Gonadal Development and Growth of Chickens and Turkeys Hatched from Eggs Injected with an Aromatase Inhibitor[1]

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ABSTRACT It was the purpose of these experiments to describe gonadal development and posthatching growth of genetic female chickens and turkeys following *in ovo* injection of the aromatase inhibitor Fadrazole® (CGS 16949A) prior to incubation. *In ovo* injection of Fadrazole® (CGS 16949A) resulted in the development of testes-like gonads in the majority of day-old genetic female chickens and turkey poults. Ninety-eight to 99% of these birds have masculine-type male genitalia at 1 d of age. Microscopic examination of the gonads of day-old genetic female chicks hatched from Fadrazole®-treated eggs showed the presence of atypical seminiferous tubules in 3 of 18 individuals and the presence of ovarian follicles in 3 of 18 individuals. No germinal elements were seen in 12 individuals. The gonads in the majority (8/11) of day-old female poults from treated eggs showed the presence of atypical seminiferous tubules. Three of 11 individuals had structures characterized as disorganized or degenerate follicles. Between the day of hatch and 6 wk, gonads in an increasing proportion of female chickens from Fadrazole®-treated eggs had normal appearing ovarian follicles. A similar trend was seen in the female turkeys between hatch and 12 wk of age. There were no differences in BW of female chickens hatched from Fadrazole®-treated eggs and those from control eggs between the day of hatch and 6 wk of age. The pectoral muscle mass and fat pad weights of these birds did not differ. In one experiment, the BW of female turkeys hatched from Fadrazole®-treated eggs was significantly greater than that of controls and equal to that of males at 3 and 6 wk of age. Thereafter, both types of females were of equal weight and significantly lighter than males. Fadrazole® treatment did not affect pectoral muscle mass of either sex of turkeys.

(*Key words*: aromatase inhibitor, gonad development, growth, chicken, turkey)

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INTRODUCTION

The phenomenon of sex-reversal of genetic female birds into phenotypic males has been recognized since ancient times. In modern times, spontaneous sex reversal of mature female chickens, presumably as a result of disease-induced ovarian degeneration, has been well described (Crew, 1923; Fell, 1923). Various types of polyploidy have also been associated with spontaneous sex reversal in commercial lines of Leghorn chickens (Abdel-Hameed and Shoffner, 1971) and in a line of chickens selected for a higher incidence of triploidy (Thorne *et al.*, 1988; Lin *et al.*, 1995). Sinistral ovariectomy in young chicks (Domm, 1929a) or in chickens of advanced age (Domm, 1927, 1929b) resulted in hypertrophy of the right gonad into a testes like structure. Reference to many early studies on experimentally induced sex reversal together with a detailed review of his own experiments can be found in the book by Masui (1967). Sex reversal has also been produced by implantation of testes from 13-d-old chick embryos into the extraembryonic coelom of 3- to 4-d-old genetic female embryos. Many recipient females develop testes or ovotestes (Stoll *et al.*, 1980; Maraud *et al.*, 1986). More recently, testicular development in genetic female chicks has been produced by interference with estradiol synthesis following *in ovo* injection of an aromatase enzyme inhibitor. Some such individuals develop secondary sex characteristics typical of males and produce spermatozoa (Elbrecht and Smith, 1992; Abinawanto *et al.*, 1997).

The plasma sex hormone milieu of male and female chicken embryos differs beginning as early as 7.5 d of incubation. Testosterone levels are higher in male embryos (Woods *et al.*, 1975), whereas estradiol levels are higher in females (Woods and Brazzill, 1981). Comparable information is not available for turkeys. Whether these differences play a role in the posthatch sex differences in BW and muscular characteristics of these two species is unknown. Limited indirect informa-

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tion, based on altering the levels or interfering with the actions of the sex hormones, is available. *In ovo* administration of estradiol transiently suppressed the early posthatch growth of female chickens and turkeys (Freeman and Burke, 1986). Although this treatment caused feminization of the gonads of genotypic male chickens and, at high doses, altered sexual development of both sexes, it did not affect growth of either sex (Etches and Kagami, 1997). Administration of testosterone *in ovo* suppresses bursal development, resulting in immunoincompetence. Consequently, this experimental approach cannot be used to study the potential impact of androgens on posthatch growth. The possible role of embryonic androgens on posthatch growth has been investigated by *in ovo* administration of an androgen receptor blocking agent (Burke, 1996). Such treatment slightly, but significantly, suppressed growth of male broiler chickens but was without effect on females.

Growth characteristics of genetic females in which sex reversal has been induced by any of the aforementioned techniques have not been well described. It was of interest to determine the posthatching growth pattern of chickens hatched from eggs injected with Fadrazole®, a nonsteroidal aromatase inhibitor. Furthermore, we wished to determine whether *in ovo* injection of this compound would alter gonadal development and growth of female turkeys.

MATERIALS AND METHODS

Egg Injection

Fadrazole® (CGS 16949A) was obtained as a gift from A. S. Bhatnagar, Ciba-Geigy, Basel, Switzerland. The compound was dissolved in sterile 0.15 m NaCl; diluted with sterile saline so that the desired dose was contained in a 50 μL volume, and then filtered through a sterile 0.2 μm filter. Air cells were located by candling the eggs. The shell surface was wiped with 70% ethanol and a hole was drilled over the air cell. Injections were made into the albumen just under the air cell using 25-gauge needles cut to 5 to 6 mm. The holes were sealed with glue. In Experiments 1 to 4, injections were done prior to incubation. In Experiment 5, eggs were injected before incubation and again on Day 8 of incubation.

Eggs

Chicken eggs were obtained from a line of Arbor Acres[3] hens carrying the sex-linked gene for slow feathering. Hens were artificially inseminated with semen from Ross[4] strain broiler breeder males. Turkey eggs were obtained from Nicholas[5] strain hens inseminated with semen from the same strain male. Eggs were incubated at 36.7 C at a relative humidity of 53 to 55% until late in incubation when the temperature was reduced to 36 C and the relative humidity was increased to 70%. Eggs were candled at 10 d of incubation to remove those that were infertile or contained dead embryos.

Sex Determination

In the first experiment, 1-d-old chicks were sexed using the sex-linked slow feathering trait. They were then killed by CO_2 inhalation and opened for examination of the gonads. At that time a sample of blood was collected. Deoxyribonucleic acid was extracted from the blood and genetic sex of each individual was determined using the DNA hybridization technique of Kodamma *et al.* (1987) as described by Mitchell and Burke (1995). Prehybridization was carried out at 62 C for 30 min followed by hybridization at 64 C for 4 h. In the subsequent two experiments with chickens, genetic sex was determined using the sex-linked slow feathering trait. In Experiment 3, phenotypic sex was also determined by examination of the genitalia by a commercial chick sexer.

Genetic sex of turkeys was similarly determined by DNA hybridization using the plasmid identified as pUMG0401 (Saitoh *et al.*, 1989), which contained a 0.4 Kb W chromosome specific turkey DNA sequence. This plasmid was provided as a gift from Professor Shigeki Mizuno (Tohoku University, Sendai, Japan). Prehybridizations of turkey samples were done at 53 C for 30 min followed by hybridization at 55 C for 4 h. Phenotypic sex was determined by examination of the genitalia by a commercial sexer.

In untreated 1-d-old chicks and poults there is a clearly distinct difference in the gross gonadal morphology of males and females. These differences have been described recently for turkey embryos (Burke, 1994) and this description, aside for size differences with age, essentially applies to posthatch neonates and adolescents. The differences between male and female are based on the number of gonads (paired or single left), the shape of the gonads, the color of the gonads and their surface appearance or texture. All of these criteria were used to evaluate gonadal normality and deviations from normality in these studies. Evaluations of the relative size of the gonads in birds hatched from Fadrazole®-injected eggs were based on a subjective comparison with age-matched controls.

Experiment 1

Experiment 1 was conducted to determine the effects of *in ovo* injection of Fadrazole® on gonadal development at the time of hatch. Two hundred forty eggs were randomly sorted in four groups of 60 eggs each. Eggs in one group received an injection of sterile saline whereas eggs in the other groups received 50, 150, or 450 μg of Fadrazole®. Upon hatching, the genetic sex of the chicks was determined using the sex linked slow feathering trait (North and Bell, 1990). Chicks were killed by CO_2

[3]Arbor Acres Farm, Inc., Glastonbury, CT 06033.
[4]Ross Breeders, Inc., Elkmont, AL 34520.
[5]Nicholas Turkey Farms, Sonoma, CA 95476.

inhalation. The yolk sacs were removed and the chicks were weighed. A sample of blood was collected from a mesenteric vein and stored frozen for future use in sex determination by DNA hybridization. The gonads were examined. If paired testes were present the gonadal sex was designated male. If a single left ovary was present the gonadal sex was designated female. In some individuals a left ovary was present together with a right gonad. The right gonad sometimes had the appearance of a small ovary, sometimes the appearance of a testes, and sometimes it appeared to have both ovarian and testicular tissue. Such individuals were designated as atypical.

Experiment 2

Experiment 2 was conducted to determine the effect of *in ovo* injection of Fadrazole® on posthatch growth of genetic female and male broilers. Three hundred and sixty chicken eggs were randomly sorted into three groups of 120 eggs each. Eggs in one group received an injection of sterile saline, eggs in the second group received 150 μg of Fadrazole®, and those in the third group received 450 μg of Fadrazole®. Injections were made prior to incubation. Upon hatching, the chicks were wingbanded and weighed. Sixty-eight chicks were obtained from saline-injected eggs, 73 from those injected with 150 μg of Fadrazole®, and 83 from those injected with 450 μg of the compound. Nineteen chicks from each treatment were randomly taken and were killed by CO_2 inhalation. Their gonads were examined and classified as in Experiment 1.

Six to eight chicks from each of the treatment groups were placed into each of eight pens. Chicks were brooded and reared according to procedures described earlier (Burke, 1996). Corn-soybean rations formulated to meet the NRC (1994) recommendations were provided for *ad libitum* consumption. A starter ration containing 3,100 kcal ME/kg, 23% CP was fed until 21 d of age. From 3 to 6 wk a ration containing 3,200 kcal ME/kg and 21% CP was used. The birds were individually weighed on the day of hatch and at 7, 14, 28, and 42 d of age. Combs were examined at 42 d and the degree of development was rated by a subjective score. A score of 3 was assigned to combs that were obviously enlarged and reddened. Combs that were small, undeveloped, and pale were given a score of 1 and a score of 2 was given to combs with intermediate development. On Day 43, the birds were killed by CO_2 asphyxiation. The gonads were examined and classified as in Experiment 1. The left *Pectoralis superficialis* muscle and the abdominal fat pad were removed and weighed.

Experiment 3

This experiment was conducted to describe gross and microscopic changes in the gonads of growing chickens that hatched from Fadrazole®-treated eggs. One hundred and two eggs were injected with sterile saline, 180 with 150 μg of Fadrazole®, and 180 with 450 μg of Fadrazole®. Injections were made prior to incubation. Upon hatch the chicks were wing-banded. All chicks were placed in a single 2.43 × 3.65 m pen and brooded as described earlier. The feeding program was as described for Experiment 2. Five genetic males from each treatment group and four or five genetic females from the control group were killed on the day of hatch and at 11, 21, 32, and 42 d of age. Twelve genetic females hatched from eggs injected with 150 μg of Fadrazole® and 12 from eggs injected with 450 μg of Fadrazole® were killed at these same times. All birds were individually weighed on the day of hatch and all survivors were individually weighed on each of the sampling days. Visual appearance of gonads of all sampled birds were described as in Experiment 1 and the gonads were then placed into 10% phosphate-buffered formalin. After fixation, pieces of gonad were processed in paraffin for histological examination. Several 3-μm thick sections from control females, all males, and all females hatched from eggs injected with the low dose of Fadrazole® were stained with hematoxylin and eosin and examined. In addition, multiple sections taken at 30-μm intervals throughout the gonads were taken from females hatched from eggs injected with 450 μg of Fadrazole®.

Experiment 4

Two hundred twenty-five turkey eggs were randomly sorted into three groups. Eggs in one group received an injection of saline, eggs in a second group were injected with 450 μg of Fadrazole®, and those in the third group received 600 μg of Fadrazole®. Injections were made prior to incubation following the procedures described earlier. Upon hatching, poults were wing-banded and phenotypic sex was determined by genital examination. The poults were placed in a single 3.0 × 11.0 m pen with pine shavings litter. Heat was provided by pancake brooders for the first 2 wk and by a room furnace thereafter. The turkeys were fed corn-soybean rations formulated to meet NRC (1994) requirements. The rations fed from 1 to 4, 4 to 8, 8 to 12, and 12 to 15 wk contained 2,881 kcal ME/kg, 28% CP; 2,953 kcal ME/kg and 26% CP; 3,078 kcal ME/kg and 22% CP; and 3,202 kcal ME/kg and 19% CP, respectively. Poults were individually weighed on the day of hatch and at 3, 6, 9, 12, and 15 wk of age. At 3 wk a blood sample was taken by venipuncture to provide DNA for determination of the genetic sex.

At 15 wk of age the gonads of 22 control birds, 27 birds hatched from eggs injected with 450 μg of Fadrazole®, and 26 birds from the higher Fadrazole® dose were visually examined. The left *P. major* muscle of these birds was removed and weighed.

Experiment 5

This experiment was designed to describe changes in the gonads of growing turkeys that were hatched from eggs injected with Fadrazole®. Control groups received an injection of sterile saline before incubation or on that day and Day 8 of incubation. One group received 600 μg of Fadrazole® prior to incubation and one group received

TABLE 1. Gonadal morphology of day-old genetically male and genetically female chicks hatched from eggs injected with Fadrazole® prior to incubation, Experiment 1

Fadrazole® dose	Genetic males			Genetic females			
	Paired testes	Ovary	Sex reversed	Paired testes	Right testis	Ovary	Sex reversed
(μg/egg)	(no.)		(%)	(no.)			(%)
0	19	0	0.0	0	0	15	0
50	19	0	0.0	10	1	8	58
150	15	0	0.0	30	1	1	97
450	14	0	0.0	21	1	1	96

the same dose prior to incubation and again on Day 8 of incubation.

Poults were wing-banded and weighed on the day of hatch and phenotypic sex was determined by a commercial sexer. Ten to 12 birds from each treatment group were killed on the day of hatch. Their gonads were examined and placed in 10% formalin. Blood was collected for sex determination by DNA analysis. Remaining birds were placed in a single 3.0 × 11.0 m pen. They were brooded and fed as described in Experiment 4. At 12 d of age all remaining birds were bled by venipuncture to provide DNA for determination of their genetic sex. At 3, 6, and 9 wk of age four to six genetic females and four to six genetic males per treatment group were killed by cervical dislocation. At 12 wk all remaining birds were killed. Individual BW of all of the turkeys were obtained at each of these ages. Gonads were examined visually and described when the birds were killed. The gonads were placed in 10% formalin and processed as described earlier.

RESULTS

Experiment 1

Sex determined by the two techniques differed in 3 of 34 chickens hatched from saline-injected eggs and in 4 of 119 chickens from eggs injected with Fadrazole®. Three chicks sexed as females by the feather sexing technique were determined to be genetic males and four chicks feather sexed as males were determined to be genetic females. The overall discrepancy between sex as determined by the two methods was 4.57%. Testicular appearing gonads were present in 58, 97, and 96% of the genetic females hatched from eggs injected with 50, 150, or 450 μg of Fadrazole®, respectively (Table 1). One genetic female in each of these treatments had both a left ovary and a small right gonad characterized as a testis. Many of the paired testes present in genetic females appeared smaller than testes of genetic males. In some cases only the right testis was found to be smaller than normal. Testes of genetic males in all treatment groups appeared normal.

Experiment 2

After determination of the genetic sex by feather examination, 19 birds from each treatment were killed to determine their gonadal appearance. All genetic males in the three treatment groups had paired testes. Nine control chicks were feather sexed as females. Two of these had gonads classified as testes. Ten chicks hatched from eggs injected with 150 μg of Fadrazole® and 10 hatched from eggs injected with 450 μg of the drug were feather sexed as female. Only two of these birds in each treatment had a single left ovary. Seven in each group had paired testes and one in each group had paired gonads that were neither clearly testicular or clearly ovarian in appearance.

At 43 d there were 19 genetic males and 26 genetic females in the control treatment. One bird sexed as a genetic male was found to have an ovary and a small undeveloped comb. One genetic female was found to have two testes and a well developed masculinized comb. In all other control birds the genetic sex, as determined by the sex-linked slow feathering trait, was compatible with

TABLE 2. Posthatching body weight[1] ($\bar{x}$ ± SE) of broiler chickens hatched from eggs injected with Fadrazole® prior to incubation, Experiment 2

	Fadrazole® dose						Probability values		
	0 μg per egg		150 μg per egg		450 μg per egg				
Age	Male	Female	Male	Female	Male	Female	Treatment	Sex	Treatment × Sex
(d)	(g)								
0	50 ± 1	49 ± 1	48 ± 1	48 ± 1	47 ± 1	48 ± 1	0.05	0.72	0.62
7	153 ± 5	150 ± 4	150 ± 5	146 ± 3	148 ± 4	146 ± 4	0.46	0.44	0.98
14	410 ± 11	391 ± 7	398 ± 15	389 ± 8	396 ± 11	380 ± 7	0.41	0.07	0.87
28	1,334 ± 22	1,161 ± 23	1,273 ± 35	1,159 ± 23	1,290 ± 23	1,130 ± 19	0.28	0.0001	0.46
42	2,440 ± 43	2,063 ± 43	2,364 ± 48	2,091 ± 52	2,368 ± 41	2,021 ± 38	0.45	0.0001	0.51

[1]The number of observations per mean ranged from 18 to 31.

TABLE 3. *Pectoralis superficialis* and abdominal fat pad weights[1] ($\bar{x}$ ± SE) of 43-d-old broiler chickens hatched from eggs injected with Fadrazole® prior to incubation, Experiment 2

	Fadrazole® dose						Probability values		
	0 μg per egg		150 μg per egg		450 μg per egg				
Variable	Male	Female	Male	Female	Male	Female	Treatment	Sex	Treatment × Sex
P. superficialis, g	139 ± 3	123 ± 3	133 ± 3	118 ± 3	137 ± 4	113 ± 2	0.11	0.0001	0.27
P. superficialis, % BW	5.8 ± 0.1	5.9 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	0.12	0.89	0.16
Fad pad, g	38 ± 1	39 ± 2	37 ± 4	38 ± 2	36 ± 2	37 ± 2	0.65	0.57	0.99
Fad pad, % BW	1.6 ± 0.1	1.9 ± 0.1	1.6 ± 0.2	1.8 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	0.87	0.0001	0.91

[1]The number of observations per mean ranged from 18 to 29.

gonadal morphology. Twenty genetic males and 28 genetic females remained in the group hatched from eggs injected with 150 μg of Fadrazole®. All of the genetic males had testes. Nineteen of the genetic females had a single left ovary; six had a left ovary and a right gonad described as a small to medium sized ovary. Three genetic females had paired testes and enlarged combs. Twenty-six genetic males and 29 genetic females from eggs injected with 450 μg of Fadrazole® remained at 43 d of age. Twenty-five of the males had paired testes and enlarged combs. One had an ovary and small comb. Eight of the genetic females had a single left ovary and 19 had both a left ovary and small to medium sized right ovary. Two genetic females had paired testes and enlarged combs.

There was a slight, but significant ($P = 0.05$), dose related decrease in BW of 1-d-old chicks in response to Fadrazole® injection (Table 2). No significant treatment related differences in BW were detected at 7, 14, 28, or 42 d of age. A sex difference in BW was first noted at 14 d age ($P = 0.07$), which was highly significant ($P \leq 0.0001$) at 28 and 42 d.

In ovo injection of Fadrazole® did not affect the absolute weight of the left *P. superficialis* or its weight when expressed as a percentage of live BW (Table 3). Muscle weight of males was greater than that of females ($P \leq 0.0001$). Abdominal fat pad weight was not affected by Fadrazole® treatment. The percentage fat pad in females was greater than that of males ($P \leq 0.0001$).

Experiment 3

All genetic females hatched from solvent-injected eggs had external genitalia classified as female by a professional sexer and all genetic males in the control group had masculine type genitalia. Only one of 63 genetic females hatched from eggs injected with 150 μg of Fadrazole® had genitalia characterized as female-like by a professional sexer. Two of 61 genetic females from eggs injected with 450 μg of Fadrazole® were so characterized. All other genetic females in these two groups were classified as males based on appearance of their genitalia.

TABLE 4. Classification of gonads based on their visual appearance in genetic female broiler chickens hatched from eggs injected with Fadrazole® prior to incubation, Experiment 3

Day	Fadrazole® dose	Left ovary no right gonad	Left ovary right gonad	Two ovaries	Two gonads testicular in shape but not in appearance	Single right testis	Two testes	No identifiable gonads
	(μg/egg)							
0	0	5						
	150		1			1	5	5
	450					1	7	4
11	0	5						
	150	1	4		7			
	450		2	2	8			
21	0	5						
	150	1	2		8			
	450		3		9			
32	0	4						
	150	1			10			
	450		2[1]		10			
42	0	4						
	150				12			
	450	1	3[2]		6			

[1]One individual had a fluid filled left ovary and a small right ovary.

[2]Both gonads were ovarian shaped and had the appearance of ovaries. In one case the left was about three times as large as the right; in one case the right was about three times as large as the left; and in one case both were small and of equal size.

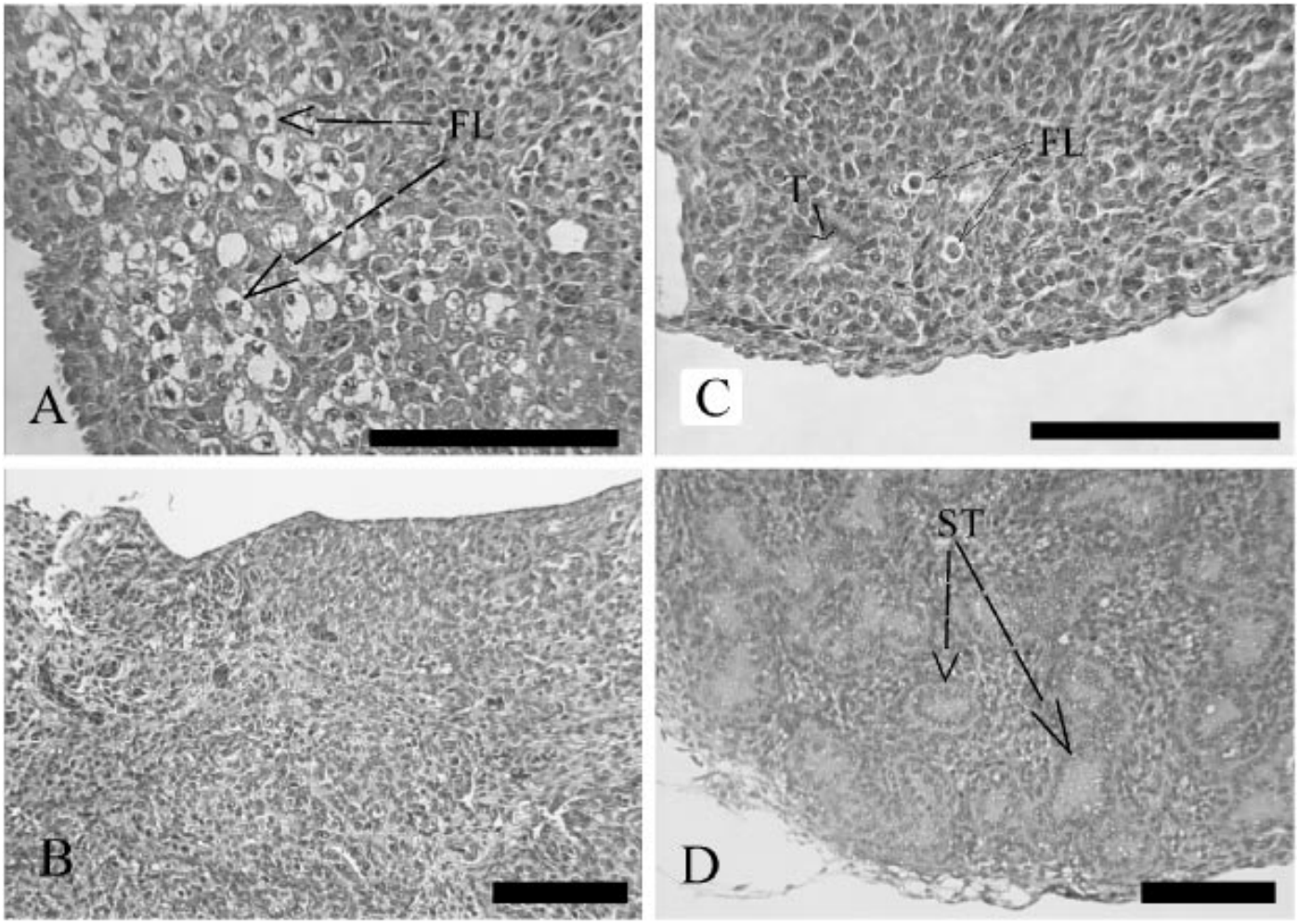

FIGURE 1. Sections of gonads from 1-d-old chicks. FL = follicles; T = tubule; ST = seminiferous tubule. Space bar = 100 μm. A. Control female with many follicles in the cortical region. The dense region to the right of the FL label is medullary tissue. B. Gonad from a genetic female hatched from an egg injected with 450 μg of Fadrazole®. No follicles or tubular structures were seen in any region of this gonad. C. Gonad from a genetic female hatched from an egg injected with 450 μg of Fadrazole®. Two structures which appeared to be follicles and a tubular structure are visible. D. Testis from a control male. Seminiferous tubules, separated by large areas of interstitial tissue are present. Compare the relative proportions of tubular and interstitial tissue in this section and in the day-old male turkey (Figure 4A).

All control females of all ages had a single left ovary and no visible right gonad. All control males and all males hatched from Fadrazole®-injected eggs had paired testes. The appearance of gonads in genetic females hatched from eggs injected with Fadrazole® is summarized in Table 4. On the day of hatch, a high proportion of the females had paired gonads that appeared to be normal testes and several had a single right gonad that appeared to be a testis. Another high proportion did not have identifiable gonads. Only one individual had a left ovary and she also had a tubular shaped right gonad that appeared testicular.

Throughout the rest of the time course of the study, the majority of genetic females hatched from Fadrazole®-treated eggs had two gonads that had the shape of testes but did not have their characteristically pearly white color and smooth surface. Rather these structures were flesh colored and had a granular surface texture similar to that of immature ovaries. A small percentage of these females had a left gonad characterized as an ovary and another structure that did not have the appearance of an ovary or a testes. One individual at each age had a normal looking left ovary with no right gonad and two 11-d-old individuals had two gonads that appeared to be ovaries.

Histological sections of ovaries of 1-d-old control chickens show a characteristic structure. The cortex and medulla are often clearly delineated (Figure 1A), but in some individuals there is no marked separation of these two regions. Clear circular structures with a diameter of 12.1 ± 1.7 μm ($\bar{x} \pm SD$) and a range in diameter of 9.1 to 17.0 μm were present in the cortical region. Generally these structures were devoid of cytoplasm but a nucleus was present in the center of the vacuolar circle. In some cases islands of pink amorphous material were present within the clear circles. In some cases these clear circular structures appeared to form a layer beneath the germinal epithelium and in other cases they appeared to extend as cords toward the medulla. In the best preserved regions a single layer of squamous cells appeared to form the outer

boundary of the circles. These structures were present in 1-d-old control females and in three of the 18-d-old females hatched from Fadrazole®-treated eggs. Based on their location in the ovary and on the fact that they were not seen in males these structures were classified as follicles.

Microscopic characteristics of gonads of 1-d-old genetic females hatched from Fadrazole®-treated eggs are summarized in Table 5. The most striking characteristic was the absence of ovarian follicles or seminiferous tubules (Figure 1B) in gonads of 13 out of 18 individuals examined. Several follicles and a single tubule were seen in the gonad of one female whose gonad was otherwise devoid of identifiable germinal elements (Figure 1C). Ovarian follicles were present in the gonads of three birds hatched from eggs injected with the lower dose of Fadrazole®. The gonads of one genetic female from each of the Fadrazole®-treated groups had a tubular appearance. The tubules, however, were less developed than those of males (Figure 1D).

At 11 d of age, gonads from five genetic females from Fadrazole®-treated eggs had a sparse number of follicles, those from 16 genetic females had neither seminiferous tubules or follicles, and those from three genetic females had structures that were termed atypical seminiferous tubules. Characterization of an abnormal or atypical structure as a variant of a normal anatomical structure is, of course, speculative. Follicles typical of those 11-d-old genetic females are present in Figure 2A. Seminiferous tubules typical of 11- and 21-d-old genetic male control chicks are present in Figures 2E and 2F, respectively. The structures characterized as atypical seminiferous tubules were much greater in diameter than normal with very loose multilayered cellular arrangement within the tubule (Figures 2B and 2D). Clear spaces were present between the cells. In contrast, at this age, normal seminiferous tubules consist of a single layer of tightly packed cells with no luminal space (Figure 2E). Several structures in the gonad of an 11-d-old genetic female from a Fadrazole®-injected egg (Figure 2C) were similar to seminiferous tubules in 1-d-old control males (Figure 1D). With increasing age, a larger proportion of the females from Fadrazole®-treated eggs showed the presence of sparse ovarian follicles (Figure 3C) or the larger number of follicles generally found in ovaries of control females (Figure 3F). The structure of these follicles appeared identical to that of control genetic females (Figure 3A). Seminiferous tubules typical of control males are present in Figure 3D. Atypical seminiferous tubules were noted in some individuals up to 42 d of age (Figure 3E). Normal appearing seminiferous tubules were seen in only two individuals from treated eggs and these being found in 32-d-old birds (Figure 3B).

As in the previous experiment there were no treatment effects on BW (Table 6). Significant sex differences in weight were present at 11 d and beyond. No treatment times sex interactions were noted.

Experiment 4

Forty-nine poults were hatched from saline-injected eggs. Twenty-five of these were classified as female based on examination of the genitalia by a commercial sexer. Twenty-four were classified as male. Fifty-one poults were obtained from eggs injected with 200 μg of Fadrazole®. Fifty of these were classified as male, based on genital appearance, and one was sexed as a female. Thirty-nine poults were obtained from eggs injected with 600 μg of Fadrazole® of which 38 were vent-sexed as males and one as a female. Eight of these poults, including

TABLE 5. Microscopic appearance of gonads from genetic female broilers hatched from eggs injected with Fadrazole® prior to incubation, Experiment 3

		Number of individuals with				
Day	Fadrazole® dose	Normal seminiferous tubules	Atypical seminiferous tubules	No seminiferous tubules or ovarian follicles	Sparse ovarian follicles	Typical number of ovarian follicles
	(μg/egg)					
0	0					5
	150		1	5		3
	450		1	8	1	
11	0					5
	150			7	5	
	450		3	9		
21	0					5
	150			2	1	5
	450		1	9		
32	0					4
	150	1	3	2	1	3
	450	1	3	1	4	1
42	0					4
	150		1	2	1	8
	450		2	4	1	2

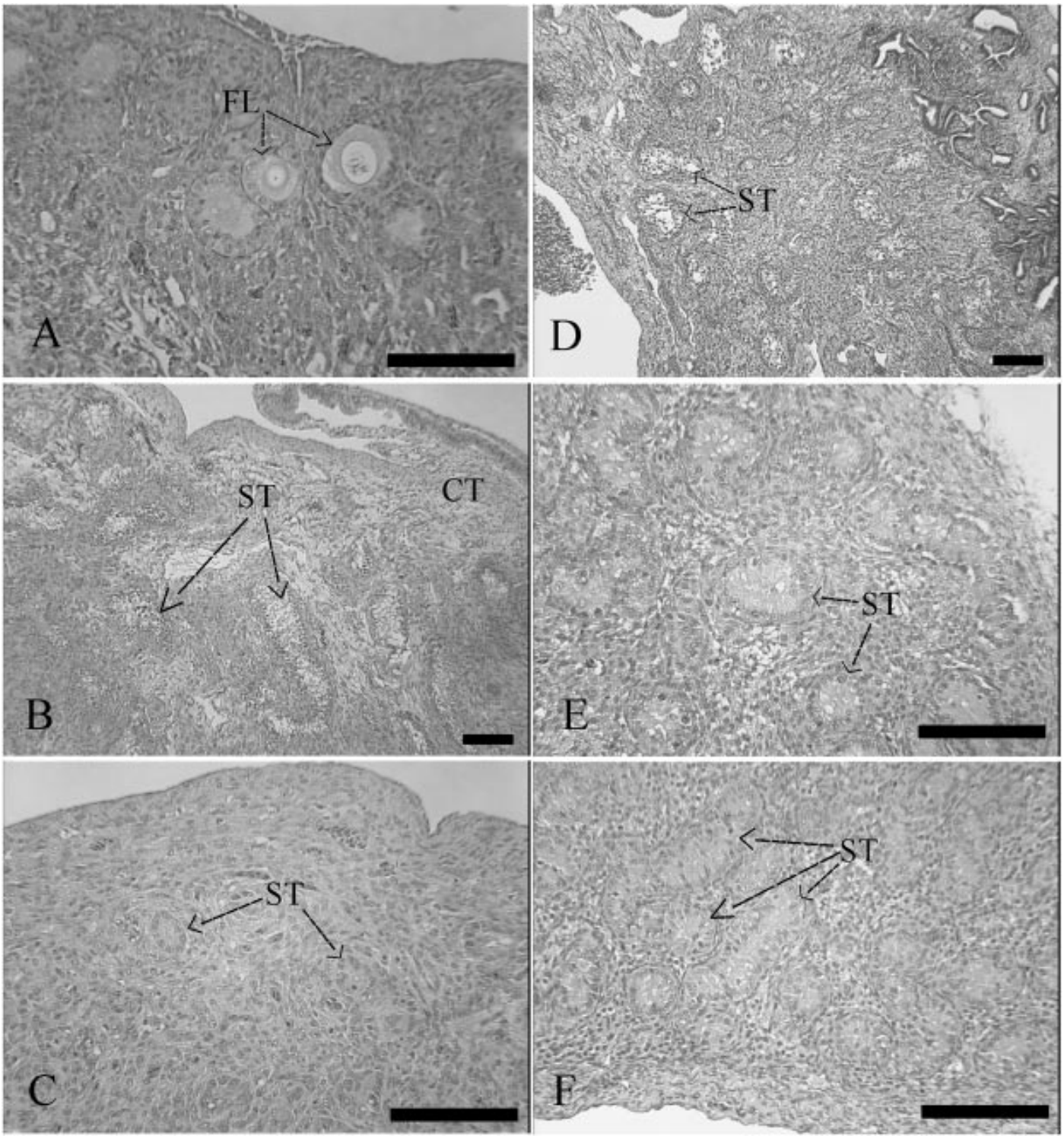

FIGURE 2. Sections of gonads from 11- and 21-d-old chickens. FL = follicle; ST = seminiferous tubule; CT = connective tissue; Space bar = 100 μm. A) Ovary from 11-d-old control female. Compare follicular diameter in this panel with Figure 1A. B) Gonad from 11-d-old genetic female hatched from an egg injected with 450 μg of Fadrazole®. Many tubular structures which appear to be abnormal seminiferous tubules are present. These tubules are of greater diameter than those of 11- or 21-d-old control males (Figures 2E and 2F). Areas of loose connective tissue are present. C) Gonad from 11-d-old genetic female hatched from an egg injected with 450 μg of Fadrazole®. Several tubules are present. These may be seminiferous tubules that are less well developed than those of normal 11 day old males (Figure 2E). No follicles were seen. D) Gonad from a 21-d-old genetic female hatched from an egg injected with 450 μg of Fadrazole®. Many large atypical tubules and areas of loose connective tissue are present. No follicles were seen. Follicles in control females of this age are large and easily identifiable (Figures 2A). E) Seminiferous tubules in an 11-d-old control male. F) Seminiferous tubules in 21-d-old control male.

the one that was sexed as a female, were killed at 1 d of age because of crippled legs. Three additional poults died within the first 24 h. All of these birds had paired gonads that appeared to be testes. The testes in the bird that was vent-sexed as a female had a substantially greater diameter than those in the other 10 poults.

At 15 wk of age, all genetic males in all treatments had gonads classified as testes. All genetic females in the

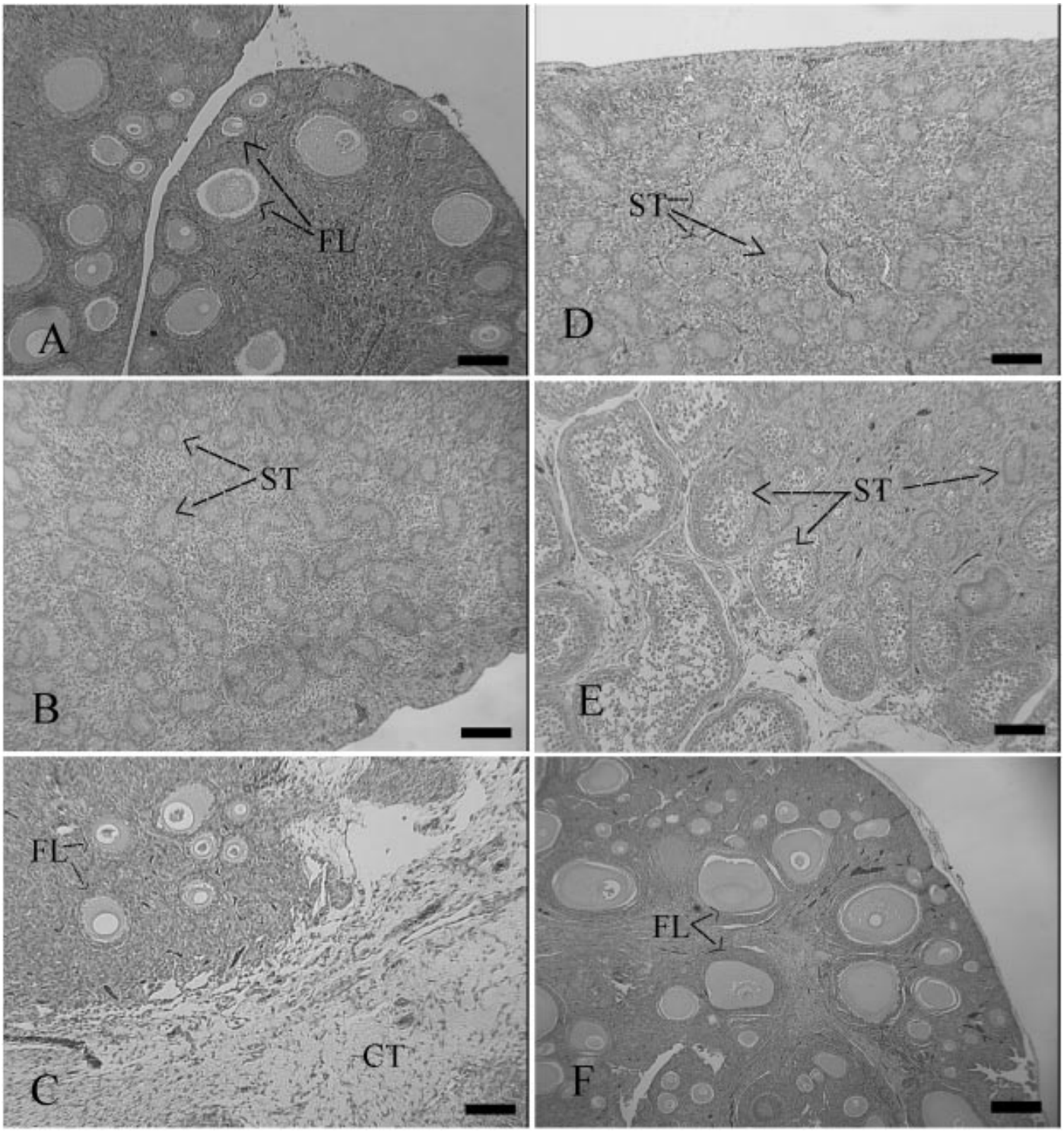

FIGURE 3. Sections of gonads from 32- and 42-d-old chickens. FL = follicle; ST = seminiferous tubule; CT = connective tissue; Space bar = 100 μm. A) Ovary from 32-d-old control female showing large number of well developed follicles. B) Gonad of a 32-d-old genetic female hatched from an egg injected with 450 μg of Fadrazole®. This individual had both a small right and small left gonad. The left appeared ovarian, the right testicular. Both contained normal appearing seminiferous tubules. C) Gonad from a 32-d-old genetic female hatched from an egg injected with 450 μg of Fadrazole®. A small island of stromal tissue with a small number of ovarian follicles was surrounded by large areas of loose connective tissue. D) Seminiferous tubules in a 32-d-old control male. E) Gonad from a 42-d-old genetic female hatched from an egg injected with 450 μg of Fadrazole®. Greatly enlarged atypical seminiferous tubules and a normal appearing seminiferous tubule are present. F) Ovary from a 42-d-old control female.

TABLE 6. Body weight[1] ($\bar{x}$ ± SE) of broiler chicks hatched from eggs injected with Fadrazole® prior to incubation, Experiment 3

	Saline		Fadrazole®—150 μg		Fadrazole®—450 μg		Probability values		
Age	Male	Female	Male	Female	Male	Female	Treatment	Sex	Treatment × Sex
(d)	(g)								
0	38 ± 0.4	39 ± 0.4	39 ± 0.4	38 ± 0.3	39 ± 0.3	38 ± 0.3	0.66	0.69	0.33
11	187 ± 4	187 ± 5	188 ± 3	175 ± 4	196 ± 3	182 ± 4	0.13	0.003	0.12
21	622 ± 13	573 ± 16	611 ± 14	546 ± 11	624 ± 11	561 ± 12	0.35	0.001	0.81
32	1,342 ± 25	1,234 ± 32	1,336 ± 38	1,240 ± 29	1,373 ± 36	1,209 ± 25	0.99	0.0001	0.50
42	2,012 ± 104	1,858 ± 75	2,132 ± 99	1,965 ± 33	2,148 ± 90	1,864 ± 32	0.36	0.004	0.70

[1]The number of observations per mean ranged from 39 to 63, 32 to 42, 25 to 37, 19 to 24, and 12 to 15 at 0, 11, 21, 32, and 42 d of age, respectively.

control group had a single left ovary. Seven genetic females hatched from eggs injected with 200 μg of Fadrazole® had a normal left ovary; five had a small but normal appearing left ovary; five had a left ovary that appeared nodular; and two had small right gonads. None of the genetic females hatched from eggs injected with 600 μg of Fadrazole® had a gonad typical of control females. Five had a small left ovary; three had a left ovary that was nodular in appearance; and four had small right gonads with no visible left gonad.

For purposes of body and muscle weight summarization all genetic females whose gonads appeared to be anything other than a normal sized left ovary were classified as atypical. There were no treatment or sex differences in BW on the day of hatch (Table 7). At 3 wk and beyond, males were heavier than females in the control group and in the group hatched from eggs injected with the low dose of Fadrazole®. Females hatched from eggs injected with the high dose of Fadrazole® were as heavy as males at 3 and 6 wk of age, but they were lighter than males thereafter. Males had significantly heavier pectoral muscles than females at 15 wk of age (Table 7) but there were no differences between treatment groups.

Experiment 5

Gonadal morphology of genetic female turkeys hatched from Fadrazole®-injected eggs changed markedly between the d of hatch and 15 wk of age (Table 8). At d of age, gonads of 11 genetic females hatched Fadrazole®-treated eggs were examined. All clearly appeared to be paired testes. At 3 wk of age and beyond, none of the genetic females that were examined had gonads that were characterized as testes. A small proportion of these females had gonads that were characterized as ovarian. Most had gonads that were not testicular in appearance nor was a single, normal-sized, normal-shaped left ovary present. In some cases two gonads that had a testicular like shape but a color and surface texture similar to that of a normal ovary were present. In some cases left gonads that were present appeared to be small, but otherwise normal ovaries. In some cases a small tubular structure was present in the gonadal region to the right of the midline.

The histological appearance of gonads from 1-d-old and 3-wk-old control poults (Figures 4A, B, D, E, and F) can be contrasted with the appearance of the gonad of a genetic female hatched from an egg injected with 600 μg of Fadrazole®. Histological sections of gonads of 1-d-old genetic females hatched from Fadrazole®-treated eggs revealed the presence of normal looking seminiferous tubules in two individuals, enlarged distorted seminiferous tubules (Figure 4C) in seven individuals and structures that appeared to be disorganized follicles in three others. At 3 wk of age, four genetic females from Fadrazole®-treated eggs showed the presence of ovarian follicles, two had a few segments of seminiferous tubules, and neither follicles nor seminiferous tubules were seen in sections from four individuals (Table 9). At 6, 9, and 12 wk of age a normal population of normal appearing follicles were present in most of the genetic females from Fadrazole®-treated eggs. Neither follicles nor seminiferous tubules were seen in sections from one 6-wk-old bird. At 12 wk of age, eight genetic females hatched from Fadrazole®-treated eggs showed the presence of a normal population of normal appearing follicles. Gonads from two individuals showed the presence of both ovarian follicles and seminiferous tubules in the same tissue section. Both gonads of one bird were described as cystic upon visual examination. This appearance was verified microscopically and germinal elements were not seen. Sections from another individual contained neither follicles nor seminiferous tubules.

Body weights of poults on day of hatch or at 3, 6, or 12 wk of age were unaffected by treatment (Table 10). Statistical analysis revealed a significant treatment effect at 9 wk that resulted from unusually low weight of males hatched from eggs injected with Fadrazole® prior to and on Day 8 of incubation. These data need to be interpreted with caution as they are based on a few observations for some means. Due to the design of the experiment the number of individuals per mean was reduced as the birds grew older. Only six males from the group injected with Fadrazole® on Days 0 and 8 remained at 9 wk and only one at 12 wk of age. Sex differences in BW were noted at 3 wk and beyond.

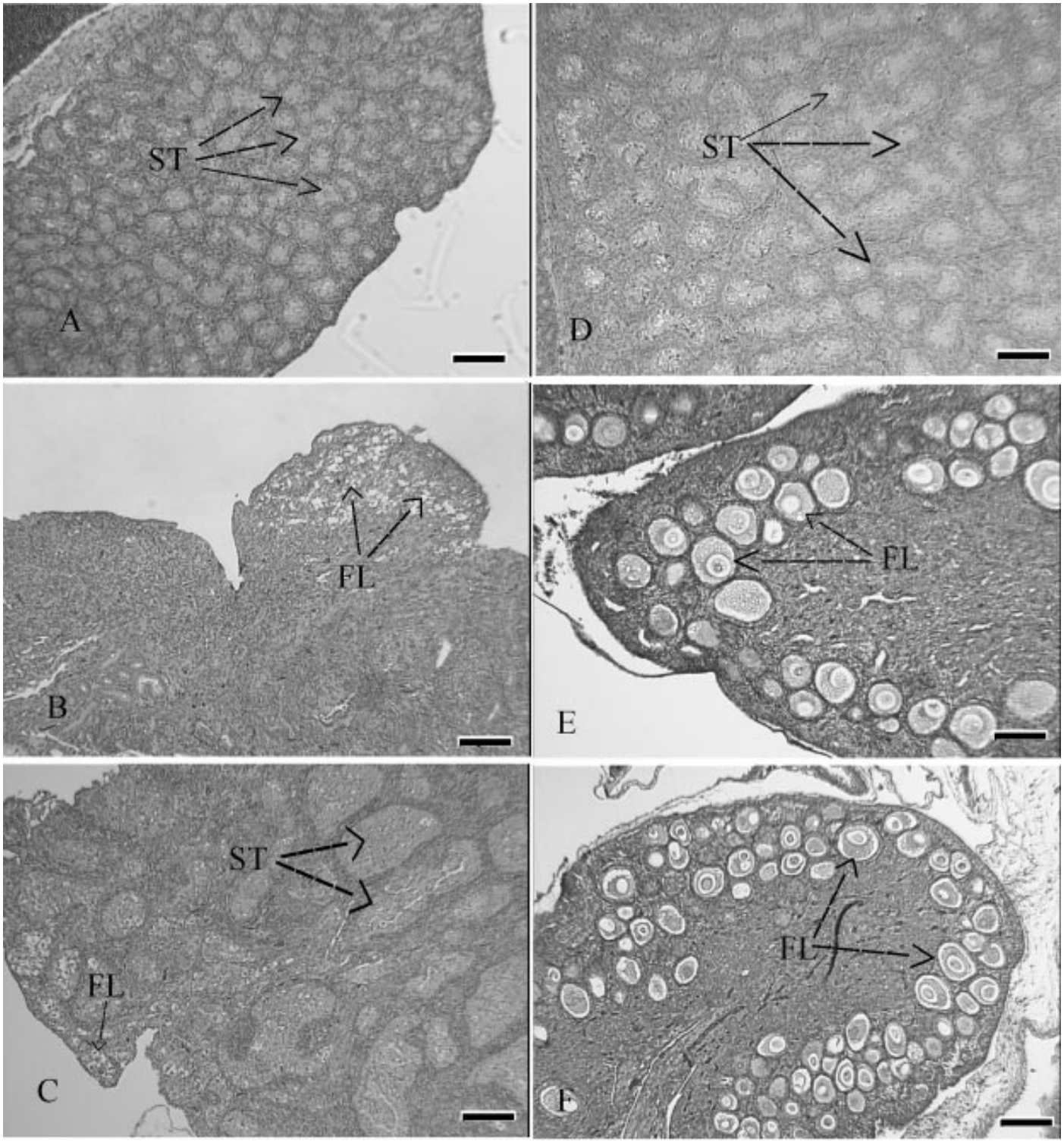

FIGURE 4. Sections of gonads from 1-d-old and 21-d-old turkey poults. FL = follicle; ST = Seminiferous tubule; Space bar = 100 μm. A) Testes from 1-d-old control male. B) Ovary of 1-d-old control female with a region containing many follicles. An adjacent region to the left is devoid of follicles. C) Gonad from a 1-d-old genetic female hatched from an egg injected with 600 μg of Fadrazole® before incubation and a gain on Day 8 of incubation. Many enlarged tubular structures identified as atypical seminiferous tubules are present. A small region which contained a few structures similar to the follicles seen in panel B are present. D) Testis from a 21-d-old control male. Seminiferous tubules underlying the tunica albuginea (left side of micrograph) often appear to have looser cellular arrangements than those toward the center of the testes (right side of micrograph). E and F) Ovaries of 21-d-old control females.

TABLE 7. Body weight[1] and *Pectoralis major* weight[2] ($\bar{x}$ ± SE) of turkeys[3] hatched from eggs injected with Fadrazole® prior to incubation, Experiment 4

	Fadrazole® dose								Probability value		
	0 μg		200 μg			600 μg					
Age	Male	Female	Male	Female	Atypical	Male	Female	Atypical	Treatment	GonSex[4]	Treatment × GonSex
(wk)	(g)										
0	66 ± 1[a]	64 ± 1[a]	64 ± 1[a]	63 ± 1[a]	61 ± 2[a]	65 ± 1[a]	. . .	65 ± 1[a]	0.21	0.44	0.44
3	589 ± 21[a]	531 ± 17[b]	620 ± 18[a]	548 ± 27[b]	521 ± 23[b]	587 ± 17[a]	. . .	582 ± 21[a]	0.60	0.01	0.14
6	2,316 ± 80[a]	1,942 ± 56[b]	2,370 ± 106[a]	1,841 ± 111[b]	1,879 ± 78[b]	2,183 ± 78[a]	. . .	2,115 ± 83[a]	0.67	0.0001	0.06
9	5,377 ± 191[a]	4,350 ± 107[b]	5,356 ± 214[a]	3,961 ± 202[b]	4,278 ± 129[b]	5,117 ± 160[a]	. . .	4,550 ± 142[b]	0.29	0.0001	0.30
12	8,647 ± 251[a]	6,852 ± 155[b]	8,587 ± 232[a]	6,386 ± 192[b]	6,666 ± 154[b]	8,288 ± 238[a]	. . .	7,040 ± 138[b]	0.29	0.0001	0.31
15	12,074 ± 343[a]	9,180 ± 202[b]	11,980 ± 250[a]	8,716 ± 145[b]	8,980 ± 163[b]	11,771 ± 319[a]	. . .	9,409 ± 155[b]	0.46	0.0001	0.54
Pectoralis weight	1,076 ± 44[a]	796 ± 27[b]	1,024 ± 43[a]	748 ± 33[b]	822 ± 34[b]	1,036 ± 40[a]	. . .	822 ± 22[b]	0.43	0.0001	0.99

[a,b]Means ± SE within age with no common superscript differ significantly.

[1]Body weights based on 17 to 26 observations per mean for the 0 dose; 7 to 14 observations per mean for the 200 μg dose; and 11 to 20 observations per mean for the 600 μg dose.

[2]Weight of the left *P. major* at 15 wk of age. Based on 8 to 15 observations per mean.

[3]Birds were classified as male, female, or atypical based on gonadal morphology at 15 wk of age.

[4]GonSex = sex determined via gonadal morphology.

DISCUSSION

In ovo treatment with Fadrazole®, a member of the azole class of aromatase inhibitors, clearly altered development of genetic female chickens in agreement with earlier studies (Elbrecht and Smith, 1992; Rickes *et al.*, 1992; Wartenberg *et al.*, 1992; Abinawanto *et al.*, 1996). We show here, for the first time, that gonadal development of genetic female turkeys is also altered by embryonic exposure to an aromatase inhibitor. Evidence for alteration of development is provided by masculinization of genitalia of 1-d-old genetic female chicks and poults; the presence of paired gonads that have the unambiguous appearance of testes in a sizable proportion of these individuals; a near absence of left ovaries and the lack of distinct, recognizable gonads in some. It is doubtful that complete, prolonged sex-reversal was produced, but it is possible that it occurred in a few individuals. One individual in the control group of Experiment 2 that was classified as a genetic female had testes and an enlarged comb at 43 d of age. Three such individuals were found in the group hatched from eggs injected with the low dose of Fadrazole® and two in the group hatched from eggs injected with the high dose of Fadrazole®. A few genetic males had an ovary at 43 d. Some of these discrepancies were seen in the control groups on the day of hatch and at 43 d as well as in the drug-treated group. These discrepancies may represent inaccuracies in the feather sexing trait as in Experiment 1 there was a 4.57% disagreement between sex determined by feather examination and true genetic sex determined by DNA analyses. The discrepancies may also be due to experimental error. The discrepancies in the drug-treated groups may in fact represent a small incidence of complete and prolonged sex-reversal. Genetic sex was not verified by DNA analysis so the latter possibility cannot be supported or discounted.

Complete characterization of the microscopic appearance of the gonads would require exhaustive examination of serial sections. Consequently, the absence of ovarian follicles or seminiferous tubules in one or two sections or even in large sample of sections from a gonad cannot be taken as proof of the absence of these structures within the organ. In contrast, the presence of even a few seminiferous tubules in a section from the gonad of a genetic female is proof of some degree of sex reversal. The majority of gonads from 1-d-old genetic females chicks hatched from Fadrazole®-treated eggs were without seminiferous tubules or ovarian follicles. A small number of individuals showed the presence of ovarian follicles. At day of age, microscopic sections of testes from control males revealed clearly demarked tubules separated by substantial amounts of interstitial tissue. Gonadal sections of two genetic female chickens from Fadrazole®-treated eggs gave the appearance of tubularity. The tubules were smaller, tightly packed with little interstitial tissue, and were less clearly defined than those of control males. More 1-d-old female

TABLE 8. Number of gonads with testicular, ovarian, or other appearance in genetic female turkeys hatched from eggs injected with saline or with Fadrazole® on Day 0 or Days 0 and 8 of incubation, Experiment 5

	Saline Day 0			Fadrazole®—600 μg Day 0			Saline Day 0 and 8			Fadrazole®—600 μg Day 0 and 8		
Age	Testicular	Ovarian	Other	Testicular	Ovarian	Other	Testicular	Ovarian	Other	Testicular	Ovarian	Other
(wk)												
0	0	5	0	5	0	0	0	5	0	6	0	0
3	0	5	0	0	1	3[1]	0	6	0	0	1	5[1]
6	0	4	0	0	0	5[2]	0	6	0	0	1	3[2]
9	0	5	0	0	1	4[3]	0	5	0	0	1	4[3]
12	0	4	0	0	4	1[3]	0	5	0	0	1	4[3]

[1]Two tubular shaped gonads with color and surface appearance of ovarian tissue.

[2]Small left ovarian structure with small tubular right structure with neither ovarian or testicular appearance.

[3]Small left ovary with no apparent right gonadal tissue.

poults than chicks showed the presence of seminiferous tubules and a smaller proportion contained what were characterized as disorganized or degenerate follicles. Seminiferous tubules in the gonads of female chicks or poults were almost always abnormal in appearance. The diameter of the tubules was substantially greater than that of genetic males. Cellular elements within the tubules were loosely packed and disorganized.

With increasing age the incidence of sections showing the presence of normal appearing follicles increased in Fadrazole®-treated females of both species suggesting that development of germinal elements appropriate to the genetic sex of the individual had been delayed rather than reversed. Seminiferous tubules were present in some females throughout the course of Experiments 3 and 5.

By 6 wk of age, male broiler chickens typically have combs and wattles that are larger and redder than those of females. By 15 wk of age the caruncles and head coloration of toms are generally distinguishable from those of hens. Little evidence of androgenization of head furnishings were seen in genetic females in these studies. The unmistakable masculinization of head furnishing shown in the 2.5 to 4-mo-old females in the study of Elbrecht and Smith (1992) and in the 10-mo-old females shown in Abinawanto *et al.* (1997) had not occurred in the 6-wk-old broiler strain birds used for these studies. This result may be due to age differences, or the use of different genetic stocks of chickens.

The changes in gonadal morphology and microscopic structure seen in these studies were not described by Elbrecht and Smith (1992); however, an abstract from the same group (Rickes *et al.*, 1992) alludes to a "mixed degree of conversion within the population". Based on our studies, it would seem that true and permanent sex

TABLE 9. Microscopic appearance of the gonads of genetic female[1] turkeys hatched from eggs injected[2] with Fadrazole®, Experiment 5

Age	Description of gonads
(wk)	
0	9 of 11 individuals had seminiferous tubules 3 of 11 individuals had disorganized or degenerate follicles
3	1 of 10 individuals had normal appearing follicles 3 of 10 individuals had a small number of normal appearing follicles 2 of 10 individuals had a few scattered seminiferous tubules 4 of 10 individuals had no apparent follicles or seminiferous tubules
6	1 of 9 individuals had no visible follicles or seminiferous tubules 1 of 9 individuals had a small number of follicles amongst disorganized stroma 7 of 9 individuals had a small left ovary with normal appearing follicles
9	10 of 10 individuals had a small left ovary with normal appearing follicles
12	8 of 11 individuals had a small left ovary with normal appearing follicles 1 of 11 individuals had a left gonad with some regions of normal appearing follicles and some regions of seminiferous tubules 1 of 11 individuals had a small left gonad with normal appearing follicles and a few scattered seminiferous tubules 1 of 11 individuals had ovarian cysts and no visible follicles or seminiferous tubules

[1]All genetic males from all groups had seminiferous tubules. A few genetic males hatched from Fadrazole®-injected eggs had some seminiferous tubules with very large, clear lumens that were surrounded by cells that appeared devoid of cytoplasm and some tubules with much greater than usual diameter.

[2]For purposes of description, the genetic females from both Fadrazole®-injected groups were combined.

TABLE 10. Body weight[1] (x̄ ± SE) of turkeys hatched from eggs injected with saline or Fadrazole® on Day 0 of incubation or on Days 0 and 8 of incubation, Experiment 5

Age	Solvent Day 0		Fadrazole®—600 μg Day 0		Solvent Day 0 and 8		Fadrazole®—600 μg Day 0 and 8		Probability values		
	Male	Female	Male	Female	Male	Female	Male	Female	Treatment	Sex	Treatment × Sex
(wk)	(g)										
0	58 ± 1	58 ± 1	60 ± 1	58 ± 1	57 ± 1	57 ± 1	57 ± 1	57 ± 1	0.22	0.51	0.83
3	612 ± 17	548 ± 18	593 ± 21	545 ± 24	589 ± 21	542 ± 22	575 ± 26	508 ± 17	0.35	0.0002	0.95
6	2,233 ± 57	2,031 ± 54	2,207 ± 80	1,981 ± 77	2,181 ± 104	1,896 ± 78	2,114 ± 117	1,886 ± 55	0.41	0.0001	0.96
9	4,911 ± 131	4,269 ± 97	4,740 ± 187	4,264 ± 140	4,803 ± 167	4,111 ± 113	4,068 ± 266	4,030 ± 74	0.02	0.0001	0.25
12	7,755 ± 251	6,496 ± 165	7,728 ± 252	6,544 ± 193	6,822 ± 708	6,205 ± 167	7,593	6,186 ± 160	0.46	0.01	0.86

[1]Means based on 21 to 36 observations at Week 0, 15 to 27 at Week 3, 10 to 21 at Week 6, 4 to 16 at Week 9, and 1 to 11 at Week 12.

reversal was not produced by aromatase inhibitor treatment. Rather it appears that regression of the primary sex cords and development of the secondary sex cords is delayed. The delay may be more prolonged in turkeys than chickens because a higher proportion of 1-d-old female poults showed the presence of seminiferous tubules. The abnormal appearance of the tubules may be a reflection of delayed regression that is underway well after hatching. It is possible that testicular development and spermatozoa production seen in the earlier studies (Elbrecht and Smith, 1992; Abinawanto *et al.*, 1997) would have developed in some individuals had they been grown to more advanced ages.

Growth and pectoral muscle development of chickens was unaffected by the *in ovo* injection of Fadrazole® in agreement with the abstract of Rickes *et al.* (1992). The first of the experiments with turkeys showed a significant stimulation in BW of females hatched from eggs injected with the highest dose of Fadrazole®. The response was present at 3 and 6 wk of age but not thereafter. There is no apparent reason why a similar response was not obtained in the second of the turkey experiments. In contrast to our results Dewil *et al.* (1998) reported that *in ovo* injection of Vorazole, another azole type aromatase inhibitor, affected BW in the early posthatching period, but not at 5 wk of age when the experiment was terminated. Percentage abdominal fat pad was reduced by the highest level of the drug in that study. The contradictory results may be explained by a variety of factors such as drug dosage, time of injection, strain of chicken, or differences in activity of the two drugs used.

It is perhaps not unexpected that manipulation of the sex hormone milieu did not affect posthatch BW or muscularity. There is only sparse indirect evidence supporting the concept that posthatch growth and muscle development is influenced by embryonic sex hormones. Furthermore, there is no evidence that physiological levels of sex steroids after hatch play any role in the normal sexual dimorphism in BW of these species. A review of the literature dealing with these topics can be found in Burke and Edwards (1994) and Burke (1996).

REFERENCES

Abinawanto, K. Shimada, K. Yoshida, and N. Saito, 1996. Effects of aromatase inhibitor on sex differentiation and levels of $P450_{17\alpha}$ and $P450_{arom}$ messenger ribonucleic acid of gonads in chicken embryos. Gen. Comp. Endocrinol. 102:241–246.

Abinawanto, K. Shimada, K. Yoshida, and N. Saito, 1997. Sex-reversal effects of non-steroidal aromatase inhibitor on aromatase ($P450_{arom}$) RNA expression in adult chicken gonads. Jpn. Poult. Sci. 34:158–168.

Abdel-Hameed, F., and R. N. Shoffner, 1971. Intersexes and sex discrimination in chickens. Science 172:962–964.

Burke, W. H., 1994. Sex differences in weight of turkey embryos. Poultry Sci. 73:749–753.

Burke, W. H., 1996. Effects of an *in ovo* injection of an anti-androgen on embryonic and posthatching growth of broiler chicks. Poultry Sci. 75:648–655.

Burke, W. H., and H. M. Edwards, Jr., 1994. Effect of early castration on body weight, muscle growth, and bone characteristics of male Nicholas strain turkeys. Poultry Sci. 73:457–463.

Crew, F.A.E., 1923. Studies in intersexuality. II. Sex reversal in the fowl. Proc. Royal Soc. B. 95:256–278.

Dewil, E., J. Buyse, J. D. Veldhuis, J. Mast, R. DeCoster, and E. Ducuypere, 1998. *In ovo* treatment with an aromatase inhibitor masculinizes postnatal hormone levels, abdominal fat pad content, and GH pulsatility in broiler chickens. Dom. Anim. Endocrinol. 15:115–127.

Domm, L. V., 1927. New experiments in ovariotomy and the problem of sex inversion in the fowl. J. Exp. Zool. 48: 31–173.

Domm, L. V., 1929a. Spermatogenesis following early ovariotomy in the brown Leghorn fowl. Proc. Soc. Exp. Biol. Med. 26:338–341.

Domm, L. V., 1929b. The effect of bilateral ovariotomy in the brown Leghorn fowl. Biol. Bull. 56:459–497.

Elbrecht, A., and R. G. Smith, 1992. Aromatase enzyme activity and sex determination in chickens. Science 255:467–470.

Etches, R. J., and H. Kagami, 1997. Genotype and phenotypic sex reversal. Pages 57–67 *in*: Perspectives in Avian Endocrinology. S. Harvey and R. J. Etches, ed. Journal of Endocrinology, Ltd., Bristol, U.K.

Fell, H. B., 1923. Histological studies on the gonads of the fowl. Br. J. Exp. Biol. 1:97–130.

Freeman, R. M., and W. H. Burke, 1986. Effect of *in ovo* administration of estradiol 17-β on post-embryonic sexual dimorphism in body weight of chickens and turkeys. Poultry Sci. 65(Suppl. 1):44. (Abstr.)

Kodamma, H., H. Saitoh, M. Tone, S. Kuhara, Y. Sakaki, and S. Mizuno, 1987. Nucleotide sequences and unusual electrophoretic behavior of the W chromosome-specific repeating DNA units of the domestic fowl, *Gallus gallus domesticus*. Chromosoma 96:18–25.

Lin, M., M. H. Thorne, I.C.A. Martin, B. L. Sheldon, and R. C. Jones, 1995. Development of the gonads in the triploid (ZZW and ZZZ) fowl, *Gallus-domesticus* and comparison with normal diploid males (ZZ) and females (ZW). Reprod. Fertil. Dev. 7:1185–1197.

Maraud, R., O. Vergnaud, and M. Rashedi, 1986. Structure of the right testis of sexually mature genetically female fowl experimentally masculinized during embryonic life and submitted to a posthatching left castration. Gen. Comp. Endocrinol. 68:208–215.

Masui, K., 1967. Sex determination and sexual differentiation in the fowl. Iowa State University Press, Ames, IA.

Mitchell, R. D., and W. H. Burke, 1995. Genotype and sexual influences on growth and muscle development of chicken embryos. Growth Dev. Aging 59:31–44.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

North, M. O., and D. D. Bell, 1990. Pages 542–543 *in*: Commercial Chicken Production Manual. 4th ed. Van Nostrand Reinhold, New York, NY.

Rickes, E. L., C. H. Chang, F. Marsilio, D. Ok, J. Spencer, R. Smith, G. Hickey, Y. T. Yang, and A. Elbrecht, 1992. Effect of aromatase inhibitor induced sexual conversion on hormone profiles, gonadal morphology, and body weight in broiler chickens. Poultry Sci. 71(Suppl. 1):26. (Abstr.)

Saitoh, H., M. Harata, and S. Mizuno, 1989. Presence of female-specific bent-repetitive DNA sequences in the genomes of turkey and pheasant and their interactions with W-protein of chicken. Chromosoma (Berl.) 98:250–258.

Stoll, R., M. Rashedi, and R. Maraud, 1980. Hermaphroditism induced in the female chick by testicular graft. Gen. Comp. Endocrinol. 41:66–75.

Thorne, M. H., R. K. Collins, B. L. Sheldon, and L. W. Bobr, 1988. Morphology of the gonads and reproductive ducts of triploid chickens. Pages 525–526 *in*: Proceedings of the World Poultry Congress. Vol. 18. Nagoya, Japan.

Wartenberg, H., E. Lenz, and H. U. Schweikert, 1992. Sexual differentiation and the germ cell in sex-reversed gonads after aromatase inhibition in the chicken embryo. Andrologia 24:1–6.

Woods, J. E., R. M. Simpson, and P. L. Moore, 1975. Plasma testosterone levels in the chick embryo. Gen. Comp. Endocrinol. 27:543–547.

Woods, J. E., and D. M. Brazzill, 1981. Plasma 17β-estradiol levels in the chick embryo. Gen. Comp. Endocrinol. 44: 37–43.