Mechanisms affecting litter sex ratio and embryo quality

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Sex ratios that deviate from 1:1 have been observed in response to a number of stimuli. In this review we will discuss sex ratio biasing, and the evolutionary and molecular mechanisms thought to underlie this phenomena in mammals. The role of embryo quality will be discussed in relation to sex ratio modulation and epigenetic programing of the embryo. Sex ratio skewing has been studied in many species and several factors have been proposed as influencing secondary sex ratios (body condition, maternal dominance, nutrition and developmental asynchrony). In swine, maternal nutrition has repeatedly been shown to influence offspring sex ratios, while maternal dominance and body condition exhibit less consistent evidence supporting their influence. Based on current evidence, we hypothesize that sex ratio biasing is the result of sexual dimorphisms that result in sex specific differences in embryo quality, and these differences lead to sex specific embryonic loss. The mechanisms through which sex specific loss occurs are not fully understood, however sexual dimorphisms in metabolism, gene expression and epigenetic mechanisms during early embryo development suggest that sex ratio modulation might be mediated through these mechanisms. We hypothesize that there are a number of mechanisms for skewing sex ratios in mammals, and that specific mechanisms are elicited in response to specific stimuli.

Introduction

Numerous factors influence embryo quality and offspring sex ratios, and to discuss all of these would be beyond the scope of this review: therefore, this review will focus on sex ratio modulation, and will specifically address embryo quality in relation to sex ratio biasing and programming mechanisms.

For many years investigators have been trying to answer the question of how adaptable sex ratios are within animal populations. Alteration of the sex ratio in mammals from the standard 1:1 ratio has implications for fields as diverse as evolutionary biology and livestock science, and while our understanding of the underlying theory and mechanisms of sex ratio skewing have improved, many answers remain to be found. Manipulation of sex ratio could have several economic advantages in the pig industry. Drickamer *et al*. (1997,1999) showed that both fertility rate and teat numbers were higher in gilts born from litters with a higher proportion of females: whereas Lamberson *et al*. (1988) demonstrated that in litters with higher numbers of males, the females in these litters had a reduced age of puberty. These characteristics are in

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addition to the obvious advantage of being able to skew the sex for breeding purposes (more females required), or for meat production (sexual dimorphic characteristic of larger males with more muscle mass). Mammalian species usually exhibit near equality between the sexes at birth, and as sperm production arises from meiotic division of the male primordial germ cell, it would logically follow that equal numbers of X and Y bearing sperm would be produced and equal numbers of male and female offspring would, therefore, result. However, evolutionary biology predicts a number of conditions in which some plasticity in sex ratios might be expected (Trivers & Willard 1973).

When discussing sex ratios and sex ratio skewing, we need to consider the sex ratio at fertilization and the sex ratio observed at birth (or even observed in utero) as potentially being different. In early studies in pigs (Parkes 1925) approximate equality between the sexes at birth was observed. However, Parkes (1925) found that the sex ratio observed *in utero* (56.8% $\pm$1.38 male fetuses) was significantly different to that observed at birth, and concluded that this skew towards males *in utero* was to ensure approximately equal numbers of males and females at birth, as fetal loss during gestation was predominantly male. This phenomenon has been extensively reported in humans (Hassold *et al*. 1983, Mizuno 2000), although Boklage (2005) contends that sex ratio at fertilization is equal and excessive early loss of female embryos then leaves an excess of male embryos earlier in gestation. Most mammalian species are reported to have secondary sex ratios of approximately 103-110:100 (males:females), and whilst these values are close to equal, the deviation from 1:1 is significant. Furthermore, in mammals the higher mortality seen in males *in utero* persists throughout life and the ratio of males to females in humans does not become equal until the fourth decade of life.

Theories of sex skewing in mammalian species

With most mammalian species showing some level of sexual dimorphism, it is easy to see that there may be a competitive advantage to being able to manipulate the numbers of males and females being born into a certain environment. Where a difference between the size of males and females exists, the extra growth potential of one sex over the other may be considered to be a competitive advantage in relation to gaining mates and food, but a disadvantage when resources are scarce in a social group. There can, therefore, be a conflict of interest between self and group in socially grouped mammals. The fact that bearing offspring is an unequal process in mammals is also of note, with the burdens and cost associated with pregnancy primarily borne by females. This means that in many mammalian species, and all domestic species, there is both sexual dimorphism and a difference in non-genetic parental investment.

Trivers and Willard (Maternal metabolic investment) (1973)

Natural selection will favour a reproductive strategy where females can bias production of their offspring from one sex to the other when their adult body condition, and by inference the expected nutritional environment of their offspring, deviates from the average. The large differences in male reproductive success mean that the mothers in a good metabolic state that have the chance to produce elite male animals will gain far more reproductive success in their offspring than if they produced females. Conversely, mothers in a relatively catabolic state, will have greater reproductive success in their offspring if they produce females. The Trivers and Willard hypothesis requires the following conditions to be met:

1. The body condition of the mother and offspring are related during the period of parental investment.

2. Where body condition of the offspring is related to maternal body condition, this must endure to adulthood.
3. Where there is an increase in offspring body condition as a result of the period of parental investment, this results in a bigger reproductive advantage for male offspring than female offspring

Local resource competition model (1978)

Clark (1978) proposed that natural selection will favour females being able to bias the sex of their offspring when there is competition for resources within small, related, single sex groups, and that the bias will be against the sex of the group (groups are female in the pig as in most mammals). Where resources are not limited, females will bias reproduction towards producing female offspring, as these will inherit maternal social rank and territory even though they would compete for local resources. Conversely, when resources are limited, mothers will produce male offspring, as males are usually more dispersing and local resources have less influence on the future reproductive success of male offspring. Assumptions required for the local resource competition model are:

1. Social rank and territory is heritable in females.
2. The shared genetics conferred by relatedness give an evolutionary advantage to groups that have reproductive strategies that benefit the group as well as the individual.
3. Males are dispersing.

Several researchers have sought to modify these theories to better fit with observed patterns of sex skewing. Hewison & Gaillard (1999) reviewed available data in ungulates which, when taken together, suggested that adherence to the hypothesis was associated with the degree of sexual dimorphism and levels of polygyny displayed by a species, although they concluded that these two factors together were insufficient to expect the Trivers and Willard model to apply. The Trivers-Willard model was designed to be applied to monotocous species, and this may explain why some of the results observed across ungulates are not consistent with the model. Silk (1983) extended the Local Resource Competition model to include large groups and unrelated individuals. The premise of Silk's (1983) inclusion of unrelated individuals is that while females modulate their own offspring sex ratios, they will also be able to influence the reproductive strategies of unrelated animals to further limit the numbers of females produced in the group. Such an influence may be exerted by aggressive behavior or limiting other females' access to resources. Evidence in a number of species suggests that aggression in mixed, single sex, groups occurs between unrelated individuals (Simpson *et al*. 1981).

Both the Trivers-Willard Model and the Local Resource Competition model predict that under certain conditions sex skewing should be observable in mammals. These hypotheses have led to the identification of a number of factors that influence sex ratio modulation in mammals.

Maternal influences on sex skewing

Maternal nutrition

Female nutrition can affect many factors that influence fertility and reproductive performance. Ovulation rate, follicular development, blastocyst development and embryo survival have all been shown to alter with changing maternal nutrition (for a review see Ashworth *et al*. 2009).

Zak *et al*. (1997a) reported differences in weaning to estrous interval, ovulation rate and embryo survival in feed restricted first parity sows, and the effect of restriction on embryo survival was confirmed in a later study by Vinsky *et al*. (2006).

Maternal body condition has been used as an indicator of how well female nutritional requirements are being met, with the assumption that poor body condition reflects poor nutrition. An association between sex ratio and body condition has been observed in sheep, reindeer, red deer and roe deer (Clutton-Brock *et al*. 1984, Kojola & Eloranta 1989, Sheldon & West 2004, Blanchard *et al*. 2005). In domestic pigs the evidence is confounded, as rank and body condition are correlated, thus an association between maternal body condition and offspring ratio is only inferred (Meikle *et al*. 1993, Meikle *et al*. 1996). Sheldon and West (2004) identified only a weak correlation between maternal body condition and sex ratio using a meta-analysis of 37 studies in ungulate mammals, indicating that maternal body condition may not be the best measure to assess the modulation of secondary sex ratios in many mammalian species. Feed restricted mice have been shown to have female biased litters when fasted for 1 week (Meikle & Drickamer 1986). Female dominant litters were also observed when mice were fed intermittently prior to breeding and during gestation in a follow-up study (Meikle & Thornton 1995). Lactational feed restriction is an experimental paradigm that has been used in the pig to investigate how caloric deficits during the very intensive period of lactation affect subsequent litter characteristics (Zak *et al*. 1997a, Vinsky *et al*. 2006, Oliver *et al*. 2011, Patterson *et al*. 2011). This model has consistently shown that feed restriction during the last week of lactation affects embryo development in the next breeding cycle: However there has been a trend over time for fewer observed effects (Table 1), and in the latest iteration of this model only embryo weights and sex ratio were different between restrict-fed and control sows. The change in response to maternal catabolism observed across these models may be attributed to the effect of genetic selection during this period. The relationships between various reproductive traits are not always well elucidated, so it is highly likely that in selecting for reproductive traits such as weaning to estrous interval and litter size, other traits will have been selected for unintentionally.

Maternal diets that are deficient in certain nutrients might also be expected to lead to skewed sex ratios in the offspring. Mice that are fed low fat diets had significantly fewer males and smaller litters than mice fed control diets (Rivers & Crawford 1974). Rosenfeld *et al*. (2003) used two nutritionally complete diets to investigate how the proportion of saturated fat in the diet influenced sex ratio in mice, and found that a very high saturated fat diet was associated

Table 1. Comparison of lactational restriction studies in primiparous sows. The table reports P-values for statistically significant results, NS indicates none-significance and N/A indicates no results available.

	Zak et al. 1997[‡]	*Vinsky et al. 2006*	*Oliver et al. 2011*
Sample size (control:treatment)	9:9	15:17	15:17*
Weaning to oestrus interval	<0.05	NS	NS
Ovulation rate	<0.05	NS	NS
Number of live embryos	<0.05	<0.05	NS
Embryonic survival (%)	<0.05	<0.01	NS
Embryonic weight	N/A	<0.005	<0.05
Litter sex ratio	N/A	<0.05	<0.05

[‡] Embryonic weight and embryo sex were not recorded by Zak *et al*. (1997).

* Oliver *et al*., (2011) selected a subset from tow forms of sows from a larger data set reported by Patterson *et al*., (2011). The Patterson data set consisted of control n = 49, and restrict treatment n = 48).

with a higher proportion of males (0.67), while a diet low in saturated fat was associated with a higher proportion of females (0.39 males). Ewes fed a diet high in polyunsaturated fatty acid (PUFA) had more males than control fed ewes (69% males) (Green *et al*. 2008).

Maternal dominance

When mammals live in social groups, hierarchies develop, and dominant animals in the social group have a competitive advantage over subordinates. The majority of evidence for the role of maternal rank on sex ratio skewing has been provided by studies in primates, and the evidence has been contradictory in many cases. In baboons and macaques, high-ranking mothers have more daughters than low-ranking mothers (Simpson & Simpson 1982, Meikle *et al*. 1984), however spider monkeys exhibit the opposite patterns of sex skewing (Symington 1987). Brown & Silk (2002) performed a meta-analysis using 35 data sets representing 15 primate species and concluded that maternal rank is not associated consistently with secondary sex ratios, although this was not to say that primates do not modulate their sex ratios. They found that small sample size correlated with finding statistically significant results; they also concluded that the distribution of positive and negative results was probably due to stochastic variation (Brown & Silk 2002).

High ranking female mice produce a greater proportion of male biased litters than low ranking females, however they also appropriate more food (Meikle & Thornton 1995). This brings into focus the issues with separating the often associated effects of dominance and nutrition. Nováková *et al*. (2009) looked at social and maternal history variables in laboratory colonies of spiny mice but did not find an association between maternal status and sex ratio. Given the potential benefits, it is somewhat surprising that the influence of maternal dominance in livestock species such as dairy cows and swine, has received little attention. Hohenbrink & Meinecke-Tillmann (2012) identified a correlation between maternal dominance index and the proportion of males born to Holstein dairy cows, with higher dominance being associated with more females. Investigations on the role of maternal rank have produced mixed results in the pig. Of three studies conducted in swine, two have reported that high maternal rank was associated with more male offspring (Meikle *et al*. 1993, Meikle *et al*. 1996), and one reported that high maternal rank was not associated with offspring sex ratio (Mendl *et al*. 1995). Meikle *et al*. (1996) suggested that the lack of agreement between these studies was due to differences in the experimental methods employed. Mendl *et al*. (1995) created groups by mixing pigs 7 weeks into their first gestation, which Meikle (1996) argued would be after the period of any sex ratio skewing influence exerted by dominance (unless females retained dominance effects from previous social groupings). However, Mendl *et al*. (1995) observed and recorded data for these pigs over 4 parities, and although some confounding effects might be expected in the observations from the first parity recorded, it would be expected that subsequent sex ratio observations would be related to the dominance level as recorded within the social group that the pigs experienced across all gestations (with the caveat that females were removed from the group pen for breeding, parturition and lactation). Furthermore, the tendency for more female biased litters in high ranking mothers observed by Mendl *et al*. (1995) was for parity 3, when rank within the social group would have been well established. The conflicting results available to date from the small number of studies completed in the pig suggests that if there is role for maternal dominance in sex ratio skewing, it will likely be complex.

Developmental asynchrony

There have been several observations that the sexual dimorphism observed in many mammalian species can be seen *in utero*, and in particular that male and female embryos do not develop at the same rate. There is some evidence that differences in development rates can be related

back to follicular development, and studies that have investigated feed restriction prior to mating have shown that reduced feed intake was associated with slowing of oocyte maturation and reduced embryo survival (Zak *et al*. 1997b). A follow up study extending the Zak *et al*. (1997b) model, found that nutritional restriction led to male biased offspring for primiparous sows, thereby demonstrating that nutritional treatments that were primarily targeted to follicular development resulted in sex ratio skewing during pregnancy (Vinsky *et al*. 2006). The role of follicular development in sex ratio modulation is interesting, with a growing body of evidence to support this as one mechanism underlying sex ratio biasing. However, the focus of this review is the embryo and aspects of follicular development will not be considered further (for review see Ashworth *et al*. 2009).

A relationship between embryo growth and sex was first illustrated by Tsunoda *et al*. (1985) who found that grouping *in vitro* cultured mouse embryos based on the time of blastocoel formation led to a segregation of males and females; fast developing embryos were skewed towards males, while slow developing embryos were skewed towards females. As in other species, there must be a level of synchronization between preparation of the uterine environment for implantation and embryonic development in the pig for pregnancy to proceed (Pope *et al*. 1988). The developmental asynchrony hypothesis proposed by Krackow (1995) predicts that the maternal environment might be more responsive to particular stages of embryo development: Therefore, if there are differences between males and females in early development, this might result in one sex being more successful than the other during the implantation stage, resulting in a sex ratio bias.

The developmental asynchrony hypothesis would suggest that the timing of insemination in relation to ovulation might also influence sex ratios. In mammals results have been mixed, with rodents and cattle exhibiting associations between sex ratios and insemination timing (Krackow 1997, Wehner *et al*. 1997), but rabbits, mice and humans showing no association (Tesh 1969, Vickers 1969, Wilcox *et al*. 1995). In gilts, although ovulation occurs simultaneously in the majority of follicles, a small proportion were reported to rupture over a longer time period, and these late ovulations are thought to develop into the less mature embryos when fertilized (Pope *et al*. 1988). Pope *et al*. (1986) showed that embryonic mortality at Day 30 of gestation was related to the stage of embryo development, with embryo loss being higher when there were proportionately more spherical blastocysts than ovoid, tubular and filamentous blastocysts. Although these factors would suggest that the timing of insemination in relation to ovulation could be involved in modulating sex ratios in swine, Soede *et al*. (2000) found no association between insemination timing and the percentage of males and have also reported little variation in the timing of ovulation among cohorts of preovulatory follicles in sows.

Positioning in utero

Many aspects of the maternal environment have been studied in relation to maternal reproductive performance, and offspring growth and reproductive performance. One interesting line of research has sought to identify how the gestational environment that a mother experienced influences her subsequent reproductive performance. Females positioned between two males *in utero* (2M) show offspring sex ratios biased towards males in mice, gerbils and pigs (Clark & Galef 1995, Vandenbergh & Huggett 1995, Drickamer *et al*. 1997). In mice, a bias has also been seen towards female offspring in mothers that were positioned between two females *in utero* (0M), while mothers positioned between one male and one female (1M) had equal numbers of male and female offspring (Vandenbergh and Huggett, 1994). It is hypothesized that testosterone, which is able to pass through the fetal membranes, is responsible for the observed skewing and the findings of Vandenbergh and Huggett (1994) would be consistent

with a dose related response. In swine, position *in utero* has also been shown to affect post natal weight gain, with 2M boars exhibiting greater weight gains than 0M females, however this was only observed in nutrient restricted conditions (Rohde-Parfet *et al*., 1990). Drickamer *et al*. (1997) reported that the numbers of males within litter was related to the success ratio of insemination, and in females that came from litters that were >67% male, conception rates at first insemination were lower.

Other factors

The evidence for the influence of maternal nutrition on modulating offspring sex ratios would suggest that other mechanisms limiting embryo access to resources could also result in skewed offspring sex ratios. In an experiment designed to investigate how the allocation of uterine length per porcine embryo influenced a number of reproductive parameters, Chen & Dziuk (1993) found that limiting space to <5cm/corpus luteum led to a tendency for fewer males in pigs. These were similar to the results reported by Wu *et al*. (1988), suggesting that if spatial allocation does affect offspring sex ratio, it is not the principal modulator of offspring sex ratios in polytocous mammals. It could also indicate that where maternal nutrition has been shown to bias towards a sex, at least part of the effect is mediated through the mother rather than within the embryo itself.

Genetic studies aiming to identify factors affecting offspring sex ratios have yielded little evidence that genetic differences influence sex ratios, with associations being small when identified (see (Clutton-Brock & Iason 1986). A recent study in red deer identified genetic differences between mothers that produced more males compared to mothers that produced more females, although this association was only found in one of the studied populations (Pérez-González *et al*. 2012). It is likely that offspring sex ratio is a complex multigenic trait, which would suggest low heritability. The requirement in this trait for plasticity that allows for environmental interactions, would also suggest that it would be resistant to phenotypic selection.

Mechanisms

Testosterone levels

Maternal testosterone concentration and *in utero* embryo exposure to testosterone are both proposed mechanisms for offspring sex ratio modulation. High maternal circulating testosterone has been associated with more male progeny in field voles (Helle *et al*. 2012). Grant *et al*. (2008) found that oocytes that developed in high testosterone follicular environments were more likely to be fertilized by Y-chromosome bearing spermatozoa and their results led them to hypothesize that testosterone might influence the composition of the zona pellucida during key points in development, favouring fertilization by X- or Y- bearing chromosomes (Grant *et al*. 2008). However, in a study by Bermejo-Alvarez *et al*. (2008) there were no differences in fertility rates between sex sorted, sex sorted and recombined, and non-sorted spermatozoa, suggesting that there was no preferential fertilization of the oocyte by spermatozoa of either sex. The role of the zona pellucida in sex ratio modulation is further brought in to question by sex ratio biases found in intracytoplasmic sperm injection (ICSI) produced embryos. Any effects of the zona pellucida is bypassed in ICSI and sex ratio biases seen at birth cannot, therefore, be the result of alterations in zona pellucida composition (Luke *et al*. 2009).

In humans, masculinization of mothers and high androgen concentrations at conception, have been associated with an increase in the proportion of male births (James 1990). As

previously discussed, the maternal gestational environment experienced confers reproductive consequences when the female reaches breeding age (Vandenbergh and Huggett, 1994; Clark *et al* 1994; Drickamer *et al*. 1997).

Glucose

It has been shown that male bovine blastocysts have twice the level of glucose metabolism of female blastocysts (Tiffin *et al*. 1991), and in humans, male embryos showed higher uptakes of glucose and pyruvate (Ray *et al*. 1995). The role of glucose as an energy substrate is well defined in somatic cells; however embryos also utilize glucose for energy and it is routinely included in embryo culture medium. Bredbacka & Bredbacka (1996) first reported an association between high levels of glucose in the culture medium and sexual dimorphism in early bovine embryos, with male embryos developing faster than females. Given the evidence for the role of developmental asynchrony in sex ratio modulation, it is likely that mechanisms shown to alter levels of sexual dimorphism *in utero* could also influence sex ratios. Larson *et al*. (2001) showed that the presence of glucose in culture medium led to a male bias in bovine embryos cultured to the expanded blastocyst stage, although interestingly they did not see any differences in growth rate between male and female embryos. Circulating levels of glucose have also been associated with offspring sex ratio biases towards increased numbers of males in several species (Machado *et al*. 2001, Cameron *et al*. 2008, Helle *et al*. 2012), thereby demonstrating that the influence of glucose on offspring sex ratio is not an artifact found only *in vitro*. Oral administration of dexamethasone resulted in lower circulating glucose levels, and the change in glucose level was predictive of offspring sex ratio (Cameron *et al*. 2008). In humans, patients with type 2 diabetes have been reported to have more sons than daughters, although this result was not seen for type 1 diabetics (James 2006). There is a feedback loop whereby glucose enhances luteinizing hormone release (LH), and LH enhances glucose availability to the oocyte by up-regulating glycolysis, and this might form the mechanistic basis for glucose modulating effects on offspring sex ratios (Zuelke & Brackett 1992, Murahashi *et al*. 1996).

Glucose-6-phosphate dehydrogenase (*G6PD*) is a housekeeping gene that encodes a key enzyme involved in the pentose phosphate pathway. Tiffin *et al*. (1991) reported that activity in the pentose phosphate pathway was four times higher in female bovine blastocysts compared to males. Wrenzycki *et al*. (2002) identified that *in vitro* produced bovine embryos exhibited higher *G6PD* expression in females than males, and this was accompanied by a significant skew towards a larger proportion of males (64:36). Similar findings have been reported in mice (Jimenez *et al*. 2003) and humans (Machado *et al*. 2001). However, as these studies were conducted *in vitro*, there is currently no direct evidence that these results are not related to the experimental methods used. Kimura & Matsuyama (2012) investigated how fructose uptake affected bovine embryo development and offspring sex ratios, and they demonstrated that fructose was not associated with alterations in the development rate, or the proportion of males and females. They suggest that the differences between the effects of glucose and fructose can be explained by differences in their metabolism: Although both substrates can be utilized for glycolysis, only glucose can also be utilized by the pentose phosphate pathway (Ketterson *et al*. 2005). In swine, there is no direct evidence for an association between either glucose concentration or *G6PD* expression and offspring sex ratios: However, as with other mammalian species, *G6PD* can be expressed in a sexually dimorphic manner (Ronis *et al*. 2011). Further studies are required in the pig to discover whether glucose and the pentose phosphate pathway play the same role in sex ratio skewing described in other species.

Epigenetics

At fertilization the principle difference between male and female zygotes is their complement of sex chromosomes. Post fertilization X-inactivation occurs in female conceptuses, and it is specifically the paternal X-chromosome that is silenced. The extra-embryonic tissues begin to develop during this time, and throughout their development these tissues retain paternal X-chromosome inactivation. However, in the embryo there follows a period of X-reactivation, before random X-inactivation occurs. Male embryos retain an active X-chromosome throughout this period. During the period of X-reactivation in female embryos, X-linked genes are likely to show sexually dimorphic gene expression, and indeed this has been shown for a number of genes. It has been reported that the DNA methyltransferases *DNMT3A* and *DNMT3B* have sexually dimorphic gene expression in bovine blastocysts (Bourc'his & Bestor 2006, Bermejo-Alvarez *et al*. 2008). These DNMTs are responsible for *de novo* methylation of CpG dinucleotides, and as such are important epigenetic regulators. The DNA methyltransferase 3-like protein (*DNMT3L*) exhibits sex-based differences in function, and although it is required for imprint establishment in female germ cells, it plays little role in imprinting in male germ cells (Bourc'his & Bestor 2006).

In humans, prenatal exposure to famine has long-term consequences on adult health and these consequences have been shown to differ between the sexes, with male offspring having increased susceptibility to the adverse effects (for reviews see Painter *et al*. 2005). These later life effects are in addition to the immediate effects on growth *in utero*. Although birth sex ratios do not deviate from expected values for women exposed to famine during gestation (Stein *et al*. 2004), postnatal mortality rates are increased in their male offspring (Painter *et al*. 2005). Tobi *et al*. (2009) demonstrated differential methylation in a number of genes in response to prenatal famine exposure, and some genes exhibited sex specific alteration in their methylation patterns. Insulin-like growth factor 2 (*IGF2*) is a maternally imprinted gene that plays an important role in embryo development and is highly conserved across species. Hypomethylation of one *IGF2* differentially methylated region (DMR) was reported in people exposed to famine (Heijmans *et al*. 2008). As in humans, maternal nutrition has been implicated in epigenetic changes in the offspring of mice. Kwong *et al*. (2006) fed mice a low protein diet during the preimplantation stage of gestation and found that mRNA expression of *IGF2* and *H19* were reduced compared to controls. Imprinted genes, and specifically *IGF2*, have also been implicated in the effects of lactational feed restriction in pigs as Oliver *et al*. (2011) showed a tendency for a sex-by-treatment interaction in the embryos, and sex differences in *IGF2* mRNA expression have also been seen in bovine blastocysts (Gebert *et al*. 2009).

Our previous studies (Patterson *et al*. 2011) have indicated that there is a large variation in response to nutritional restriction during lactation, with some sows becoming highly catabolic and having greatly compromised embryo weights, whilst others show little catabolism and non-compromised embryo weights. Having classified sows by the level of catabolism they displayed in response to lactational feed restriction, Vendramini (2012) performed microarray analysis using the EmbryoGENE EMPV1 array (Tsoi *et al*. 2012) on day 9 embryos to identify 340 genes differentially expressed between High and Low catabolic sows. Ingenuity pathway analysis indicated that macromolecule metabolism, cell morphology and cellular assembly and organization were highly represented processes. Furthermore, confirmation by q RT-PCR indicated sex by sow-catabolism interactions for several genes, consistent with our previous observations for day 30 embryos (Oliver *et al*. 2011). We have also recently investigated global methylation in day 30 embryos from our lactational restriction model using methyl-seq and found DMRs associated with treatment: Moreover, the greatest methylation changes

were observed in partially methylated sequences (G Oliver unpublished). Overall, using the cohort of samples generated from primiparous sows feed restricted during lactation, we have been able to demonstrate physiologic alterations in the embryos (Patterson *et al*. 2011), gene expression differences related to treatment (Oliver *et al*. 2011), gene expression differences related to sows lactational catabolic state (Z Ren *et al*. manuscript in preparation), and DMRs related to sows lactational catabolic state (G Oliver unpublished). We interpret these studies as demonstrating a link between maternal nutrition, epigenetic alterations and embryo quality, and would suggest that the epigenetic changes are responsible for the phenotypic differences in embryo quality, and as these responses are possibly sex specific, the observed sex ratio skewing may be mediated through this mechanism.

Summary

Three mechanisms could account for the modulation of sex ratios in mammalian species: 1) Sperm production could favour production of one sex. However the meiotic division that generates sperm would suggest that equal numbers would be produced and evidence supports this. 2) The oocyte might be able to recognize whether a sperm contains an X or Y chromosome and respond such that fertilization favours one sex. Again, there is little evidence that this happens. 3) There is sex selective loss during gestation, the mechanism(s) of which are not fully understood and may very between species. We hypothesize that sex specific loss is due to sex specific differences in embryo quality that result from sexually dimorphic responses to environmental stimuli. Sex selective loss has a large body of evidence supporting it, and as a mechanism for adaptive manipulation of sex ratios, it has a plasticity that would allow for multiple influences and responses.

There is clear evidence for maternal mechanisms mediating offspring sex ratio manipulation in swine, and sex ratio skewing has been reported in response to glucose, caloric restriction and high fat diets. It is probable that where maternal dominance has been reported to affect offspring sex ratios in pigs, this would be at least in part related to how rank affects access to nutrition. Swine models of sex ratio skewing mostly do not adhere to the Trivers-Willard hypothesis. The pig is a litter bearing species where males are dispersing and females live in groups, and it would seem that there is more evidence supporting the local resource competition model. The mechanisms that mediate sex ratio biasing in mammals are still not fully known, although the plethora of available studies would suggest that multiple mechanisms are involved (Figure 1). It might be expected that specific treatments would elicit specific mechanisms, and this might certainly be true for glucose influenced sex biasing. However, some of the longer-term consequences of offspring sex ratio skewing, such as the dip in adult reproductive performance seen in female pigs that are positioned between two males during gestation, would indicate that the embryos are being programmed *in utero*, and that the mechanism for these long-term outcomes is epigenetically based.

Declaration of interest

The authors have no conflicts of interest that could be perceived as prejudicing the impartiality of this review article.

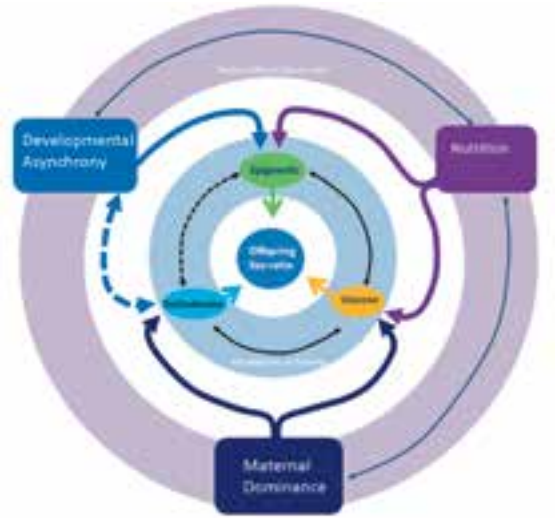

Fig. 1 Interactions between component factors influencing offspring sex ratio. Factors that have been consistently implicated in sex ratio modulation are shown in the purple outer circle, and mechanisms that may be mediating the actions of these factors are shown in the blue inner circle. The solid lines indicate direct evidence for an interaction between factors and mechanisms, and the dashed lines indicate hypothetical relationships.

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