EFFECTS OF MATERNAL CONDITION ON SEX RATIO IN QUAIL AND HUMANS

by

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(Under the Direction of Kristen J. Navara)

ABSTRACT

If sex determination is random, offspring sex ratios are expected to be 0.50 (i.e., 50% males). However, there are examples in nature where this is not the case. A common theme is that maternal condition and nutrition can influence sex ratio through various mechanisms such as sex-specific laying or implantation. What dietary component triggers sex ratio manipulation and when sex ratio changes occur are still unknown. We tested whether dietary fat is a key mediator of sex ratio adjustment in birds, and whether human embryos are sensitive to variation in maternal condition in a sex-specific manner. A flock of Japanese quail were given feed high in fat, but no significant changes in sex ratio were observed compared to the control diet. In humans, data from multiple embryo transfers were reviewed and the rates of successful implantation were not correlated with maternal body mass index or sex of the embryo.

INDEX WORDS: Sex ratio determination, maternal condition, human, avian

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The ability to choose the sex of one's offspring has been of interest to humans both past and present. The process of sex allocation has historically been presumed to occur according to random chance, resulting in the equal production of males and females. However, there is now a large body of evidence indicating that offspring sex ratios are often skewed, and both birds and mammals have the ability to facultatively adjust sex ratios according to environmental and social conditions. The ability to take control of these potential mechanisms in order to increase sex ratios (more males) or decrease sex ratios (more females) can benefit a large number of people.

An understanding of how sex ratio adjustment may occur in birds may have many important implications. For example, the poultry industry is greatly interested in the potential to control sex ratio, as sex is determined before eggs are laid in birds. The commercial egg industry euthanizes and discards millions of newly hatched chicks because they are male and are therefore unable to lay eggs (Klein et al., 2003). If industry workers could purposefully manipulate chick sex ratios, there would be benefits in both welfare concerns and industry profits. Sex ratio manipulation also has a large effect on our efforts to preserve endangered species. In the Kakapo Parrot, supplemental feeding led to females overproducing males which hindered their potential breeding output (Clout et al., 2002). However, conservationists applied sex allocation theory in order to increase the Kakapo population by limiting feed access to birds of higher maternal body condition to keep their sex ratios closer to 0.50 (Robertson et al., 2006).

Humans can also greatly benefit from further understanding of sex ratio adjustment. If adjustment is significant, it could be of great use to those seeking natural family planning (i.e. outside of *in vitro* fertilization treatments) as well as benefiting those undergoing fertility treatments. In embryo transfers where the sex of the embryo is known, doctors may choose one sex over the other based on these differential survival rates. The potential for natural family planning may be especially relevant in countries with "missing women" where the male:female ratio is much higher than elsewhere in the world. This high sex ratio is present at birth and is likely due to sex-specific abortions (Sen, 2003). If natural family planning is more available, this can reduce the number of abortions performed solely for family planning purposes.

Does it make sense for mammals and birds to skew sex ratios?

Sex ratios are measured at three general timeframes: at or before fertilization (primary sex ratio), at birth or hatch (secondary sex ratio), and at sexual maturity (tertiary sex ratio). If sex determination is truly random, the expected sex ratio is 1:1, i.e. one male per female, but this ratio is not always the case. While tertiary sex ratio can be skewed due to a large number of outside factors, primary and secondary manipulation are primarily influenced by maternal and paternal effects. There are many different ideas as to why sex ratio skewing occurs and how it can increase reproductive success.

Trivers and Willard (1973) suggested that it would be beneficial for parents to influence primary and secondary sex ratios depending on how much investment they are contributing to those offspring. In a mating system where one sex has more variance in reproductive success, it is advantageous to invest more resources into the sex that has the greatest variance if the parents are in good condition with an abundance of resources. The sex with a greater variance in reproductive can only reach its maximum potential with an abundance of resources. Therefore, in

times of high resource availability, this becomes a more reliable investment. However, in times of limited resources, it is more beneficial to have offspring of the sex that has the more stable reproductive success regardless of the availability of energy reserves.

On the other hand, Fisher (1930) proposed the equal investment hypothesis. He argued that parents will invest the same amount of resources into male and female offspring. If one sex is more costly to produce than the other, this may not equate to a 1:1 ratio of males:females. The sex that is less expensive to produce will be produced in greater numbers, and the strength of that skew will be dependent on the difference in cost between the sexes. The greater the difference, the stronger the skew.

There is also Düsing's model (1884), which assumes that parents with the ability to have more of the minority sex will do so to maximize their fitness. This follows Darwin's suggestion that if the population sex ratio for a species is not roughly balanced, some members of the majority sex will be "superfluous" (Darwin, 1871). Therefore, Düsing suggested that it is advantageous to produce more of the minority sex, as they will have less competition for mating. This may be particularly applicable to a species with a monogamous mating system. This may not always hold true, however. For example, in a species that follow a lek mating system, the males will compete in a mating display and the females will choose one or two males to mate with. In this case, males always have more competition for mating even if they are the minority sex. In more monogamous mating systems where one male will only mate with one female, producing more of the minority sex would be an advantage. Assuming that this ability is heritable, their offspring will produce more of the minority sex and thus will continue to have increased fitness.

These hypotheses explore how sex ratio manipulation might have come about by providing evolutionary benefits to species. However, they do not explain the specific triggers behind sex ratio skews. A continuing theme across examples of both avian and mammalian sex ratio manipulation is maternal condition and resource availability.

Dietary Cues

Food Availability and Maternal Body Condition

Food availability and body condition (increased or better condition defined as increased weight or body fat content) are highly correlated (Kitaysky et al., 1999) and therefore it is difficult to determine which is the one that has significant effects on sex ratio in both birds and mammals. In fact, some studies will alter maternal body condition by restricting access to food (Pike, 2005). Regardless, many studies have shown skews in sex ratios in response to diet and maternal condition, but the direction of these skews is not always consistent. More work needs to be done to examine how food availability and food quality influences sex ratios in birds and mammals. For summaries of dietary effects on avian species, see Table 1.1, and see Table 1.2 for mammalian species.

Birds

When food is limited or in times of poor maternal body condition, blue-footed boobies, Ural owls, and American kestrels were all shown to have male-biased sex ratios (Torres and Drummond, 1999a; Wiebe and Bortolotti, 1992; Brommer et al., 2003). Other species show the opposite effect and produce male-biased sex ratios in times of food abundance and/or female-biased sex ratios in times of food scarcity. Some examples include zebra finches, Kakapo parrots, pigeons, and yellow-legged gulls (Bradbury and Blakey, 1998; Clout et al., 2002; Pike, 2005; Alonso-Alvarez and Velando, 2003). While these species appear to skew sex ratios in

opposite directions in response to the same cues, they all skew sex ratio towards the larger sex in times of food abundance and towards the smaller sex in times of food scarcity. This indicates that factors related to body size (of one sex or the other) may mediate the effects of diet on offspring sex ratios.

There are two main thoughts to why maternal body condition causes these skews in sex ratios. The first follows the Trivers-Willard Hypothesis (1973), in that the increased body condition allows for increased investment into offspring. This affects the offspring's reproductive success, and it is therefore advantageous to invest more into the offspring with the greater variance in success. The other thought process is that one sex is more expensive to produce (presumably the larger sex), and therefore will be produced in a lesser number according to Fisher's equal investment theory (Fisher, 1930). The question that separates these two ideas is whether the parents skewing sex ratios out of a drive to increase reproductive success (Trivers and Willard, 1973) or if they are skewing because of their own physiological constraints (Fisher, 1930).

Mammals

There is an abundance of evidence that mammals also skew offspring sex ratios according to food availability. Gese et al. (2016) conducted a feed restriction experiment on captive coyotes. Females were given excess food (110% of maintenance value) or restricted food (65% of maintenance value) for seven months prior to mating. After mating, both groups were fed a normal diet for pregnancy and lactation. This was done for two reproductive cycles, with some females switching diets while others were maintained on the same diet treatment for both cycles. It was found that coyotes on the excess food diet whelped more pups, had more implantation sites, and had higher sex ratios than food restricted coyotes. There was not a

difference in fetal death (implantation sites versus number of pups whelped) between the groups, so the change in sex ratio was due to an increased number of male pup embryos, not sex-specific embryo death. Additionally, these effects persisted to the second reproductive cycle for the feed-restricted coyotes. This study suggests that there is preferential implantation or fertilization of male versus female embryos depending on nutrition levels. While the number of male embryos and pups changed according to pre-pregnancy diet, the number of female pups did not. Perhaps there is a mechanism in place in coyotes to preferentially implant male embryos over female embryos after a certain number have been implanted, and before this critical number has been reached, there is no preference for male or female embryos.

In Arctic foxes, it was found that mothers in areas with high food availability produced lower proportions of male offspring (Goltsman et al., 2005). While other studies mentioned here have theorized that changes in sex ratio are due to physiological restraints, Goltsman and colleagues suggested that changes in sex ratio were made as female offspring are less likely to migrate away from the area they were born in and will therefore stay in an area with abundant food.

While humans have not been under the pressure of natural selection for decades, they still appear to retain patterns of sex allocation that comply with those seen in non-human mammals. A study in humans of over 10,000 births across four years was conducted observing the relationship between body weight and sex ratio. The researchers looked at the effect of both pre-pregnancy weight and total weight gain over the course of the pregnancy. They found that women weighing less than 54.6kg (120.4lbs) before pregnancy produced a lower proportion of male babies than women that weigh more than 54.6kg before pregnancy (Cagnacci et al., 2004). However, the same study showed that women in the highest quartile of weight gain during

pregnancy also produced decreased sex ratios. This was corroborated by a study on IVF patients that found higher body mass indices (BMI) had lower success rates (Lintsen et al., 2005).

This suggests that male embryos survive best within an optimal range of both body weight and weight gain through pregnancy, and that weights outside this range may be detrimental to male embryonic survival. This study does have its limitations, as pre-pregnancy weight was not measured but simply based on each patient's memory, and therefore has the potential to be inaccurate. Further, it is possible that patients with low pre-pregnancy weight gain more weight on average than patients with high pre-pregnancy weight. This could mean that pre-pregnancy weight is what causes the shift in secondary sex ratio and the weight gain is merely correlational. A similar study showed that women with higher energy intake gave birth to a higher proportion of males than women with lower energy intake around the time of conception (Mathews et al., 2008). Unlike Cagnacci et al. (2004), the authors did not find a correlation of sex and maternal energy intake during pregnancy, suggesting that pre-pregnancy nutrition has a greater impact than nutrition during pregnancy.

Influences of micro- and macro-nutrients on sex ratios

As of now, it is unclear whether sex ratio biases in the studies described above occurred due to differences in total energy intake, or due to changes in the intake of specific macro-nutrients. Despite the many examples of diet affecting avian sex ratio, no studies to our knowledge have specifically researched specific dietary components in correlation to sex ratio. In mammals, however, there have been many attempts to discover effects of specific micro- and macro-nutrients on offspring sex ratio. For example, Mathews et al. (2008) found that intake of a number of macro- and micro-nutrients were correlated with fetal sex, suggesting that diet quality can have a significant effect on sex ratio rather than just quantity.

Micronutrients

There is a potential influence of micro-nutrients on sex ratio, as Mathews et al. (2008) found that pre-pregnancy intake of vitamin C, vitamin E, folate, iron, zinc, calcium, and potassium in humans can all be correlated with offspring sex ratio in humans. However, given that these are micronutrients, it is possible that these are just correlations and the true cause of sex ratio adjustment lies in macronutrients. Macronutrient intakes before conception that were correlated with sex ratio in this study included fats and protein.

Protein

No studies to our knowledge have specifically studied potential effects of protein on offspring sex ratio. Some studies have noted protein, but lack conclusive evidence of protein's effects. For example, in humans, Mathews et al. (2008) found that pre-conception protein intake can be correlated with fetal sex, but this was not isolated and could have been correlation due to other nutrients or total dietary intake. In zebra finches, different quality diets were found to have subtle impacts on offspring sex ratios, but only when looked at in conjunction with clutch sizes (Arnold et al., 2003). Protein was altered in both quantity and quality in these diets, but this was done in conjunction with other nutrients. Additionally, the diets were designed to alter female quality in general rather than identify effects of specific nutrients.

Carbohydrates

Some studies have suggested that carbohydrate intake is what triggers a shift in sex ratio. In Tammar wallabies, an increase in glucose levels was associated with a decrease in sex ratios (Schwanz & Robert, 2013). However, this study was done in a wild population and thus the blood glucose measurements may not be affected by other factors unknown to the authors. The stress of being captured and handled can alter blood sugar levels and the animal's food

consumption is unknown. This is a possible explanation for the inconsistencies this study found in maternal glucose and sex ratio in different years of the study.

Additionally, in rats, increased fructose in drinking water caused a significant increase in sex ratio (Gray et al., 2013). It was concluded that this effect was not due to sex-specific fetal death, implantation location, or effects on motility of X vs Y bearing sperm. Therefore, it is likely that the effect was due to impact(s) on the ovary and/or oocytes. Further, the sex ratio change was not correlated with body weight or food intake differences, as all four treatment groups had very similar intake and body weights. While secondary sex ratio was the focus of this study, Gray et al. did observe primary sex ratio in a subset of their test subjects and found that the sex ratio changes were also present then.

In a study conducted by Cameron and colleagues (2008), experimentally decreased levels of glucose were associated with a lower proportion of male offspring. However, the authors emphasized that the change in glucose levels were a more accurate predictor of sex ratio rather than the absolute levels. Cameron et al. argued that the change in levels is more informative of the maternal body condition during pregnancy and at the time of birth. Therefore, it is more advantageous to look at the direction body condition is going in rather than the current, static condition. Since the majority of maternal investment comes during pregnancy rather than at conception, it follows that the body should be looking to future condition more than current condition.

While these studies provide support for carbohydrates as mediators of sex ratio adjustment in mammals, some studies show that they do not always have a significant effect. In a study on Richardson's ground squirrels, there was no correlation between glucose levels during gestation (Ryan et al., 2014). It must be noted that these samples were taken during gestation, not

conception. Therefore, investment had likely already been made into offspring while the only available method of sex ratio manipulation would be sex-specific fetal death, which is considered to be a large waste of resources at this point and overriding the benefit of sex ratio manipulation. Additionally, a study on mice found that sex ratios of mothers on a high-fat, high-sucrose diet did not differ significantly from a control diet group (Chin et al., 2017).

Fat

Fats may be better mediators of offspring sex ratios because the quantity of fat can indicate availability of food resources over the course of a breeding season, rather than a short-term snapshot of current resource availability. In mice, differences in dietary fat content exerts a strong effect on sex ratio. Females fed a diet high in saturated fat produced a male-biased sex ratio (0.67) while those on a low-fat diet but high in carbohydrates produced a female-biased sex ratio (0.39) (Rosenfeld et al., 2003). These sex ratios were calculated over the course of multiple pregnancies and were not correlated with body mass. The increased fat content of the diet caused a male bias that had no correlation to the weights of the mothers.

In another study, mice on fat-restricted diets experienced increased incidences of stillborn births, decreased litter sizes, higher rates of postpartum cannibalism, and female-biased sex ratios (Rivers and Crawford, 1974). There was no difference in the number of female offspring between the low fat and the control group. It was therefore concluded that either the Y-bearing sperm was less likely to fertilize eggs when the female was on a low-fat diet (which was considered unlikely), or that not enough fat was particularly detrimental to male embryos during gestation.

Polyunsaturated fats appear to exert similar effects to saturated fat in that they can skew offspring sex ratio towards males. When cows were given supplemental omega-6 fatty acids,

there was an increase in the proportion of males produced (Marei et al., 2018). Cows that were given 3% and 5% levels of fatty acids listed above (protected so as to not be broken down in the rumen) had 14/20 and 17/20 males compared to the 8/19 of the control. Further, Marei et al. did an *in vitro* study where they matured oocytes in the presence of polyunsaturated fats before *in vitro* fertilization. Oocytes fertilized in the presence of linoleic acid and conjugated linoleic acid produced male-biased sex ratios. This shows that the fats were likely affecting the oocytes directly and not just the uterine environment. Additionally, neither the *in vivo* nor *in vitro* sex ratio effects appear to be due to sex-specific fetal death. Since the *in vitro* fertilization eggs matured in linoleic acid and conjugated linoleic acid had higher sex ratios than the controls (and alpha-linoleic acid), the effect could not have been due to sex-specific differential implantation rates. Instead, these results suggest that there is selective fertilization of X- and Y-chromosome bearing sperm. It must be noted that the supplementary fats before conception did reduce or eliminate weight loss seen in the control group, so body weight cannot be completely ruled out as the source of sex ratio skews.

Mechanisms of Sex Ratio Manipulation

At this point, there is abundant evidence that food quantity and/or quality influences offspring sex ratios in both mammals and birds. However, how this may happen still remains unclear. There are a number of potential mechanisms that could underlie the ability to skew offspring sex ratio. The mechanisms boil down to one of three strategies: altering the proportion of male to female gametes, selective fertilization of oocytes, differential survival rates and differential implantation of male and female embryos. Each of these strategies has advantages and disadvantages, and each has supporting evidence in literature. Further, it is possible that multiple strategies are implemented to increase the success rate of sex ratio manipulation.

Primary Manipulation

Since sexes of mammals and birds are determined via sex chromosomes, this is the target for primary manipulation. In mammals, sex is determined by the male gamete. Therefore, the male can produce more X-chromosome-bearing sperm or Y-chromosome-bearing sperm, skewing the probability of what type of sperm fertilizes the egg. This has been shown to occur in humans. Some men will produce more Y-chromosome-bearing sperm than X-chromosome-bearing sperm, leading these men to produce a high proportion of male offspring (Gellatly, 2009). Females can potentially exhibit control around this same time. Fluctuating levels of hormones during the estrus cycle affect vaginal environment and can thus affect sperm motility rates (Orihuela et al., 1999). This could be a method of selective fertilization or another method of altering the ratio of male and female gametes. Since sex ratio and timing of insemination have been correlated in multiple studies with multiple species, this is likely a method of sex ratio manipulation (Verme & Ozoga, 1981; Huck et al., 1989; Harlap, 1979).

The heterogametic sex in avian species is female, and therefore females determine offspring sex ratio. During meiotic segregation, either the Z (male) or W (female) chromosome is expunged into the polar body and degraded. The surviving chromosome then determines the sex. Skewing meiotic segregation or selective ovulation and/or selective fertilization of follicles based on their sex are all potential mechanisms for primary control in birds. Since acute injections of certain hormones prior to ovulation (and therefore prior to meiotic segregation) can significantly affect sex ratio without disrupting ovulation or fertility of laid eggs, maternal control of meiotic segregation is plausible (Pinson et al., 2015; Pinson et al., 2011; Gam et al., 2011).

Secondary Manipulation

Secondary sex ratio manipulation is also a potential mechanism and would primarily be caused by differential survival rates of male and female embryos. When a female bird enters reproductive state, some follicles are selected and grow steadily over a long period of time before entering a seven to ten-day period of rapid yolk deposition. Skewing sex ratio after ovulation would result in the loss of all of the nutrients invested into the egg. However, Pike (2005) proposed a model of follicular development and re-absorption that allows birds to skew the sex of their first laid egg in a clutch while not losing significant resources. The first ovulation is canceled if the follicle is the wrong sex, and the follicle is then resorbed. This prevents gaps in the laying sequence in addition to preventing unnecessary loss. It is very possible that there are multiple mechanisms in place to allow for sex ratio manipulation at multiple levels. For example, the follicular development model proposed by Pike (2005) could be the first layer of manipulation, followed by differential mortality of male and female embryos. This could act as a failsafe, if one mechanism fails, the other may take over. This ensures that any further investment beyond egg laying and incubation would be made into the most "profitable" sex given the current environment and parents.

In mammalian species, the bulk of maternal investment is done over a long period of time during pregnancy (Wade & Schneider, 1992). Since the sex of an embryo is determined before implantation, it is possible to have a reactive method of manipulation while still conserving resources. For example, differing implantation rates, differing embryo survival rates, and differing miscarriage rates are all viable as they can prevent investment into the "wrong" sex. Additionally, control over this mechanism for mammals could be much simpler than in birds, as there is no need for control over selective ovulation or resorption of yolk. Differential survival

rates of male and female embryos in multiple species support the selective abortion model as a mechanism for sex ratio manipulation (Cichoń et al., 2005; Orzack et al., 2015). Further, Yosef and colleagues (2019) were able to use genetic engineering to cause a sex ratio bias via male-specific fetal death. Since this shows that a genetic mechanism may be responsible for sex ratio manipulation, it is possible that such a mechanism could be heritable as well. This is in line with the Trivers-Willard Hypothesis, as the advantageous mechanisms must be heritable.

Could Hormones Mediate these Effects?

In order for sex ratio adjustment to occur in response to environmental and social variables, there must be communication between the part of the body where the trigger occurs and the reproductive system. This is most likely done via hormones, as the endocrine system is how the body communicates between its various systems (Molina, 2018). This is further supported by the fact dietary and social cues can have significant effects on hormone levels (Pike & Petrie, 2005b, Chin et al., 2017). Multiple studies have indicated that hormones may be important in the process if sex ratio adjustment in both birds and mammals, especially testosterone and corticosterone.

Birds

Pike and Petrie (2006) studied chronic hormone effects in Japanese quail utilizing silastic implants. The hormones tested included testosterone, 17-beta-estradiol, and corticosterone (Pike & Petrie, 2006). Of these three hormones, only corticosterone was shown to have an effect on sex ratio. Higher corticosterone levels (verified through fecal corticosterone analysis) were correlated with lower sex ratios. Further, levels of estradiol and testosterone in fecal matter were unaffected when corticosterone was increased. This suggests that corticosterone altered sex ratio

through a pathway that did not involve estradiol or testosterone levels, such as directly affecting the female's ovaries in ways that do not directly involve steroidogenesis.

Acute hormone levels have been shown to have significant effects on sex ratio as well. In birds, sex is determined at the time of meiotic segregation (i.e. which sex chromosome is passed from the mother to the offspring). Thus, hormone levels at this point are plausibly what determines offspring sex. Therefore, it should be possible for an acute elevation in hormone levels during meiosis to cause a bias in sex ratio. Meiosis is assumed to occur five hours before ovulation, which occurs shortly after oviposition (Olsen & Fraps, 1950). Injections given during the window of meiotic segregation caused a strong male bias in sex ratio (Pinson et al., 2011). While Pike and Petrie (2006) showed no correlation between chronic testosterone elevation and sex ratio, Pinson and colleagues (2011) showed that an acute elevation in testosterone can be correlated with sex ratio. It is possible that the chronic elevation of testosterone was not at a high enough level to cause a skew in sex ratio, or that the change in testosterone has a stronger effect than just the overall level.

In homing pigeons, it was found that chronic elevations of corticosterone and testosterone exerted a significant effect on sex ratio (Goerlich-Jansson et al., 2013). Testosterone treated birds produced a higher proportion of male offspring hatched from first-laid eggs within a clutch while corticosterone treated birds produced a higher proportion of female offspring hatched from first-laid eggs. Additionally, the bias for the testosterone-treated birds persisted to the next year, despite treatment having stopped at that point. However, these results do not match what has been seen in an earlier study in homing pidgeons, where it was found that testosterone implants did not affect sex ratios (Goerlich et al., 2010).

In a free-ranging population of white-crowned sparrows, it was found that females with higher levels of corticosterone had lower sex ratios (Bonier et al., 2007). However, this population was small (n=19). Further in their study, Bonier and colleagues (2007) gave some of the females corticosterone implants, which did cause a significant bias towards females when compared to the placebo group.

In a study correlating sex ratio with body condition, 17-beta estradiol, testosterone, and corticosterone levels, it was found that females with better body condition, lower corticosterone levels, and higher testosterone levels tended to have male-biased sex ratios, as well as invested more nutrients into the male eggs (Pike & Petrie, 2005a). Experimental manipulation of hormone levels has shown significant effects on sex ratio, but Pike and Petrie (2005a) showed that naturally occurring levels of corticosterone and testosterone can be correlated with sex ratio. Additionally, the fact that peahens invested more nutrients into male eggs when giving rise to a high sex ratio clutch gives an interesting insight into sex ratio studies. The relationship between investment and sex is a curious one. Did body condition and hormone levels cause increased investment into follicles (the bulk of investment happening days before ovulation and sex determination) that caused that follicle to become male? Or was the follicle destined to become male and selected because increased investment was possible?

These effects are not universal, however. Zebra finches were shown to not have any correlation between baseline corticosterone levels and sex ratio, and experimentally stressed birds did not alter sex ratios when compared to control groups (Gam and Navara, 2016). Yet, in an experiment where corticosterone levels were acutely elevated around the time of meiotic segregation, zebra finches were shown to skew their sex ratios towards males (Gam et al., 2011), which is opposite of the usual direction corticosterone treatments skew sex ratios.

Mammals

These hormones are not limited to having a sex ratio effect in birds, as they also appear to influence sex allocation in mammals as well. In rats exposed to a stress-inducing factor (presence of a cat) for many days, the stress caused a decrease in many reproductive parameters, decreased testosterone and 17-beta estradiol levels, and a decrease in sex ratios (Mahmoodkhani et al., 2018). In ground squirrels, mothers that had high corticosterone levels during gestation were more likely to produce males than females in their litters (Ryan et al., 2011). This again suggests that the timing of the hormone levels is just as crucial as the actual levels themselves.

Testosterone can also influence offspring sex ratios. In a study in nutria, high testosterone levels were associated with a female-biased sex ratio (Fishman et al., 2018). While this does not follow the recurrent theme of high testosterone being associated with a high male sex ratio, the fact that it can still cause an effect on sex ratio is interesting, as birds and mammals have very different sex determination pathways.

In cows, it was found that eggs with higher levels of follicular testosterone are more likely to become male embryos rather than female embryos (Grant, 2008). This provides important evidence that hormones specifically around the site of fertilization can have a significant impact on sex ratios.

While the effects of hormones on sex ratios are somewhat noisy and inconsistent, these hormones may be interacting with dietary effects. For example, Pike and Petrie (2005a) showed that maternal body condition, sex ratio, and hormone levels are correlated, which brings in the question of which factor is affecting sex ratio. Given that food availability can significantly affect both corticosterone levels in birds (Jenni-Eirmann et al., 2008; Kitaysky et al., 2007) and

body condition in birds (Kitaysky et al., 1999) and mammals (Altmann et al., 1993), multiple variables should be observed when studying sex ratio on mammalian or avian species.

Hypotheses and Model Systems

The above research provides strong support that sex ratio manipulation occurs in mammals and birds, and that food quantity and/or quality are significant cues that can influence the process of sex allocation in both groups. We hypothesized that fat, in particular, is an important mediator of offspring sex ratios in both birds and mammals. We chose fats given that total caloric intake has significant effects on sex ratios in both mammalian and avian species and that fat is dense in calories. Additionally, fat is untested to our knowledge in avian species and has been shown to have significant effects on mammalian species' sex ratio. We tested this idea on primary sex ratios using an experimental dietary treatment in Japanese quail, and on secondary sex ratios using body condition index as a proxy for fat content in humans.

To test the impacts of dietary fat composition on primary sex ratios in birds, we provided a diet high in fat and low in carbohydrates to Japanese quail and analyzed sexes of eggs laid during this dietary treatment. Then, to test whether maternal body condition predicts sex-specific embryonic survival in humans, we conducted a correlational study examining the relationship between maternal body condition and survival of male and female embryos to the implantation stage after intracytoplasmic sperm injection (ICSI). Based on previous studies showing that high food availability leads to the production of more male offspring in both birds and mammals, we predicted that the quail fed a diet high in fat and low in carbohydrates would produce a significantly higher proportion of male offspring. In addition, based on prior findings that mid-stage fetal losses in women who gain less weight during pregnancy are more likely to be male,

we predicted that body condition index at the start of an *in vitro* fertilization cycle would correlate with rates of male survival to implantation.

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Tables and Figures

Table 1.1: Summary of dietary effects on sex ratio in avian species.

Species	Variable	Sex Ratio Skew	Larger or Smaller Sex	Reference
Blue Footed Booby	Low maternal body condition	Male	Smaller	Torres and Drummond, 1999a
Ural Owl	Low maternal body condition	Male	Smaller	Wiebe and Bortolotti, 1992
American Kestrel	Low maternal body condition	Male	Smaller	Bromer et al., 1996
Kakapo Parrot	Supplemental food	Male	Larger	Clout et al., 2002
Zebra Finch	High food abundance	Male	Larger	Bradbury and Blackey, 1998
Yellow-legged Gull	High food abundance	Male	Larger	Alonso-Alvarez and Velando, 2003
Pigeon	High food abundance	Male	Larger	Pike, 2005

Table 1.2: Summary of dietary effects on sex ratio in mammalian species.

Species	Variable	Sex Ratio Skew	Larger or Smaller Sex	Reference
Humans	Low pre-pregnancy weight	Female	Smaller	Cagnacci et al., 2004
Humans	High pregnancy weight gain	Female	Smaller	Cagnacci et al., 2004
Humans	High energy intake	Male	Larger	Mathews et al., 2008
Coyote	Excess diet	Male	Larger	Gese et al., 2016
Mice	High sugar and high fat diet	None	N/A	Chin et al., 2017
Tammar Wallaby	High Glucose	Female	Smaller	Schwanz and Robert, 2013
Mice	High saturated fat diet	Male	Larger	Rosenfeld et al., 2003
Mice	Low fat high carbohydrate diet	Female	Smaller	Rosenfeld et al., 2003
Mice	Fat restricted diet	Female	Smaller	Rivers and Crawford, 1974
Cows	Supplemental Fat	Male	Larger	Marei et al., 2018
Cows	IVF Oocytes matured with supplemental fat	Male	Larger	Marei et al., 2018
Arctic Fox	High food availability	Female	Male	Goltsman et al., 2005
Rats	Increased fructose intake	Male	Larger	Gray et al., 2013
Rats	Decreased glucose intake	Female	Smaller	Cameron et al., 2008
Richardson's ground squirrels	Glucose levels	No correlation	N/A	Ryan et al., 2014

CHAPTER 2

DOES DIETARY FAT SUPPLEMENTATION SKEW OFFSPRING SEX RATIOS IN JAPANESE QUAIL?

Introduction

Reproduction is one of the most energetically expensive processes that an animal can undergo. Birds use maternal effects to maximize the chances of offspring survival. A maternal effect is a general term to describe numerous alterations a mother can make to ensure her offspring have the best chance at survival and eventually reproductive success, such as altering yolk hormone levels to alter offspring behavior later in life (Ruuskanen and Laaksonen, 2010). One particularly potent programming that a mother can provide is modulation of offspring sex.

Trivers and Willard (1973) proposed that when a parent can invest the maximum amount of resources into their offspring, it is more beneficial for that offspring to be the one with greater variance in reproductive success, because those offspring have the highest fitness. It is thus not surprising that a plethora of studies have shown impacts of food availability and food quality on offspring sex ratios in birds. Total caloric intake and its relationship with sex ratio has been studied, and the results have been inconsistent. Many species will bias sex ratios towards the larger sex when resources are abundant and/or will bias towards the smaller sex when resources are scarce (Torres and Drummon, 1999a; Wiebe and Bortolotti, 1992; Clout et al., 2002; Bromer et al., 2003; Alonso-Alvarez and Velando, 2003). Given that food availability can be strongly correlated with maternal body condition (Kitaysky et al., 1999), it is difficult to pinpoint which variable is causing the effects on sex ratio. Regardless, the effects of food availability and/or

maternal condition are clearly present. However, it is unknown whether one dietary component in particular triggers this effect. Dietary fat content has been shown to have a significant effect on mammalian sex ratios (Rivers and Crawford, 1974; Rosenfeld et al., 2003) but has yet to be specifically studied in avian species.

We tested whether a high fat and low carbohydrate diet provokes changes in offspring sex ratios produced by Japanese quail in comparison to a low fat and high carbohydrate diet. We hypothesized that the fat composition of the diet, as opposed to the carbohydrate content, influences primary sex ratios produced by birds. We increased the fat content of a quail diet by supplementing with safflower and flaxseed oils, with the overall effect of increasing the fat:carbohydrate ratio in the diet while remaining isocaloric. This diet had a higher fat content and a lower carbohydrate content compared to a control diet. Based on the findings that food limitation decreases the proportion of male offspring produced by birds (Bradbury and Blakey, 1998; Alonso-Alvarez and Velando, 2003), we predicted that birds fed a diet high in fat and low in carbohydrates would produce male-biased sex ratios. Alternatively, if carbohydrate content of the diet drives sex ratio skews in birds, we would predict the opposite, where birds on the high fat:low carbohydrate diet produce a higher proportion of female offspring.

Material and Methods

Housing and Bird Care

Sexually mature Japanese quail (n=130) were pair-housed in wire cages (6”x12”x10”) with one male and one female per cage. Quail had *ad libitum* access to water and feed throughout the entire experiment through nipple drinkers and trough feeders. The quail cages were housed in a single, climate-controlled room with a light clock schedule of 14:10 hours of light to dark.

Design and Dietary Treatments

The control diet was the standard quail layer diet used at the University of Georgia Poultry Research Facility (Table 2.1). Our high fat diet was a different formulation that included 5% safflower seed oil and 5% flaxseed oil by weight. We selected safflower seed oil and flaxseed oil because they are high in Omega-3 and Omega-6 fatty acids, these fatty acids have previously been shown to be important in sex allocation in mammals (Marei et al., 2018), and they are actively deposited into egg yolk, meaning that the follicles and germinal disc are exposed to these fats (Raes et al., 2002). The formulation effectively elevated the fat content of the diet while simultaneously decreasing the carbohydrate content. We monitored egg production for all paired quail for two weeks to identify the most consistent layers. After the two weeks, 30 pairs were allocated to the high fat:low carbohydrate diet and 35 pairs were allocated to stay on the control diet. The two groups remained on these diets for the remainder of the experiment. We monitored egg laying patterns for another two weeks while the experimental group adjusted to the new diet. Eggs were then collected for another two weeks after the adjustment period for molecular sexing analyses (Fig. 2.1). Female body weights were taken weekly during the experiment. Feed intake was unable to be monitored during the course of this experiment.

Sexing of Offspring

After collecting eggs, we stored them in a cooler at 4℃ for a maximum of seven days before transferring them to an incubator at 37.5℃ at 58% relative humidity for four days. The incubated eggs were then removed and frozen at -50℃. While some suggest that sexing unincubated eggs is a better method of detecting primary sex ratios (Klein et al., 2003), this method can be less reliable for molecular sexing as it is not always successful for embryos at this early stage of incubation (Arnold et al., 2003). We opted to incubate for four days because this

provided ample embryonic tissue for DNA extraction. DNA sexing was utilized over sexing via dissection later in development to increase accuracy and reduce incubation time.

To extract DNA from embryos, we used a standard salt extraction according to procedures described in Lambert et al. (2000). While eggs were still frozen, we removed their eggshells and weighed out 10-20mg of embryonic tissue. DNA amplification was focused around the CHD-1 alleles to visualize male and female sex chromosomes (Fridolfsson and Ellegren, 1999). PCR primers and reaction concentrations were the same as specified in Pinson et al. (2011). Reaction times and temperatures were as described in Fridolfsson and Ellegren (1999). Primers used were 2550F (5'-GTTACTGATTCGTCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3') (Fridolfsson and Ellegren, 1999). PCR products were visualized utilizing ethidium bromide staining of a 3% agarose gel. Male products presented as a single band while female products presented as two bands (Fig. 2.2).

Statistical Analyses

We utilized a generalized linear mixed model in order to account for the multiple factors possibly influencing the sex of each individual egg. Fixed effects included treatment (as a factor), initial weight, change in weight, number of eggs the female laid, and the percentage of eggs that hatched (arcsine transformed). Random effects included individual bird ID number and cage row. Analyses were run in R using the lme4 package (R Core Team 2018, Bates et al. 2015). Body weight and condition has been correlated with sex ratio (Pike 2005, Pike and Petrie 2005), so we included initial weight and change in weight. We also included laying rate (number of eggs collected divided by the number of days collected) and percent fertile (number of fertile embryos divided by number of eggs laid) to account for male and female fertility. This measure also helped to account for any differences in copulation rates, which is important because mate

attractiveness has been shown to have an effect on sex ratio (Burley 1981, Rutstein et al. 2004, Rutstein et al. 2005). In this analysis, each individual egg was considered to be an experimental unit.

To compare body weights between the two groups and before and after the experiment, we used Welch two sample t-tests, run in R (R Core Team, 2018). To compare laying rates, we ran proportion tests in R (R Core Team, 2018).

Results

Neither the experimental group nor the control group significantly changed weight over the course of the experiment (Welch Two Sample t-test $p=0.31$ and $p=0.48$, Table 2.2) and when comparing within timepoints, there were no differences between experimental and control birds before (Welch Two Sample t-test $p=0.29$, Table 2.2) or after treatment (Welch Two Sample t-test $p=0.52$, Table 2.2).

However, the average *change* in weight was significantly different between the control group (-3.9g) and the experimental group (+4.4g) (Welch two sample t-test $p<0.001$, Table 2.2), indicating an effect of the dietary treatment.

At the start of the experiment, the control group and experimental group had similar laying rates (0.79 and 0.78 respectively, Welch two sample t-test $p=0.93$, Table 2.3). At the end of the experiment, the control group showed no significant change (0.85, Welch two sample t-test $p=0.62$, Table 2.3) while the experimental group was laying at a significantly lower rate (0.59) compared both to the control group and the experimental group at the start of the experiment (Welch two sample t-test $p<0.01$ and $p<0.01$, Table 2.3).

The generalized linear mixed model showed no significant effects of dietary treatment, initial or final maternal weights, the change in maternal weight, laying rates, or fertility rates on offspring sex (all $p>0.05$, Table 2.4).

Discussion

Based on previous studies in which food availability was positively correlated with the proportion of male offspring produced, we predicted that females on our experimental high-fat diet would produce a higher proportion of male offspring. Alternatively, if carbohydrate content was the driving factor in sex allocation, we would have predicted the opposite. Contrary to predictions, there was no influence of dietary treatment on offspring sex ratios during the course of this study, nor were there relationships between female body weights, or the change in their weights, with sex ratios.

It is possible that the quail were not on the experimental diet long enough for changes to sex ratio to take place. However, given the significant change in laying rates and the differences in the change in weights over the course of the experiment, physiological changes did have enough time to happen. It is also possible that the fats selected to add to the diet were not the optimal ones to trigger changes in offspring sex ratios. To our knowledge, there have been no previous studies examining the influences of dietary fat on sex ratios in birds. We used a combination of safflower seed and flaxseed oil, but it is possible that if we provided a more comprehensive fat supplement, we may have seen influences on offspring sex ratios.

What we *can* conclude from these data is that decreasing total carbohydrate content in comparison to fat content of the diet did not influence sex ratios, which indicates that carbohydrates as a macro-nutrient may not be important in the process of sex ratio adjustment. This conflicts with a study in mice, where it was found that a low fat:high carbohydrate diet

caused decreased offspring sex ratios (Rosenfeld et al., 2003). While the lack of an effect is not what was expected, it is unsurprising to find another example where different species (especially those within different classes of animals) have different responses to the same variables.

We were surprised by the finding that birds in the experimental diet group laid fewer eggs during the time they were on the diet. The experimental diet used was formulated to achieve all of the quail's needs. However, this was a new diet and as such the quail may not have responded to it as predicted, i.e., they might not have eaten enough of the diet to fulfill all nutritional requirements. Unfortunately, measuring feed intake was not done during this experiment and thus is unknown, however the quail did not lose a significant amount of weight during the experiment despite almost daily egg production. Broilers genetically selected to have more fat have been shown to have decreased fertility when compared to broilers bred to be leaner (Zhang et al., 2017). While these differences were due to genetics and not differences in diet, it still suggests that excess body fat has a negative impact on reproduction in birds. Additionally, this suggests that body composition may be more influential than diet. Despite this reduction in laying, there was not an impact of offspring sex ratio, suggesting that one sex of eggs was not specifically targeted to not be laid.

While our high fat:low carbohydrate diet did not have a significant effect on offspring sex ratio, there was a decrease in laying rate. This suggests that fat:carbohydrate ratio does not have a significant impact on sex ratio in Japanese quail, but it may have an effect on laying rates. The excess fat in the experimental diet is likely what caused the decreased laying rates. These results do not suggest that altering dietary fat:carbohydrate ratio has a significant effect on sex ratio in Japanese quail.

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Tables and Figures

Table 2.1: Diet table. Formulated percentages of diets by weight for control and experimental diet. Control diet used was a low fat and high carbohydrate diet, while the experimental diet was high fat and low carbohydrate.

Ingredient	Control Diet	Experimental Diet
Corn	57.00%	28.05%
Soybean Meal	20.40%	42.39%
Limestone	4.70%	0.52%
Salt	0.60%	0.40%
Dicalcium Phosphate	1.30%	1.50% (Defluorinated, not dical
Trace mineral	0.08%	0.08%
Vitamins	0.25%	0.25%
D.L. Methionine	0.05%	0.50%
Fish Meal	5.00%	0%
Alfalfa Meal	5.00%	0%
Wheat Midds	5.00%	0%
Soybean Oil	0.68%	0%
Safflower Seed Oil	0%	5.00%
Flaxseed Oil	0%	5.00%
L. Lysine	0%	0.34%
Threonine	0%	0.50%
Coban 90	0%	0.05%
Solka flock	0%	15.42%

Table 2.2: Welch two sample t-tests comparing the average weights (±SE) of each dietary treatment group before and after treatment.

	Initial Weight	**Final Weight**	**p-value**	**Change in Weight**
Control Diet	161.59±22.78	157.69±23.49	0.48	-3.9±8.4
Experimental Diet	155.47±22.82	161.29±21.34	0.31	5.8±11.2
p-value	0.29	0.52		<0.001

Table 2.3: Average laying rates (±SE) of each dietary treatment group before and after treatment with Welch two sample t-tests for comparison

	Initial Laying Rate	**Final Laying Rate**	**p-value**
Control Diet	0.79±0.16	0.85±0.20	0.62
Experimental Diet	0.78±0.22	0.59±0.20	<0.01
p-value	0.93	<0.01	

Table 2.4: Results of the generalized linear mixed model of the impacts of dietary treatment, initial weight, change in weight, number of eggs laid, and percent hatch (fixed effects) plus Bird ID and row (random effects) on egg sex.

Variable	**Fixed vs Random Effect**	**p value**
Treatment (factor)	Fixed	0.328
Initial Weight	Fixed	0.405
Change in Weight	Fixed	0.546
Number of Eggs Laid	Fixed	0.161
Percent Fertile	Fixed	0.203
Bird ID	Random	N/A
Row	Random	N/A

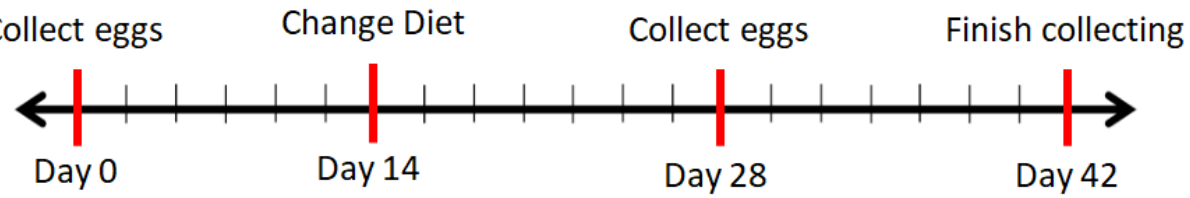

Figure 2.1: Timeline of experiment.

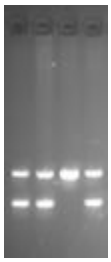

Figure 2.2: Example of PCR Products. Lanes 1, 2, and 4 are female products and lane 3 is a male product.

CHAPTER 3

DOES BODY MASS INDEX DOES PREDICT SEX-SPECIFIC SURVIVAL TO IMPLANTATION?

Introduction

Human sex ratios at birth are expected to be 0.50 (50% males). However, sex ratios across the world can be as low as 0.48 in Europe and as high as 0.51 in Asia (United Nations, 2019). A large number of physiological, environmental, and societal factors have been correlated with sex ratio skews in humans. These correlations include war time, timing of fertilization within the menstrual cycle, monetary wealth, and country's latitude to name a few (Russell, 1936; Gray, 1991; Cameron and Dalerum, 2009; Navara, 2009). While birth sex ratios are likely impacted by a combination of these factors and even interactions between the factors, nutrition stands out as a significant mediator of offspring sex ratios in both humans and in non-human mammals. For example, feed restriction decreased sex ratio in coyotes and high fat diet can increase sex ratio in mice (Gese et al., 2016; Rosenfeld et al., 2003). In humans, sex ratio has been shown to decrease in times of famine and eating disorders that increase or decrease food intake can increase or decrease sex ratios respectively (Song, 2012; Bulik et al., 2008). Further, dietary changes may explain other correlations with sex ratio such as war time and wealth. These may be correlated with food availability and energy intake, which in turn causes the change to sex ratios.

A trend exists where calorically dense diets give rise to more male offspring in several mammalian species, including mice (Rosenfeld et al., 2003) and cows (Marei et al., 2018; Gese

et al., 2016) while fat restricted diets caused a decrease in the proportion of male offspring produced by mice (Rivers and Crawford, 1974). A similar effect was also seen in humans where higher pre-pregnancy weights and increased energy intake during pregnancy were correlated with a higher proportion of male offspring (Cagnacci et al., 2004; Navara 2014; Bulik et al., 2008). Additionally, increased energy intake around the time of conception can also be correlated with a higher proportion of male offspring (Mathews et al., 2008). Despite these multiple examples, the mechanism of sex ratio manipulation is unknown.

There are many opportunities for a sex ratio bias to occur throughout the process of reproduction. These can occur during one of three timeframes: at fertilization, at implantation, and during pregnancy. Differential production of X- versus Y-bearing sperm, and/or differential abilities of these sperm to survive and reach the egg would impact offspring sex ratios. Some studies support these mechanisms. Yet, sex ratio skews in humans are more likely to occur after fertilization, due to high rates of potential miscarriage (Roberts, 1975). Most of these studies show evidence of sex-specific vulnerability to adverse condition later in fetal development (Cagnacci et al., 2004, Navara, 2014). However, the potential for sex ratio adjustment earlier in development is not as well documented. Given the numerous studies relating energy intake with sex ratio, this is a plausible explanation. For example, gestational weight gain was found to be correlated with sex ratio (Navara 2014). If a mother does not have enough energy intake, it is possible that male embryos are not getting enough nutrients and do not survive to term. However, when looking at fetal deaths before birth, sex ratios were different from those at birth, which suggests that fetal death is sex specific. Unfortunately, there was no data available for gestational weight gain for fetal deaths prior to six months of age, so it remains to be seen when the differential survival rates occur.

There are potential mechanisms by which survival of embryos may be different for males and females as early as during implantation. The interactions between the embryo and the uterine wall during implantation are complex and the uterine environment is affected by nutrition (Elrod and Butler, 1993). Specifically, the pH of uterine environment of cattle decreased in response to excess fed protein which was correlated with a loss of fertility. Further, it was shown that this change in pH only took place in the uterine environment and not in blood, urine, or saliva that was also tested (Elrod et al., 1993).

Successful implantation does not a guarantee the embryo will be carried to term, however. There are many differences in male and female embryos at the early stages of life that may render them differentially vulnerable to physiological insults. In particular, males have faster growth rates, metabolism, and cell division rates (Tsunoda et al., 1985; Ray et al., 1995). It has been found that males are more susceptible to adverse conditions, which may be due to the fact that males require more energy to support their increased growth and growth rate compared to their female counterparts (Tsunoda et al., 1985; Ray et al., 1995). In addition, miscarriage rates are at the highest early in development (Roberts, 1975). It is possible that suboptimal nutrition during this sensitive early stage could influence survival of males and females differently.

We hypothesize that, since body mass index (BMI) is a rough indicator of food intake (Altmann et al., 1993), maternal BMI may predict implantation success in a sex-specific manner. We predict that this will follow a similar trend to those seen in previous studies where low BMI is correlated with a female biased sex ratio (Cagnacci et al., 2004; Navara 2014; Bulik et al., 2008; Mathews et al., 2008), most likely due to male embryo's increased metabolic needs (Ray et al., 1995). Additionally, this sex ratio change should occur around implantation, so female

embryos will implant successfully more often than male embryos in mothers with low BMI. Additionally, due to obesity's negative impacts of fertility, we expect to see mothers with extremely high BMI to have lower male embryo implantation and fetal survival given males' higher susceptibility to adverse conditions.

Materials and Methods

Data on *in vitro* fertilization attempts that occurred between 2014 and 2018 were pulled from medical records of a local fertility clinic (Reproductive Biology Associates, Atlanta, Georgia). From those, we collected most recent height and weight measured at the clinic, sex of embryo transferred, and embryo transfer outcome for each patient for which all was available. Fetal cardiac activity (FCA) was used to determine the outcome of implantation, and was marked as either positive or negative. Live birth reports were frequently unavailable, and the aim of this study was to find differences in embryo survivability early in pregnancy, so final outcomes were not recorded. Only the first transfer for each patient was used in our analysis. While multiple transfers are commonly done in fertility treatment, it was important to avoid increasing the sampling of patients that have excessive trouble conceiving. This list was narrowed to only patients who had genetic testing done prior to transfer, so the sex of the embryo transferred was known. Patients that had more than one embryo transferred at one time were excluded because the implantation of multiple embryos could change uterine dynamics. A random selection was done of the remaining patients for a final total of 223 transfers.

We calculated maternal BMI from listed height and weight measures and then split patients into four categories according to their BMI measures: <18.5, 18.5-24.999, 25-29.999, ≥30. We used generalized linear models were run in R with age, embryo sex, and BMI as

predictors of embryo transfer success. BMI was once again tested as a continuous variable and as a factor at four levels.

Results and Discussion

Results of embryo transfers are shown in Table 3.1. Generalized linear models (Table 3.2) showed no significant effect of embryo sex, BMI, or age on embryo transfer success rates. These data suggest that maternal BMI and sex of the embryo are not significant factors in success rates of embryo transfers. While these findings do not rule out the possibility that maternal BMI can affect sex ratios, it does suggest that BMI does not affect implantation or survivability of implanted embryos.

It should be noted that the embryos in this study cannot be considered a random sampling of the general population. By observing embryos at a fertility clinic, we impose two biases on these data. First, fertility treatment is usually prohibitively expensive and therefore reduces the sample size based on socio-economic factors. It has been shown that socio-economic class can influence sex ratio, potentially through food availability (Russell, 1936). Second, many couples seeking fertility treatment have fertility issues, which may cause any potential sex ratio manipulation mechanisms normally present to not take place, as any offspring is better than no offspring, genetically speaking.

Further, most embryos at the participating clinic are created through intracytoplasmic sperm injection (ICSI), which bypasses sex-specific sperm selection, which may be a method of sex ratio manipulation. Additionally, we did not observe all embryos at this fertility clinic. We only observed those that underwent pre-implantation genetic testing (PGT) (so we could know the sex of the embryo before implantation). During an IVF/Assisted Reproductive Technology cycle, embryos are created from the female's harvested oocytes. Typically, multiple embryos are

created so only the highest quality embryos are selected for transfer. This further separates our data set from a random selection of embryos from the general population. So, our sample size is not from the general population, but from a certain subset mostly within a certain socio-economic class that is selected for the highest quality embryos available.

If sex ratio manipulation through differential implantation success rates is to prevent investment into low-quality embryos or those that are less likely to survive in the current environment, it is possible that the high-quality embryos typically used in PGT embryo transfers are able to bypass this selection. Finally, the fact that each patient is actively trying to get pregnant once again changes the sample set of our research. ART patients are typically on medication to alter hormone levels to make their uterine environment particularly receptive to embryos. This may override any reactionary/post-fertilization sex ratio manipulation pathways.

It has also been shown that ART itself has a significant effect on offspring sex ratios (Maalouf et al., 2014). Given this, it is possible that a BMI effect is still present but overridden by the effects of ART. Specifically, ICSI bypasses natural selection of sperm twice. Semen is filtered to remove dead and immotile sperm before use in ICSI, and during ICSI individual sperm are selected to be placed inside of oocytes for fertilization. During this selection, motile sperm and morphologically normal sperm are preferentially selected by embryologists. This allowed us to measure whether maternal BMI predicted embryo survival during a very specific stage – between transfer and fetal cardiac activity. In humans, a majority of miscarriages occur during this early stage (Roberts, 1975). Thus, we predicted that if males are differentially vulnerable to nutritional and/or energetic factors related to maternal BMI, it would be most likely to occur at this stage when all embryos are particularly vulnerable to adverse conditions (Roberts, 1975). We did not find evidence that the two sexes are differentially sensitive to

maternal BMI at this stage. In addition, we found that success rates of the procedure in general were quite high for all groups (78%), compared to a study that found the pregnancy rates for ART cycle start was 42.6% (Luke et al., 2017). However, this was comparing number of pregnancies from total number of cycles started where our study only observed patients whose cycles made it to embryo transfer. A number of the cycles that do not result in successful pregnancies may not produce enough quality embryos to merit an embryo transfer. The reason for the very high rate of successful implantations that we observed at this clinic remains unclear, but this high success rate could explain why we did not see a relationship between maternal BMI and survival of transferred embryos. Perhaps a particular practice used by this clinic was able to circumvent the mortality that would normally occur in an ICSI procedure, removing the potential for a sex-specific effect that we may have observed otherwise.

Finally, it is possible that the nutritional profile of food taken in by these women once the ICSI cycle began did not match what they took in beforehand (and thus could not be predicted by the single measure of maternal BMI). This is a major shortcoming of this study, as sometimes the listed height and weight values for patients were taken long before embryo transfer. Additionally, BMI as the only predictor for embryo transfer success does not allow for a comprehensive prediction. For example, BMI can reflect similar values for low caloric intake and low caloric output (e.g., exercise) and for high caloric intake and high caloric output. However, without increased patient interaction and data collection on a more extensive study, it was not possible to observe additional factors.

In conclusion these data do not provide support for sex ratio manipulation happening during embryo implantation solely based on BMI. However, given that our sample size is has multiple differences from the general human population, it does not rule out this as a possibility,

or at least does not rule out this mechanism in all mammals. These data are useful to fertility clinics, as it shows that male and female embryos do not have different success rates overall or within any of the tested BMI categories, at least using the practices employed at this particular fertility clinic. More work needs to be done to examine how BMI relates to embryo survival in males and females in clinics that exhibit lower rates of successful implantation. In addition, future efforts should be made to follow these embryos further along in development to determine whether there are more windows later in development during which one sex is more sensitive to maternal BMI than another.

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Tables:

Table 3.1: Embryo transfer outcomes. Results of embryo transfers divided into BMI category as well as male and female embryo transfers.

	Underweight (BMI = < 18.5)	**Normal** (BMI = 18.5-24.9)	**Overweight** (BMI = 25-29)	**Obese** (BMI >30)	**Overall**
Male	100% (5/5)	79.6% (43/54)	79.4% (27/34)	70.6% (12/17)	79.1% (87/110)
Female	100% (2/2)	78.2% (43/55)	73.8% (31/42)	78.6% (11/14)	77.0% (87/113)
Overall	100% (7/7)	78.9 (86/109)	76.3% (58/76)	74.2% (23/31)	78.0% (174/223)

Table 3.2: Generalized linear models results. P-values for three separate generalized linear models of Embryo Transfer Success affected by age and BMI factor for male embryo transfers, female embryo transfers, and all embryo transfers

	Underweight (BMI = < 18.5)	**Normal** (BMI = 18.5-24)	**Overweight** (BMI = 25-29)	**Obese** (BMI >30)	**Age** (Continuous Variable)
Male	0.992	0.875	0.425	0.689	0.144
Female	0.989	0.551	0.828	0.719	0.455
Overall	0.986	0.554	0.486	0.829	0.124

CHAPTER 4

CONCLUSIONS

A running theme throughout literature is that nutrition can have a significant effect on sex ratio, both in current diet as well as using maternal body condition as a proxy. Many previous studies have shown that food availability, dietary composition, and maternal body condition can impact offspring sex ratios in a variety of avian and mammalian species. However, it is currently unknown if there is a single dietary component that can skew sex ratio or if the effect is due to general overall caloric intake. The work described herein was geared towards testing whether dietary fat, both via direct dietary manipulations and using maternal BMI as a proxy, may serve as a key mediator of sex ratio adjustment in birds and humans

The results of these experiments show no impact of a high fat and low carbohydrate diet on offspring sex ratios produced by quail, and no correlation between maternal mass and offspring sex ratios in these birds. Quail on the experimental (high fat:low carbohydrate) diet did not yield significantly different sex ratios than the quail on the control diet. There was not any significant difference in body weight, laying rates, and sex ratio. Additionally, BMI was not predictive of the success of embryo transfer of male or female embryos in humans.

Despite the results of the Japanese quail study, it is still possible that fat can influence sex ratios in avian species. However, while there have been studies on how elevated levels of dietary fat affects avian reproduction in general, there has never been a report of skewed offspring sex ratios. Given the plethora of studies on caloric intake on sex ratios, it may be more likely that total caloric intake is a more important dietary factor than any one specific nutrient. Future

experiments should be designed to reinforce or refute this finding, possibly thought testing two different high fat diets rather than comparing one to a control. Also, comparisons using different kinds of fatty acids could show different results than those seen here.

While our human sex ratio study did not show maternal BMI to be an accurate predictor of embryo transfer success rates, it is still possible that maternal diet and body condition is a factor influencing sex ratios in humans. The fertility clinic we studied had a very high success rate of embryo transfers. Therefore, any differences in male and female embryo survival may not have been seen. Future studies should observe multiple clinics so that a variety of transfer techniques are observed, allowing for a more complete picture of embryo transfers. Additionally, getting more information from patients, particularly about their diet and exercise habits, would allow researchers to narrow down what specifically may affect embryo survival rates.