

EFFECT OF L-TYROSINE ON GROWTH PERFORMANCE, AGE OF SEXUAL PUBERTY, SEMEN QUALITY AND TESTOSTERONE LEVEL IN FRIESIAN BULLS

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SUMMARY

Thirty Friesian bull calves averaged 8.5 ± 0.16 month of age and 195 ± 15 kg body weight were used in this study. All bulls were fed similar diets. The bulls were divided into three similar groups (10 each), according to their weight and age. Bulls in the 1st group were considered as control (G1), while those in the 2nd group were received single oral dose from L-tyrosine (50 g/bull) at 8.5 months of age (G2). Bulls in the 3rd group were received two oral doses from L-tyrosine (50 g/bull) at 8.5 and 10.5 months of age (G3). The results revealed that L-tyrosine treatment increased ($P < 0.01$) the live body weight (LBW) in G3 and G2 compared to G1 at 12 month, first appearance of spermatozoa, sexual puberty, and at 24 months of age. Age at first appearance of spermatozoa and puberty age were ($P < 0.001$) earlier by about 1.2 and 1.5 and 0.9 and 1.7 months, respectively, for bulls in G2 and G3 than the control. Changes in testosterone concentration during the study period were higher ($P < 0.05$) in G3 and G2 than in G1. L-tyrosine increased ($P < 0.01$) percentages of progressive motility and livability of spermatozoa, and sperm cell concentration compared to control at first appearance of spermatozoa, puberty, and at 24 months of age. In general, semen quality improved ($P < 0.01$) at 24 month of age as compared to puberty age. Sperm motility and livability and sperm cell concentration significantly increased ($P < 0.01$) with advancing age in all groups. Morphology defects in the head of spermatozoa were lower ($P < 0.05$) in G2 and G3 than in G1 at first appearance of spermatozoa, sexual puberty and at 24 months of age. Also the proximal droplets were lower ($P < 0.05$) in G2 and G3 than in G1 at puberty and at 24 months of age. There was a significant increase in number of normal sperm cells with age advancement as well as significant decreases in proximal droplets and head defects. The present results indicated that treated bulls by one or two doses of L-tyrosine (50 gm/bull calves) induced an improvement of growth performance, earlier sexual puberty and improved semen quality of Friesian bull.

Keywords: Friesian bull, L-tyrosine, puberty, testosterone and semen quality

INTRODUCTION

Enhancement the release of sex hormones was suggested to improve growth and reproductive performance. The amino acid (L-Tyrosine) is involved in formation of catecholamine from adrenal gland and thyroxin from thyroid gland (Hammerl and Russe, 1987a). In this respect, Abo-Elroos (1992) reported that L-Tyrosine possessed to improve the reproductive efficiency in rabbits, through enhancing the release of sex hormones. Gabr (2009) found that the double oral dose from L-Tyrosine (100 mg/kg live body weight) at 15 days-interval induced early puberty in rabbit bucks.

Exogenous tyrosine decreased age at puberty in rats (Hammerl and Russe, 1987b); increased litter size in rats (Hammerl and Muller, 1988) and swine (Hammerl and Russe, 1987a); increased LH pulse frequency in growth-restricted lambs (Hall *et al.*, 1992); induced follicular growth, estrus, and ovulation in anovulatory dairy cows (Wetzel, 1985; Hammerl, 1986 and Munsterer, 1987); and improved expression of estrus in sows (Hammerl and Russe, 1987a). The amino acid tyrosine, may be involved in stimulating GnRH release because availability of tyrosine influences on synthesis of norepinephrine (Wurtman *et al.*, 1981; Gibson and Wurtman, 1986

and Acworth *et al.*, 1988), a neurotransmitter that stimulates GnRH release (Ramirez *et al.*, 1984 and Terasawa *et al.*, 1988).

Tyrosine hydroxylase is an enzyme involved in the synthesis of catecholamine (Nagatsu *et al.*, 1964). Alonso *et al.* (1982) found that production of the tyrosine hydroxylase is controlled by tyrosine level in the blood.

Dopamine is an important hypothalamic catecholamine activating the release of growth hormone (Muller, 1973). Catecholamine stimulates the hypothalamus to release GnRH via adrenergic receptor mechanism (Arthur, 1989). Release of GnRH stimulates anterior pituitary gland to release FSH and LH, which control folliculogenesis and spermatogenesis.

The aim of this experiment was to study the effect of L-tyrosine on characterization reproductive development, testosterone level, age of puberty and semen quality of Frisian bull calves.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Production Research Station, Sakha, Kafer

El-Sheikh Governorate, Egypt, belonging to the Animal Production Research Institute.

Animals and management:

Thirty Friesian bull calves averaging 8.5 ± 0.16 month of age and 195 ± 15 kg live body weight (LBW) were divided into three equal groups (10 each), according to their LBW and age. All bulls were fed similar diets. Bulls in the 1st group were considered as a control group (G1). Bulls in the 2nd group were received single oral dose from L-tyrosine (50 g/bull) at 8.5 months of age (G2), while bulls in the 3rd group were received two oral dose from L-tyrosine (50 g/bull) at 8.5 and 10.5 months of age (G3). All bulls were judged to be free of physical defects and diseases and had normal external genitalia. Animals were kept freely under semi-open sheds and were fed according to the recommendations of Animal Production Research Institute (APRI, 2002) throughout the experimental period.

Feeding system and management

Concentrate feed mixture (CFM) used in feeding the bulls of all groups was composed of 37.5% yellow corn, 20% soybean meal, 15% corn gluten, 22.5% wheat bran, 3% molasses, 0.5% premix (mixture vitamins and minerals) and 1.5% common salt. Rice straw and fresh berseem (during winter season) or corn silage (during summer season) according to the recommendation of the APRI, (2002).

Experimental procedures

Throughout the experimental period, semen was collected from all bulls twice weekly using an artificial vagina from 10 up to 24 month of age. Immediately after collection, the percentage of progressive sperm motility, livability and abnormalities of spermatozoa, as well as, sperm cell concentration were evaluated according to Barth (2002). Abnormal sperm were classified into four categories according to descriptions used for bovine evaluations (Barth, 2000), head defects, s midpiece defects, proximal and tail defects. The bull was considered at the age of puberty when produced an ejaculate containing minimum of 50×10^6 sperm cells having at least 10% progressive motility according to Wolf *et al.* (1965).

Blood samples were monthly collected in clean test tubes via the jugular vein and centrifuged at 3000 rpm for 10 minutes to separate blood serum which stored at -20°C for determination of testosterone concentration in blood serum.

Total serum testosterone assay was conducted by radioimmuno assay using - Pontex 335 kit (I^{125}). Types of testosterone assayed were (A) total testosterone (direct extraction-coated tubes) and (B) free testosterone. According to the instructions of the

producing company (Pantex Santa Monica), the solvents used in this assay break the protein binding during extraction process. The standard curve of testosterone ranged between 0.1 and 25.6 ng/ml.

Statistical analysis

Results (age and LBW) were statistically analyzed according to Snedecor and Cochran (1980). A factorial design (3 groups x 4 ages) was used and the statistical model was:

$$Y_{ijk} = U + A_i + B_j + AB_{ij} + e_{ijk}.$$

Where:

Y_{ijk} = Observed values

U = Overall mean

A_i = group (3 groups)

B_j = age (4 ages)

AB_{ij} = Interaction due group x age

e_{ijk} = Random error

The significant differences among means were tested using Duncan Multiple Range Test (Duncan, 1955). The percentage values of semen characteristics were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

Results presented in Table (1) revealed that L-tyrosine treatment has significantly positive effect ($P < 0.01$) on live body weight (LBW) in G3 and G2 than in G1 at the studied four ages, 12 month of age, first appearance of spermatozoa, puberty, and 24 months of age. Increase of LBW after treatment with tyrosine is mainly due to production of tyrosine hydroxylase enzyme. This enzyme involved in the synthesis of catecholamine (Nagatsu *et al.*, 1964). Dopamine is an important hypothalamic catecholamine activating the release of growth hormone (Muller, 1973). Also it has been found that catecholamine stimulates the hypothalamus to release GnRH via adrenergic receptor mechanism (Arthur, 1989). Release of GnRH stimulates anterior pituitary gland to secrete FSH and LH, which control the spermatogenesis (Hafez, 1993 and Salisbury *et al.*, 1978).

Age at first appearance of spermatozoa and puberty were significantly ($P < 0.001$) earlier by about 1.2 and 1.5 and 0.9 and 1.7 months, respectively, for bulls in G2 and G3 groups than the control group (Table 1 and Figure 1). The present results were agreed with Noseir *et al.* (2007) results who found that, L-tyrosine induced puberty in the rabbit two weeks earlier than control, and Grasa *et al.* (2009) who reported that L-tyrosine induced puberty in treated rams earlier than the untreated rams.

Table 1. Effect of L-tyrosine on age, body weight and serum testosterone level (Mean±S.E.) at first appearance of spermatozoa, puberty, and 12 and 24 months of age

Items	Group	12 months	First sperm	Puberty*	24 months
Body weight (kg)	G1	226	247	291 ^b	459 ^b
	G2	246	261	312 ^a	481 ^a
	G3	262	271	311 ^a	498 ^a
	S.E.	±12.50	± 13.5	± 7.20	± 11.5
Age (months)	G1	-	13.8 ^a	15.5 ^a	-
	G2	-	12.6 ^b	14.6 ^{ab}	-
	G3	-	12.3 ^b	13.8 ^b	-
	S.E.	-	± 0.24	±0.51	-
Testosterone (ng/ml)	G1	6.25 ^b	8.56 ^b	7.27 ^b	6.54 ^b
	G2	7.23 ^a	9.57 ^a	8.56 ^a	7.69 ^a
	G3	7.95 ^a	9.68 ^a	8.56 ^a	8.01 ^a
	S.E.	± 0.25	± 0.32	± 0.27	± 0.17

*Sexual puberty was recorded when the first ejaculate contained a minimum of 50×10^6 sperm cells and had at least 10% progressive motility (Wolf *et al.*, 1965).

^a and ^b: Means within the same column with different superscripts are significantly different at (P<0.05).

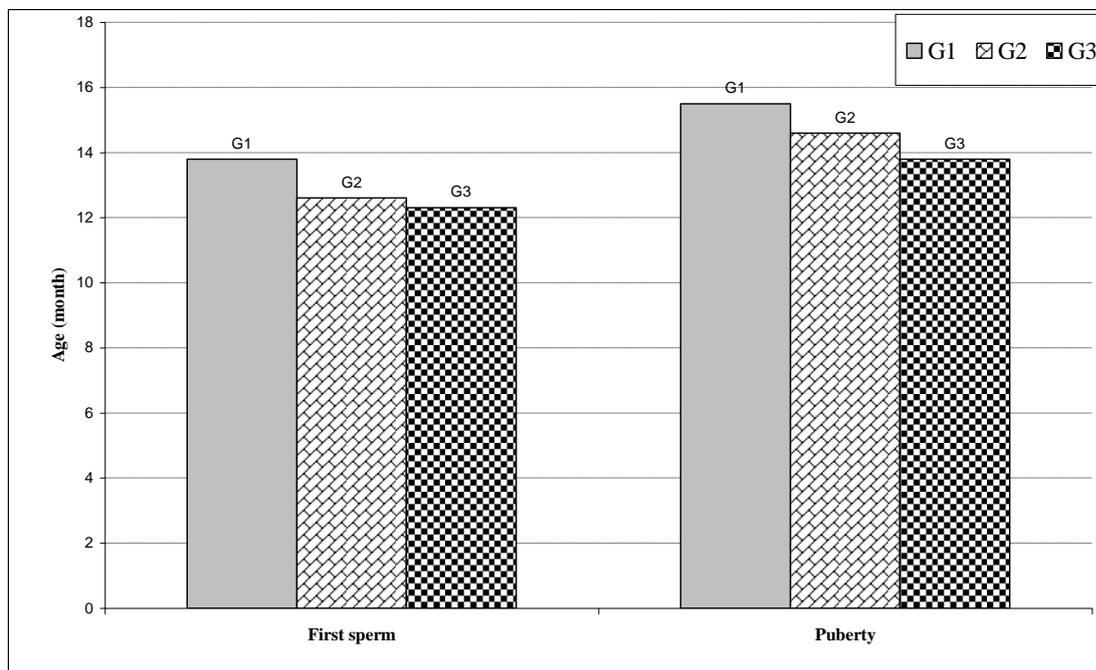


Figure 1. Effect of L-tyrosine on age at first sperm and sexual puberty in bull calves

Overall changes in testosterone concentrations during the study period were significantly higher in G3 and G2 than in G1 at 12 month, first appearance of spermatozoa, puberty, and at 24 months of age (Table 1 and Figure 2). The present results are agreed with Saad *et al.* (2005) who found that serum testosterone concentrations increased significantly in rabbit treated by L-tyrosine compared to control. Testosterone concentration levels increased with advancing age (Figure 1). In growing male cattle, serum testosterone increased in linear fashion as the testes develop and spermatogenesis progresses (McCarthy *et al.*, 1979 and Schams *et al.*, 1981).

Data presented in Table (2) shows that bulls treated with L-tyrosine increased significantly (P<0.01) percentages of progressive motility and livability of spermatozoa and sperm concentration compared to the control at first appearance of spermatozoa, puberty, and 24 months of age. In

general, semen quality of the bulls showed significant (P<0.01) improvement at 24 months compared to puberty age. However, ejaculate volume did not significantly affected by treatment groups. Also, sperm motility and livability, as well as, sperm cell concentration were significantly (P<0.01) increased with advancing age in all groups. Sperm cell concentration was increased by advancement of age (Figure 3). The present findings are in agreement with those reported by Helbig *et al.* (2007) who found that the first production of sperm cells occurred at approximately 14 months of age (13–15 months), but almost no spermatozoa exhibited normal morphology at this age. The earliest sperm were extremely malformed but by the second collection, cellular morphology began to improve, the greatest improvements occurring from 19 to 23 months of age. Additionally, the appearance of first sperm in beef bulls is known to be earlier, at 8–12

months of age and, as bulls advance in age from 13 to 24 months, the percentage of morphologically normal sperm cells increases (Lunstra and Echternkamp, 1982 and Arteaga *et al.*, 2001). It was reported that

exogenous L-tyrosine increased frequency of LH pulses (Hall *et al.*, 1992) this conclusion may explain the improvement of semen quality in L-tyrosine treated bulls in the present study.

Table 2. Effect of L-tyrosine on semen characteristics (Mean±S.E.) at first appearance of spermatozoa, puberty, and at 24 months of age

Items	Group	First sperm	Puberty*	24 months
Ejaculate volume (ml)	G1	1.6	2.4	2.42
	G2	2.1	2.9	2.75
	G3	2.2	3.1	3.03
	S.E.	± 0.2	± 0.32	± 0.25
Sperm cell concentration (x 10 ⁶ /ml)	G1	36.5 ^b	554 ^b	1087 ^b
	G2	41.5 ^{ab}	628 ^{ab}	1242 ^{ab}
	G3	44.5 ^a	747 ^a	1347 ^a
	S.E.	± 2.2	± 60	± 70
Sperm mass motility (%)	G1	5.2 ^b	52.45	76.51 ^b
	G2	7.3 ^a	58.54	81.5 ^{ab}
	G3	7.9 ^a	60.39	87.47 ^a
	S.E.	± 0.19	± 4.5	± 3.6
Live sperm (%)	G1	29.5 ^b	45.51 ^b	65.24 ^b
	G2	32.5 ^a	52.34 ^{ab}	74.25 ^{ab}
	G3	33.4 ^a	54.25 ^a	78.24 ^a
	S.E.	± 0.6	± 2.4	± 4.3

*Sexual puberty was recorded when the first ejaculate contained a minimum of 50×10⁶ sperm cells and had at least 10% progressive motility (Wolf *et al.*, 1965).

^a and ^b: Means within the same column with different superscripts are significantly different at (P<0.05).

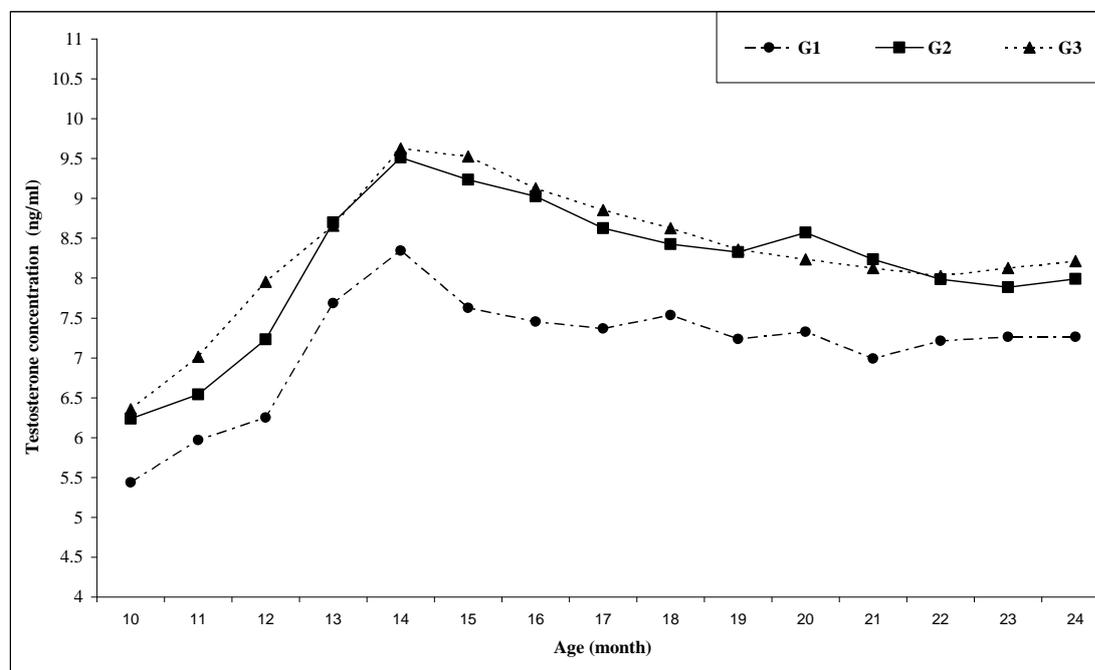


Figure 2. Effect of L-tyrosine on serum testosterone concentration in bull calves

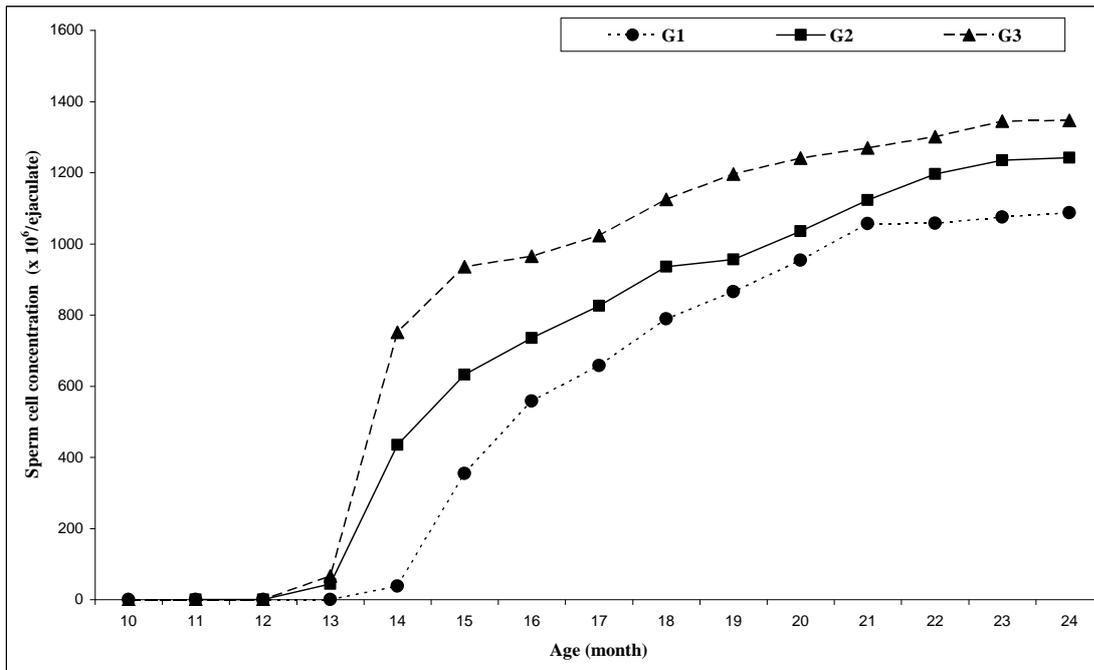


Figure 3. Effect of L-tyrosine on sperm cells concentration in bulls

Table 3. Effect of L-tyrosine on sperm morphology (%) (Mean±S.E.) at first appearance of spermatozoa, puberty, and at 24 months of age

Sperm morphology (%)	Group	First sperm	Puberty*	24 months
Normal	G1	6.21 ^b	22.52 ^b	71.81 ^b
	G2	11.18 ^a	29.87 ^a	79.54 ^a
	G3	12.75 ^a	30.15 ^a	81.42 ^a
	S.E.	± 1.2	± 1.3	± 1.5
Head defects	G1	51.82 ^a	42.85 ^a	12.57 ^a
	G2	47.4 ^b	39.1 ^b	9.7 ^b
	G3	47.1 ^b	39.2 ^b	8.1 ^b
	S.E.	± 1.2	± 1.2	± 0.6
Midpiece defects	G1	15.2	14.13	7.5 ^a
	G2	14.7	12.9	5.26 ^b
	G3	14.6	12.7	5.28 ^b
	S.E.	± 0.8	± 0.7	± 0.4
Tail defects	G1	7.23	7.3	5.9
	G2	8.92	7.63	4.5
	G3	8.35	7.15	4.3
	S.E.	± 0.6	± 0.8	± 0.7
Proximal droplets	G1	19.54	13.2 ^a	2.24 ^a
	G2	17.8	10.5 ^b	1.0 ^b
	G3	17.2	10.8 ^b	0.9 ^b
	S.E.	± 0.8	± 0.7	± 0.4

^a and ^b: Means within the same column with different superscripts are significantly different at (P<0.05).

*Sexual puberty was recorded when the first ejaculate contained a minimum of 50×10⁶ sperm cells and had at least 10% progressive motility (Wolf *et al.*, 1965).

Data presented in Table (3) shows that normal sperm morphology was significantly (P<0.05) higher in G2 and G3 than the control group at first sperm, puberty and 24 months of age. However, the defects in sperm head morphology were significantly (P<0.05) lower in G2 and G3 than in the control group at first appearance of spermatozoa, puberty and 24 months of age. However, proximal droplets were significantly (P<0.05) lower in G2 and G3 than in the control group at puberty and at 24 months of age. Midpiece defects were significantly (P<0.05) lower

in L-tyrosine groups than in control group at 24 month of age, but no significant differences at first appearance of spermatozoa and puberty of age.

The present results are in agreement with Ho and Suarez (2001) who proposed that, L-tyrosine induced puberty in small ruminants with an improvement of semen characters. The increase in protein tyrosine associated with improvement of puberty and semen characteristics in rams (Kulanand and Shivaji, 2001 and Baker *et al.*, 2004).

The same trend was observed by Perez-Pe *et al.* (2002) and Grasa *et al.* (2009); they demonstrated that protein tyrosine improved puberty, fertility and semen characteristics in rams.

Changes in semen morphology were significantly decreased in proximal cytoplasmic droplets ($P < 0.01$) and head defects ($P < 0.05$) with advancing bull age, but no significant changes in midpiece and tail defects Table (3). These results are similar to that by Helbig *et al.* (2007). They found that an increase in number of normal sperm cells and a decrease in proximal droplets and head defects with increasing bull age.

CONCLUSION

In conclusion, an oral single or two dose of L-tyrosine (50 g/bull calves) showed an improvement in growth performance, induced earlier puberty and had beneficial effects on semen quality of Friesian bulls calves.

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تأثير التيروسين علي النمو و البلوغ الجنسي وجودة السائل المنوي ومستوى هرمون التستوستيرون في عجول الفريزيان

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أستخدم في هذه الدراسة ٣٠ عجل فريزيان متوسط أوزانها 195 ± 15 كجم ومتوسط أعمارها 8.5 ± 0.16 شهر. وقسمت إلى ثلاث مجموعات متماثلة (ن = ١٠) على حسب وزن الجسم والعمر. كل العجول تم تغذيتها على نفس المواد. العجول في المجموعة الأولى تركت بدون معاملة كنترول (المجموعة الأولى) وعجول المجموعة الثانية تم تجريبيها عن طريق الفم ٥٠ جم تيروسين لكل عجل مرة واحدة عند عمر ٨,٥ أشهر (المجموعة الثانية) وعجول المجموعة الثالثة تم تجريبيها مرتين عن طريق الفم ٥٠ جم تيروسين لكل عجل في كل مرة عند عمر ٨,٥ و ١٠,٥ شهر (المجموعة الثالثة).

وأظهرت النتائج المتحصل عليها أن المعاملة بالتيروسين أدت إلى زيادة وزن الجسم الحي معنوياً مقارنة بالكنترول عند عمر ١٢ شهر والعمر عند ظهور أول حيوانات منوية وعمر البلوغ و ٢٤ شهر. كما أدت المعاملة بالتيروسين إلى تكبير العمر عند ظهور أول حيوانات منوية بـ ١,٢ و ١,٥ شهر وعند البلوغ بـ ٠,٩ و ١,٧ شهر للمجموعة الثانية والثالثة مقارنة بالكنترول علي الترتيب. وكذلك ازداد تركيز التستوستيرون في المجموعتين الثانية والثالثة مقارنة بالكنترول عند ظهور أول حيوانات منوية و البلوغ و ١٢ و ٢٤ شهر من العمر. كما ازداد معنوياً كل من نسبة الحركة والحي وتركيز الحيوانات المنوية للعجول المعاملة بالتيروسين مقارنة بالكنترول عند ظهور أول حيوانات منوية و البلوغ و ٢٤ شهر من العمر. ازدادت جودة السائل المنوي معنوياً عند عمر ٢٤ شهر مقارنة بعمر البلوغ كما ازداد معنوياً كل من نسبة الحركة والحي وتركيز الحيوانات المنوية مع زيادة العمر في كل المجموعات. كما أدت المعاملة بالتيروسين إلى تحسين الصفات المرفولوجية للحيوانات المنوية حيث قلت نسبة الحيوانات المنوية الشاذة بالرأس ووجود نقط بروتوبلازمية بجسم الحيوان المنوي خلال جميع المراحل مقارنة بالكنترول. وازدادت أعداد الحيوانات المنوية الطبيعية كما نقصت نسبة الحيوانات الغير طبيعية بالرأس ووجود نقط بروتوبلازمية بجسم الحيوان المنوي مع زيادة عمر العجول. ويتضح من الدراسة أن معاملة العجول الفريزيان بالتيروسين مرة واحدة أو مرتين بمعدل ٥٠ جم لكل حيوان بكرت عمر البلوغ وحسنت صفات السائل المنوي.