

The Androgenic Action of Gibberellic Acid (GA₃) on Reproductive Performance of New Zealand White Rabbit Bucks

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Abstract: An experiment was carried out to investigate the hormonal effect of GA₃ on sexual system and reproductive performance of adult male rabbits. Twenty-male New Zealand white rabbits at 7-months old were randomly divided into equal 4 groups. Groups 2, 3 and 4 were injected subcutaneously (under nick skin) with 200, 400 and 800 µg GA₃/kg B.W./week for 6 weeks treated period, while group 1 was served as a control group. Male rabbits treated with GA₃ at all studied doses caused a significant increase in semen ejaculate volume (EV), sperm concentration (SCon), total sperm out-put (TSO) and sperm motility (%) compared to the control group. The medium dose (400 µg GA₃/kg B.W./week) had the best effect for the most of the pervious semen characteristics compared to low and high doses. Furthermore, GA₃ increased libido (decrease reaction time RT), normal sperm and live sperm significantly compared with the control group, while, dead sperm was decreased. There were an increase in seminal plasma total lipids and total protein in the treated groups while, urea concentration was decreased. GA₃ administration caused a decrease in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) Enzymes activities in seminal plasma for treated groups but Alkaline phosphatase (ALP) and acid phosphatase (AcP) enzymes activities were increased. Treated male rabbits with GA₃ doses resulted in a gradual and significant decrease in serum testosterone concentration compared to the control group.

Key words: Rabbits • Gibberellic acid • Fertility • Enzymes • Seminal plasma components

INTRODUCTION

Gibberellic acid (actually a group of related substances called gibberellins) was discovered as a metabolic byproduct of the fungus *Gibberella fujikuroi* [1]. Gibberellic acid (GA₃) has many effects regulating various physiological processes including seed germination, the mobilization of endosperm storage reserves, shoot growth, flowering, floral development and fruit set [2,3]. Moreover, El-Mofty *et al.* [4] reported that GA₃ is a plant growth regulator used in many countries, including Egypt, to increase the growth of fruits and vegetables.

Recent evidence indicated that GA₃ might has biological actions in animals. Alkhiat *et al.* [5], Madacsi *et al.* [6] and Abd-Elhamid *et al.* [7] reported positive influences of GA₃ on body weights of rats, poultry, pigs and calves.

In addition, Gawienowski *et al.* [8] and Gawienowski and Chatterjee [9] reported that GA₃ has demonstrated in mammals a number of estrogenic hormone-like actions. It is worthy noting that, in the castrated male rats, GA₃ partially restored the weight of the prostate but did not significantly change the epididymis and seminal vesicles [10]. Gawienowski *et al.* [8,11] have clearly demonstrated that, GA₃ not only exhibits synergistic uterophic effect with estradiol in the immature mouse, but it also possesses androgenic properties in male chicks based on the chicks comb bioassay. Elkomy [12] found that treated mature cockerels with GA₃ conducted to improve semen quality traits (sperm concentration, sperm motility, increase live sperm and decrease abnormal sperm). Also, who reported that, the GA₃ doses had low serum testosterone concentration compared with the control group. Elkomy *et al.* [13] mentioned that, gibberellic acid can have testosteric biological effects on male chicks,

whereas, gibberellic acid treatments induced effects on chick comb and testes' weights that was similar to testosterone effects, as both reduced testes weights significantly. Also, GA₃ was capable of inducing testosterone secretion in male chicks.

The main purpose of this study was to investigate the androgenic action of GA₃ (as a phyto-hormone) on reproductive performance and some biochemical seminal plasma parameters of mature New Zealand white rabbit Bucks

MATERIALS AND METHODS

This study was carried out at El-Sabahia Poultry Research station, Animal production Research Institute, Agriculture Research Center and Arid Lands Cultivation And Development Research Institute, Mubarak City for Scientific Research and Applied Technology, Egypt.

Twenty mature male New Zealand white rabbits 7-month old with average initial weight (3.100±25 kg) were used during spring season. Animals were divided into 4 equal groups of 5 rabbits. The rabbits were individually housed in cages. Feed and water were provided *ad libitum*. The composition of the ingredients of pellet concentrated feed (% on a dry mater basis) is shown in Table 1.

Groups 2, 3 and 4 were injected subcutaneously (under nick skin) with 0.2 ml/kg of ethanol-sesame oil solution containing 200, 400 or 800 µg GA₃/kg B.W./week, while group 1 served as a control group which was treated in a like manner except that the injected solution contained the ethanol-sesame oil mixture only for 6 weeks

treated period. GA₃ was dissolved in The solution that prepared as 1:11 ethanol- sesame oil mixture with the addition of 2 mg NaHCO₃ /0.2 ml of injection solution.

Semen from each rabbit was collected weekly using an artificial vagina and a teaser doe [14]. The volume of each ejaculate was recorded nearest 0.1 ml (using a graduated collection tube) after removal of the gel mass. A weak eosin solution [15] was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH+Co., Brandstwierte 4, 2000 Hamburg 11, Germany). Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration. The percentage of motile sperm was estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage. Total number of motile sperm (TMS) was calculated by multiplying percentage of motile sperm and total sperm outputs. Assessments of live, dead and abnormal spermatozoa were performed using an eosin-nigrosine blue staining mixture [16]. Initial hydrogen ion concentration (pH) of semen samples was determined just after collection using a pH comparative paper ranging from 0 to 14 with 1 grades (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Reaction time was determined as the moment of subjecting a doe to the buck until the completion of erection using a stopwatch, it was measured in seconds.

Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4 °C and was stored at -20 °C until later analysis. Seminal plasma samples were analyzed for total protein (TP) by the Biuret method according to Henry *et al.* [17]. Albumin (A)

Table 1: Proximate analysis of pelleted concentrate feed (% on a dry matter basis)

Pellet composition (%)		Chemical analysis**	
Berseem hay	30.0	Crude protein (%)	17.5
Yellow corn	25.0	Crude fiber (%)	14.0
Wheat bran	26.2	Crude fat (%)	2.7
Soybean meal	14.0	Nitrogen free extract	56.4
Molasses	3.0		
CaCl ₂	1.0		
NaCl	0.4		
vitamin and mineral mixture.*	0.3		
Methionine	0.1		

*The vitamin and mineral premix/kg contained the following IU/gm for vitamins or minerals: A-4,000,000, D3-5000,000, E-16,7 g, K-0.67 g, B1-0.67 g, B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantoic acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.).

**The chemical analysis of the pellets (AOAC, 1990).

concentrations was determined by the method of Doumas *et al.* [18]. Total lipids and Total cholesterol were measured according to Frings *et al.* [19] and Richmond [20], respectively. Glucose concentration was determined by the method of Trinder, [21]. The activities of seminal plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel [22]. For assaying acid phosphatase (AcP) activity, the method of Moss [23] was used. Alkaline phosphatase (AIP) activity was measured at 405 nm by the formation of para-nitrophenol from para-nitrophenylphosphate as a substrate [24].

Blood samples were withdrawn from the ear's vein from each animal under each treated group. Blood samples were centrifuged at 3500 rpm for 20 min to obtain plasma and stored at -20 °C until later analysis. Testosterone concentration in plasma was measured using immunoassay commercial kit [25].

Histo-pathological examinations for testes were done by using three males from each group which scarified by slaughter at the end of the treatment period. Testes were dissected and fixed in Bwan staining solution for at least two days. Paraffin processing machine, ten microns thickness sections, standard hematoxylin and eosin stain were used for paraffin sections [26].

Data were analyzed as a complete randomized design [27] using the general linear model procedures of SAS [28]. Significance of the effects was tested at level $p < 0.05$ (*) and $p < 0.01$ (**) with the appropriate F statistic. Duncan's multiple range tests was used to detect any significant differences among the experimental means [29].

RESULTS AND DISCUSSION

Semen Characteristics: Data of semen ejaculate volume (EV), initial hydrogen ion concentration (pH), reaction time (RT), sperm concentration, total sperm output (TSO), sperm motility (%), total motile sperm per ejaculate (TMS), total motile normal sperm (TMNS) and live, dead and abnormal sperm of rabbits injected subcutaneously with 0.2 ml/kg of ethanol-sesame oil solution containing 0, 200, 400 or 800 µg of GA₃ /kg B.W./week for 12 weeks are presented in Table (2). Results showed that treated male rabbits with Gibberillic acid (GA₃) at any dose was resulted a significant increase ($P < 0.05$) in their semen ejaculate volume compared to the control group and this increase was necessary for sperms to provide them by nutrient elements that are needful for their live. Since, the increase semen ejaculate volume was correlated with a significant increase in sperm concentration (SCon.) and

total sperm out-put (TSO). Within the GA₃ treated groups it can be noticed that the effect of the medium dose (400 µg GA₃) was significantly higher than the other two doses, while the high dose (800 µg GA₃) was the most decline. These results are in corresponding with the result of Elkomy [12], who found that treated adult cocks with GA₃ conducted to a significant increase in EV and S Cons and this increase was correlated with increase in serum testosterone level.

There was a gradual and significant increase in sperm motility (%) due to treated bucks with GA₃ compared to the control group and this effect was GA₃ doses dependent manner. Increasing sperm motility in the GA₃ doses due to that GA₃ may be activated enzymes controlling the flagellar system. Similar results found by Elkomy [12], he reported that sperm motility was increased significantly in adult cocks, which treated with GA₃.

A significant decrease effect ($P < 0.05$) on the percentage of dead and abnormal sperm resulted in treated bucks with GA₃ doses were found and this effect was evident with the highest dose. Increase percentage of motile sperm and decrease the percentage of dead and abnormal sperm in the GA₃ treated groups were reflected on increase live sperm that showed a gradual and significant increase with increase GA₃ dose. Increase percentage of live sperm and decrease percentage of dead and abnormal sperm in the GA₃ groups may be attributed to increase the activation of spermatogenesis in seminiferous tubules to produce a complete sperm due to effect of GA₃ on testicular germinal epithelium in seminiferous tubules, which are responsible for the formation of complete sperm.

Improvement percentage of live and motile sperm, which happened in the GA₃ groups, were conducted to a significant increase in Percentage of total motile sperm per ejaculate (TMS) and total motile normal sperm (TMNS) compared to the control male rabbits. Increase TMS and TMNS means in The medium groups, which treated with 400 µg GA₃ than the other GA₃ groups was due to increase its EV and sperm concentration, while, the high dose (800 µg GA₃) had the lowest means for these measurements.

Increase sperm concentration and sperm motility were influenced seminal plasma hydrogen ion concentration. Since, the seminal plasma pH was significantly decreased in treated groups compared to untreated group and these differences may be due to increase sperm metabolite activities, which affected on seminal plasma hydrogen ion concentration.

Table 2: Effect of treatment male rabbits with different doses of GA₃ on semen ejaculate volume (EV), PH, reaction time (RT), sperm concentration (SCon), total sperm output (TSO), sperm motility (MOT), total motile sperm (TMS), total motile normal sperm (TMNS), live, dead and abnormal sperm (X±SE)

Parameters	GA ₃ doses (µg / kg body weight/ week)			
	Control	200	400	800
EV (ml)	0.62±0.07 ^c	0.79±0.09 ^b	0.87±0.10 ^a	0.76±0.09 ^b
pH	8.00±0.94 ^a	7.79±0.92 ^b	7.42±0.87 ^c	7.50±0.88 ^c
RT (Sec)	15.40±1.82 ^a	8.05±0.95 ^c	9.19±1.08 ^b	9.51±1.12 ^b
S Con (X*10 ⁶ /ml)	295.0±34.8 ^c	359.6±42.4 ^a	363.5±42.8 ^a	318.1±37.5 ^b
TSO (X*10 ⁶ /ml)	182.5±21.5 ^d	286.0±33.7 ^b	315.6±37.2 ^a	241.2±28.4 ^c
sperm motility (%)	72.3±8.52 ^c	79.1±9.32 ^b	79.5±9.37 ^b	84.9±10.00 ^a
TMS (X*10 ⁶ /ml)	132.1±15.57 ^d	227.8±26.80 ^b	252.1±29.70 ^a	205.6±24.23 ^c
TMNS (X*10 ⁶ /ml)	106.6±12.56 ^d	204.4±24.09 ^b	219.5±25.86 ^a	185.2±21.82 ^c
Live sperm (%)	73.3±0.49 ^d	83.38±0.41 ^c	85.5±0.65 ^b	87.4±0.46 ^a
Dead sperm (%)	26.7±3.15 ^a	16.6±2.34 ^b	14.5±2.29 ^c	12.7±1.49 ^d
Abnormal sperm (%)	19.4±2.29 ^a	10.4±1.23 ^c	12.9±1.53 ^b	9.8±1.16 ^c

^{abc} means the same row have the different superscript are significantly different at (p≤ 0.05)

Table 3: Effect of treatment male rabbits with different doses of GA₃ on seminal plasma: glucose, total protein, albumin, urea, high density lipoprotein (HDL), cholesterol and total lipids (X±SE)

Parameters	GA ₃ doses (µg / kg body weight/ week)			
	Control	200	400	800
Glucose (mg/dl)	62.1±2.17 ^a	50.4±0.89 ^b	42.9±1.80 ^c	38.1±1.65 ^d
Total protein (g/dl)	4.9±0.07 ^b	5.1±0.07 ^b	6.4±0.12 ^a	5.0±0.12 ^b
Albumin (g/dl)	3.0±0.03 ^c	3.1±0.04 ^b	3.7±0.05 ^a	3.0±0.03 ^c
HDL (mg/dl)	12.0±0.15 ^c	19.3±1.16 ^a	21.2±0.77 ^a	14.3±0.35 ^b
Cholesterol (mg/dl)	116.9±2.86 ^c	154.3±2.15 ^a	158.6±3.75 ^a	128.8±2.16 ^b
Urea (mg/dl)	40.2±0.71 ^a	33.2±0.47 ^c	32.8±0.45 ^c	36.8±0.34 ^b
Total Lipids (g/dl)	255.0±12.91 ^d	351.2±9.20 ^b	386.1±13.41 ^a	301.2±11.05 ^c

^{abc} means the same row have the different superscript are significantly different at (p≤ 0.05)

From Table 2 it can be seen that there was a decline in reaction time (increase Libido) due to treated bucks with GA₃ at any dose compared to control group. The previous effect may be due to increase testosterone hormone level for that the treated rabbits, which had a higher, level from testosterone hormone than the control rabbits. Also, the medium GA₃ dose had the lowest RT that correlated with the highest testosterone level than the other doses. This result was corresponding with that of Elkomy *et al.* [30] they reported that there was a relationship between increase fertility and testosterone hormone level and increase libido in male rabbits. From Table 2 it can be concluded that treatment with medium dose of GA₃ showed the best results in the most of the pervious semen characteristics compared to low and high doses.

It is clear from the present results that the administration of gibberillic acid improved semen characteristics and has positive effect on semen quality

and quantity. These observations refer to that GA₃ may be caused stimulating and supporting spermatogenesis process in seminiferous tubules to produce spermatozoa and also, sex accessory glands to secrete seminal plasma. Our results are in agree with that of Elkomy [12] how reported that in adult cockerels GA₃ may be have a direct testosterone like action on testes or/ and stimulate activation of their testicular germinal epithelium in seminiferous tubules.

Seminal Plasma Characteristics: Effect of injected rabbits with different doses of Gibberellic acid for 12 weeks on some seminal plasma constituents (glucose, total proteins, albumin, HDL, cholesterol, total lipids (TL) and urea) are presented in Table 3.

Seminal plasma glucose concentration showed a decrease (P < 0.05) in the GA₃ groups compared to the control group and this decrease was a GA₃ dose-dependent.

Group treated by medium GA_3 dose showed a significant higher seminal plasma total protein and albumin level ($P < 0.05$) than the other GA_3 doses or the control group, in spite of, there were no-significant differences were found between low and high doses GA_3 compared to the control group, the total protein was still higher in the GA_3 groups. It is known that seminal plasma proteins coat and protect spermatozoa during ejaculation. A positive relationship between increase seminal plasma total protein and albumin concentration and increase semen quality was found in the present study and this result was corresponding with Elkomy *et al.* [30], they reported that an increase in seminal plasma total protein and albumin concentrations were showed in high fertile male rabbits compared to low fertile rabbits and this increase was associated with increase their seminal quality measurements. Many studies have shown that low content of seminal plasma proteins is associated with poor semen quality [31, 32]. Taha *et al.*, [33] revealed that there was a positive relationship between semen quality and level of seminal plasma total proteins. Similar results were found by Osama and El-Sahn [34], they found a positive relationship between increasing seminal plasma total proteins and albumin and increasing total number of sperm output. Kulkarni *et al.*, [35] showed that, seminal plasma total protein is mainly composed of albumin and globulin, in addition to small quantities of nonprotein nitrogen, amino acids and peptides. these compounds make up the amphoteric property of seminal plasma proteins, thus, low protein content in seminal plasma reduce its buffering capacity and in turn semen quality [36].

A significant decrease ($P < 0.05$) in seminal plasma urea concentration was observed in the GA_3 treated groups at any dose compared to the control group. On the other hand, within the GA_3 groups, the highest dose of GA_3 had the highest mean in seminal plasma urea concentration when compared with the other GA_3 groups. Reduce urea concentration in the seminal plasma for GA_3 groups may be attributed to decrease transaminases activities, since, increase transaminases activities was related to amino acid imbalance that initiates protein catabolism [37]. And this decrease was reflected on increase seminal plasma total protein level for GA_3 groups.

Data showed that administration with low, medium and high doses of GA_3 increased ($P < 0.05$) seminal plasma HDL, cholesterol and total lipids concentration (Table 3) compared to the control and the highest effects were recorded for the medium dose then the low dose. Seminal plasma total lipids play important roles in the membrane

structure of spermatozoa, sperm metabolism, sperm capacitating and fertilization of the female gamete [38, 39]. In addition, some investigators Taha *et al.*, [33] and reported that the reductions in sperm concentration and motility were associated with a decrease in seminal plasma lipids content and also with sperm aging (poor semen quality). In the present study, a high level of total lipid and cholesterol, which resulted in GA_3 treatments (Table 3), was simultaneous with high values of sperm motility and sperm concentration for the same groups (Table 2).

There was a significant ($P < 0.01$) decreased in seminal plasma activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) due to treatment bucks with GA_3 (Table 4) compared to untreated bucks. The transaminases activities (AST and ALT) in semen are a good indicator of semen quality because it measures sperm membrane stability [40,41]. Boehnke *et al.* [42] found that among 22 different criteria used for evaluating heat stressed bulls, the least useful method were the determination of GOT activity. Also, the increments of the activities of AST and ALT in seminal plasma are mainly due to the leakage of these enzymes [43]. Yousef and Zeitoun [44] found that there were negative correlation coefficients between decrease sperm motility on one side and AST and ALT release on the other side. They reported that the activities of these enzymes could be used as an indicator of sperm integrity. Yousef *et al.* [45] reported that there was a negative correlation between increased ALT and AST activities and decrease ejaculate volume, sperm concentration, total sperm output, sperm motility index, total motile sperm, Therefore, the decrease in the activities of these enzymes coincided with the increase of semen quality. Similar results found by Elkomy *et al.* [30] and El-Sebiey *et al.* [46]. At the same time, the reduction seminal plasma total protein (Table 3) in the control group may be correlated with increase transaminase activities compared to the GA_3 treated group, whereas, Reson *et al.* [37] reported that increase transaminases activities were related to amino acid imbalance that initiates protein catabolism.

Seminal plasma alkaline phosphatase (ALP) and acid phosphatase (AcP) enzymes (Table 4) were recorded an increase in their activities in all groups that treated by GA_3 compare to the control group. And this increase was significant ($P < 0.01$) with AcP only. Previous studies showed that rabbit's seminal plasma contained a number of enzyme activities [45]. These enzymes play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism,

Table 4: Effect of treatment male rabbits with different doses of GA₃ on seminal plasma: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), acid phosphatase (AcP) and serum testosterone (X±SE)

Parameters	GA ₃ doses (µg / kg body weight/ week)			
	Control	200	400	800
ALT	17.2±0.87 ^a	10.6±0.52 ^c	10.7±0.62 ^c	14.3±0.52 ^b
AST	42.9±1.76 ^a	32.4±1.64 ^b	31.6±1.37 ^b	33.6±0.87 ^b
ALP	52.8±0.56 ^{N.S}	53.2±0.55 ^{N.S}	53.7±0.33 ^{N.S}	52.7±0.82 ^{N.S}
AcP	26.6±0.52 ^c	33.5±0.25 ^{a b}	33.8±0.42 ^a	32.7±0.33 ^b
Serum Testosterone	2.87± 0.68 ^a	1.97±0.61 ^{a b}	1.17±0.20 ^b	1.57±0.22 ^{ab}

^{abc} means the same row have the different superscript are significantly different at ($p \leq 0.05$).

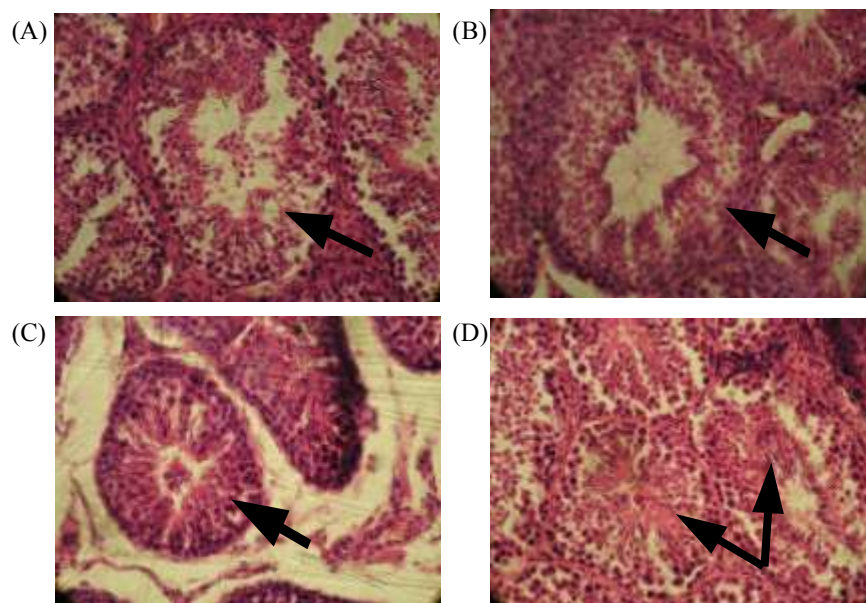


Fig. 1A,B,C,D: **A:** Cross section of testes from control group at the end of treatment period (H&E 400 X). **B:** Cross section of testis from bucks treated with 200 µg GA₃ (low dose) at the end of treatment period (H&E 400X). **C:** Cross section of testis from bucks treated with 400 µg GA₃ (medium dose) at the end of treatment period (H&E 400X). **D:** Cross section of testis from bucks treated with 800 µg GA₃ (high dose) at the end of treatment period (H&E 400X).

in fertilization process and in the maintenance of constant osmotic pressure during preservation [47]. The phosphatases enzymes in semen play an important role in transamination and phosphorylation processes in sperm metabolism and thus explain the differences observed in the semen quality [36]. Also, Kamel [48] found that, in rabbit groups, which have higher seminal quality index the AcP, activity was increased in their seminal plasma. Moreover, El-sebiey *et.al.* [46] found that the high fertile male rabbit strains had phosphatase enzymes activities higher than the low fertile strains. A similar result found

by Elkomy *et al.* [30] between the high and low fertile male rabbit under the same strain.

Results presented in Table 4 indicated that there was a significant effect ($P < 0.05$) of GA₃ treatment on decrease serum testosterone concentration in the treated groups compared to the control group. Furthermore, the medium GA₃ dose (400 µg GA₃) had significantly lowest mean for serum testosterone concentration than the other two GA₃ doses or the control group. In spite of, serum testosterone concentration was decreased significantly in the all GA₃ doses, the semen quality and quantity was

increased compared to control group. This improvement in semen characteristics may be due to the direct testosterone-like action of GA_3 , which inhibit the testosterone secretion and replacement the hormone in its action. Spermatogenesis process depends on the action of testosterone [49]. And testosterone is needed to initiate spermatogenesis at puberty and for the maintenance of this process in the adult. It also required for the compilation of meiosis and for the differentiation of the spermatids [50]. From our results it can be demonstrated that GA_3 may have a direct androgenic-like action on testes and / or stimulate activation of their testicular germinal epithelium in seminiferous tubules. The present results are in agreement with that of Elkomy [12] who mentioned that semen characteristics were improved, in spite of, serum testosterone concentration was decreased in cockerels, which treated with GA_3 and GA_3 may have an androgenic-like action on stimulate spermatogenesis process. As well as, Gawienowski *et al.* [8] Gawienowski *et al.* [11] and Elkomy *et al.* [13] have clearly demonstrated that, GA_3 possesses androgenic properties in male chicks based on the chicks comb bioassay.

Histological Examination for Control and Treated Rabbit's Testes: Histological examination of testes sections for control male rabbits revealed histological structures of the seminiferous tubules that were lined by spermatogenic epithelium with spermatids and sperms in the Lumina (Figures 1 A). However, testes sections for treated rabbit bucks that injected subcutaneously with 200 μg GA_3 (Figures 1 B), 400 μg GA_3 (Figures 1 C) or 800 μg GA_3 (Figures 1 D) revealed that GA_3 supports and stimulates completion of spermatogenesis in the seminiferous tubules that were lined by spermatogenic epithelium with all stages of spermatogenesis. The same results of GA_3 on cocks' testes was found by Elkomy [12], how reported that, treated cocks with GA_3 conducted to stimulate spermatogenesis process in the seminiferous tubules to produces complete sperm.

CONCLUSION

From our results, it can be concluded that, GA_3 has a direct androgenic-like action on testes in male rabbits and it has a positive effect on semen quality and quantity. Since, GA_3 may be caused stimulating and supporting spermatogenesis process in seminiferous tubules to produce complete spermatozoa and also, sex accessory glands to secrete seminal plasma.

REFERENCES

1. Riley, J.M., 1987. Gibberellic acid for fruit set and seed germination. CRFG Journal, 19: 10-12.
2. Seetharim, A. and P. Ksuma-Kumari, 1975. Induction of male sterility by gibberellic acid in sunflower. Indian J. Genet. Breed., 35: 136-138.
3. Aswathanarayana, S.C. and M. Mahadevappa, 1977. Determination of optimum stage of gametocyte application in inducing pollen sterility for production of hybrid rice. Mysore J. Agric. Sci., 25(3): 284-287.
4. El-Mofty, M.M., S.A. Sakr, A.M. Rizk and E.A. Moussa, 1994. Carcinogenic effect of gibberellin A3 in Swiss albino mice. Nutr. Cancer, 21(2): 183-190.
5. Alkhiat, A.A., H. Morsy, E. Shehata and A. Abdellatif, 1981. Veterinary pharmacology and toxicology. Ministry of High Education and Scientific Research. Iraq.
6. Madacsi, J.P., F.W. Parrish and J.L. Mc Naughton, 1988. Anim. Feed Science and Technol., 20: 69.
7. Abd-Elhamid, A.M., T.M. Dorra, M.A. Ali and E.H. Abuo-Egla, 1994. Effect of gibberellic acid on broiler chickens performance and some metabolic parameters. Arch. Anim. Nutr., 46: 269-276.
8. Gawienowski, A.M., S.S. Stadnicki and M. Stacewicz-sapuntzakis, 1977a. Synergistic uterotrophic effect of gibberellic acid and estradiol in the immature mouse. Life Sciences, 20: 785-788.
9. Gawienowski, A.M. and D. Chatterijee, 1980. Effect of prostaglandin inhibitor on the uterotrophic response of estradiol and gibberellic acid. Life Sciences, 27: 1393-1396.
10. Bouton, C., M. Maillet and M.J. Feintuch, 1969. C.R.Soc. Biol., 163: 1484-1487.
11. Gawienowski, A.M., S.S. Stadnicki and M. Stacewicz-sapuntzakis, 1977b. Androgenic properties of gibberellic acid in the chicks comb bioassay. Experientia, 33: 1544-1545.
12. Elkomy, A.E., 2003. Physiological studies on Gibberellic acid (GA_3) and reproductive functions of adult fowl. Ph.D. thesis. Faculty of Agriculture, Alexandria University.
13. Elkomy, A.E., Samar A. Elnagar and Azza El-Sebai, 2007. Steroidogenic effects of gibberellic acid (GA_3) on chicks. Egypt Poult. Sci., 27(4): 1239-1255.
14. Tesh, S.A. and J.M. Tesh, 1971. Artificial insemination in the rabbit and its use in routine teratogenic studies. Excerpta medica (Amsterdam) Intl. Congers Series, 220: 332-336.

15. Smith, J.T. and D.T. Mayer, 1955. Evaluation of sperm concentration by the haemocytometer method. *Fertil.*, 6: 271-275.
16. Blom, E., 1950. A one-minute live-dead sperm stain by means of eosin-nigrosin. *J. Fertil. Steril.*, 1: 176-177.
17. Henry, R.J., D.C. Cannon and W. Winkelman, 1974. *Clinical chemistry principles and techniques*, 11th ed. Happer and Row Publishers, pp: 1629.
18. Doumas, B.T., W.A. Watson and H.G. Biggs, 1977. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinic. Chem. Acta.*, 31: 87-96.
19. Fringes, C.S., T.W. Fendly, R.T. Dunn and C.A. Queen, 1972. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. *Clin. Chem.*, 18: 673-674.
20. Richmond, W., 1973. Colorimetric method for the determination of plasma cholesterol. *Clin. Chem.*, 19: 1350-1356.
21. Trinder, P., 1969. *Ann. Clin. Biochem.*, 6: 24.
22. Reitman, S. and S.A. Frankel, 1957. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clinic. Pathol.*, 28: 56-63.
23. Moss, D.W., 1984. In: *Methods of Enzymatic Analysis*, 3rd Ed. Bergmeyer, H.U., Ed., Verlag-Chemie, 4: 92-106.
24. Principato, G.B., M.C. Asia, V. Talesa, G. Rosi and E. Giovannini, 1985. Characterization of the soluble alkaline phosphatase from hepatopancreas of *Squilla mantis* L. *Comp. Biochem. Physiol.*, B80: 801-804.
25. Biosource-Europe S.A. 8, rue de L'Industrie.B-1400 Nivelles. Belgium.
26. Steven, A., 1977. The hematoxyline in theory and practice of histological techniques. J.D. Bancroft, A. Steven and I.M.P. Dauson (eds). Churchill living stone, Edinburgh., pp: 85-94.
27. Steel, R.G.D. and J.H. Torrie 1980. *Principle and procedures of statistics. A Biochemical Approach* (2nd Ed.) Mc Graw-Hill Book Company, New York, USA.
28. SAS. *SAS User's Guide: Statistics*, version 5 Edition SAS Inst., Inc., Cary, NC, USA, 1986.
29. Duncan, D.B., 1955. Multiple ranges and multiple F testes. *Biometrics*, 11: 1-42.
30. Elkomy, A.E., M.E. El-Seiby and K.I. Kamel, 2008. Comparative study of semen quality and free amino acid content in seminal plasma between high and low motile sperm rabbit bucks. *Egypt. Poult. Sci.*, 28(II): 633-649.
31. Ashworth, P.J., R.A. Harrison, N.G. Miller, J.M. Plummer and P.F. Watson, 1994. Survival of ram spermatozoa at high dilution: protective effect of simple constituents of culture media as compared with seminal plasma. *Reprod. Fert. Dev.*, 6:173-180.
32. White, I.G., P. Goh and J.K. Voglmayr, 1987. Effect of male reproductive tract fluids and proteins on the metabolism and motility of ram spermatozoa. *Arch. Androl.*, 19: 115-125.
33. Taha, T.A., E.I. Abdel-Gawad and M.A. Ayoub, 2000. Monthly variations in some reproductive parameters of barki and awassi rams throughout 1 year under subtropical conditions. 2- Biochemical and enzymatic properties of seminal plasma. *Anim. Sci.*, 71: 325-332.
34. Osama, M. Aly and A. El-Sahn, 2006. Effect of crossing on the performance of local strains. 3. Seminal quality, electrophoretic pattern of seminal plasma proteins, fertility and hatchability in Bandara, Gimmizah and their reciprocal crosses. *Egypt. Poultry Sci.*, 26: 123-136.
35. Kulkarni, B.A., S.G. Dhamds and D.D. Patankar, 1996. Seminal plasma protein profiles of Murrah and surti buffalo bulls. *Ind. J. Anim. Sci.*, 66: 900-903.
36. Dhami, A.J., K.L. Sahni, G. Mohan and R.P. Tripathi, 1994. Comparative evaluation of initially static and motile semen ejaculate from Friesian and Murrah buffalo bulls for physico-morphological, biochemical, enzymatic and mineral constituents of seminal plasma. *Indian J. Animal Sci.*, 64: 926- 932.
37. Reson, F., N.R. Roberts, L.F. Buchnick and G.A. Nichol, 1958. An enzymatic basis for the gluconeogenic action of hydrocortisone. *Sci.*, 127: 289.
38. Demirci, E., 2002. Evcil Hayvanlarda Reprodüksiyon, SunÖi Tohumlama ve Androloji Ders NotlarÝ. F... Vet. Fak. Ders Teksiri, No: 53, ElazÝÚ.
39. Hafez, E.S.E., 1987. *Reproduction in Farm Animals*. Lea & Febiger, Philadelphia.
40. Kelso, K.A., A. Redpath, R.C. Noble and B.K. Speake, 1997. Lipid and antioxidant changes in spermatozoa and seminal plasma throughout the reproductive period of bulls. *J. Reprod. Fertil.*, 109: 1-6.

41. Zedda, M.T., P.P. Bini, S. Pau and U. Sbernardori, 1996. Constituents of seminal plasma and blood serum of the ram. *Bull. Soc. Ital. Biol. Sper.*, 72: 227-230.
42. Boehnke, H.J., U. Schlüter, H. Marré and J. Pfeilsticker, 1978. Sperma of reproduction bulls from the biological and economical aspects. *Dtsch Tierarztl Wochenschr.* May 5, 85(5): 160-4. German.
43. Navarro, C.M., P.M. Montilla, A. Martin, J. Jimenez P.M. and Utrilla, 1993. Free radicals scavenger and antihepatotoxic activity of rosmarinus. *Plant Med.*, 59: 312-314.
44. Yousef, M.I. and M.M. Zeitoun, 1998. Bovine ovarian follicular fluids modulate the release of transaminases, acrosome reaction and motility of the rabbit's sperm in vitro. *Alexandria J. Agric. Res.*, 43: 17-26.
45. Yousef, M.I., G.A. Abdallah and K.I. Kamel, 2003. Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Animal Reproduction Sci.*, 76: 99-111.
46. El-Seiby, M.E., A.E. Elkomy and K.I. Kamel, 2008. Evaluation of spermatological parameters and free amino acids composition of Black Baladi, New Zeland White and V-Line rabbits buck under winter egyptian condition. *Egypt. Poult. Sci.*, 28(2): 617-631.
47. Dhami, A.J. and S.B. Kodagali, 1987. Correlation between biochemical and enzymatic constituents of semen of Suerti buffalo bulls. *J. Anim. Sci.*, 57: 1283-1286.
48. Kamel I. Kamel, 2005. Effect of folic acid on semen quality and biochemical parameters of rabbits. *Egypt. Poult. Sci.*, 25: 373-394.
49. Sharpe, R.M., K. Donachie and I. Cooper, 1988. Re-evaluation of the intratesticular level of testosterone required for quantitative maintenance of spermatogenesis in the rat. *J. Endocrinology*, 117: 19-26.
50. Poccia, D., 1994. Intercellular signaling systems. In: Poccia D (Editor), *Molecular Aspects of Spermatogenesis*. R.G. Landes Company, Austin, TX, USA.