SPERMATOGENESIS AND SPERM MATURATION IN MALE POULTRY: TARGETS FOR IMPROVING MALE REPRODUCTIVE PERFORMANCE

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Overview
Spermatogenesis and sperm maturation are complex processes which are affected by a myriad of endocrine, paracrine and neural factors. In birds, regulation of testicular and excurrent duct growth, maturation and function are only poorly understood. As the quality and quantity of sperm produced may be altered at any of several points during sexual maturation, spermatogenesis or during extragonadal sperm maturation, understanding these processes could have profound effects on male reproductive performance. In this paper, the effects of transient prepubertal hypothyroidism on adult testis size and sperm production, as well as the effects of the sperm degeneration allele on sperm function, will be discussed. These two models strongly suggest that male reproductive performance can be altered during development, positively by increasing sperm production via transient hypothyroidism and negatively by altering the pattern of excurrent duct differentiation and, ultimately, function. In the future, breeders may be able to exploit the genetic components of these traits to increase the reproductive capacity of breeder males.

Transient neonatal hypothyroidism
In the adult rat, testis size and sperm production can be

1The research summarized in this report was supported by grants from the PHS (R01-DK45821) and the USDA (91-37203-6890, 95-37203-2072), and the Arkansas Agricultural Experiment Station.
increased by 80 and 140%, respectively, following neonatal
treatment with the reversible goitrogen 6-n-propyl-2-thiouracil
(PTU) with no apparent effects on either circulating testosterone
levels or sperm fertilizing ability (1-3). These effects have been
shown to be due to hypothyroidism, as thyroid hormone replacement
abolishes the observed increases (1). The mechanisms by which PTU
induced hypothyroidism stimulate testis growth and adult sperm
production are unknown, however ultimately Sertoli cell numbers
are increased 1.57-fold (4-6). In the domestic fowl, little is known
of the temporal aspects or trophic stimulation of Sertoli cell
proliferation. However, as total sperm production is closely
correlated with the number of Sertoli cells present in the testis
(4,7,8), efforts to increase sperm production in the fowl's testis
may best be directed at increasing the number of Sertoli cells
present in the adult. Based on previous work in mammals (4, 9-11),
transient hypothyroidism could provide us with a useful tool for
studying the temporal and endocrine factors involved in the
regulation of Sertoli cell proliferation and ultimate cellular
composition of the fowl's testis.

The effects of neonatal PTU treatment on testis size and
sperm production in rodents are far greater than those achieved
using any alternative methodology (reviewed in 12,13). Previous
efforts to increase testis size or sperm production, including
juvenile hemicastration (14), immunization of males against
estradiol (15) or inhibin-α (16), have had only modest effects. In the
rat and boar, hemicastration must occur prior to postnatal days 15
and 56, respectively, for compensatory growth to occur (17,18).
Thus, it appears that hemicastration must occur during the period of
Sertoli cell proliferation in order to be effective (19). Interestingly,
unlike all of the previously described methodologies (14-16) the
effects of transient neonatal hypothyroidism on the testis are not
mediated by increased levels of circulating FSH, which are actually
depressed throughout a treated individual's life (20, 13). In order to
be effective, PTU treatment must commence after birth but prior to
postnatal day 11 in the rat (3,21). The effects of PTU treatment on
testis growth can be further augmented with exogenous FSH (21), a
treatment that mimics the many of the effects of hemicastration.
Taken together these studies suggest that there is only a narrow,
species specific, window of opportunity in which to positively alter
the cellular composition and spermatogenic capacity of the testis.
Investigators have studied the effects of thyroid hormone suppression on testicular function and reproductive performance in the domestic fowl with varied results (22-26). These early studies demonstrated in adults that chronic hypothyroidism resulted in reductions in spermatogenesis, ejaculate volume and sperm fertilizing ability. Furthermore, it was shown that prepubertal goitrogen treatment reduced testis growth to 8 weeks of age in the fowl, after which time precocious puberty was observed and the testes of treated birds grew rapidly to 16 weeks of age (25). Abnormal spermatid attachment was one reported side effect of hypothyroidism in the fowl(25), suggesting that either precocious puberty or thyroid hormone deficiency had some effect on spermatogenesis in treated males. However, none of these studies was based on treating prepubertal males with goitrogen for a very limited period of development and then allowing treated males to recover to euthyroidism and become sexually mature.

We have recently evaluated the efficacy of using transient prepubertal hypothyroidism to increase testis size and sperm production in the male fowl (27). In an early study male leghorn chicks were treated with PTU (.1% w/w of the diet) from hatch to 6 weeks of age with no apparent effects on adult testis size or sperm production. Based on the more recent observation that in order to be effective PTU treatment must occur during a defined period of development, e.g. corresponding to Sertoli cell proliferation, we investigated the effects of PTU treatment over a number of treatment intervals. As a result of a previous report (25), we also evaluated testicular morphology at time points during development. Our results demonstrated that transient prepubertal hypothyroidism, if induced at an appropriate stage of development, can lead to increases in adult testis size and sperm production in the domestic fowl (27).

Briefly, treatment of prepubertal male fowl with 0.1% PTU in the diet between 6 and 12 weeks of age resulted in a 96% increase in adult testis size, a 37% increase in spermatogenic efficiency (sperm/g testis), and a 115% increase in daily sperm production (27). While PTU treatment between 8 and 14 or 10 and 16 weeks of age resulted in approximately a 35% increase in testis weight, there was no apparent increase in spermatogenic efficiency and thus the increased testis weight observed in these males may be attributable to an increase in caloric intake (Mankar and Kirby, submitted). As in
rodents (3, 9-11, 21), there appears to be a limited window of effectiveness for PTU treatment in the domestic fowl. While the absolute period of Sertoli cell proliferation is unknown in the fowl (28,29), hemicastration is known to result in compensatory hypertrophy until 8 weeks of age (8). Thus, the period of maximal effectiveness of PTU treatment, 6 to 12 weeks of age, in the work reported here treatment encompasses this period.

Hypothyroidism induced with methimazole and the thiouracil compounds has been reported to result in precocious puberty in the fowl when administered from hatch to 16 weeks of age (25,26). In the present study, males treated with PTU between 6 to 12, 8 to 14 or 10 to 16 weeks of age all had elevated serum testosterone levels during the period of treatment. At 16 weeks of age males treated from 8 to 14 or 10 to 16 weeks of age also had pachytene and/or zygotene spermatocytes in their enlarged seminiferous tubules. As testosterone is required to maintain spermatogenesis (reviewed in 30,31), elevated serum testosterone levels in these groups may have led to accelerated seminiferous tubule maturation and a concomitant increase in the rate of survival of germ cells which have entered meiosis. It appears that the period of cellular proliferation in the fowl's testis may be influenced by a number of external factors, as treatment with sulfamethazine (32) or treatment with tamoxifen (33) results in the production of viable spermatozoa by 9 weeks of age. However, in the case of both Sulfamethazine and tamoxifen, adult testicular size and sperm production appear to fall within the range observed in non-treated animals (32,33).

The induction of precocious puberty in prepubertal male fowl treated with PTU is in contrast to the effects observed in most mammals. In rodents, early hypothyroidism is inhibitory to testicular development during the treatment period (34,35), with the stimulatory effects of transient hypothyroidism on gonadal function not apparent until adulthood (1,2). In the rat, transient neonatal hypothyroidism suppresses the pubertal increases in serum levels of FSH and LH and delays the pubertal rise in serum testosterone levels by 10-20 days (20). In both the rat and the golden hamster, neonatal PTU treatment results in a permanent suppression of serum gonadotropin levels in the presence of normal circulating testosterone levels (9,13,20). In the fowl, prepubertal hypothyroidism induced between 6 to 12, 8 to 14, or 10 to 16 weeks of age results in an elevation of serum testosterone levels, which is
in sharp contrast to the effects observed in rodents. Interestingly, precocious puberty is frequently observed in young male humans during prepubertal hypothyroidism, however the effects of hypothyroidism on testicular development and on secondary sexual attributes can be ameliorated by a return to euthyroidism (reviewed in 36).

In summary, transient prepubertal PTU treatment can result in increases in adult testis size and sperm production in the fowl. The effective treatment window appears to be between 6 and 12 weeks of age. While the precise mechanisms of testicular hypertrophy remain unknown, there are several similarities in treatment effects on the fowl's testis to those seen in rodents. First, the effective treatment window is of relatively short duration in both groups, with PTU treatment commencing early or late having little, if any, effect on adult testicular attributes. Second, when effective, transient prepubertal PTU treatment results in both an increase in adult testis size and spermatogenic efficiency (sperm produced per g of testis). Finally, PTU treatment resulting in enhanced testis size and sperm production does not result in elevated serum testosterone levels in the adult. However, unlike the rodent, transient prepubertal PTU treatment can result in precocious puberty in the male fowl. The observed increases in serum testosterone levels during treatment, coupled with the appearance of pachytene and/or zygotene spermatocytes and the effects of the later treatment intervals on the organization of the adult seminiferous tubule are in contrast to the effects observed in rodents. Taken together, these results suggest that PTU treatment may be working via distinctly different mechanisms in rodents and the domestic fowl. Transient PTU treatment may prove to be a valuable tool for studying the mechanisms involved in regulating testicular maturation and function in the domestic fowl. From a practical perspective, while the cost of PTU is prohibitive, a derivative of the successful treatment protocol may prove useful for increasing the reproductive efficiency of avian species, such as the domestic turkey where reproduction is primarily accomplished by artificial insemination and viable sperm numbers may be limiting.

Heritable sperm degeneration in the domestic fowl

Froman and Bernier (37) described a unique, heritable,
reproductive disorder in the Delaware rooster. Affected males were characterized by a high proportion of dead sperm in their ejaculates, which resulted in poor overall fertility. Dead sperm were found in the ductus deferens, with the first significant numbers of dead sperm found in the mid-ductus deferens; with increasing proportions of dead cells in the caudal regions of the ductus deferens and the highest proportion of dead cells were found in the receptaculum (37). Furthermore, when daily semen collection was conducted over a week to ten day interval, the proportion of dead sperm in the ejaculates of affected males was reduced to that observed in normal males. These data suggested that the observed phenomenon was due to some dysfunction during extragonadal sperm maturation or storage, but did not completely rule out a defect in the seminiferous epithelium.

Kirby and coworkers (38) evaluated the effects of the duration of sperm storage in the female on sperm survivability by using sperm competition assays. In these trials, equal numbers of live sperm from affected males and from normal brown leghorn males were mixed and used to inseminate New Hampshire females. Following a single insemination, eggs were collected for 2 weeks, incubated and the proportion of chicks sired by the affected males determined by chick color. Following both intravaginal and intramagnal inseminations, the proportion of chicks sired by the affected males declined rapidly over the period of egg collection. These results suggested that sperm continued to die even after being removed from the male and placed within the female (38).

Subsequent studies have shown that the trait is not due to an autoimmune condition (39), aberrant sperm metabolism (40) nor is it due simply to inbreeding (41). In one of these studies (41), Delaware males were mated to Single Comb White Leghorn females and the crossbred F\textsubscript{1} male progeny evaluated. These males possessed the same proportion of dead sperm as their purebred sires and were used to found the subsequent generations of males possessing the sperm degeneration allele. After eight generations of crossing, once to New Hampshire hens and all subsequent crosses with SCWL females, it appears that this trait is due to a single, dominant, gene. Furthermore, it was clearly demonstrated that this trait is not due to the same sperm dysfunction as is caused by homozygosity for the rose comb allele, as the effects of these two defects have been shown to be distinct and cumulative in the male fowl (42). Current
efforts are directed at identifying the gene associated with this trait through the use of contemporary molecular genetic and mapping techniques.

In an earlier study (39), it was noticed that the normally carefully and uniformly coiled ductus deferens appeared to be irregularly formed in males affected with the sperm degeneration allele. A subsequent histomorphometric analysis of the entire extratesticular ductal system (rete testis; connecting ducts; efferent ducts; epididymal duct; and ductus deferens) of normal and affected males was completed (41). This study revealed that the proximal efferent ducts of affected males were malformed and were much less highly folded than seen in controls. In affected males there was more than a doubling of luminal area and a 40% decrease in the luminal periphery. As the proximal efferent ducts are the site of fluid absorption (they arise from the mesonephric duct), extracting more than 90% of the water present in seminiferous tubule fluid, they play an important role in the concentration of seminal fluid (43). Furthermore, as the epithelium of the efferent ducts is the most complex to be found in the excurrent ducts, it also appears to be the principal site of protein secretion (44-46). It should also be noted that proteins associated with sperm in the excurrent ducts appear to be important for sperm survival within the hen's oviduct (47-49), thus the apparent reduction in efferent duct capacity may result in suboptimal exposure to essential proteins.

The research reported to date has strongly suggested that the defect in affected males resides in the excurrent ducts. However, Froman and Bernier (37) clearly demonstrated that the sperm found in the excurrent ducts of affected males were highly vesiculated, with aberrantly formed axonemes and mitochondria. The question persisted as to whether an additional defect, in addition to the observed defect in the efferent ducts, could be identified in the seminiferous epithelium. Such an abnormality could result in imperfectly formed, perhaps weakened, sperm that are more susceptible to the less than optimal excurrent duct environment that had been proposed. Recently we conducted an electron and light microscopic evaluation of the seminiferous epithelium of normal and affected roosters. After considerable evaluation, we were unable to identify any apparent defect in the seminiferous epithelium or in the ultrastructure of sperm in affected males.

Taken together, the studies involving males with the sperm
degeneration allele strongly suggest that proper excurrent duct function is required for normal sperm survival in the fowl. Sperm that are not exposed to an appropriate environment in the excurrent ducts appear to have a reduced life-span both within the male's excurrent ducts as well as within the oviduct. As the excurrent ducts are regulated by a number of different genes, the identification of those genes/gene products associated with sperm maturation could provide breeders with markers for enhancing the survivability and fertilizing ability of fowl sperm.

ACKNOWLEDGEMENTS
The author thanks Dr. David Froman and Ms. Maithili Mankar for their tremendous contributions to the research contained in this review, Ms. Marsha Rhoads for superb technical assistance and Cheryl Looper for assistance with animal care. The author is truly appreciative of the donation of breeder male chicks by Peterson Industries, Decatur, AR.

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Question: R. Reddy

Have you seen other methods other than PTU treatment for increasing testis size or inducing precocious puberty in roosters?

Answer: J. Kirby

I have not seen any other pharmacological method for increasing testis size in the fowl, however we have observed that interval feeding of male breeders, that is releasing them from feed restriction during critical periods of prepubertal development, appears to enhance adult testis size and sperm production. Dr. Wayne Kuenzel at the University of Maryland has recently demonstrated that precocious sperm production can be induced in male fowl through the feeding of sulfamethazine.

Question: I. Pevzner

Have you ever observed the sperm degeneration allele or anything like it in commercial strains of broiler breeder males?

Answer: J. Kirby

We have evaluated the seminal attributes of a number of commercial broiler breeder males. We, as have others, have found that the semen quality of broiler breeder males is not as good, perhaps, as we see in commercial leghorns or in dual purpose breeds. From several commercial lines we have selected males that consistently produce less than 90% intact sperm and have mated them to SCWL females and the resulting crossbred males were evaluated. In each of the lines tested to date, some males have sired crossbred offspring that produce ejaculates containing a high proportion (>25%) dead sperm. However, I must caveat this response by saying that until we have a PCR based test to identify the presence or absence of the allele, we really don't know if these birds have the same defect. Dr. Doug Rhoads, Dr. Butch Sizemore, and myself are collaborating on a project to develop such a PCR based test.