Kcal %) of major nutrients in mouse diets.

Diet	D12450B[a] (LF)	D12492[a] (VHF)	Purina 5015[b] (CLC)
Protein	20	20	18
Carbohydrates			
Starch	31	0	51
Maltodextrin	4	13	NS
Sucrose	35	7	1
Total carbohydrates	70	20	56
Fats			
Soybean oil	6	6	NS
Lard	4	54	NS
Total fat	10	60	26

[a] Defined Research Diet (Research Diets, Inc., New Brunswick, NJ) with equivalent amounts of casein, cellulose, minerals, vitamin mixes [31]. D12450B diet had a caloric density of 3.8 kcal/g, D12492, 5.2 kcal/g.
[b] Purina Complete Life Cycle (CLC) 5015 diet, 4.4 kcal/g. (Purina Inc., St. Louis, MO.) NS, Not specified.

spite the fact that the Trivers and Willard hypothesis [12] has often been liberally and sometimes overinterpreted, that some sex-ratio skewing may be nonadaptive, and that there is much literature that is conflicting [13, 18–21], the hypothesis has provided a useful theoretical framework to begin to study sex-ratio deviation.

Maternal Nutrition and Sex Ratio of Offspring in Various Species

This section will discuss what is known about how nutrition of the mother can affect the sex ratio of her progeny, first in the large artiodactyl species, second in rodents, especially mice, and finally in other animals.

Large ruminants. The prediction that females in better body condition would produce more male than female progeny has been observed in red deer [14, 22, 23], roe deer [24], mature ewes [25], reindeer [26], Barbary sheep [27], domestic pigs [28], and a number of other species, although there are exceptions [13, 29]. Dairy cows, but not heifers, on a high plane of nutrition give birth to proportionately more bull than female calves than cows on a poorer diet [30]. Repeat breeder cows, i.e., ones that have problems becoming pregnant by artificial insemination, also tend to produce more males [31]. The data on roe deer [24] were obtained with farmed animals on a diet controlled for low- and high-energy intake by varying the oil content. In that study, 75% of the calves born to the high-energy does were male, while the low-energy group produced only 46% males. Most other studies have been performed on wild populations, which are less well-controlled.

Rodents. There have been surprisingly few studies aimed directly at testing the Trivers and Willard hypothesis [12] in mice, although there are several reports that are consistent with its applicability in this species. Numerous studies have shown that maternal nutrition, particularly a diet that is inadequate, can affect litter size and viability [16, 32–34]. Rivers and Crawford [32] fed mice either a low-fat or control diet. Females on the low-fat diet had litters with a significant sex-ratio distortion (~1:3, males:females) relative to controls, where the sex ratio was 1:1. Females on the low-fat diet also had smaller litters, suggesting that there had been selective loss of male embryos or fetuses. Drickamer [33] noted that dominant female mice could appropriate more food than lower ranking females and produced a greater proportion of male-biased litters. Meikle and Drickamer [34] found that both wild and laboratory mice deprived of food 1 wk before mating produced fewer males than control wild and laboratory females. In a follow-up study, Meikle and Thornton [16] showed that intermittent feeding of wild mice both prior to and during gestation gave female-biased litters relative to controls. Food restriction of female rats results in a skewing of offspring sex ratio, which has been attributed to a decrease in uterine glycerylphosphorylcholine diesterase activity [35]. In rats, a maternal diet high in sodium and potassium but low in calcium affects the sex ratio of offspring [36, 37]. Interestingly, hamsters dosed with caffeine have significant skewing of the sex ratio toward females [38], speculatively attributed to inhibition of cAMP phosphodiesterase activity.

In our experiments, we chose to examine the effects of two defined, nutritionally complete diets [39; Table 1], which differ primarily in their sources of energy, on the sex of offspring born to female NIH Swiss mice [39]. Diet 1 was low in saturated fat (LF), with the majority of calories provided as sugars and complex carbohydrate. The second was very high in saturated fat (VHF), with 54% of its energy provided as lard (Table 1). The goal was to determine whether these diets could influence the sex ratio of pups born.

NIH Swiss mice maintained on the two diets from 30 days of age delivered four successive litters of pups after being bred at approximately 10, 20, 28, and 40 wk of age, resulting in 1048 young born over 108 pregnancies (Table 2). The effects of diet on litter size, maternal weight, gestation length, and sex ratio were tested by using a mixed model procedures with a repeated measures design [40, 41]. Because each female had multiple correlated records within

TABLE 2. Weight at conception, litter size, gestation length, fraction male pups, and number of male-biased litters over four successive pregnancies in mice maintained on the LF and VHF diets.

Diet	Litter[a]	Conception weight (g)[b]	Litter size[b]	Pregnancy gestation length (d)[b]	Fraction male pups	Number of male-biased litters
LF	1 (n = 15)	20.8 (±1.4)	9.4 (±1.7)	20.0 (±1.4)	0.48	3
	2 (n = 14)	26.7 (±2.2)	10.8 (±2.9)	19.8 (±1.4)	0.45[c]	4
	3 (n = 15)	29.4 (±5.0)	9.1 (±2.3)	19.3 (±1.5)	0.35[d]	1
	4 (n = 10)	30.8 (±2.3)	9.1 (±4.8)	20.0 (±1.4)	0.38[c]	0
VHF	1 (n = 16)	23.1 (±2.2)	9.5 (±2.0)	19.6 (±2.1)	0.51	10
	2 (n = 15)	30.6 (±4.3)	10.7 (±2.8)	18.8 (±1.9)	0.66[d]	12
	3 (n = 14)	35.7 (±5.9)	9.9 (±2.3)	20.0 (±1.2)	0.65[d]	12
	4 (n = 9)	38.0 (±5.8)	8.6 (±4.3)	19.9 (±1.5)	0.71[d]	7

[a] Cannibalism, death of three females, and failure of some females to conceive account for the reduced litter numbers over the course of the study.
[b] Values for maternal weight at conception, litter size, and pregnancy length are means, with SD provided in parentheses to indicate extent of variability.
[c,d] Sex ratio deviated significantly from 0.5; $P < 0.05$[c]; $P < 0.01$[d].

TABLE 3. Effect of diet on sex ratio of first litter born to mature mice, aged 20–27 wk before breeding.

Diet	Conception weight (g)[a]	Litter size[a]	Gestation length (d)[a]	Sex ratio	No. of male-biased litters
LF (n = 14)	31.0 ± 4.9[b]	9.2 ± 3.6	20.4 ± 1.5	0.38[c]	2
VHF (n = 11)	41.4 ± 7.4[b]	9.1 ± 3.4	20.4 ± 1.7	0.64[c]	10

[a] Values for maternal weights at conception, litter size, and gestation length are means ± SD.
[b] Mothers on VHF diet were significantly heavier ($P < 0.001$) than ones on LF diet.
[c] Sex ratios deviated significantly from 0.5 ($P < 0.05$).

treatment, the pooled variance of values for the females in the two treatments was used to determine the effect of the diets. Parity and treatment by parity interactions were tested with residual error.

Sex ratio (fraction of male pups) for the VHF and LF groups was tested against the expected value of 0.5 by using a *t*-statistic [42]. Gestation length (~20 days) and litter size (~9 pups) did not differ between the VHF and LF groups and did not change as the mice aged, although the mice did become progressively harder to breed. The sex ratio of pups (fraction of males) born to mothers on the VHF diet was unusually high (0.67) and to the mothers on the LF diet very low (0.39), spanning litters 2–4. Importantly, this skewing of the sex ratio was related to the diets fed and not to the individual weights of the mothers.

Mice that were first bred at 10 wk of age delivered similar numbers of sons and daughters, whereas virgin mice bred later than 20 wk of age produced pups for which the sex ratio was skewed according to diet (Table 3). The experiments showed that the source and possibly amount of calories provided to mature female mice on a nutritionally complete diet can influence sex of offspring born and are consistent with the Trivers and Willard sex-allocation theory [12]. The second set of experiments, which employed older females, clearly showed that age of the mother rather than parity order affected offspring sex ratio. Only mature females showed a significant response to the diets in terms of the sex of the offspring they produced.

Marsupials. Austad and Sunquist [43] performed an experimental field study with the American opossum (*Didelphis marsupialis*), in which randomly selected females had their diet supplemented with sardines. The provisioned group produced a male-biased sex ratio of pouched young, while the controls produced males to females in about the same number. This study is of particular interest because, in opossums, the young make their way to the pouch within 14 days after conception so that sex selection must occur early in development. Also, the supplement was high in lipid and rich in *n*-3 essential fatty acids, which has been suggested to influence sex ratio toward males in humans [44].

Primates. Although in most societies, humans are not generally considered to be polygynous, retrospective census studies have indicated significant, although somewhat inconsistent, changes in sex ratio associated with particular socioeconomic conditions, geographic areas, and social groupings [45–48]. Crawford et al. [44] speculated that a high content of essential fatty acids in the diets of pregnant females favors boys and suggested that male fetuses are more susceptible to fatty-acid deficiencies than females. Williams and Gloster [49] concluded that a there is a positive correlation of male births and food availability, and that if caloric availability declines, so does the male to female ratio, although the changes are generally quite small and certainly not of the magnitude noted by us for mice. A large group of African women, most of whom were malnourished as determined by their height and weight, for example, produced more daughters than sons [50]. A study examining birth rates of women from rural Ethiopia also demonstrated that a positive correlation existed between women who were in better nutritional state, as determined by body mass and muscle indices, and percent of male births [51]. Analysis of over 10 000 children born in Modena, Italy, revealed that thinner mothers were less likely to give birth to sons [52]. In humans, males appear to have higher in utero caloric demands than females [54]. Vulnerability of male offspring to in utero malnutrition and other environmental stressors might, therefore, have arisen through natural selection, by maximizing the mother's reproductive success, so that she tends to give birth to the more energy-demanding male offspring during auspicious environmental cycles [53].

Male births have also been loosely correlated with a masculine phenotype of the mother, high estrogen and androgen levels at the time of conception, and android patterns of fat distribution in women [55, 56]. As in rodents, electrolytes within the pregnant mother's diet might also affect sex ratio in humans [57].

Studies on sex-ratio biases in nonhuman primates, many of which are polygynous, have been as controversial as those with humans [14, 58], and many of the outcomes noted have been obtained on small sample sizes where stochastic variation can easily lead to erroneous conclusions [58]. High-ranking females of some species tend to produce mainly males, but in others, e.g., baboons, the opposite occurs, and thus, another hypothesis, that of the advantaged daughter has been proposed because daughters of high-ranking females tend to inherit the elite social status of their mothers [14, 59]. Indeed, the complex social structure and intensity of competition for local resources in primate populations means that the predictions of Trivers and Willard [12] would likely not apply in many instances. Nevertheless, it still seems likely that nutritional status of the mother, and the cost of reproduction, play a significant role in adjusting sex ratios among primates [58].

Possible Nonnutritional Causes of Sex-Ratio Skewing

Distortions in the sex ratio have been attributed to factors other than nutrition of the mother [see 14]. In rodents, females stressed in some manner tend to produce fewer sons than nonstressed females [60–65]. Housing pregnant females under crowded conditions reduces sex ratio (fraction of male offspring) [63], while mating at first postpartum estrus tended to produce more males [66]. When subordinate female hamsters are paired with dominant females, they produce smaller litters and fewer sons than their dominant counterparts [64]. Treating such subordinate females with either dexamethasone [64] or progesterone [67] abrogates this stress-induced selective loss of male pups, sug-

gesting an endocrine basis for the phenomenon. Parity has been observed to influence sex ratio of pups born to golden hamsters [68]. Litter sizes and sex ratio increased until the third litter and then declined in subsequent litters. For females bred only once in their lifetimes, male-biased litters occurred in hamsters aged between 100 and 455 days but was female-biased in younger and older animals. Body condition and amount of food consumed, which could have been important variables, were not considered in the statistical models used to analyze the data in any of these studies.

The timing of insemination has long been held to affect sex ratio in species that ovulate spontaneously, particularly in livestock [14, 69]. Early studies with rabbits suggested that fewer male offspring are produced from early matings [70]. In hamsters, more male births occur if fertilization occurs late in estrus, possibly as the result of low vaginal pH [71]. Data from cattle have been mixed, with many older studies indicating that breeding early in the estrous period favored females [72]. More contemporary experiments have generally shown little effect of early insemination [73, 74], but the method used for estrus synchronization may have influenced the outcome of recent work [74]. Gutiérrez-Adán et al. [75] presented evidence that, in cattle, the timing of insemination relative to maturation stage of the dominant follicle influences sex ratio. Moreover, there is little doubt that, in deer [69, 76] and sheep [69], early insemination skews the ratio toward females, while late insemination favors males.

Timing of mating in mice may affect sex ratio in some strains of mice, but not others [77]. B6/CBA F1 hybrid mice produce more females when they are mated early and more males when mating is delayed by a few hours [78]. In addition, the timing of embryo transfer to such mice was found to skew the sex ratio. If embryos were allowed to develop in vitro to the two-cell stage and then transferred to females the morning after they had copulated, i.e., the embryos were 24 h advanced of the recipients, more female fetuses resulted, whereas early or synchronous transfers tended to favor males. The experiments of Jimenez et al. [78] revealed an additional interesting fact, namely that late-stage absorptions were essentially randomized between males and females in the asynchronously bred mice, indicating that selective late-stage abortion could not provide the basis for the sex skewing.

Not unexpectedly, the effect of timing of insemination on sex of offspring in humans is unclear. Some studies indicate that more males are born with natural insemination 3 or more days before or 1 or more days after ovulation [79–83]. However, other results dispute whether timing of intercourse, artificial induction of ovulation, and artificial insemination has any affect on sex ratio in humans [84].

Studies of birth rates from preindustrial Finland (1775–1850) indicate that more sons were born during periods when adult males declined in the population [85]. After the industrial revolution, female births began to outnumber male births in more developed countries. In humans, one reason for the recent upsurge in female relative to male births in Western societies may be age of the mother. Gutiérrez-Adán et al. [86] analyzed birth records in Spain from 1945 to 1997 and showed that only two variables—mean age at marriage and the older age at which women give birth—correlated ($P < 0.01$) with the reduction in the ratio of male to female births. Similar findings, especially for nonwhites, have been found in a U.S. study [87]. Analysis of baboon births in Gombe National Park reveals subordinate females had more sons at a younger age, while dominant females produced more sons as they aged [88].

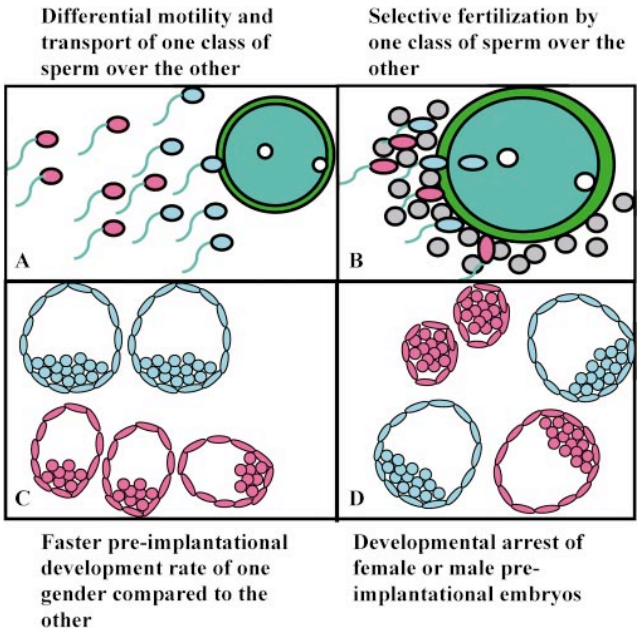

FIG. 1. Proposed preimplantational mechanisms resulting in skewing of offspring sex ratio.

One particularly interesting cause of sex-ratio variation in rodents arises from the mother's prior intrauterine position [89, 90]. Mothers born between two males (2M) tend to be masculinized, assume a dominant social role, and produce male-biased litters, while females born with no adjacent males produce litters biased toward females. The basis of this epigenetic phenomenon is unclear but may be caused by high androgen concentrations encountered by the 2M females. In light of the above rodent studies, it is interesting to note that women who have high testosterone levels tend to score high on dominance measure tests and conceive more sons than those women who score low on these tests [91–93].

While this review has focused on nutrition and related factors that can affect offspring sex ratio, it should be recognized that a multitude of other factors might affect the primary sex ratio. Examples of additional factors include sexual behavior, hormonal concentrations, natural disasters, environmental pollutants, endocrine disrupters, and genetic factors [11, 63].

Theoretical Mechanisms of Sex-Ratio Skewing

Several hypotheses, none of which have been rigorously tested, have been proposed to explain skewing of sex ratios in mammals. These hypotheses are not necessarily mutually exclusive because more than one mechanism could operate within a single species, and mechanisms might well vary between species. The proposed mechanisms fall into two classes: those that operate prior to conception and those that favor one sex over the other after fertilization has occurred (Fig. 1).

1. Sperm of one sex might have differential motility or make their way more directly to the oocyte than the other depending on the conditions prevailing in the reproductive tract of the impregnated female, e.g., state of

cervical mucus, nutrient/energy status of tract secretions, vaginal pH relative to the precise time at which copulation occurred in relation to estrus (Fig. 1A) [71, 94]. One class of sperm might have intrinsic physiological differences in viability, capacitation, or the dynamics of the acrosome reaction [75, 95].
2. Sperm of one sex might be more capable of effecting fertilization once the egg has been reached, depending on factors such as the condition of the female reproductive tract and the penetrability of the zona pellucida, which likely vary according to the time of ovulation relative to time of insemination. Depending on the maturational state at the time of fertilization, the oocyte might preferentially bind X- or Y-bearing sperm (Fig. 1B) [96].
3. Differences in the rate of development or in the sensitivity to conditions of XX versus XY embryos within the female reproductive tract cause a selective loss of embryos of one sex prior to placentation (Fig. 1, C and D). Such selection might be favored by particular nutritional components or developmental asynchrony between the embryos and uterus. For example, faster growth of embryos of one sex in a litter-bearing species, where the space available limits the number of fetuses that survive, could provide a competitive advantage to that gender.
4. Selective fetal resorption/abortion is the final possible means of skewing offspring sex ratio. It would appear to provide a relatively costly means for adapting sex ratio to maternal and environmental selective pressures and was not found to be the cause of sex-ratio skewing in the recent studies of Jimenez et al. in mice [78]. Nevertheless, as a result of fetal resorption following implantation, the Norway rat produces litters biased toward females if mating occurs at first postpartum estrus following removal of the first litter [97]. Induced uterine crowding also leads to a female bias in these rat litters. In each case, the bias arose from absorption of male fetuses after they had implanted but within the first half of pregnancy. Examination of implantation sites in subordinate female hamsters reveals a preferential fetal loss of male pups between Days 5 and 10 of pregnancy [65]. Analysis of preterm mortality records from the Medical Birth Registry in Norway revealed that human male embryos appear to be more sensitive to uterine stress and thus likely to be aborted than females [98].

In some species of birds, changes in sex ratio have been observed relative to abundance of food [99–101]. Because the female is the heterogametic sex in birds, preovulatory gamete selection must provide the means whereby avian sex bias within the resulting clutch is adjusted [101].

Sexual Dimorphism in Development of Preimplantation Embryos

Male and female preimplantational embryos differ in their mRNA expression patterns. For instance, some genes located on the X chromosome are expressed more robustly in bovine and human female versus male embryos [102–105]. Several autosomal genes expressed in trophoblast, such as IFN-τ [106] and hCG [107], and a variety of imprinted genes [108–110] are also not expressed or methylated identically across the sexes.

The most frequently reported manner in which early male and female embryos differ is in their rates of cleavage in the first few days after fertilization. Embryos produced in vitro in a number of species seem to fall into fast-cleaving and slow-cleaving groups, which are predominantly male and female, respectively. This phenomenon has been observed for bovine [111–115], murine [116], and ovine embryos [117, 118]. Male in vivo-produced porcine embryos, both prior to and subsequent to blastocyst hatching, have also been reported to be larger and to have more cells than female embryos [119, 120]. That male embryos develop faster is by no means universally accepted, however, as some studies have reported no differences in human [121], bovine [122], and cultured mouse embryos in the time to reach the blastocyst stage [122 and unpublished work from this laboratory on bovine and mouse]. Similarly, male and female porcine embryos have been reported to grow at similar rates in vivo [123, 124]. Nonetheless, male bovine blastocysts have significantly more cells than females immediately posthatching [125].

There could be several explanations for these contrasting observations. One is species and breed/strain differences. Another is that the culture conditions employed for the in vitro studies influenced the results. For example, the presence of glucose in the medium may preferentially favor either the growth or the development of male over female bovine embryos [106, 126–128]. A third explanation may relate to the manner in which growth rates are measured. In many cases, the end-point employed for in vitro studies has been the time taken to reach a readily observable stage in development, most usually the formation of the blastocyst. By such a standard, all embryos could have equivalent growth rates during the early cleavage stages, but the female embryos might be less capable than male embryos in making a particular developmental transition, e.g., to form a blastocoel or to advance from early to late blastocyst (see Fig. 1D). Thus, a failure to develop or to grow at the same rate as the other sex is probably due to inadequacies of the culture medium or to other environmental stresses. There are several studies indicating that IVP male bovine embryos predominate among blastocysts and that this skew in sex ratios becomes more exaggerated at the expanded and hatched stages [129–132]. Meanwhile, embryos arrested in development prior to the blastocyst stages have been shown to be predominantly female [128, 133, 134]. Our laboratory has shown that the block to female bovine embryo development in a glucose-containing medium occurs at about the time the blastocoel cavity begins to form [106]. Moreover, the data show no differences in growth rate between male and female embryos up to Day 6 of development and that the females that advance to expanded blastocyst do so at the same rate as the male embryos. The cohort of females that fail to advance to expanded blastocyst appear to be less tolerant of the high glucose concentrations in the medium than the successful females. In mouse embryos, a high concentration of glucose (5.56 mM) in the media does not detrimentally effect female or male embryonic development (unpublished observations), which is consistent with the finding that glucose does not always inhibit preimplantational murine embryo development [135].

Implications of Sex-Ratio Skewing to Agricultural and Human Medicine

If there is a difference in the relative numbers of male and female IVP embryos at the blastocyst stage, a skew toward males born after embryo transfer might be anticipated unless, of course, female blastocysts have some advantage over males posttransfer. A preponderance of bull

calves has been noted in at least one such study with cattle [136]. Usually, however, transfer of embryos in cattle is carried out with a mixture of compact morulae and early, rather than expanded, blastocysts. Under such a regimen, it is unlikely that a marked difference in sex ratios would be noted.

Importantly, many successful human IVF programs now utilize blastocyst-stage embryos because it ensures that the embryos are developmentally competent through the cleavage stages. In earlier days, IVF embryos were cultured only through the very early cleavage stages before they were transferred [137]. While such early studies showed no skew in the sex ratio [e.g., 138], several recent reports show a distinct male bias after selection of the most advanced embryos for transfer [139–141]. In other words, inadvertent sex selection may be occurring in human IVF programs. These data suggest that, in the human as well as in the bovine, male embryos make the transition to blastocysts better than females. It also seems possible that, if embryos are selected at the expanded-to-hatched blastocyst stage in either species, the bias toward males will be exaggerated.

Maternal skewing of offspring sex ratio might have important agricultural implications. Offspring of one gender may be preferred over the other. For instance, females are preferred in the dairy industry, whereas males are favored in the beef industry. Altering the diet content prior to breeding might provide a means of manipulating the sex ratio, e.g., a lower plane of nutrition might result in more female offspring.

In summary, sex-ratio skewing occurs in some mammalian species under both field and laboratory conditions, and these alterations might be adaptive, particularly to the mother who bears most of the lifetime burden of caring for the young. The underlying mechanisms are likely to be complex and are still not well understood. However, by combining field and laboratory results, reasonable inferences may be drawn. Our studies in the mouse indicate that maternal diet, possibly its caloric content, can play a directive role in skewing offspring sex ratio. As Sheldon and West [29] discuss, past studies testing the sex-allocation theory of Trivers and Willard [12] in various animal population have employed the nebulous term maternal condition. Maternal condition in wild populations has been assessed either by the animal's dominance behavior or has been based on morphological/physiological characteristics. A unifying definition of maternal condition needs to be established before proper inferences can be drawn across populations and among various species. Importantly, the diet of the mother, both before and after conception, needs to be considered as causative factors in skewing offspring sex ratio in animals.

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