Hormones Rule the Roost: Hormonal Influences on Sex Ratio Adjustment in Birds and Mammals

7

In view of the apparent lack of genetic variance in the sex ratio in many species, a hormonal mechanism mediated by environmental factors provides a plausible explanation of many trends

Clutton-Brock and Iason (1986)

The purpose of this note is to persuade endocrinologists that mammalian sex ratios merit their attention

James (2008)

There is now abundant evidence supporting the idea that mammals and birds can facultatively control the sexes of offspring in response to environmental and social conditions, and we also know that there are some likely targets within the reproductive system during the process of gamete production and offspring development that may be manipulated to bias the sex ratios of the offspring produced. What we are missing now are the physiological transducers that act to convert environmental and social information into physiological signals that then act on either the developing gametes or the growing embryos to alter offspring sex. These transducers would need to interact with and coordinate a suite of both regulatory and responsive body tissues, and there are three main body systems known to act in this manner: the nervous, immune, and endocrine systems. There is now mounting evidence that these three systems are intimately interconnected, even leading to the renaming of the three as the neuroendocrine immune system (Wilder 1995). Together, these three systems control perception of external stimuli that an animal may encounter, the translation of that perception into a chemical messenger, and the reaction of target tissues. It is no wonder, then, that some have hypothesized that activities of this system underlie the mechanism behind sex ratio adjustment in vertebrates. In this chapter, I will present mounting evidence that hormones, steroid hormones in particular, are key players in the mechanisms responsible for sex ratio adjustment in both birds and mammals.

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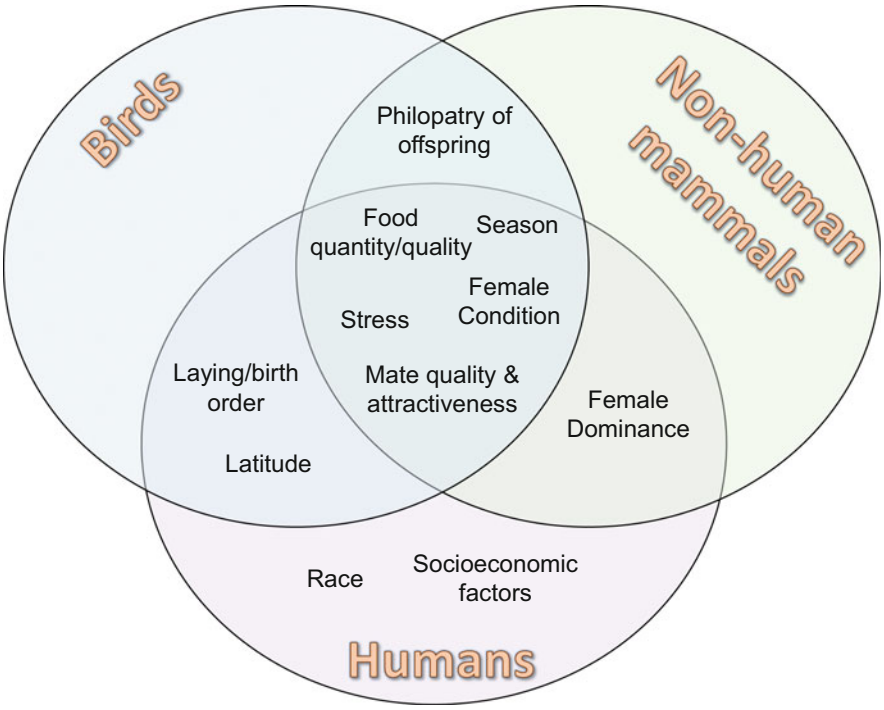


Fig. 7.1 A Venn diagram of the overlapping factors that have been shown to influence sex ratios in birds, humans, and nonhuman mammals

7.1 Links Between Hormones and Factors that Alter Sex Ratios

In Chaps. 2, 3, and 5, I outlined the factors that have been shown to influence sex ratios in humans, nonhuman mammals, and birds. Strikingly, despite the fact that birds and mammals utilize different modes of sex determination (WZ versus XY), nearly all factors that drive sex ratio biases are shared among the three groups (Fig. 7.1). These factors include food quality and quantity, female condition, progression through the breeding season, stress, and mate attractiveness and/or quality. Whether all of these factors have the capacity to trigger sex ratio biases on their own or whether a single factor that interrelates all of the others is responsible for the documented sex ratio skews in these systems is unknown. What *is* known, however, is that the physiological responses to a majority of these cues involve the same set of endocrine signals—steroid hormones. Figure 7.2 highlights the fact that while each of the cues known to trigger sex ratio biases influences physiological changes via a complex network of mediators, all of these mediators interact with the adrenal and reproductive steroid hormones, corticosterone in birds and cortisol in mammals, testosterone, estrogen, and progesterone.

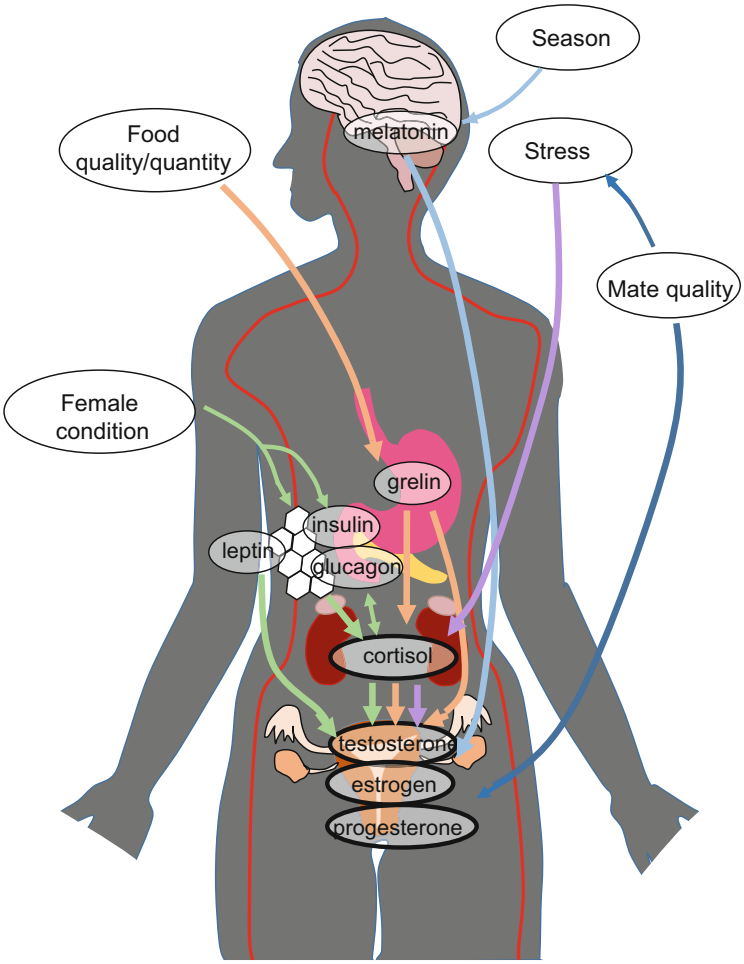


Fig. 7.2 A diagrammatic representation of how multiple factors that have been shown to influence avian and mammalian sex ratios ultimately interact through the same hormonal pathways involving actions of glucocorticoids and/or reproductive hormones

For example, the physiological response to a lack of food or a decrease in maternal body condition triggers the responses of mediators that directly interact in a reciprocal fashion with the adrenal glands, because a main action of the adrenal glucocorticoids is to stimulate the breakdown of energy reserves when the individual is in a stress state. This is true for both birds and mammals. We also know that many of these mediators directly interact with the reproductive system to stimulate or inhibit the function of the reproductive organs and the production of the sex steroids as a result. When looking at how these different cues collectively influence reproduction in both birds and mammals, it becomes clear that most of the responses funnel through the adrenal system, alter the production of

glucocorticoids, and ultimately trigger changes in levels of sex steroids to influence the reproductive system. As a result, four prime candidates as mediators of sex ratio adjustment in both birds and mammals are cortisol/corticosterone, testosterone, progesterone, and estrogen. Below, I will present the evidence for and against these hormones as prime mediators of sex ratio adjustment in birds and mammals.

It is now well known that when the production of steroid hormones is triggered, developing gametes and offspring are exposed to those steroid hormones. First, several of these hormones are produced in the cells surrounding the developing gametes; in both birds and mammals, the granulosa and theca cell layers surrounding the oocyte produce androgens, estrogens, and progesterone. In addition, stress hormones (i.e., glucocorticoids such as cortisol in mammals or corticosterone in birds) are produced by the adrenal glands which lie in relatively close proximity to the ovary in both birds and mammals. Studies have documented accumulation of testosterone, estradiol, and corticosterone in the yolks of avian eggs (Schwabl 1993; Sockman and Schwabl 1999). Concentrations of these hormones can be influenced by conditions that the female experiences during rapid yolk deposition. For example, female Eastern bluebirds that experienced simulated territorial intrusions and/or high breeding densities deposited more testosterone into their eggs (Navara et al. 2006; Bentz et al. 2016b). Bluebirds are not the only species in which this occurs. Similar elevations occur in house sparrows, American coots, tree swallows, European starlings, and others (Schwabl 1997; Reed and Vleck 2001; Whittingham and Schwabl 2002; Bentz et al. 2013; Pilz and Smith 2004) reviewed in (Bentz et al. 2016a). Along the same lines, exposure to stressful experiences drives females to elevate concentrations of corticosterone in yolks. Barn swallows exposed to a predator during egg laying produced eggs with significantly higher concentrations of corticosterone in the yolks (Saino et al. 2002). Hayward and Wingfield (2004) showed in quail that when concentrations of corticosterone are elevated in circulating plasma, concentrations in egg yolks rise as well. These yolk hormones then go on to exert potent programming influences on offspring growing within the eggs (Groothuis and Schwabl 2008; Navara and Mendonça 2008; Hayward and Wingfield 2004). This paradigm is not restricted to birds. Abundant research suggests that hormones produced by mammalian mothers reach and influence offspring during development. For example, female spotted hyenas with higher dominance ranks produce higher concentrations of testosterone during gestation, and exposure to these higher hormone levels programs cubs to exhibit higher levels of aggression (Dloniak et al. 2006). It is also well known that stress-induced glucocorticoids reach and program developing mammalian embryos (e.g., Drake et al. 2005; Tangalakis et al. 1992). Thus, it is clear that there is a pathway by which steroid hormones can act to communicate information about the environment to the reproductive organs in a way that influences gamete and/or offspring growth and development. Given such a pathway, it is logical to hypothesize that these steroid hormones may also mediate changes in sex ratios in response to the same types of environmental and social conditions.

7.2 Evidence that Steroid Hormones Influence Avian Sex Ratios

7.2.1 Case Study: Peafowl

Some of the first solid evidence that steroid hormones play a role in the adjustment of avian sex ratios came from two studies on peafowl conducted by Thomas Pike and Marion Petrie in 2005. First, Pike found that when they manipulated the attractiveness of the peacocks that the peahens were mating with by removing eye spots on the train, females mating with these males deposited higher concentrations of corticosterone into egg yolks and also produced a significantly higher proportion of female offspring (Pike 2005). They then more directly examined the correlation between maternal hormone concentrations, body condition, and offspring sex ratios in peafowl; they found that females in lower body condition had higher concentrations of corticosterone in circulation, lower concentrations of testosterone, and they produced a significantly higher proportion of female offspring. A direct test of how the concentrations of the two steroid hormones related to sex ratios produced showed a significant positive correlation between sex ratio and testosterone concentrations and a significant negative correlation between sex ratios and corticosterone concentrations (Pike and Petrie 2005). Because corticosterone can influence levels of testosterone and vice versa, it was not clear whether both steroids participated in the determination of offspring sex, or whether one was related to sex ratios indirectly due to an interaction with the other. However, these two studies opened up a flood of subsequent experiments examining the roles of these two hormones in sex ratio adjustment.

7.2.2 Case Study: Japanese Quail

Following the peacock studies, the same authors continued examining the role of steroid hormones in adjustment of sex ratios, but this time in a different model—Japanese quail (Pike and Petrie 2006). To experimentally test whether elevated concentrations of corticosterone, estradiol, and testosterone influence sex ratios, they implanted quail with silastic implants containing one of the following: estradiol, fadrozole (an aromatase inhibitor that prevents production of estrogen), corticosterone, metyrapone (a corticosterone synthesis inhibitor), or testosterone. After implantation, they collected eggs for 10 days and compared sex ratios to pretreatment sex ratios. They found that females treated with corticosterone significantly reduced the proportion of male offspring they produced in comparison both to sex ratios of offspring that they produced prior to treatment and to sex ratios produced by females in the other treatment groups. None of the other treatment groups triggered a significant sex ratio bias compared to pretreatment sex ratios. It is interesting that elevating corticosterone concentrations exerted a significant effect on sex ratios, while inhibiting corticosterone production via treatment with metyrapone did not. Perhaps this indicates that the default sex ratio produced by

quail females is always 50:50 unless a part of the sex determination process responsible for producing males is disrupted by exposure to high concentrations of corticosterone. Whether this is, in fact, true still remains unknown, and the effects of metyrapone have not been tested in any other additional species. It is interesting that testosterone did not exert an influence, given the positive relationship between testosterone and sex ratios produced by peahens. This could indicate that quail utilize a different mechanism of sex ratio adjustment or that the link between testosterone and sex ratios in peahens was due to an indirect linkage with corticosterone.

7.2.3 Influences of Corticosterone in Other Systems

Corticosterone shows great potential as a mediator of offspring sex ratios, not only because the results of the work in peafowl and quail strongly support corticosterone as a candidate mediator but also because the key function of this hormone is to respond to changes in surrounding stimuli and coordinate body systems to deal with those changes. As shown above (Fig. 7.2), corticosterone lies at the nexus of all of the responses to cues that have been shown to stimulate sex ratios in birds. In response to food restriction, corticosterone concentrations elevate and act to liberate energy reserves. When experiencing a stressful event, corticosterone is the primary responder that acts to help the animal maintain homeostasis in the face of that event. Corticosterone concentrations even change seasonally and could underlie the seasonal changes seen in avian sex ratios. A body of work now supports the idea that corticosterone plays a role in offspring sex ratios, though the story is not quite as simple as we might expect.

Following the quail study by Pike and Petrie (2006), Bonier et al. (2007) conducted a two-prong experiment in white-crowned sparrows. First, they measured corticosterone concentrations in naturally breeding females and showed that those with higher levels of corticosterone produced significantly higher proportions of female offspring. They then tested this relationship experimentally using time-release corticosterone pellets. Females implanted with corticosterone produced significantly more female offspring compared to controls, just as female quail did. What's more, a similar study in homing pigeons showed the same effect; females implanted with corticosterone produced more female offspring. Thus, elevating corticosterone over a period of a week or longer, either endogenously or via corticosterone implantation, stimulates the production of a higher proportion of female offspring in all four of these avian species. However, when during sex determination does corticosterone act to skew sex ratios, and does corticosterone act alone or in combination with other modulators?

Examining the timing of the treatments in these four studies may tell us a bit more about how and when corticosterone is acting (Fig. 7.3). In the white-crowned sparrow study, implants were given to birds after the first clutch, prior to re-nesting. The birds began their second clutches on average 10 days after implantation, which would mean that concentrations of corticosterone would have been elevated during

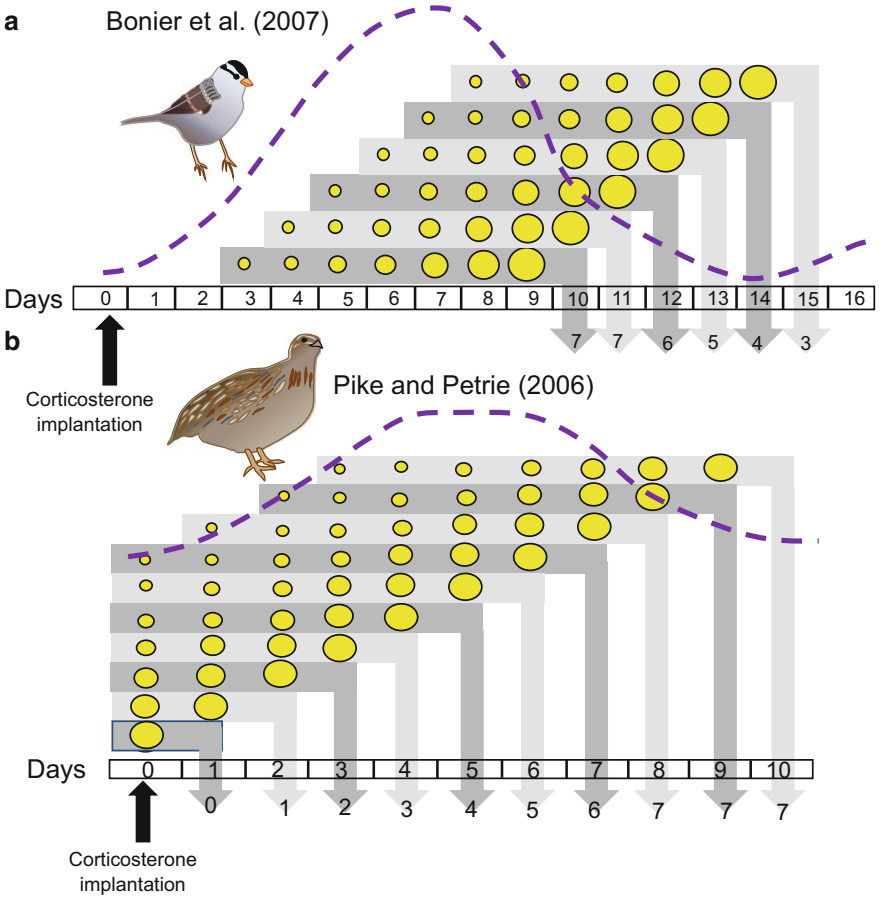


Fig. 7.3 Illustrations of the timing of corticosterone treatment relative to rapid yolk deposition within ovarian follicles in two key studies in which corticosterone implants were used (**a**) White-crowned sparrow: Bonier et al. (2007), (**b**) Japanese quail: Pike and Petrie (2006). In both cases, the dashed purple line indicates the estimated profile of corticosterone concentrations in circulation, downward pointing arrows indicate when the indicated follicle ovulates, and numbers within the arrows indicate the number of days that the developing follicle would likely have been affected by elevated concentrations of corticosterone while undergoing rapid yolk deposition. In the white-crowned sparrow study (**a**), the first egg of the second clutch was laid, on average, 10 days after implantation of corticosterone pellets, and the day of clutch completion was, on average, at 15 days after implantation. By day 15, corticosterone concentrations had returned to normal, which suggests that meiotic segregation and ovulation of the later few follicles occurred when corticosterone concentrations were low, yet resulting sex ratios were still female biased. In the quail study (**b**), eggs were collected for 10 days after implantation. As a result, corticosterone concentrations were likely elevated during meiotic segregation and ovulation for nearly all follicles, but because egg collections started quickly after implantation, only the final three follicles would have been exposed to elevated levels of corticosterone during the entire phase of rapid yolk deposition

a majority of rapid yolk deposition (which takes about 5–7 days) for these clutches. However, concentrations of corticosterone in corticosterone-implanted birds were already down to baseline levels by the time those clutches completed (on average 15 days after implantation). Because meiotic segregation completes just hours prior to ovulation (see Chap. 6), corticosterone concentrations in circulation were likely very low at the time that at least the last few eggs in the clutch were completing meiotic segregation prior to their ovulation. This could mean that sex ratio adjustment happens as a result of an effect during rapid yolk deposition, perhaps by altering follicle growth rates (a possibility described in detail in Chap. 6). On the other hand, if enough corticosterone accumulates in yolk to influence meiotic segregation, then there still could be a direct effect at that stage as well.

The quail study (Pike and Petrie 2006) could shed further light on the timing of corticosterone action. In this case, the authors reported the average sex ratio for 10 days following implantation with corticosterone. They further reported that there was no evidence that sex ratio skews became more dramatic towards the end of that 10-day period. Yet, as shown in Fig. 7.3, only the last three eggs collected had been exposed to the treatment during the entire time of follicle growth. This could indicate that, in this case, corticosterone was not acting on sex ratios by influencing the contents of the egg during rapid yolk deposition, because if that were the case, we would have expected the female-biased sex ratio to be driven by a very high proportion of females towards the end of that 10 day period, because eggs laid in the first few days after implantation would have had little to no time to deposit yolk under the influence of the elevated levels of corticosterone. This points to a mechanism by which corticosterone acted directly on the process of meiotic segregation. However, it is also possible that only a short time of corticosterone elevation is required during rapid yolk deposition to exert the effects that would ultimately influence offspring sex.

There was another study that tested the influences of long-term treatment with corticosterone that further supports the idea that corticosterone may act at the time of meiotic segregation. Aslam et al. (2014) provided feed that contained corticosterone to laying hens for 14 days and examined the resulting sex ratio in eggs (Fig. 7.4a). In this case, despite treatment that spanned a similar time period to the previous two studies discussed, corticosterone treatment did not appear to influence sex ratios, though it did alter the relationship between body mass and sex ratios. Corticosterone was provided through the diet and this treatment produced significantly higher concentrations of corticosterone in circulation during the day by day 4 of treatment and extending through at least day 10. However, because birds do not take in food at night, concentrations of corticosterone likely dropped substantially during the night. Given that meiotic segregation often takes place in the very early hours before lights go on, levels of corticosterone would have been much lower at this time than during the time that blood was collected for corticosterone measurement. It is possible that this method of supplying corticosterone did not bias sex ratios because concentrations were not high enough during meiotic segregation to influence the segregation process.

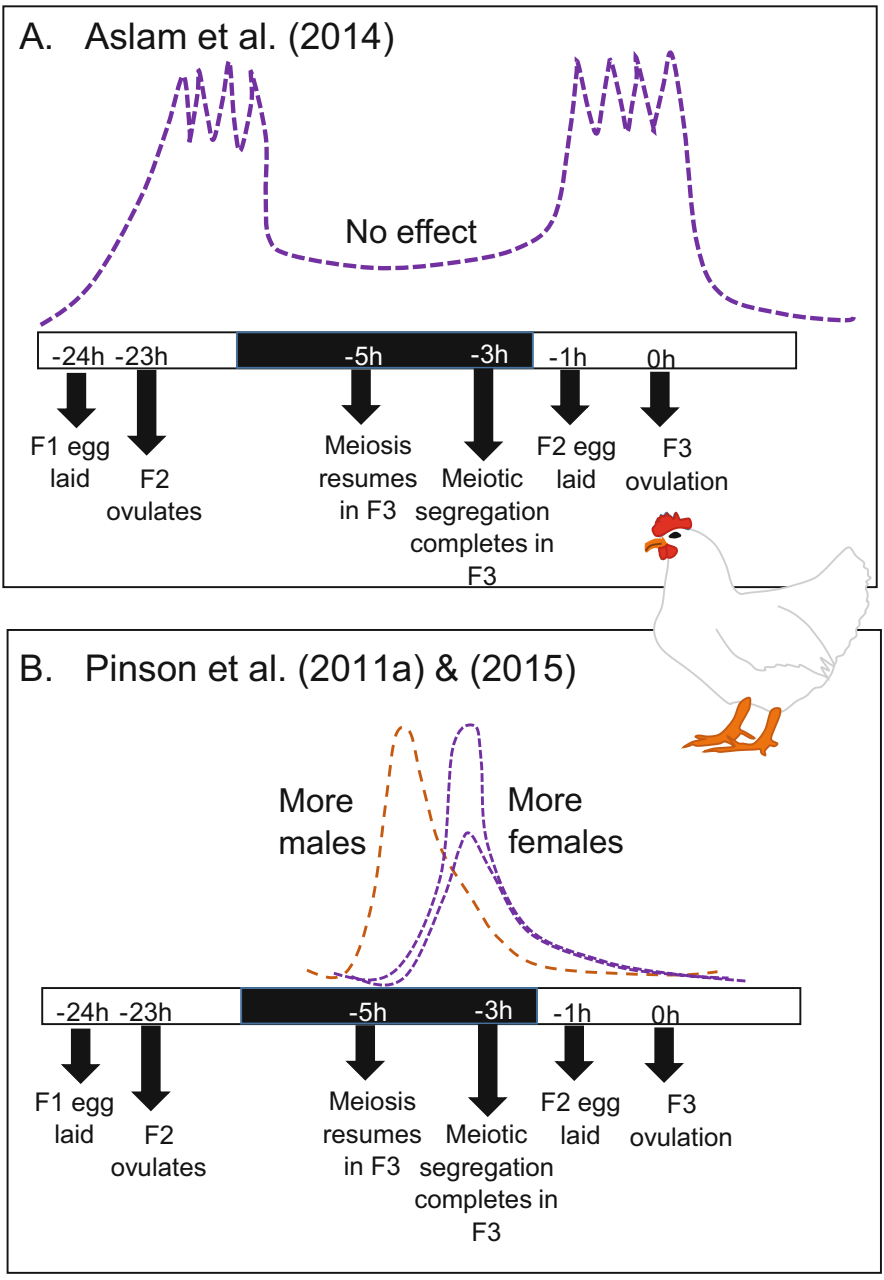


Fig. 7.4 Illustration of the timing of corticosterone treatment relative to the timing of meiotic segregation during three key studies in which corticosterone was administered to white leghorn laying hens (**a**) Corticosterone administered in feed over 14 days—Aslam et al. (2014), (**b**) Corticosterone administered as injections 5 and 4 h prior to ovulation—Pinson et al. (2011a) and Pinson et al. (2015). In both cases, dashed lines indicate the estimated profiles of corticosterone concentrations in circulation, and purple dashed lines indicate profiles that ultimately

In an attempt to further elucidate the target time at which corticosterone acted to skew sex ratios, we experimentally elevated corticosterone concentrations on a shorter timescale, using injections that elevated concentrations for a period of hours rather than implants or feed treatments that elevated concentrations for days (Fig. 7.4b). We used laying hens as our model because they have been selected for their precise timing of ovulation and egg laying; these hens lay eggs at a rate of one egg per day, and ovulation of the next follicle in the sequence takes place approximately 30 min prior to oviposition of the egg that came before it. Because the egg spends 24 h in the reproductive tract after ovulation, we can then use the timing of an egg to predict when the ovulation of future eggs will occur. This allowed us to pinpoint corticosterone treatments to the precise time when sex chromosomes were segregating. For our first attempt at this, we used a pharmacological dose of corticosterone that elevated concentrations well outside of the physiological range, and the result was that hens injected with this high dose of corticosterone just prior to meiotic segregation produced significantly more *male* offspring, rather than the predicted bias towards females (Pinson et al. 2011a). A simultaneous study in zebra finches was conducted in a similar manner and also using a pharmacological dose, and the results were the same—corticosterone-treated birds produced more *male* offspring (Gam et al. 2011). Despite the surprising direction of these effects, these studies tell us that corticosterone does appear to have the ability to influence which way the sex chromosomes segregate during the completion of meiosis I, albeit in the opposite direction as the one we would have predicted.

The next step was then to examine the influences of an elevation of corticosterone within the physiological range. For zebra finches, we triggered a physiological stress response, resulting in an endogenous elevation within the physiological range, just prior to meiotic segregation using a bag stress. This treatment, during which we placed female zebra finches in cloth bags for 5 min, stimulated elevation in corticosterone concentrations, but did not stimulate a bias in sex ratios (Gam and Navara 2016). This treatment may have failed to trigger an effect on sex ratios either because an elevation of corticosterone within the physiological range is not sufficient to do so or because the elevation at this lower level did not last long enough to overlap with the critical time necessary to influence meiotic segregation. To conduct a similar test in laying hens, we continued to rely on injections to

Fig. 7.4 (continued) produced female-biased sex ratios, while the orange dashed line indicates the profile that ultimately produced a male-biased sex ratio. In the Aslam et al. (2014) study (**a**), corticosterone was provided in daily feed, and blood samples taken during the day indicated that concentrations of corticosterone were elevated in plasma at days 4 and 10 after treatment began. However, since hens do not eat during the night, we would expect that concentrations of corticosterone dropped during the night, when meiotic segregation was occurring for each follicle. In the Pinson et al. studies (**b**), an injection containing a pharmacological dose of corticosterone at 5 h prior to ovulation resulted in male-biased sex ratios, while a physiological and a pharmacological dose given at 4 h prior to ovulation resulted in female biases. Note that the individual treatments at 4 h did not produce significant effects on their own, but when combined, did stimulate a significant female bias in offspring sex ratios

maintain better control over the level of corticosterone elevation. We provided high and low physiological doses at 5 h prior to ovulation, the same timing as was used in previous experiments, and there was no influence on offspring sex ratios, similar to what we saw in zebra finches. However, when we treated birds with corticosterone one hour later (4 h prior to ovulation), we did see a sex ratio skew but this time towards *females* (Pinson et al. 2015). Thus, it appears that the timing and the dosage influence not only whether a sex ratio skew occurs but the direction of the sex ratio skew. How this might happen is still unknown, but based on these studies, it appears that there is a short critical window during which corticosterone concentrations must be elevated for sex ratio adjustments to occur.

7.2.4 Influences of Testosterone

It is possible that the observed effects of corticosterone on offspring sex ratios are actually occurring via another downstream hormonal mediator. In the peafowl study mentioned above, sex ratios were not only negatively correlated with corticosterone concentrations, they were also positively correlated with testosterone concentrations. Testosterone concentrations have also been shown to differ between eggs that produce males versus females in some species (e.g., peafowl—Petrie et al. 2001), and when mated with more attractive males, female zebra finches both deposit higher amounts of testosterone into their egg yolks (Gil et al. 1999) and produce a higher proportion of male offspring (Burley 1981). Further, corticosterone is a well-known suppressor of reproduction and the corresponding reproductive hormones (including testosterone) in both male and female birds (Shini et al. 2009). As a result, it is possible that the effects on sex ratio after corticosterone administration actually occur due to downstream changes in circulating levels of reproductive hormones, testosterone in particular, and there are now studies to support this idea (Fig. 7.5).

Veiga et al. (2004) were the first to test this idea experimentally. They implanted spotless starlings with silastic implants containing testosterone and monitored sex ratios for up to 3 years afterward. Females implanted with testosterone produced a significantly higher proportion of male offspring during the year of manipulation and also for 3 years afterward. It is unlikely that testosterone directly manipulated sex ratios by influencing meiotic segregation in this instance, because first, females treated with testosterone delayed laying, perhaps until testosterone concentrations had lowered enough to restore normal reproductive physiology. In addition, when the implants were collected from the birds, there was no hormone remaining in them, and it was unlikely that the hormone in the implants lasted beyond the manipulation year, even though sex ratios produced by implanted birds remained biased at this time. The authors suggest two possibilities for how the treatment exerted such long-term effects on sex ratios. First, the treatment may have permanently influenced testosterone production by the ovaries such that higher concentrations of testosterone were produced by testosterone-implanted birds compared to control birds during all 3 years. Second, the testosterone treatment appeared to affect dominance status in female starlings; testosterone-implanted

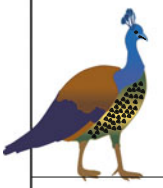
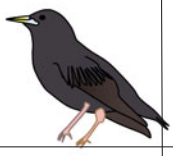
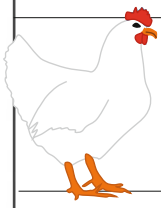
Species	Treatment	Outcome
 Peafowl	High endogenous levels of testosterone	More males
Spotless starling 	Silastic implant containing testosterone propionate	More males for 3 years
 Homing pigeon	Silastic implant containing testosterone	More males in 1st eggs for 2 years
Zebra finch 	Injection of testosterone enanthanum after 1st egg	More males in eggs 3,4,& 5
 White leghorn	Testosterone injection (1.5mg) 5h prior to meiotic segregation	More males in eggs ovulated after treatment
Japanese quail 	Silastic implant containing 2.4mg testosterone	No effect

Fig. 7.5 A summary of studies in birds testing the influences of testosterone on offspring sex ratios

females tended to be more dominant than controls. If this dominance status then remained the same going forward, a resulting increase in aggressive interactions, and, in effect, a higher number of testosterone spikes, could underlie the long-term effects on sex ratios. Neither of these possibilities has yet been tested.

There are additional studies that support the role of testosterone in adjustment of offspring sex ratios in birds. In homing pigeons, an implant containing testosterone stimulated the production of more male offspring in first eggs of the clutch

(Goerlich et al. 2009). As in the starling study, these effects lasted beyond the manipulation year, into 1 year after manipulation. Unlike the starling study, however, the pigeons were held in cages and were not able to participate in conspecific interactions that were hypothesized to influence sex ratios in the starling study. Hence, in this case, it appears more likely that the testosterone treatment permanently influenced the ovarian physiology and/or receptor dynamics in the ovary in such a way that sex ratio skews continue beyond the point when the hormone in the implant ran out.

Finally, Rutkowska and Cichoń (2006) provided even further evidence that testosterone treatment not only has the ability to influence sex ratios but exerts effects beyond when the concentrations of testosterone have dropped to baseline levels. They administered a single injection of testosterone to females just after the appearance of the first egg in the clutch and then monitored the sexes of eggs across the clutch and compared those to the sexes of eggs produced by control females. They showed a striking effect, testosterone-treated females producing significantly more male offspring towards the end of the clutch compared to the beginning. This effect is not likely due to direct influences on meiotic segregation because testosterone concentrations from an injection generally drop within a day.

The three studies above implicate testosterone as a driver of sex ratio adjustment in birds; however, they don't provide much insight into how or when testosterone is acting. Despite the fact that testosterone was not elevated from the treatments at the time of meiotic segregation, it is still possible that passage and storage of that testosterone in the yolk influenced the process of meiotic segregation. Rutkowska and Cichoń (2006) did find an elevation in concentrations of yolk testosterone in the eggs 3, 4, and 5 laid by the testosterone-treated females. Those were the same eggs in which elevations in the proportion of male offspring were observed. Goerlich et al. (2009) found no difference in yolk testosterone concentrations in pigeon eggs from testosterone- and control-treated females but suggest that the outer rings of the yolk could have had elevated levels that were not detected when homogenized with the other layers for the assay. Therefore, the next step was to test whether testosterone exposure at the time of meiotic segregation could influence offspring sex.

We took on this question using a similar design to that described above for corticosterone treatment of laying hens. We treated laying hens with a high dose of testosterone 5 h prior to ovulation, which was likely 1–3 h prior to meiotic segregation of the sex chromosomes. The result was a significant increase in the proportion of male offspring in the eggs ovulated hours after the testosterone injection, significantly higher proportions than controls and than sex ratios in the eggs ovulated a day prior to treatment (Pinson et al. 2011b). Since rapid yolk deposition ceases approximately 24 h prior to ovulation (well before our treatment) (Johnson 2015), the skew did not occur due to differences in yolk testosterone concentrations but could result from a similar mechanism if exposure of the germinal disk from either side is enough to trigger the same process that influences meiotic segregation. The details of how this might occur, however, remain unknown.

7.2.5 Influences of Progesterone

Given the effects of testosterone on sex ratios as well as the fact that two other sex steroids, progesterone and estradiol, are much more prevalent and closely associated with the process of ovulation, it is reasonable to think that progesterone and/or estradiol may also be mediators in the process of sex ratio adjustment in birds. In fact, Correa et al. (2005) were the first group to attempt to target a hormone to the time of meiotic segregation, and they did this by providing injections of progesterone to laying hens. In this case, progesterone was injected 4 h prior to the end of the light phase, well before hens would ovulate, and the injection acted to speed up the process of ovulation, stimulating ovulation of those eggs 6 h after the progesterone injection. They tested the effects of a low (0.25 mg) and a high (2 mg) dose on sexes of the eggs ovulated after injection and found that the high dose significantly reduced the proportion of male offspring in those eggs (25% males were produced in the high progesterone group compared to 61 and 63% in the other two groups). Progesterone concentrations in these hens were significantly elevated at 2 h prior to ovulation when sex chromosomes were likely segregating. This suggests that changes in progesterone concentrations have the ability to influence sex ratios; however, because progesterone is a precursor to other sex steroids, including testosterone, it is unclear whether progesterone was acting directly or via downstream effects of testosterone. Given that elevations of progesterone concentrations potently disrupt and interfere with the timing of the ovulation process in this and other studies (Etches and Cunningham 1976; K. Navara pers obs.), it is unlikely that birds would use elevations of progesterone to influence offspring sex ratios in a natural context. However, this study spurred the idea for testing for hormonal influences at the time of meiotic segregation and also provides support for the idea that downstream mediators, such as other sex steroids, may instead be the mediators used in a natural context.

7.2.6 Influences of Estrogen

We've now seen that two sex steroids (progesterone and testosterone) have the capacity to influence sex ratios in birds, but what about the other major player in the ovulatory process, estrogen? Given that the majority of endocrine-disrupting chemicals that animals are exposed to in the environment are estrogen mimics, it is particularly important for physiological ecologists and toxicologists to know whether these compounds that are already known to influence the reproductive systems of developing offspring and eggshell quality can also influence sex ratios (Giesy et al. 2003). In fact, female-biased sex ratios have been observed at the population level in gull colonies breeding in DDT-contaminated environments (reviewed in Giesy et al. 2003), and it is also now well documented that exposure to contaminants that mimic estrogens can disrupt spindle fiber formation and function in oocytes (e.g., Can and Semiz 2000). Yet, it is unknown whether these skews occurred at the primary or secondary sex ratio level.

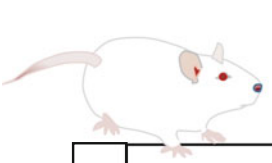
During their study examining the influences of progesterone on sex ratios in laying hens, Correa et al. (2005) also measured estradiol concentrations in blood. When included in the full model with all other parameters, estradiol was unrelated to offspring sex ratios, but when included alone in the model, estrogen was negatively correlated with the proportion of male offspring produced, and estradiol concentrations were higher in the group that received the high-dose progesterone injection and produced more female offspring. This is the only evidence to date that estrogen plays a role in the process of sex ratio adjustment in birds. In the Pike and Petrie (2006) study described above, neither estrogen nor fadrozole exerted significant influences on offspring sex ratios, and in an additional study, von Engelhardt et al. (2004) administered 30 mg estradiol injections to female zebra finches on four consecutive days. This treatment significantly elevated estradiol concentrations in female circulation during egg laying but did not influence the sex ratios within those eggs. The results of these studies and the fact that female biases in primary sex ratios are not observed in more avian species that have been exposed to compounds that are estrogen mimics suggest that estrogen does not likely play a role in the process of sex ratio manipulation.

7.3 Evidence that Hormones Influence Sex Ratios in Mammals

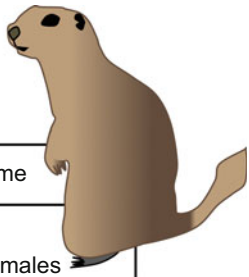
While the progress towards understanding the potential hormonal influences on the process of sex ratio adjustment in birds has been steady, evidence for the same in mammalian systems is mostly indirect, and experimental approaches to answer such questions are generally lacking. In three reviews, William James has done an excellent job compiling the evidence that most of the factors that have been observed to influence sex ratios in mammalian systems do so by altering parental concentrations of gonadotropins and sex steroids near the time of conception (James 1996a, b, 2008). As shown in Fig. 7.2 above, many of the cues that skew offspring sex ratios do indeed trigger endocrine responses, particularly in the production of adrenal glucocorticoids and sex steroids within the reproductive system. Below, I will examine the evidence that these hormones play key roles in the adjustment of offspring sex ratios in humans and nonhuman mammals.

7.3.1 Influences of Glucocorticoids

There is now a large body of work showing that exposure to stressful events influences birth sex ratios in humans (reviewed in Chap. 2 and Navara 2010), and there is also evidence of a similar pattern in nonhuman mammals as well (Lane and Hyde 1973; Ideta et al. 2009). In general, stress appears to decrease the proportion of male offspring. Stressful stimuli are transduced into physiological signals via the production of glucocorticoids, primarily in the form of cortisol in most mammals. Yet, there are only a handful of studies that address the potential relationship between glucocorticoid concentrations and sex ratios in mammals (Fig. 7.6). In the first study to test the role of the stress hormone axis in sex ratio adjustment,



Influences of Glucocorticoids on Sex Ratios in Mammals



	Species	Treatment	Outcome
Before Conception	Albino Rat	Injection with long-acting ACTH prior to estrus	Fewer males
	Human	High levels of salivary cortisol	Fewer males
	Human	High cortisol in circulation	No relationship
	Field vole	Corticol in female circulation	No relationship
During Gestation	Golden hamster	Dexamethasone treatment in drinking water	Abolished stress-induced female bias
	Richardson's ground squirrel	High fecal glucocorticoid metabolites	More males
		High bound cortisol in circulation	More males

Fig. 7.6 A summary of studies in mammals testing the influences of glucocorticoids on offspring sex ratios

Geiringer (1961) found that injecting albino rats with a long-acting form of adrenocorticotropic hormone (ACTH), a hormone that stimulates glucocorticoid production from the adrenal glands, reduced the proportion of male offspring in the litters of treated mothers. Much later, in a study of humans, Chason et al. (2012) showed that women who had higher concentrations of cortisol in saliva prior to conception were less likely to produce a male baby. These patterns comply with the multitude of studies that show female biases after exposure to stressful stimuli (see Chaps. 2 and 3).

The remaining studies that address the role of glucocorticoids on sex ratio adjustment in mammals, however, show that the story is likely much more complicated than a simple effect of cortisol on the sex determination process. A study in field voles showed no relationship between corticosterone concentrations in blood collected prior to conception and the sex ratios of litters produced afterward (Helle et al. 2008). In another study of humans, Bae et al. (2017) and colleagues tested anxiety levels and salivary cortisol concentrations in women prior to conception, and in this case, there was no association between cortisol and the sexes of babies produced. This could perhaps have been due to the fact that the saliva was collected on day 6 of the menstrual cycle in the Chason et al. (2012) study but on day 1 of the cycle in the Bae et al. (2017) study. Perhaps cortisol concentrations closer to conception are more indicative of the linkage between concentrations of the hormone and the resulting sex of the baby. In the Bae et al. (2017) study, it was instead men who were diagnosed with an anxiety disorder that produced a biased sex ratio; those men had a 76% increase in the chances of producing a male baby. Perhaps cortisol concentrations interact in a different way with sex ratios in men compared to women. Cortisol concentrations were not measured in men, but the relationship itself is the opposite of what we would predict based on the opposite pattern that emerges in the majority of previous literature. In particular, men with high-stress jobs produced *fewer* male offspring in two previous studies (Lyster 1971; Snyder 1961). Cortisol concentrations were not measured in those cases either, however, and it is possible that both jobs may be occupied by a category of men who are somewhat resistant to stress. More studies directly measuring cortisol concentrations in men over time and relating those concentrations to sex ratios produced by those men would be helpful towards determining whether a link is there.

The final studies examine the relationship between glucocorticoid concentrations during gestation and the resulting sex ratio at birth. In golden hamsters, Pratt and Lisk (1990) showed that inducing a social stress caused females to reduce litter sizes and skew litter sex ratios towards females, likely via male-biased fetal death. They then repeated this effect but provided stressed females with dexamethasone in drinking water. Dexamethasone is a synthetic glucocorticoid that acts to inhibit the release of adrenocorticotropic hormone from the pituitary gland, completely abolishing the downstream release of endogenous glucocorticoids from the adrenal glands. This treatment prevented the female bias in females experiencing social stress, indicating that glucocorticoids may be a mediator that controls sex ratios by triggering fetal death in a sex-specific manner.

Two additional studies examine the relationship between gestational glucocorticoids and sex ratios, both in Richardson's ground squirrels. In these two studies, fecal glucocorticoid metabolites and free and bound cortisol in circulation were measured during gestation. While the previous studies were testing whether preconception glucocorticoid concentrations could determine whether a male or female baby was *conceived*, the two studies in ground squirrels tested whether glucocorticoid concentrations predicted sex-specific fetal loss during pregnancy. In the first study, mothers with high amounts of glucocorticoid metabolites

in feces produced more male-biased litters (Ryan et al. 2011). In the second study, concentrations of bound cortisol in blood were higher for mothers that produced male-biased litters, while concentrations of free cortisol in blood and amounts of glucocorticoid metabolites in feces were not related to sex ratios within litters (Ryan et al. 2014). It is unclear why fecal glucocorticoid metabolites were not related to sex ratios in both studies, although the authors suggest it may result from the collection method; in the first study, an average over multiple collections was used, while data in the second study were collected from only one fecal sample per animal. Still, it is also unclear why only the concentrations of bound cortisol were associated with sex ratios in the second study, given that cortisol bound to binding globulins is thought to be relatively inactive. Additionally, the fact that higher indicators of glucocorticoid concentrations in these two systems were associated with *male*-biased sex ratios is the opposite of what we would predict given the large number of studies showing that stress during gestation appears to result in the production of a *lower* proportion male offspring.

The studies described above give us only a taste of how glucocorticoids may interact in the process of sex determination in mammals. What the results indicate is that we need to test for influences of glucocorticoids at several stages of gamete and fetal development and test for effects in both males and females. We need to conduct experimental studies that test whether elevating glucocorticoid concentrations above baseline concentrations, as is seen in stressful situations, influences offspring sex. This is a wide open field with a great need for additional study before questions about how glucocorticoids interact with offspring sex ratios in mammals can be answered.

7.3.2 Influences of Estrogen

As in birds, most of the cues that appear to influence offspring sex ratios in mammals have the capacity to directly and/or indirectly modulate the production of reproductive hormones. However unlike in birds, estrogen shows more promise as a mediator of sex ratio adjustment in mammalian systems. Some of the first evidence was indirect; mice fed a diet high in fat produced significantly more male pups (Rosenfeld et al. 2003), and it was later shown that mice on high fat diets have significantly higher concentrations of estradiol in circulation compared to those on low fat diets (Whyte et al. 2007). Since then, more direct tests have been done. Zhang et al. (2006) conducted in vitro fertilization using mouse gametes while incubating with and without estradiol. When estradiol was supplied, the resulting offspring was more likely to be male. In addition, when Holstein dairy cows were treated with estradiol just prior to insemination, this treatment increased the probability of birthing a male by 12.1% (E2 = 63.8% male offspring, Control = 51.8% male offspring) (Emadi et al. 2014).

Unfortunately, not all studies examining the influence of estradiol on sex ratios show skews in the same direction. In 2005, Perret tested urinary estradiol levels both during the follicular phase (i.e., as ovarian follicles were maturing) and at ovulation

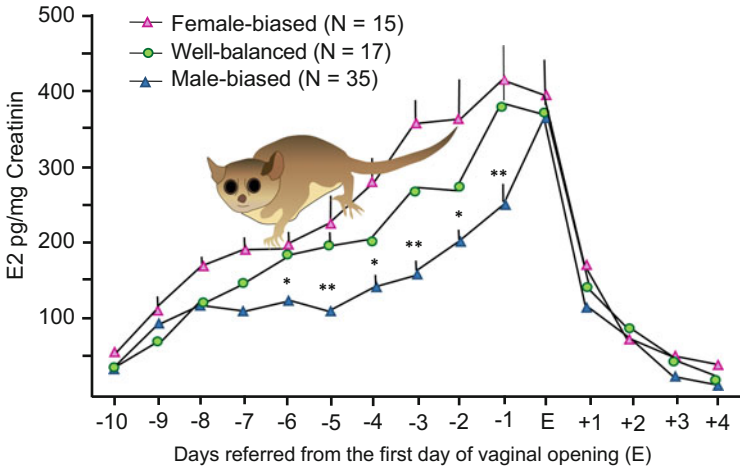


Fig. 7.7 Profiles of urinary estradiol concentrations in gray mouse lemurs during the days before and immediately after vaginal opening and in relation to whether litters produced afterward were female biased, well balanced, or male biased

in gray mouse lemurs (Perret 2005). Resulting sex ratios were positively related to estradiol levels during the follicular phase but not at ovulation (Fig. 7.7). In fact, concentrations of estradiol during this phase were over 150 pg/mg lower when resulting litters were male biased (338 pg mg) compared to when the litters were female biased (181 pg/mg). When litter sex ratios were balanced, concentrations of estradiol in urine were intermediate (281 pg mg). It is unclear why the direction of the skew seen in the lemur study is the opposite of those seen in mice and cows. Perhaps the directional influence of estradiol is species specific. Alternatively, the relationship in lemurs could result from a discrepancy between concentrations in the urine and concentrations in the blood and/or follicular fluid. Interestingly, while the mouse and cow studies showed influences of estradiol at the time of fertilization, the lemur study showed no association between sex ratios and urinary estradiol concentrations at ovulation. However, this study still supports a role for estradiol in the determination of offspring sex ratios in mammals.

How might estradiol be acting on sex ratios in these cases? Emadi et al. (2014) suggest that estradiol treatment could influence the timing between ovulation and fertilization, a factor that has been shown to influence sex ratios in previous studies involving both cows (Martinez et al. 2004) and mice (Krackow 1995). Given that estradiol treatment did not affect oviductal transport of oocytes in cows (Crisman et al. 1980), and the fact that, in the mouse study, fertilization was done directly by the researchers, this possibility is not the likely explanation behind the estradiol-induced sex ratio skews in these two systems. Instead, it is likely that estradiol somehow increased the likelihood that Y-bearing sperm would successfully bind to and fertilize the egg. Perret (2005) pointed out that the development and maintenance of the cumulus oophorus, which functions in sperm sequestering, is

influenced by gonadotropin treatment (Bedford and Kim 1993) and, as a result, may also be sensitive to estradiol. Alternatively, it is possible that the estradiol in the medium and/or the follicular fluid influences the sperm, themselves. Human sperm have a biologically active membrane receptor for estradiol and binding this receptor results in a release of intracellular calcium (Luconi et al. 1999). Whether X- and Y-bearing sperm contain different quantities of these receptors remains unknown.

These studies indicate a clear role for estradiol to influence sex ratios at or prior to fertilization in mammals. More work needs to be done to determine whether estradiol is influencing the oocyte or the sperm to affect fertilization in a sex-specific way. In addition, all of these studies measured sex ratios at birth and did not account for potential sex-specific influences on blastocyst survival/loss. Finally, the influences of elevated estradiol concentrations during gestation on sex ratios have never been tested. More work on the potential role of estradiol is badly needed. In addition, given the high number of environmental contaminants that show estrogenic properties, it is important to examine the impacts of estrogen mimics on sex ratios in mammals.

7.3.3 Influences of Testosterone

In Chap. 3, I highlighted the evidence that maternal dominance rank influences the sex ratios produced by those mothers. Given the linkage between dominance and testosterone concentrations in males, it has been suggested that testosterone may also be a candidate in the regulation of sex ratios in mammals. Valerie Grant (2007) provides a nice review addressing this idea. Indeed, women who produce males rank higher on a dominance scale than those who produce females (Grant 1994), and women who are more dominant have higher concentrations of testosterone in circulation (Grant and France 2001). In 2008, Shargal showed in ibexes that dominance rank positively correlated with offspring sex ratios and that the most dominant individuals not only had more sons but also had higher amounts of testosterone in feces (Shargal et al. 2008). Additional indirect evidence for the role of testosterone in the determination of offspring sex in mammals was provided by two studies conducted in house mice and Mongolian gerbils; in both studies, female mice that developed between two male siblings in utero were more likely to produce a higher proportion of male offspring as adults (Vandenbergh and Huggett 1994; Clark and Galef 1995). The female mice that gestated between two male siblings also had shorter anogenital distances at birth compared to other females that did not have males as neighbors in utero, which suggests exposure to higher concentrations of androgens during development. This indicates that androgens can program the physiology of offspring in a way that influences the sex ratios of their offspring. Whether those females produce elevated concentrations of androgens in circulation when conceiving offspring remains unknown; however, these two studies have been cited as supportive evidence of a role for testosterone in the process of mammalian sex determination.

Since this time, there have been more direct examinations of how testosterone concentrations relate to mammalian sex ratios. Helle et al. (2008) showed in field voles that females with high testosterone and glucose concentrations produce a higher proportion of male offspring. In this case, it is unclear whether it was the high testosterone or glucose concentrations that were responsible for the influence on sex ratios; however, in a study conducted in mice, injections of flutamide, an androgen receptor blocker, just prior to induced ovulation resulted in female-biased offspring sex ratios (flutamide = 45.3% male, control = 58.4% male) (Gharagozlou et al. 2016). So overall, it appears that high concentrations of testosterone result in male-biased sex ratios, while blocking androgen action reduces the proportion of male offspring.

In an attempt to pinpoint the mechanism by which testosterone might act, Valerie grant and her colleagues performed some elegant work examining the role of testosterone in the fluid contained within the ovarian follicle on the resulting sex of the offspring (Grant and Irwin 2005; Grant et al. 2008) (Fig. 7.8). First, in 2005, they collected primary and subordinate ovarian follicles from cows and aspirated the follicular fluid, granulosa cells, and cumulus–oocyte complexes. Some of the follicular fluid was used to measure testosterone and estradiol concentrations, and the cumulus–oocyte complexes were fertilized in vitro and were then cultured to the 6–8 cell stage, after which the cells were sexed using molecular methods. Follicular concentrations of estradiol were not related to the ultimate sex of the offspring; however, testosterone concentrations in subordinate follicles were; follicles that had higher concentrations of testosterone were more likely to produce a male offspring (Grant and Irwin 2005). The reason that this was only seen in subordinate and not primary follicles is likely because concentrations of testosterone decrease as the follicle nears the point of ovulation. Thus, there may be a sensitive period during which testosterone concentrations in the follicular fluid may influence offspring sex. In an additional study primarily focused on those subordinate, antral follicles, Grant et al. (2008) verified this effect; follicles with higher concentrations of testosterone were significantly more likely to produce male offspring.

This idea has been challenged. García-Herreros et al. (2010) used the same techniques to repeat the experiment and found no significant relationship between testosterone concentrations in follicular fluid and offspring sex; however, Grant and Chamley (2010) argue that if you add the data from the García-Herreros et al. (2010) study to those of the Grant et al. (2008), the result is a significant positive relationship between follicular testosterone concentrations and the proportion of male offspring that result. Still, in two additional studies, oocytes were incubated with exogenously supplied testosterone in attempts to stimulate the production of male blastocysts, and there was no effect of testosterone treatment in either study (Diez et al. 2009; Macaulay et al. 2013). Thus, it is possible that the true effector of sex ratio is an upstream precursor or downstream metabolite of testosterone. This remains to be tested.

Overall, a majority of studies examining influences of testosterone have tested levels prior to conception. To my knowledge, only two studies, one conducted in marmosets and one in ground squirrels, have tested whether androgen levels during

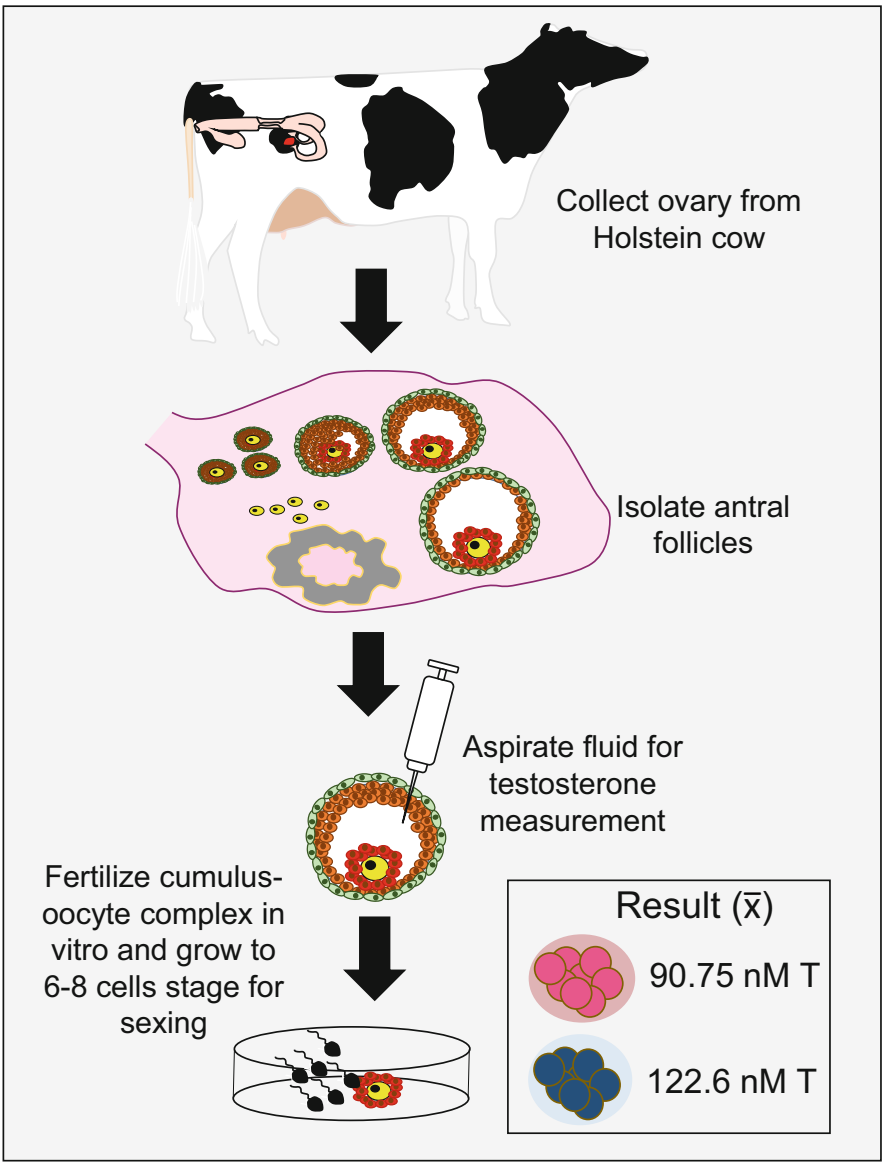


Fig. 7.8 Visual depiction of the experimental design used by Grant et al. (2008). Antral follicles were collected from cows, follicular fluid was aspirated from the follicles, and the cumulus–oocyte complexes were transferred to a dish where they were fertilized using sperm from bulls. At the 6–8 cell stage, blastocysts were collected and sexed molecularly. Results above show the average testosterone concentrations in follicular fluid for follicles that ultimately resulted in male versus female blastocysts

gestation affect embryonic survival in a sex-specific way. No effect was found in either case (French et al. 2010; Ryan et al. 2014). In both of these studies, the phases of gestation were well represented. French et al. (2010) collected urine from the female marmosets 2–5 times per week over the entire course of gestation, and Ryan et al. (2014) collected blood for androgen analyses both early and late in gestation. More studies are needed, however, to test for influences of testosterone during gestation, particularly in species where maternal dominance has been shown to exert an effect on sex ratios.

7.3.4 Evidence that Multiple Reproductive Hormones Act Together

To date, most researchers addressing the role of hormones in sex ratio adjustment have aimed to find a "holy grail" mediator that ties all of the cues known to influence sex ratios in various systems together. Indeed, we have identified some candidate hormones that have the potential to be potent regulators of sex ratios in both avian and mammalian systems. However, some researchers have instead hypothesized a multitier system of regulation, involving multiple mediators that act in concert to adjust sex ratios. An example of this was posited in several reviews by William James (James 1996a, 2004, 2008). James was among the first researchers to suggest that reproductive hormones play a role in the process of sex ratio adjustment, and he further theorizes that these hormones act in both males and females to exert their effects. Specifically, he hypothesized that high concentrations of both testosterone and estradiol in both male and female parents result in the production of more male offspring, while high concentrations of gonadotropins and progesterone have the opposite effect. He postulates that sex ratios correlate positively with R, where R is a function of the equation:

$$(\text{testosterone} + \text{estrogen})/(\text{gonadotrophins} + \text{progesterone})$$

As cited above, there is evidence suggesting that both testosterone and estradiol have the capacity to induce male biases in offspring sex ratios. In addition, occasions during which gonadotropins were provided to men and women for fertility reasons can offer additional insight. For example, men treated with human chorionic gonadotropin (HCG) have elevated concentrations of both testosterone and estradiol and produce male-biased sex ratios (Sas and Szöllösi 1980). While this effect could result from either the testosterone or estradiol elevation independently, James (2008) uses this study as support for the idea that these hormones work in concert. In another study, women treated with clomiphene citrate produce a higher proportion of female offspring. Clomiphene citrate is a chemical that blocks estrogen receptors in the brain and results in elevated levels of the gonadotropins (luteinizing hormone and follicle-stimulating hormone) in the body. In addition, women treated with gonadotropins show a similar female bias in the offspring they produce (James 1985). Still, the fact that high levels of gonadotropins should elevate estradiol concentrations in blood is problematic in terms of supporting the hypothesis.

How, exactly, might these hormones coordinate, in both sexes, to control sex ratios? James provided a potential answer to this as well (James 1997). In males, the epididymis produces a secretory product called glycerylphosphorylcholine (GPC) (Mann and Lutwak-Mann 2012). The role of this molecule is unknown and is unlikely to participate in fertilization or activation of sperm (Jeyendran et al. 1989; Wallace and White 1965). However, the female reproductive tract produces glycerylphosphorylcholine diesterase (GPCD), which splits GPC into free choline (Wallace and White 1965). In men, GPC concentrations are correlated positively with testosterone, and in female rats (Wallace et al. 1964), GPCD concentrations are correlated positively with estrogen and negatively with progesterone concentrations (Cooper et al. 1988). James suggests that this mechanism, controlled by reproductive hormones, may provide a control of offspring sex ratios that is coordinated between males and females. To date, this idea has not been tested empirically. It does, however, point out that we should be considering the possibility that multiple hormones may act together to influence sex ratios in both mammals and birds.

7.4 What About Nonsteroid Hormones?

To date, researchers have focused almost solely on the steroid hormone as mediators of sex determination in both birds and mammals; however, we know that there are multiple other hormonal regulators that interact to control physiological responses to the many cues that have been shown to adjust offspring sex ratios. Below I discuss some other potential regulators of offspring sex ratios in birds and mammals.

7.4.1 Factors that Regulate Blood Glucose

In mammals, there is evidence that circulating glucose concentrations contribute significantly to the determination of offspring sex. For example, Helle et al. (2008) showed that field voles with high concentrations of circulating glucose produce more male offspring, and Mathews et al. (2008) showed in humans that higher intake of energy in general increased the proportion of male offspring born. Cameron et al. (2008) were able to experimentally reduce sex ratios of litters produced by mice while treating them with dexamethasone. While this treatment is known to inhibit glucocorticoid production, as described above, it also reduces circulating glucose concentrations. In the Cameron et al. (2008) study, circulating glucose concentrations were directly related to litter sex ratios, and when glucose concentrations were experimentally reduced by the dexamethasone treatment, fewer male offspring were produced. This suggests that the results in response to dexamethasone treatment described in golden hamsters above Pratt and Lisk (1990) may have resulted from changes in circulating glucose concentrations rather than changes in glucocorticoid production. The link between blood glucose concentrations and sex ratios in birds is less clear.

Given the potential for glucose to be a potent regulator of offspring sex ratios, it makes sense to consider the effects of the hormones that regulate glucose concentrations in circulation. Insulin is the first hormone that comes to mind when thinking about glucose regulation, and mammalian ovarian follicles are sensitive to insulin (Louhio et al. 2000). In fact, there is growing evidence that insulin resistance is involved in a major ovarian dysfunction, polycystic ovary syndrome (Dunaif 1997), and insulin receptors are present and active in mammalian ovaries (Willis and Franks 1995). Thus, insulin could act to coordinate information about food availability with modulation of offspring sex ratios at the level of the ovary.

Perhaps even more tantalizing is the idea that insulin-like growth factors mediate the process of sex ratio adjustment. Insulin-like growth factors (IGF-1 and IGF-II) are molecules that are homologous to pro-insulin in its primary, secondary, and tertiary structures. IGF-1 binds to insulin receptors to help regulate concentrations of glucose in the blood and tissues (Clemmons 2004), and both IGF-1 and IGF-II have well-known functions in controlling the growth and differentiation of granulosa and theca cells in both mammals and birds (Onagbesan et al. 1999) and have been found in the mammalian oocyte as well (Armstrong et al. 2002). In fact, it has been suggested that IGFs are major regulators of follicle selection in mammals (Mazerbourg et al. 2003) and birds (Onagbesan et al. 2009). Thus, there is potential for IGFs to act in the process of sex ratio adjustment during mechanisms that would occur at the level of the ovarian follicle. If we instead consider sex ratio adjustment triggered by the male in mammals, both IGFs and insulin are active in the process of spermatocyte differentiation in the testes (Nakayama et al. 2004). In addition, IGFs are also known to function during early embryonic development (Wang et al. 2006) and are expressed by preimplantation embryos (Doherty et al. 1994) Given that IGFs coordinate the body's energetic reserves with reproductive functions at the level of the ovary, the testis, and the developing embryo, it is a seductive hypothesis that IGF may mediate the process of sex ratio adjustment at more than one developmental level. Studies examining how levels and activity of IGFs vary with offspring sex ratios are needed.

7.4.2 Leptin and Ghrelin

Two of the most striking drivers of sex allocation that emerge in both birds and mammals are the availability of food and the body condition of females. Yet, in studies of sex ratio adjustment, hormones and other factors that control appetite, fat deposition, and other responses to changes in food intake have been virtually ignored. It is now well known that factors produced by the stomach and adipose tissue not only regulate the intake of food and breakdown and storage of energy, they also directly interact with reproductive tissues in both the male and female to regulate reproductive physiology. I will highlight two of these factors below:

Leptin is a hormone that is secreted from adipocytes and acts to increase metabolic rate and decrease appetite in both mammals and birds (Denbow et al. 2000; Barash et al. 1996). Leptin receptors are expressed in the mammalian and

avian ovaries (Cassy et al. 2004; Karlsson et al. 1997), and in addition to its actions on metabolism and appetite, leptin regulates reproductive function in both males and females (Barash et al. 1996). In the granulosa and theca cells of the mammalian ovary, leptin acts as an antagonist to IGF-1 (Agarwal et al. 1999; Zachow and Magoffin 1997; Spicer and Francisco 1997). Leptin also directly affects testicular function (Tena-Sempere et al. 2001), is present in seminal fluid, and is secreted by spermatozoa as well (Jope et al. 2003). In fact, there is evidence that leptin can influence sperm function (Lampiao and Du Plessis 2008). It is also known that leptin is secreted by the human endometrium (González et al. 2000) and directs the process of implantation (Yang et al. 2006). As a result, it is easy to see how the pleiotropic effects of this hormone could act to integrate information about food availability and maternal condition into a mechanism to skew offspring sex ratios.

Another hormone known to be intimately involved in both metabolic regulation and reproduction is ghrelin. Ghrelin is produced by the hypothalamus and the stomach and acts as an appetite suppressant. Receptors for ghrelin are present in both the mammalian and avian ovaries (Gaytan et al. 2003; Sirotkin et al. 2006), and ghrelin is now known as a second factor that regulates reproductive function based on energy status within the body (Barreiro and Tena-Sempere 2004). This hormone also influences sperm function and morphology (Kheradmand et al. 2009) and regulates early blastocyst development as well (Steculorum and Bouret 2011). Ghrelin is yet another potential regulator of sex ratios that has, to date, gone unexplored.

7.5 Conclusions

Clearly, our understanding of how hormones are involved in mediating the adjustment of offspring sex ratios is only in its infancy for both mammals and birds. In birds, in which females are heterogametic, hormones are likely acting at the level of the female ovary to influence which sex chromosome is retained in the oocyte. This could occur via influences on the processes of follicle selection and/or growth, or via direct effects on meiotic segregation of sex chromosomes. Many of the hormones identified above show activity during each of those stages. In mammals, where the males are heterogametic, there is a wider range of targets for manipulating sex ratio adjustment. Hormones may act at the level of male sperm production, female receptivity of the egg, blastocyst implantation, and embryonic survival. Many of the hormones described above are active at each level. Given the evidence to date, it appears likely that sex ratio adjustment may not only act at multiple levels in an animal but may also be regulated in different ways by different hormones. As a result, the manipulation of offspring sex ratios is not likely a one-step process. Instead, sex ratio skews likely result from the complex interplay involving a web of hormones that act on several reproductive targets.

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