Physiological Factors Affecting the Reproductive Performance of Commercial Broiler Breeder Males

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Introduction

The tremendous improvements in growth rate, body conformation and carcass yield of commercial broiler chickens have dramatically reduced the interval from hatch to slaughter. Concomitant with these changes has been the implementation of rigorous growth restriction programs for the development of parent stock. Over the past several years efforts have been made to “optimize” the reproductive performance of broiler parent stock, and the overall results have generally been satisfactory. However, the biological basis for the interactions between rapid growth rate, increased breast muscle yield and reduced reproductive performance are, at best, only poorly understood. As the conformation of broilers becomes more extreme (e.g., higher proportion of lean mass becomes breast muscle), the potential for negative interactions with reproductive performance becomes greater. The purpose of this overview is to highlight some of the important aspects of breeder male reproductive maturation and fecundity.

The presentation outlined here (and presented in detail as slides at the end) was designed to overview three of the components that affect male reproductive performance:
1. Circadian biology and the interactions of multiple circadian oscillators on the daily rhythms of activity, temperature and reproductive performance;
2. The role of FSH as a critical regulator of sperm production and sexual maturation in broiler breeder males; and
3. The development of epididymal stones in a wide range of male fowl from commercial leghorns to broiler breeders.

Biological Rhythms and Reproduction
Biological rhythms are found in all living organisms, plants and animals. Several different categories of rhythms are recognized including ultradian (extremely short cycle rhythms of seconds, minutes to hours), circannual (rhythms which tend to approximate a yearly pattern of variation) and the most commonly studied circadian (24 hour ("circa" around a "dian" day or dial)) rhythms. These biological rhythms have multiple components, most important of which is that they tend to persist under constant conditions, that is under circumstances where no apparent daily changes in the external environment occur (e.g., constant light). In this presentation we are concerned with three different rhythms: 1) the "circadian" rhythm of body temperature; 2) the circadian rhythm of activity; and 3) the ultadian/circadian rhythm of LH secretion in female poultry. The take home message here is that while the circadian rhythms of temperature and activity in both young and old females can be easily disrupted with constant light, the profound drive of the reproductive rhythm is not disrupted. However, the presence of a light dark cycle directly affects the interval of reproductive events. For more information see Beaupre et al 1997; Yang et al, 2000 and Bacon et al, 1999.

Sexual Development of Male Breeders

When cockerels hatch they have a complement of both somatic and germinal precursor cells in their testes. The somatic cells eventually develop into "Sertoli" (nurse) "Leydig" or other structural cells. Sertoli cells function to protect the developing sperm cells and are primarily regulated by FSH (follicle stimulating hormone) from the pituitary and testosterone from the Leydig cells. The Leydig cells are primarily regulated by LH (luteinizing hormone) which is released from the pituitary in a pulsatile fashion. The germinal precursor cells subsequently become "stem cells (spermatogonia)" which provide an ever renewing population of cells for meiosis (the haploid reduction of nuclear DNA) and subsequent maturation into spermatozoa. This self renewing population of stem cells is a profound difference between males and females, who hatch with a finite number of potential follicles. Thus, while female fowl may produce a few hundred to a thousand or so eggs in a lifetime, males can theoretically produce trillions of spermatozoa (about one billion per day). The theoretical maximum number of sperm is most likely established by the number of Sertoli cells, cells which proliferate during only a very limited period of time. This "window" of Sertoli cell proliferation appears to be between 2-12 weeks of age, however the precise period has not yet been established. Unlike Sertoli cells, Leydig cell populations appear to change over time and reproductive status. The take home message is: The maximum potential for sperm production is established early in breeder males- that is sometime during the first 8-10 weeks of age.

At the same time the testis is beginning to develop, and partly responsible for directing its maturation, the brain (hypothalamus, primarily) and pituitary are establishing their hard-wired relationship. That is, the hypothalamus, pituitary and testes establish communications via their respective hormones. The hypothalamus talks with the pituitary using a number of neurotransmitters, including GnRH (gonadotropin releasing hormone) that stimulates the production and release of LH and FSH which travel via the circulation to the testes where they bind to receptors on Leydig and Sertoli cells, respectively. Leydig cells then produce testosterone which feeds back on the hypothalamus to decrease GnRH secretion decreasing LH and FSH production. The early stages of maturation that help to establish the feed back loop are important
in establishing the “gonadostat” settings that will regulate pituitary function over the life of the male. Furthermore, the early period of FSH secretion is thought to be coupled with Sertoli cell proliferation in the developing testis. From a management perspective, Dr. Wayne Kuenzel at the University has suggested that some regions of the hypothalamus associated with the regulation of feed intake may be co-localized with areas controlling pituitary function.

Recently we have been working to determine the relationship between FSH and testis growth and maturation in cockerels. First we have studied the relationship between circulating hormone (FSH) levels, testis weight and FSH receptor mRNA levels within the developing testis. Many investigators have shown a strong correlation between testis weight and function (in the absence of any known pathology) in a number of mammalian species and in egg type chickens. We have reevaluated this relationship, and as expected, have shown that testis weight, and by interpolation testis size, is highly \( r^2 > .9 \) related to daily sperm production. Daily sperm production is thus a measure we can use to estimate the theoretical maximum level of reproductive performance for any given male. In our original FSH studies, looking at adult (28-30) week old breeder males, we have shown that circulating FSH levels are highly related \( (r^2 > .8) \) to adult testis size and sperm production. Plasma FSH levels were also significantly reduced in adult males who had been heavily feed restricted following 6 weeks of age. Interestingly, unlike FSH neither plasma LH nor testosterone levels were significantly related to testis weight, daily sperm production or fertility in broiler breeder males.

Further studies on development of the reproductive tract in cockerels has led us to believe that FSH has a profound impact on testis development. The relationships between FSH, testis weight and FSH receptor mRNA levels are shown for SCWL cockerels reared from hatch to the end of the experiment on a 16L:8D photoperiod are also presented. The take home message from this figure is that the rapid pubertal increase in testis growth is preceded by about two weeks with an increase in plasma FSH levels, followed by an increase in FSH receptor levels and finally by the tremendous increase in testis mass. The increase in testis mass can be associated with the onset of sperm production associated with puberty. When we look at Broiler breeder males reared under traditional management conditions, that is a short photoperiod (8L:16D, or a declining natural day length) and moderate feed restriction, the exponential phase of testis growth occurs within 2-3 weeks of photostimulation. Gradually, after peak semen production is achieved, testis weight and sperm production slowly decline in aging males. We currently are evaluating these effects on circulating FSH levels in commercial broiler breeder males.

**Reduced Reproductive Performance in Males**

We have suggested here that the set-point for establishing the regulatory thermostat of gonadotropin secretion is at least partially completed within the first few weeks of a males life. We have also suggested that circulating FSH levels are highly related to testis size, sperm production and the theoretical maximum level of reproductive performance. We have further implied (though less strongly) that management conditions can alter circulating FSH levels and ultimately reduce sperm production. So, what does all of this have to do with raising commercial broiler breeder males in the field?
In our ongoing studies with broilerized males we have data that strongly suggests that mild stressors, those which may cause a male to either lose a little weight or to cause a disruption in water intake, such as by increasing feed restriction to "grow a male back to the curve" at a critical point in development or by placing males in cages with cups instead of bell waterers, can lead to a complete shut down of testis function. In commercial males these changes will be more subtle. In our hands, we can induce puberty (that is the presence of meiotic cells, the onset of spermatogenesis) with feed alone by seven weeks of age. Further, it is possible to disrupt the normal pattern of testis development by implementing more stringent feed restriction between 6 to 8 weeks of age, resulting in reduced testis size, sperm production and the theoretical maximum of sperm production. Additionally, we have been able to reduce reproductive performance by initiating more stringent feed restriction in males between 18-23 weeks of age, suggesting that the resources and endocrine changes required for normal testis function can be negatively and permanently affected around the time of photostimulation. The take-home message from this should be that critical points in development exist, and that stressing males during these periods can have a profound effect on the reproductive potential of those males. Further, careful management of males will include thoughtful consideration of issues above and beyond simply body weight. Ours, and many others', studies have shown that while roosters do not know their own body weight, their ability to perceive their condition can have a profound effect on reproductive performance. Our measurements are simple physiological measurements that do not even take into account the potential effects of altered condition on the behavioral component of sexual and other social activities of the commercial broiler breeder male.

**Epididymal Stones in Diverse Rooster Populations**

In addition to potential problems in the central regulation of male reproductive performance, we have identified (along with Dr. Janice Bahr and colleagues at the University of Illinois) an additional anomaly in males. We have found epididymal stones in males ranging from Delaware crosses, to pure-line leghorns and commercial type broiler breeder males. These calcified stones occur within the efferent ductules of the male’s epididymal region and may result in the complete blockage of sperm transit through the ductal system and dramatically reduced fertility. While we do not yet have a direct cause for this problem, we are looking at the interaction between nephrotactic viruses and this condition in commercial type males. Please see Janssen et al (2000) for more information.

**Acknowledgements**

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Additional Reading


Kirby, JD, CJ Tressler and YK Kirby (1998) Evaluation of the duration of sperm fertilizing ability in five lines of commercial broiler breeder and Delaware cross males. Poultry Science 77:1688-1694


Slides Presented
Arkansas's Populations (in millions)
- People: 2.53 (+2.5%)
- Horses: 0.16
- Swine: 0.85
- Cattle: 1.80
- Turkeys: 26.00
- Chickens: 1,400.00

Animal Models for Improving our Understanding of Fundamental Processes in Biology

Circadian Rhythms (aka: Biological Clocks)
Circadian rhythms are those processes/activities whose period (cycle) approach 24 hours and which persist under constant conditions.

In mammals tremendous progress has been made in our understanding of circadian rhythms due to the presence of a powerful and relatively obvious central pacemaker: the suprachiasmatic nucleus (SCN). In some birds, the SCN (if actually present) is at best a small, less highly developed/organized pacemaker for daily entrainment, suggesting multiple pacemakers. We have studied the relationships between body temperature, activity rhythms, and reproductive function in roosters and in collaboration with Dr. Wayne Bacon at OSU we have studied rhythms in turkey hens.

Body temperature rhythms in adult male fowl

Ovarian interactions with the circadian pacemakers? Are there interactions between daily rhythms of body temperature, locomotor activity and the ovulatory cycle in turkey hens?
Sexual Maturation in Males

- Transient neonatal hypothyroidism in rats
- Transient post-hatch PTU treatment in roosters
- Impaired (?) testis development in broiler chickens
- Pulsatile gonadotropin secretion in males

![Graph showing plasma concentration over time with data points indicating transient effects.](image)

![Graph showing effects of neonatal PTU treatment on pituitary function in adult male rats.](image)

![Graph showing the time required to produce a 2 Kg broiler.](image)
Plasma FSH concentration and testis weight in adult broiler breeder males

Relationship between body weight and testis weight in broiler breeder males at 28-40 WOA

Why study pubertal development in breeder males?

Seminiferous tubules of normal 'fertile' male (FSH >10 ng/ml) (A) and from an adult male with low (< 5ng/ml) mean circulating plasma FSH levels (B).

Unlike mammalian species of farm animals, we can't measure testis size in live birds without using surgery or an imaging technology. Ultrasound, while expensive, may provide useful information on the maturation sums of males.
What are the critical windows of male sexual development?

- 2-12 weeks is the normal period of Sertoli cell proliferation. As each Sertoli cell can support only a limited number of germ cells, it sets the theoretical limit on DSP.
- 18-25 weeks period of clonal germ cell proliferation and fine tuning of pituitary "gonadostat" (pituitary governor). Probably sets the pattern for long-term testis function.
Effect of SMZ treatment on pituitary and testis function in broiler breeder males

So where is the axis affected in "bad" males?

SMZ in Control Males at Photostimulation

Days

FSH (mIU/mL)

Control (TW=21.5g)
SMZ (TW=26.3g)

FSH mRNA

SC

Testis?

Pituitary?

Hypothalamic "time keeper"?

GnRH

GnRH

FSH

LH

Inhibin

Testosterone
Effect of jugular cannulation on plasma corticosterone levels in adult male fowl

Relationship between FSH and LH concentrations in the plasma of samples collected at 15 minute intervals from the jugular vein of a chronically cannulated adult male fowl.

Relationship between the episodic pattern of plasma LH and testosterone concentrations in a normal (mean FSH = 11 ng/ml) 40 week old breeder male with an average testis weight of greater than 25 grams per testis.
Are all avian sperm equal?

Sperm fertilizing ability in commercial breeder males

<table>
<thead>
<tr>
<th>MIGRATION</th>
<th>Pulse</th>
<th>Time Series</th>
<th>Mean ± S.D</th>
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<tbody>
<tr>
<td>LIF</td>
<td>Frequency</td>
<td>0.54 (0.05)*</td>
<td>0.63 (0.03)*</td>
</tr>
<tr>
<td></td>
<td>Amplitude</td>
<td>0.59 (0.10)*</td>
<td>0.58 (0.08)*</td>
</tr>
<tr>
<td>TSH</td>
<td>Frequency</td>
<td>0.23 (0.07)*</td>
<td>0.16 (0.07)*</td>
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<tr>
<td></td>
<td>Amplitude</td>
<td>2.57 (0.71)*</td>
<td>3.96 (1.50)*</td>
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<tr>
<td>TESTOSTERONE</td>
<td>Frequency</td>
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<td>0.22 (0.07)*</td>
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<tr>
<td></td>
<td>Amplitude</td>
<td>1.23 (0.32)*</td>
<td>0.27 (0.17)*</td>
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</table>

<table>
<thead>
<tr>
<th>Male Line</th>
<th>Total Eggs</th>
<th>Fertility (e)</th>
<th>Fertility (d)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.245</td>
<td>33.5 ± 2.3</td>
<td>43.2 ± 2.3</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>3.265</td>
<td>33.5 ± 2.3</td>
<td>43.2 ± 2.3</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>3.245</td>
<td>33.5 ± 2.3</td>
<td>43.2 ± 2.3</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>3.265</td>
<td>33.5 ± 2.3</td>
<td>43.2 ± 2.3</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>3.415</td>
<td>33.5 ± 2.3</td>
<td>43.2 ± 2.3</td>
<td>10.8 ± 0.3</td>
</tr>
</tbody>
</table>

Number of eggs: 30
We have recently identified a previously uncharacterized condition of calcium stones in the proximal efferent ducts of the male. These ducts perform essentially all of the functions typically associated with the epididymis of mammals. We have found stones in the ducts of males from birds on both coasts, the midwest as well as from Japan. Rates of occurrence range from 10-95%. Janssen et al. 1998.

Stones eventually result in the exfoliation of germinal cells from the seminiferous epithelium and reduced fertility following both natural mating and artificial insemination.

Stones start out as small fluid filled cysts, with age depending on strain.

Acknowledgements

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- U of A: David Kreider, Rick Rote and Doug Rhodes
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  - Technicians: Martha Rhodes and Janet Wasington
  - Undergraduate Students: James Bates, Brian Woodworth, Dietrich Thurrell, Cha Bright, Shadi Hall, Reema Perwal, and Jason Warren
Audience Questions and Answers for Kirby

Question: Can one use a naloxone challenge to evaluate breeding capacity?

Response: It works in mammals but does not work with birds.