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The Effects of Genistein on the Cockerel Testis during Embryonic Development [1]

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Summary

There is no in vivo study on the effect of phytoestrogens on the development of testis in domestic fowl; therefore the aim of this study was to examine the effect of genistein on the cockerel testis during embryonic development. Genistein from soybean were injected into yolk via a small hole at the blunt end of the egg on day 4 of incubation. Cockerels were decapitated on post-hatching day 3 and paraffin sections of the testes were prepared. Nuclear volume density and absolute volume of Sertoli cells and germ cells, seminiferous tubule volume density and absolute volume were determined using the standard point counting method. Genistein did not cause any inhibitory effect on the embryonic development of cockerel testis. On the contrary it had a stimulatory effect on germ cell development.

Keywords: *Testis, Genistein, Cockerel*

Genisteinin Embriyonik Gelişim Sırasında Civciv Testisleri Üzerine Etkisi

Özet

Bu çalışmada, embriyonik gelişim sırasında uygulanan genisteinin civciv testisleri üzerinde göstereceği etkilerin belirlenmesi amaçlandı. İnkübasyonun 4. gününde, soya fasulyesinden elde edilmiş genistein, yumurtanın küt ucundan küçük bir delik açılarak yumurta sarısına enjekte edildi. Çıkımdan 3 gün sonra erkek ciccivlerin testisleri alınarak paraffin kesitleri hazırlandı. Standart nokta sayım yöntemiyle Sertoli hücreleri ve germ hücrelerinin nukleus hacim yoğunlukları ve toplam hacimleri belirlendi. Çalışma sonucunda embriyonik dönemde uygulanan genisteinin, civciv testisleri üzerinde olumsuz bir etki göstermediği saptandı. Aksine germ hücrelerinin gelişimini olumlu yönde etkilediği belirlendi.

Anahtar sözcükler: *Testis, Genistein, Horoz*

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INTRODUCTION

Several studies have shown that estrogens play a significant physiological role in male reproductive development and function [1]. Phytoestrogens are plant compounds which have estrogen-like biological activity. They are similar to endogenous estrogens, in both structure and function and present in a wide variety of feed sources [2]. Genistein is a phytoestrogen present in soybean products as well as in other legumes, constituents of the most commercial poultry feeds [3]. Genistein shares structural features with the 17β-estradiol (the phenolic ring and the distance between some hydroxyl groups); therefore, it can bind to vertebrate estrogen receptors (ERs) and sex hormone binding proteins [4,5].

There is growing evidence that neonatal exposure of the developing male to phyto-estrogens and environmental estrogens can result in a range of abnormalities in reproductive development and function in mammals [6-9]. Since there is no in vivo study on the effect of phyto-estrogens on the developing testis of domestic fowl, the aim of this study was to examine the effect of genistein on the cockerel testis during embryonic development.

MATERIAL and METHODS

Fertile White Leghorn eggs (Lohmann LSL) were obtained from local hatcheries (Has Tavuk, Turkey). The eggs were incubated at 37.5ºC and 60% relative humidity and turned every third hour. Since the gonads commence to differentiate after incubation day 6 [10], genistein from soybean, (purity=99.4%, Sigma Chemical, St. Louis, MO, USA) in the oil/lecithin/propylene glycol emulsion were injected into the yolk via a small hole at the blunt end of the egg using insulin syringes and disposable needles (500 µg/egg) on day 4 of incubation. The control groups were given a vehicle only. After injection, the shell was sealed with paraffin wax.

Eight cockerels from each group were decapitated on day 3 of hatching and the testes were removed and fixed in Bouin's solution for 24 h and then embedded in paraffin. 4 µm thick sections were taken from the testes and stained with Harris' haematoxylin and eosin.

The body and testis weights of all birds were recorded at death and the gonado-somato indices (testis weight/ body weight) and testis weight asymmetry (right testis weight/left testis weight) were calculated.

Nuclear volume density and absolute volume of Sertoli cells and germ cells, seminiferous tubule volume density and absolute volume were determined using the standard point counting method [1,11]. This involved using a systematic clock-face sampling pattern from a random starting point and evaluating 40 fields of the testis section for each animal by using a Nikon 100X objective fitted with a 100-point eye-piece graducule. The grid points falling over each interested structure were counted. The total number of grid points over each structure was expressed as a percentage of the total number of grid points (=4000). For each animal, the percentage volume (volume density) was converted to absolute volume per testis by multiplying by the testis volume, as measured testis weight. This was justified as shrinkage was minimal, that is, the testis weights before and after fixation were comparable.

The significances of the differences between groups were obtained by one-way analysis of variance (ANOVA) and followed by a Duncan test using the SPSS 11.0 program.

RESULTS

Figure 1 shows a typical immature testis on the post hatching day 3. In this stage, germ cells with spherical and large nuclei and Sertoli cells with considerably smaller and irregular nuclei can be clearly differentiated in the seminiferous tubules. Histopathological alterations were not observed in the testis sections of genistein injected group *(Figure 1)*.

The left testis weight and testis weight asymmetry was increased significantly by the treatment. The mean testis weight and body weight increased but this was not statistically significant. The gonado-somato indice was not affected *(Table 1)*.

Non significant decrease was observed in the volume densities of the seminiferous tubule, Sertoli cells and germ cells as a result of the genistein administration. Because overall testis size increased, this reduction converted to an increase in absolute volume. However, the increase was significant only for germ cells *(Table 2)*.

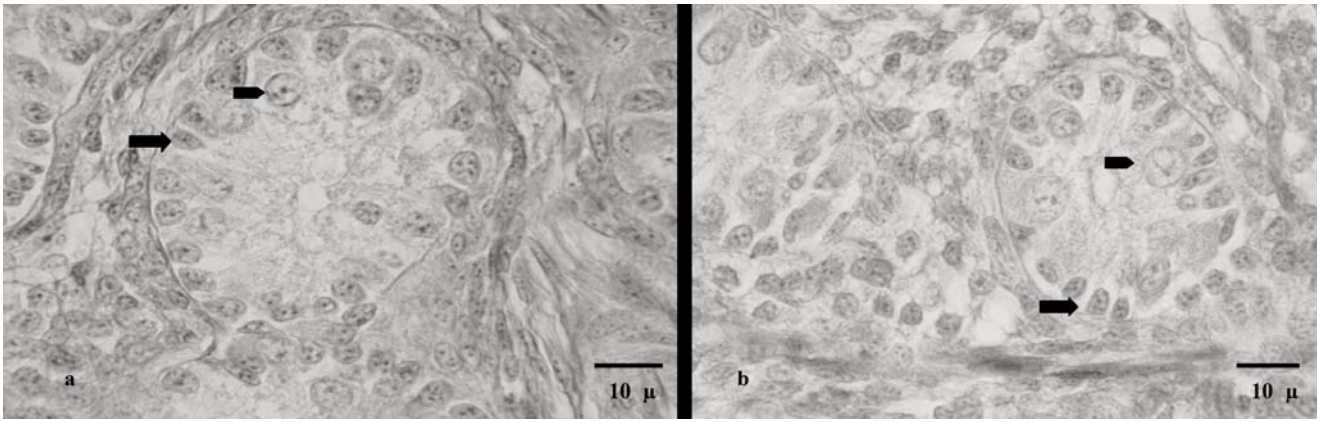

Figure 1. Sections of 4 days old cockerel testis showing germ cells (arrow head) and Sertoli cells (arrow). H&E a) Genistein injected group b) Control group.

Şekil 1. Dört günlük civcivlere ait tesis kesiti, HE. Germ hücreleri okbaşı, Sertoli hücreleri ok ile gösterilmiştir. a) Genistein enjeksiyonu yapılmış grup b) Kontrol grubu

Table 1. *The body and testis weight parameters of the birds on post hatching day 3*
Tablo 1. *Üç günlük civcivlerin vücut ve testis ağırlıkları*

GROUP	Body weight (g)	Left testis weight (g)	Right testis weight /Left testis weight	Mean testis weight (g)	Testis weight /body weight index
Genistein	39.72±1.27	0.006±0.0006*	0.85±0.12***	0.005±0.0004	0.0001±0.000010
Control	36.14±0.76	0.004±0.0003	0.88±0.04	0.004±0.0003	0.0001±0.000008

**P < 0.05, ***P < 0.001, relative to respective control group. Values are presented as means ± SEM (n = 8 birds)*

Table 2. *The volumetric left testis parameters of the birds on post hatching day.*
Tablo 2. *Üç günlük civcilere ait sol tesis hacim parametreleri ve testis ağırlıkları*

GROUP	S. tubule volume density (%)	S. tubule absolute volume (mm^3)	Sertoli cell nuclear volume density (%)	Sertoli cell absolute nuclear volume (mm^3)	Germ cell nuclear volume (%)	Germ cell absolute nuclear volume (mm^3)
Genistein	51.80±1.04	300±30	6.87±0.23	39±4	3.48±0.20	20±2*
Control	52.70±1.39	220±20	8.3±0.21	35±3	3.54±0.20	14±0.8

**P < 0.05, relative to respective control group. Values are presented as means ± SEM (n = 8 birds)*

DISCUSSION

Previously we have reported the histological appearance of immature testis in domestic fowl [11]. In the present study, the same appearance was also observed in 3 days old cockerel testis without any histopathological alterations in both experiment and control groups.

In rodents, the effect of genistein on developing testis is equivocal. In some of the studies no significant effects on testis weights of animals were reported by genistein administration [9,12]. Atanassaova et al.[1] reported that for rats at day 18 of age and adult, those on a soybean free diet had significantly higher testis weights than those on a diet containing soybean. Contrary to those findings, Kang et al.[13] reported that testis weights increased in the neonatal period and did not change in the adult period in animals receiving genistein. In the present avian study, the left testis weight increased significantly.

A stimulatory effect of genistein administrated during embryonic development on germ cell development was observed. Previous studies have shown the stimulatory effect of estrogens on the germ cells of mammalian testis [14-16]. We have also observed a stimulatory effect of other weak estrogenic compounds on germ cell development in the cockerel testis (unpublished data). In a rodent study, the stimulatory effect of weak and low dose potent estrogens was also reported [1]. However, in the same study, although genistein is also a weak estrogenic compound, its adverse effect on the testis was observed.

On the right testis there were no differences between the control and genistein administrated

animals with respect to any parameters (data not given). Since there are no estrogens receptors observed on the right testis [17,18], it can be speculated that, in the present study, the observed effect on the left testis might have occurred via estrogens receptor. The effect on the testis weight asymmetry by the treatment also supported this theory. However, other hormonal pathways should not be underestimated since the left gonad will differentiate into a testis or an ovary depending on the relative concentrations of estrogen and testosterone [19] and, in intact embryonic chick testes, the testosterone levels are invariably higher than the estrogen levels, whereas the converse is true for the ovary [20]. In ovo administration of weak estrogenic compounds at high dose has been reported to cause the left gonad to form an ovotestis in fowl and quail [21]. Since genistein is a weak estrogenic compound, the dose we used in this study seems to be insufficient to trigger ova testis formation.

Genistein is present in commercial poultry feeds and can be deposited in the egg [22]. Thus, it is possible for it to pass to the embryo via egg yolk. The result of this study showed that genistein administration did not cause any inhibitory effect on testis development; on the contrary it has a stimulatory effect on testis development. However, in an in vivo study, the inhibitory effect of genistein on the testosterone secretion of in rooster Leydig cells has been observed [23]. Also California quail fed a diet with a high level of phytoestrogens had poor breeding success [24]. Nevertheless, according to our result it can be concluded that genistein present in poultry feeds did not show a negative effect on embryonic testis development. On the contrary, it had stimulatory effect on germ cell development during embryonic development.

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