World human population is expected to grow to 9.3 billion in 2050, an increase of nearly 33% from the current level of 7 billion (USA Census Bureau 2012; FAO 2009). Most of this growth is forecast to take place in the developing countries. It is estimated that feeding a population of 9.3 billion people in 2050 would require raising overall food production by 70% from the current levels. Foods derived from animal sources are expected to be in great demand due to their nutritive value, and increased affluence of people in developing countries. In particular, the global demand for poultry meat and eggs is expected to grow exponentially over the next several decades. In the year 2011, global production of broiler meat stood at 80,420 Mt, of which 63,726 Mt (or 79%) was produced in the USA. Similarly, nearly one-half of the global turkey meat production of 5,312 Mt was produced in the USA (USDA, Foreign Agricultural Service, http://www.fas.usda.gov/livestock_arc.asp). Increasing global poultry production in the future would require significant improvements in genetics, nutrition, and managerial practices, including reproduction. Some of the existing...
challenges and future prospects for improving poultry production from the view of a reproductive biologist are discussed in this chapter.

Reproductive Dysfunction in Broiler Breeder Chickens

Approximately 8.5 billion broiler chickens are reared in the USA annually for meat production. Broiler breeder chickens are genetically selected for faster growth, higher feed intake, and greater muscle yield in their progenies. Just like their progenies, the parental line of broiler breeder chickens also displays hyperphagia that leads to reproductive problems (Robinson et al. 2007). Consequently, broiler breeder hens have the poorest reproductive efficiency of all commercial avian species. Current management practices involve cumbersome and often imprecise feed restriction methods to limit body growth in an effort to increase egg production. Despite adopting laborious methods, egg production remains suboptimal due to excessive follicular recruitment that often leads to internal or double (nonviable) ovulations. It is not clear, however, what factor(s) promotes this excessive follicular recruitment in the broiler breeder hen ovary. The following review covers some of the significant research areas that hold promise for improving reproductive efficiency in broiler breeder chickens.

Broiler Breeder Hens Have Excessive Visceral Adiposity and Multiple Ovarian Follicular Hierarchies

Broiler breeder female chickens are hyperphagic. When allowed unrestricted access to feed, they gain twice as much weight as feed-restricted chickens. A major part of this excess body weight is due to the increased deposition of visceral adipose tissue (abdominal fat pad, mesenteric fat, and fat around visceral organs). Accumulation of excessive visceral adipose tissue due to unrestricted feeding, as seen in Fig. 2.1, often leads to hypertrophy of adipocytes with excess triglycerides. Coincidentally, the ovary of the ad libitum-fed broiler breeder hen also develops multiple follicular hierarchies. A normal ovary has a typical hierarchy of 4–6 preovulatory follicles (F1–F4 in Fig. 2.2) that are greater than 10 mm in diameter. The largest follicle (F1) ovulates every 26–28 h and the preovulatory follicular hierarchy is maintained by sequential recruitment of prehierarchical follicles. However, in broiler breeder hens that had unrestricted access to feed, the normal follicular hierarchy is disrupted by selection and growth of more than one follicle resulting in multiple hierarchy, multiple ovulations, and internal ovulations. This is one of the main reasons for poor reproductive efficiency of broiler breeder hens. As in females, male broiler breeder chickens that are fed ad libitum were found to have reduced duration of fertility, possibly contributing

Fig. 2.1 Photographs of a part of small intestine in situ in a broiler breeder hen (18 weeks-old) showing excessive mesenteric adipose tissue accumulation due to unrestricted feeding (right) compared to lesser level of adipose tissue following restricted feeding (left) (Ramachandran, unpublished data)
Fig. 2.2 Ovarian follicular hierarchy in leghorn (left) and broiler breeder hen (right). F1–F5 denote preovulatory follicles. Note double hierarchy of preovulatory follicles in broiler breeder hen ovary (picture courtesy: Dr. Alan Johnson, Department of Animal Science, Pennsylvania State University).

Gonadotropins

Gonadotropins are critical for ovarian follicular development as well as egg production, initiation, and maintenance. Several studies have attempted to determine if gonadotropin secretion was altered in broiler breeder chickens in response to feed restriction. One of the studies suggests that plasma LH and FSH concentrations in Shaver Starbro broiler breeder pullets were significantly higher in ad libitum-fed chickens compared with feed-restricted hens (Renema et al. 1999). Similarly, ad libitum-fed broiler breeder pullets showed the highest responsiveness to ovarian hormones and to cLHRH-I in releasing FSH prior to sexual maturity compared with feed-restricted pullets, suggesting that feeding regimen can modify pituitary sensitivity to cLHRH-I and to gonadal hormones (Bruggeman et al. 1998b). Feed restriction of Hybro G broiler breeder pullets between 7 and 15 weeks-of-age followed by ad libitum feeding led to improved reproductive performance, although pituitary and plasma LH and FSH concentrations, and median eminence levels of cLHRH-I, were not different compared with pullets fed ad libitum (Bruggeman et al. 1998a). This raises the possibility that FSH responsiveness in the pre-hierarchical follicles is increased in response to overfeeding in broiler breeder chickens. FSHR were found to be expressed in both theca and granulosa cells of the developing ovarian follicle (You et al. 1996) but altered FSH signaling in broiler breeder ovaries in response to overfeeding has not been investigated. While it is unequivocally clear that ad libitum feeding reduces reproductive efficiency in broiler breeder hens, the underlying mechanisms involving hypothalamic–pituitary–ovarian axis and sensitivity to FSH at the ovarian level remain to be elucidated.

Metabolic Hormones

The root-cause(s) for the ovarian dysfunction in broiler breeder hens most likely lies within the ovarian follicles and visceral adipose tissue that tend to accumulate excessive triacylglycerol and fatty acids as a result of overeating (Chen et al. 2006). There are evidences to suggest that ad libitum-fed broiler breeder hens suffer with lipotoxicity leading to upregulation of proinflammatory cytokines expression in the liver and an increase in circulating levels of ceramide and sphingomyelin (Pan et al. 2012). In another study, ad libitum feeding of broiler breeder hens was associated with an increased apoptosis of granulosa cell and suppressed Akt activation (Xie et al. 2012). Furthermore, treatment of
granulosa cells with palmitic acid, a saturated fatty acid, was found to activate apoptotic machinery in the granulosa cells. Leptin is an adipocytokine hormone that affects various metabolic and reproductive functions mediated through the hypothalamic–pituitary–gonadal axis in mammals (Barash et al. 1996; Bluher and Mantzoros 2007). Although existence of leptin in avian species is debatable (Sharp et al. 2008; Simon et al. 2009), leptin receptor is unequivocally expressed in various tissues including the thecal layer of the ovarian follicles in chickens (Cassy et al. 2004; Ohkubo et al. 2000). Injection of leptin-like substance to fasted laying hens was found to delay cessation of egg laying, attenuate regression of yellow hierarchical follicles, altered ovarian steroidogenesis (Paczoska-Eliasiewicz et al. 2003). Discovery of chicken leptin or endogenous ligand(s) for leptin receptor will improve our understanding on the role of leptin in ovarian dysfunction in broiler breeder hens.

The role of IGF on excessive adipose tissue deposition and ovarian dysfunction in broiler breeder hens has been investigated. Systemic levels of IGF-I and IGF-II were found to be elevated in broiler breeder pullets in response to feed restriction (Bruggeman et al. 1997; Hocking et al. 1994). In another study, the proportion of carcass fat in ad libitum-fed chickens was found to be positively correlated with plasma glucagon, IGF-II, and 17β-estradiol but negatively correlated with plasma insulin, insulin/glucagon ratio, IGF-I, thyroxine, and triiodothyronine suggesting that ad libitum feeding favors fat deposition (Sun et al. 2006). Consequently, excessive accumulation of carcass fat is likely to be detrimental to overall metabolism and in particular, to the reproductive system. Treatment of granulosa cells isolated from F1, F2, and F3 preovulatory follicles of broiler breeder hens with IGF-I alone or in combination with LH significantly increased granulosa cell proliferation in birds fed ad libitum more than feed-restricted hens suggesting that IGF-I may play an important role in accelerating the rate of maturation of follicles (Onagbesan et al. 1999). The precise role of IGF, GH, or insulin on ovarian follicular development in broiler breeder hen ovaries remains to be elucidated.

**Inhibin/Activin**

Inhibin, a hormone secreted predominantly by the granulosa cells of the ovarian follicle, acts as a negative feedback regulator of pituitary FSH secretion (Johnson et al. 1993; Vanmontfort et al. 1992, 1995). Expression of the inhibin α-subunit and inhibin/activin βA and βB subunits, as well the activin type II receptor have been documented in the developing follicles of broiler breeder hen ovaries suggesting a paracrine role for inhibin and activin within the ovary (Slappey and Davis 2003). Plasma inhibin levels were negatively correlated with FSH and positively correlated with progesterone levels in female chickens (Lovell et al. 2001; Vanmontfort et al. 1992). A practical application exists in modifying inhibin action to improve egg production in chickens. Active immunization of chickens against inhibin in broiler breeder hens was found to increase cumulative number of eggs produced by 9.5 % at the end of week 40 (Satterlee et al. 2002). Immunoneutralization of inhibin in chickens is likely to control the entry of ovarian follicles into preovulatory hierarchy (Lovell et al. 2001). Further studies are required to determine whether inhibin signaling can be altered in commercial settings to improve egg reproduction efficiency.

**Anti-Müllerian Hormone**

Anti-Müllerian hormone is predominantly secreted by the granulosa cells of the ovarian follicle in adult female chickens (Wojtusik and Johnson 2012). Recently, a possible role for AMH in excessive follicular recruitment in broiler breeder hens has been reported. As expected, AMH gene expression was found to be significantly higher in broiler breeder hen ovaries compared to Leghorn chicken ovaries (Johnson et al. 2009). Similarly, AMH gene expression was higher in the ovaries of fully fed broiler breeder hens compared with feed-restricted hens. AMH was postulated to enhance granulosa cell proliferation in an autocrine or paracrine mechanism but excessive AMH is likely to inhibit follicle development (Johnson et al. 2009). The ovarian AMH gene expression
appears to be susceptible to vitamin D levels since a dose-dependent decrease in AMH mRNA levels was detected to vitamin D treatment (Wojtusik and Johnson 2012). Increased serum levels of AMH are associated with polycystic ovarian syndrome in women (Cook et al. 2002), a condition that resembles excessive follicular recruitment as occurring in broiler breeder hens that are fed ad libitum. At the present time, methods to quantify circulating levels of AMH in chickens are not available and therefore, a correlation between plasma AMH levels and egg production are not known. Future studies are required to determine if the manipulation of ovarian AMH levels leads to normalizing ovarian follicular hierarchy and higher egg production in broiler breeder hens.

Artificial Insemination

Comprehensive reviews on the history, methods, and challenges of AI in commercially important avian species can be found elsewhere (Blesbois 2007; Donoghue and Wishart 2000; Long 2006). AI technology is critical to turkey meat production as AI is almost exclusively used for turkey breeding. This is due to a disparity in the sizes of toms and hens as toms often exceed 33 kg in body weight while the hens are only 9 kg thus rendering mating challenging (Donoghue and Wishart 2000). In contrast, AI is not commonly used in chickens as broiler breeder hens are typically reared in floor pens instead of cages and due to low fertility of cryopreserved chicken semen (Donoghue and Wishart 2000). However, AI may become essential and practically relevant if future genetic selection of broilers favors a body conformation that limits physical mating. AI technology in turkeys utilizes fresh liquid semen (Donoghue and Wishart 2000) as storage of liquid semen greater than 6–24 h greatly reduces fertility. Poor fertilizing ability of the frozen/thawed avian spermatozoa can be attributed to several factors including greater sensitivity to the freezing/thawing process, deleterious effects of the cryoprotectant on survival, and the ability to withstand longer storage/seLECTION in the SST of the female reproductive tract.

Recent studies have focused on mitochondrial function (Froman and Feltmann 2010) and the composition of the plasma membrane (Long 2006) with a view to develop cryopreservation methods that maintain the integrity of spermatozoa upon freezing/thawing and longevity once inside the SST. Mitochondria provide energy for sperm mobility and survival in the female reproductive tract and as such, conservation of mitochondrial integrity and function are critical for successful sperm cryopreservation. The function of chicken spermatozoa mitochondria can be temporarily inactivated using a calcium ion chelator prior to cooling to 10 °C and can be reactivated within a 5-h period (Froman and Feltmann 2010). In this study, a fertility rate of 88% was achieved when the spermatozoa stored for 3 h were reactivated and used for insemination. A mass spectrometric analysis of proteins extracted from chicken spermatozoa revealed that expression levels of proteins related to ATP metabolism and glycolysis differ in high-versus low-sperm-mobility New Hampshire chicken lines (Froman et al. 2011). This suggests that mitochondrial function and energy levels are critical for sperm mobility.

Carbohydrates on the spermatozoa plasma membrane are found to be significantly altered during cryopreservation, and the degree of such modification was influenced by the type of cryoprotectant and freezing–thawing rates (Pelaez et al. 2011). In this regard, the type of cryoprotectant and freezing process was also found to alter the ability of chicken spermatozoa to undergo acrosome reaction (Moce et al. 2010). Fluidity of the avian spermatozoa plasma membrane was found to be affected with a significant decrease in cholesterol/phospholipid ratio following cryopreservation (Blesbois et al. 2005). Membrane fluidity is also one of the predictors for the success rate of semen cryopreservation in the chicken (Blesbois et al. 2008). Cryopreservation of spermatozoa of turkeys and sandhill cranes (Grus canadensis) using dimethylacetamide as the cryoprotectant resulted in greater viability for the frozen/thawed crane semen compared with turkey semen emphasizing the need for optimization of protocol suitable for each species (Blanco et al. 2012).
More studies are required to develop an appropriate cryopreservation method taking into consideration the anatomy and physiology unique to avian spermatozoa.

Sex Selection

Male chicks in egg producing flocks are not useful for the egg production and therefore, approximately, one-half of the chicks hatched in the poultry egg industry are typically culled. This represents an enormous waste of resources that were used in breeding and rearing parent chickens, fertile egg handling and hatching of eggs. Unlike mammals, sex in avian species is determined by the female and favoring female offspring would provide tremendous cost-saving to the poultry egg industry. Despite the commercial importance of sex selection to the poultry industry, there are only a few studies that have investigated the possibility of altering the sex ratio. Sex determination in avian species occurs during the first meiotic division that typically happens 2–4 h before ovulation (Olson and Fraps 1950). Among various factors coincident during this period, a dramatic surge in circulating progesterone levels, predominantly emerging from the preovulatory follicles, is highly critical for the induction of ovulation. Using commercial Leghorn chickens, Correa et al. attempted to further elevate circulating progesterone levels during the critical window of sex determination (Correa et al. 2005). In this study, administering progesterone 2 mg (high dose) or 0.25 mg (low dose) to White Leghorn hens (Babcock B300 strain) 4 h prior to the end of the light cycle resulted in far fewer males (25 %) from hens treated with high dose of progesterone compared with the number of males from low dose progesterone or sesame oil-treated hens (61–63 %). While this study confirms the proof-of-principle that progesterone can affect sex ratio in the first egg, more research needs to be done to determine the effect of progesterone on sex ratio and its impact on egg production efficiency over a longer time period. Two recent studies adopted a similar approach of elevating circulating testosterone (Pinson et al. 2011b) or corticosterone (Pinson et al. 2011a) levels in White Leghorn hens (Hyline strain) during the predicted window of sex determination. A single dose of testosterone or corticosterone was administered to hens 5 h prior to the predicted time of ovulation and sex of the resultant offspring was determined. Interestingly, testosterone treatment resulted in a significantly higher proportion of male chicks compared to the control (Pinson et al. 2011b). Similar to testosterone, corticosterone treatment resulted in over 80 % of the chicks being male compared to only 40 % in untreated control hens (Pinson et al. 2011a). Based on the foregoing, altering sex ratio and hatching more females in the commercial poultry egg industry seems plausible in the future. It is, however, important that the overall egg production efficiency is not compromised while we attempt sex selection. Future studies should focus on using non-hormonal feed supplements that will accomplish sustained sex selection.

Transgenic Chickens

Modifying the chicken genome through transgenic technology has tremendous potential for imparting disease resistance and for expression of novel compounds in meat and eggs. Development of tools used in transgenic technology will also facilitate conservation and long-term preservation of PGC and embryonic and stem cells. Comprehensive reviews of various methods used to create transgenic chicken can be found elsewhere (Han 2009; Mozdziak and Petitte 2004; Park and Han 2012a; Petitte et al. 2004). Replication incompetent retroviral vectors including lentiviral vectors have been used for integrating transgenes into the chicken genome. Using this technique, several elegant studies have demonstrated the stable integration, germ line transmission, and expression of transgenes in chickens. Transduction of transgene constructs was typically achieved by infecting blastodermal cells or PGC derived from embryonic gonads or embryonic blood circulation with retroviral vectors (Harvey et al. 2002; Kamihira et al. 2005; Lillico et al. 2007; McGrew et al. 2004; Scott and
Lois 2006). Using lentiviral vector, a novel strategy to develop chickens that are genetically resistant to avian influenza was described (Ding et al. 2005). In this study, transgenic chickens were created overexpressing a short-hairpin RNA driven by the U6 promoter that inhibits and blocks influenza virus polymerase and prevents virus propagation. Although viral methods of creating transgenic chickens are feasible, such methods would render the transgenic chicken unsuitable for agriculture use. To overcome this disadvantage, recent studies have used DNA transposons to integrate transgenes into the chicken genome. PiggyBac, a DNA transposon isolated from the cabbage looper moth Trichoplusia ni (Cary et al. 1989), has been widely used for creating genetic modifications in mice (Ding et al. 2005) and in chicken embryos (Lu et al. 2009). Recently, transgenic chickens were successfully produced by microinjecting DNA constructs encoding GFP and piggyBac transposase into the sub-germinal cavity of newly laid eggs (Liu et al. 2012). Using non-virally transfected gonadal PGC with GFP and piggyBac DNA elements, transgenic chickens overexpressing GFP were created at a very high rate of transgene integration (437 transgenic chickens created out of 459 total hatched chicks; Park and Han 2012b). Similarly, piggyBac or Tol2, another transposon isolated from the medaka fish genome, was used to integrate transgene into PGC derived from embryonic blood that was then utilized to develop transgenic chickens (Macdonald et al. 2012). Taken together, non-viral methods for creating transgenic chickens offer tremendous potential for improving nutritive value of poultry meat or egg and for imparting disease resistance to chickens.

**Conclusion**

In conclusion, there is tremendous potential for improving the reproductive efficiency of broiler breeders and to help meet the increasing global demand for poultry meat. Understanding the role of hormones in ovarian follicular recruitment and ovulation is critical for improving reproductive efficiency. Selecting for female chicks in the egg production industry will help to conserve resources and lower costs. Some of the emerging technologies for cryopreservation of semen and PGC are likely to improve poultry production efficiency. Successful development of transgenic chickens that selectively overexpress certain microRNA and enzymes can prevent disease epidemics and disease-free flocks to allow uninterrupted food production.

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