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# EFFECT OF AGE, AND VITAMIN E AND SELENIUM ADMINISTRATION ON SEMEN CHARACTERISTICS OF NEW ZEALAND WHITE RABBIT BUCKS

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**ABSTRACT:** The aim of this study was to investigate the effect of age and supplementation with vitamin E and selenium combination on semen characteristics of New Zealand White (NZW) rabbit bucks. Thirty six NZW rabbit bucks were divided into three equal experimental groups according to their age. Group 1: young age (4-5 months), Group 2: middle age (9-10 months) and Group 3: old age (20-24 months). Each group was divided into two subgroups (treatment and control).Treated group was subcutaneously injected with 50 mg vitamin E and 0.1 mg selenium per kg live body weight, while the other was given saline solution and served as control. Experimental animals were injected once a week for 12 weeks. After six weeks of treatments, semen was collected weekly for six successive weeks. Reaction time was recorded; semen characteristics and plasma testosterone were estimated. Results showed that the young age group had significantly ($p<0.05$) lower sperm cell concentration/ml, total motile sperm/ml and insignificantly lower initial motility and sperm viability than those recorded in other ages. The middle age group had significantly ($p<0.05$) higher whole ejaculate volume than other groups. Also, they had significantly ($p<0.05$) higher sperm membrane integrity and significantly ($p<0.05$) lower fructose level than those recorded in the old age group, but not significant with the young age group. Animals treated with Vit. E and selenium showed significantly ($p<0.05$) improvement in most semen quality traits compared with control animals. Averages of sperm viability, motility, sperm concentration, total motile sperm per ml, ejaculate volume, sexual libido and sperm membrane integrity were significantly higher in bucks received Vit. E and selenium than those recorded in control bucks. In addition, best results were observed in the middle age group, meanwhile, semen characteristics of young and old rabbit bucks did not decrease dramatically and still in acceptable range. It could be concluded from our study that young and middle age bucks with supplementing with Vit. E and selenium could be used successfully and efficiently in rabbit breeding systems.

**Key words:** Age - Vitamin E Selenium - Semen Quality - Testosterone - Rabbit Bucks

## INTRODUCTION

Environmental conditions and age of animals are two important factors affecting productive and physiological performance of rabbits (Askar and Ismail, 2012 and Khalil et al., 2014). Vitamin E is an important antioxidant, it is a free radical scavenger on the cell membrane (Mahmoud et al., 2013). Moreover, selenium constitutes a necessary part of glutathione peroxidase an enzyme responsible for protecting cell internal structures from free radicals and is considered an antioxidant for cellular membrane lipids (Gutierrez et al., 2008).

Many studies reported that Vitamin E and selenium have a synergistic effect when used together, and they affect many biological processes including reproduction (Koyuncu and Yerlikaya, 2007), metabolism (Awadeh et al., 1998), spermatogenesis and semen quality and protecting against oxidative stress (Yousef et al., 2003). Mahmoud et al. (2013) found that combination of Vitamin E and selenium improved semen characteristics and the reproductive performance in Ossimi rams. It is well known that semen quality is reduced with increasing age of rabbit bucks, because of degradation of semen quality with increasing age lead to decrease in fertility, conception rates and the productivity of the does (Cherfaoui et al., 2013). Also, Tanemura et al. (1993) reported that there was narrowing and sclerosis of the tubular lumen, a decrease in spermatogenic activity, degeneration of germ cells, and decreased number and function of Leydig cells with increasing age of male mouse.

Therefore, the objectives of the present study were to investigate the effect of buck's age on some physical and biochemical semen characteristics, and to study the impact of vitamin E and selenium administration in different ages of NZW rabbit bucks on semen traits.

## MATERIALS AND METHODS

### Animals and experimental design

This experiment was carried out at the Rabbitry Experimental Farm belonging to Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Thirty six New Zealand White rabbit bucks were used in this study. All animals were healthy and free of any external parasites or skin diseases and they were kept continuously under the same managerial and environmental conditions during the whole experimental period. Animals were divided into three equal experimental groups (12 bucks each) according to age. The 1$^{st}$ group aged from 4 to 5 months (young age), the 2$^{nd}$ group aged from 9 to 10 months (middle age) and the 3$^{rd}$ group aged from 20 to 24 months (old age). Each main group was divided into two subgroups (6 bucks each); one subgroup from each group was subcutaneously injected with 50 mg vitamin E and 0.1 mg selenium per kg live body weight weekly through the experimental period, while the other group was injected with saline solution and considered as control. All bucks were individually housed in galvanized wired cages, where feed and water were provided *ad libitum*. The diet contained 17% crude protein, 2.8% fat, 10% crude fiber and 2600 KCal digestible energy/kg diet. Lighting system was 16 hrs light/8 hrs dark in the rabbitry during all experimental period. The experimental period extended for 3 months during summer season 2015.

Ambient temperature and relative humidity inside the rabbitry were recorded daily during the experimental period by using digital thermo-hygrometer equipment. The temperature–humidity index (THI) was estimated according to thermal comfort level of an animal environment according to Marai et al. (2002) for rabbit is a function of temperature and relative humidity of the enclosure. It was measured according to the following equation: THI = dbºC- [(0.31 - 0.31 RH) (dbºC - 14.4)],

where dbºC = dry bulb temperature in Celsius and RH = RH%/100. The values obtained are then classified as absence of heat stress during experimental period.

**Studied traits**

**Semen collection and evaluation:** After five weeks from treatment, bucks were trained for semen collection by artificial vagina. Data were collected after the six$^{th}$ week of treatment, the period required to complete spermatogenesis process in rabbit bucks. Semen was collected weekly from each buck in each group for successive six weeks. Time of sexual libido (TSL) was measured in terms of reaction time in seconds and was estimated between introducing the teaser doe to the buck's cage up to the point, when the buck started to mount the doe and give ejaculation (Khalil et al., 2015).Semen characteristics were estimated immediately after collection in each semen sample such as; semen whole ejaculate volume (with gel), net ejaculate volume (without gel), initial motility (IM), sperm cell concentration/ml (SCC/ ml), total motile sperm/ ml (TMS/ ml), sperm viability % (SV), sperm membrane integrity %(SMI) and initial fructose (IF, mg/dl).Sperm motility percent was estimated microscopically at 600X to the nearest 5%. A drop of 10 µl of semen was delivered onto a clean glass slide and covered with a coverslip (Roca et al., 2000). The percentages of total sperm motility were estimated after viewing five microscopic fields. Percentage of sperm viability was measured by using the live/dead stain (Eosin-Nigrosine-based) and phase contrast microscope. Percentage of live/dead spermatozoa was estimated by counting the number present in 100 sperm in different fields. Sperm Cell Concentration was assessed spectrophotometrically according to Castellini et al. (2007).Sperm membrane integrity percent (SMI %) was assessed according to Hypo-Osmotic Swelling Testby observing the response of sperm cell to hypotonic medium (Jeyendran et al., 1984). The hypo-osmotic solution (150 m Osm/l) was prepared by dissolving 7.35g sodium citrate and 13.51g fructose in 100 ml of distilled water. The solution was stored at 4ºC till used. A volume of 10µl of undiluted semen was gently mixed in each of the 2 ml hypo-osmotic solution and incubated at 37ºC for one hour. Number of swollen sperm was counted by placing a drop of incubated semen suspension on a glass slide, covered with a coverslip and examined under a lightmicroscope at 600X magnification. At least 100 sperm cells on each slide were counted randomly. Initial fructose was measured by a colorimetric methodas described by Foreman et al. (1973).

**Plasma testosterone concentration:** Blood samples were collected from each animal from the ear vein in heparinized tubes between 8:00-10:00 am. Blood samples were collected at 6 and 12 weeks from beginning of experiment. Plasma was obtained by blood centrifugation at 3000 rpm for 20 min. and stored at -20ºC until analysis. Plasma testosterone was analyzed by ELISA kits manufactured by DiaMetra, Spello-Perugia, Italy.

**Statistical Analysis:** Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 2004). Differences among means were detected using Duncan's new multiple test (Duncan, 1955). Two-way analysis of variance was carried out for all traits using the following model:

$$Y_{ijk} = \mu + A_i + T_j + AT_{ij} + e_{ijk}$$

Where:

$Y_{ijk}$ = Observation on the $k^{th}$ individual from the $i^{th}$ age in $j^{th}$ treatment

$\mu$= Overall mean

$A_i$= Fixed effect of the $i^{th}$ age

$T_j$ = Fixed effect of the $j^{th}$ treatment

$AT_{ij}$ = Interaction between $i^{th}$ age and $j^{th}$ treatment

$e_{ijk}$ = Random error associated with the $ijk^{th}$ individual

## RESULTS

**Temperature-humidity index (THI) during Experiment:** Collected metrological data inside rabbitry showed averages of ambient temperature ($^oC$), relative humidity (%) 28.52 and 50.91 respectively. According to Marai et al. (2002) equation for calculation of temperature humidity index (THI), it was found to be 26.37 THI for the present experiment. Marai et al. (2002) stated that THI value below 27.8 in rabbits is considered absence of heat stress.

**Semen quality traits:**

Results showed significant differences among treatments and their interactions in most studied traits (Tables 1 and 2). Sexual libido showed inferior ($p<0.001$) results in the old age group compared with other groups. The middle age group had significantly ($p<0.001$) higher whole ejaculate volume than other groups and significantly ($P<0.01$) higher net ejaculate volume (0.51 ml) than the young age (0.36 ml) but not significant with the old age (0.45 ml). However, the young age had significantly lower SCCML, TMSML and TSL, and insignificantly lower IM% and SV% than those recorded in other ages. The middle age had insignificantly higher SMI % and significantly ($p<0.01$) lower fructose level than those recorded in the old age, but not significant with the young age. The young males had significantly higher ($P<0.05$) plasma level of testosterone (354.51 ng/dl) than the middle age group (242.55 ng/dl) and insignificant with the old age group (283.85ng/dl).

Treating bucks with Vit. E and selenium improved significantly most semen quality traits compared with control animals. Average of IM%, SV%, SCCML, TMSML and SMI% (74.02, 86.9, 369.71, 278.36 and 91.86, respectively) were significantly higher in bucks treated with Vit. E and selenium than those recorded in control bucks (65.18, 81.15, 261.84, 175.6 and 89.87, respectively). Moreover, Bucks received Vit. E and selenium insignificantly improved net and whole ejaculate volume and fructose level compared with control group. Moreover, treated animals showed insignificantly shorter TSL and lower plasma testosterone level than those recorded in control animals (Table 2).

In this context, the interactions among treatments had significant effects on all studied traits. The old control bucks had significantly ($p<0.01$) delayed TSL compared with all experimental groups except with the old treated bucks. The highest net and whole ejaculate volume, IM%, SV%, SCCML, TMSML and TMSEJ were obtained in the middle treated animals (0.52, 0.83, 76.59, 88.64, 392.88, 305.91 and 215.47, respectively) but the lowest values were recorded (0.35, 0.41, 0.64, 81.44, 230.57, 155.45 and 92.32, respectively) in the young control animals (Table 2). The highest SMI % and fructose level were estimated in the old treated males (93.45 and 247.16, respectively) but the lowest values were recorded in both the young control and the middle control bucks (88.77 and 225.65, respectively), respectively. Moreover, the

highest plasma testosterone was estimated in the young control males (358.05 ng/dl) but the lowest value was obtained in the middle treated males (220.83 ng/dl).

## DISCUSSION

The rabbit's farm profitability depends on the males and females fertility. Cherfaoui et al. (2013) stated that because of the fertilizing capacity of semen, the male can influence fertility, conception and the productivity of the rabbit does. Ebeid (2009) proved that Vitamin E and selenium keeping of male fertility by development of spermatozoa and maturation in the epididymis and viability of sperm to complete the fertilization process. In the present study, we investigated the effect of age and administration of vitamin E and selenium on some semen characteristics and testosterone concentration of NZW rabbit bucks. The results showed clearly that age of rabbit bucks had significant effect on most of semen characteristics. The young males had significantly higher plasma level of testosterone (354.51 ng/dl) than the middle age males (242.55 ng/dl) and insignificant with the old males (283.85 ng/dl), consequently, they had the highest sexual libido (TSL) than that observed in other ages. However, bucks of the middle age had significantly higher value whole ejaculate volume (0.73 ml) than those obtained from other groups (0.42 ml and 0.49 ml for young and old, respectively). Also, they had significantly lower fructose level (201.32 mg/dl) than those recorded in the old age (245.66 mg/dl), but not significant with the young age (22.98 mg/dl). The results are in agreement with Cherfaoui et al. (2013), who recorded that age of male affected aversely semen characteristics in rabbit.

In fact, degradation of semen quality with the increase of male age leads to decrease in fertility. Many studies suggested that there are some mechanisms of how male age could affect semen quality. Parkening et al. (1988) showed in mice that semen volume decreased by the advanced age and this may be attributed to the seminal vesicles insufficiency. Also, Christopher et al. (2003) reported that increasing male age may affect the epididymal functions which adversely affect sperm motility. So, with increased male age there is narrowing and sclerosis of the tubular lumen, a decrease in spermatogenic activity and decreased number and function of Leydig cells in mouse as described by Tanemura et al. (1993). In addition, Kathleen and Cliff (1984) reported that there was an inherent loss in steroidogenic function of the rat testis in aging. The old rats (25 months) exhibited reduced testosterone levels than in the middle-aged (10 months). Sperm output (total motile sperm/ml) was higher in middle group (adult bucks) than the young and old bucks. This may be due to testicle size are still developing in the young buck and have reached maturity in the adult. Otherwise, the advance in age of bucks, testicular tissues may be broken down faster than being replaced (King, 1993).

The present results clearly confirmed that using combination of vitamin E and selenium led to improvement semen characteristics for bucks of NZW rabbit under experiment conditions. Results indicated that bucks treated with vitamin E and selenium had significantly higher in SV%, IM %, SCC/ml, TMS/ml and SMI % than those recorded in control bucks. However, slightly decreased testosterone level in treated group, time of sexual libido was faster in treated animals than those recorded in control animals. These results agreed with El-Sheshtawy et al. (2014) results, who recorded that administration of vitamin E and selenium complex had markedly improved the sexual desire in buffalo-bulls. Our results are in agreement with the many previous studies which recorded improvements in semen characteristics and reproductive performance of farm animals when treated with vitamin E and selenium in vivo or in

vitro such as; in rabbit (Yousef, 2010) in cattle (Awadeh et al., 1998) in buffalo-bulls (El-Sheshtawy et al., 2014), in ossimi rams (Mahmoud et al., 2013), in boars (Horky et al., 2012). These improvements in males performance reflect the physiological role of vitamin E and selenium to increase performance of male reproductive system and female productivity especially under heat stress conditions by many suggested points: 1) Antioxidant effect for testes and spermatozoa. Vitamin E is a free radical scavenger on the cell membrane (Yousef, 2010) and metabolic functions of selenium are closely linked to vitamin E which not only protects biological membranes from oxidative degeneration but also it constitutes a necessary part of glutathione peroxidase an enzyme responsible for protecting cell internal structures from free radicals and is thus considered an excellent antioxidant for cellular membrane lipids (Dhingra et al., 2004). Therefore, vitamin E and selenium provide biological stability to the spermatozoal plasma membrane (Gutierrez et al., 2008). Treatment with vitamin E led to inhibited hydrogen prodexide as a reactive oxygen metabolites (ROMs) in seminal plasma by 80% compared to control group in growing rabbit bucks (Cesare et al., 2003). 2) Vitamin E and selenium provide the protection to seminiferous tubules of the testes, and they are essential for the germ cells and Sertoli cell development in testes, also, they have positive effects on the number of germ cells in adults (Abdel-Hasseb et al., 2004). 3) Vitamin E and selenium stimulates Leydig cells to testosterone biosynthesis of testes by stimulating the anterior pituitary hormones secretion, and increase testicular cholesterol content (Abdel-Hasseb et al., 2004) and increase the level of testicular zinc content (Abdel-Hasseb et al., 2004) and zinc play an important role in enhancement of testosterone retention within the testes (Kellokumpu and Rajaniemi, 1981). 4) Vitamin E and selenium are involved in the synthesis and production of prostaglandins (Ahmed et al., 2001). Prostaglandin F2$\alpha$ administration has increased the number and motility of spermatozoa in farm animals (Hafs et al., 1974). 5) Selenium acts as selenocysteine; an amino acid that is present in several enzymes; which is a co-factor for hepatic enzyme type 1, 5-Iodothyronine-deiodinase and increases the ability of deiodination of $T_3$&$T_4$ and increases the ability to degrade $rT_3$ (Dhingra et al., 2004).

The present study showed significant effects due to the interactions among treatments on all studied traits. Brown and Arthur (2001) suggested that selenium is important during early testicular development. This may explain results of our study regarding the quick response observed in the highest net and whole ejaculate volume, IM%, SV%, SCML, TMSML and TMSEJ obtained in the middle age treated animals but the lowest values were recorded in the young control animals. Cesare et al. (2003) recorded that the reactive oxygen metabolites (ROMs) level increased with increasing age of bucks. This increment with ageing is probably due to a physiological increase of the anabolic processes (Zarhevsky and Reznick, 1998) which may explain the inferior results in the old age group in our study.

Generally, control animals in each age group were higher but not significant in testosterone level than their counterparts. The reason for this observation is not clear but could be due differences in spermatogenic cycle between age groups and also to sampling time distance (6 and 12 weeks). Moreover, the highest plasma testosterone was estimated in the young control males but the lowest value was obtained in the middle treated males. This reduction in plasma testosterone level in middle treated males may be due to a shift occurred in testosterone from the blood to important tissues of the testes.

## CONCLUSIONS

The present study showed that age of rabbit bucks is considerable factor on rabbit industry. The obtained results were comparable between rabbit age groups, and they could be used in rabbit breeding. Generally, semen characteristics and sexual libido of young and middle age groups were superior to old rabbit bucks. Administration of vitamin E and selenium to rabbit bucks could improve reproduction in rabbit farms.

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**Table(1):** Effects of age and vitamin E and selenium supplementation on sexual libido (TSL) and ejaculate volume and sperm motility of NZW rabbit bucks (mean).

| **Main effects** | **TSL (Sc)** | **Ejaculate volume (ml)** | | **Initial sperm motility (%)** | **Sperm Viability (%)** |
|---|---|---|---|---|---|
| | | **Net** | **Whole** | | |
| **Age Group (A)** | | | | | |
| Young | $5.62^{b}$ | $0.36^{b}$ | $0.42^{b}$ | 68.64 | $84.21^{ab}$ |
| Middle | $6.74^{b}$ | $0.51^{a}$ | $0.73^{a}$ | 71.77 | $85.38^{a}$ |
| Old | $8.67^{a}$ | $0.45^{ab}$ | $0.49^{b}$ | 68.38 | $82.49^{b}$ |
| MSE | 0.32 | 0.02 | 0.02 | 0.71 | 0.43 |
| *P.Value* | 0.001 | 0.010 | 0.001 | 0.095 | 0.026 |
| **Treatment (T)** | | | | | |
| Control | 7.34 | 0.43 | 0.51 | $65.18^{b}$ | $81.15^{b}$ |
| Vit E+ Selenium | 6.69 | 0.45 | 0.59 | $74.02^{a}$ | $86.90^{a}$ |
| MSE | 0.31 | 0.02 | 0.03 | 0.70 | 0.437 |
| *P.Value* | 0.306 | 0.593 | 0.136 | 0.001 | 0.012 |
| **Interaction Effect (A*T)** | | | | | |
| Young * Control | $5.58^{b}$ | $0.35^{b}$ | $0.41^{c}$ | $64.30^{b}$ | $81.44^{c}$ |
| Young * Vit E+ Selenium | $5.65^{b}$ | $0.37^{ab}$ | $0.45^{bc}$ | $72.98^{a}$ | $86.97^{ab}$ |
| Middle * Control | $6.87^{b}$ | $0.49^{ab}$ | $0.63^{b}$ | $66.94^{b}$ | $82.11^{c}$ |
| Middle * Vit E+ Selenium | $6.63^{b}$ | $0.52^{a}$ | $0.83^{a}$ | $76.59^{a}$ | $88.64^{a}$ |
| Old * Control | $9.50^{a}$ | $0.43^{ab}$ | $0.49^{bc}$ | $64.30^{b}$ | $79.89^{c}$ |
| Old * Vit E+ Selenium | $7.77^{ab}$ | $0.46^{ab}$ | $0.50^{bc}$ | $72.43^{a}$ | $85.08^{b}$ |
| MSE | 0.32 | 0.02 | 0.03 | 0.71 | 0.44 |
| *P.Value* | 0.003 | 0.050 | 0.001 | 0.001 | 0.001 |

a,b,c Mean within a column and within a source not sharing a common superscript differed significantly ($p \leq 0.05$ or $p \leq 0.001$)
MSE= Mean of standard error

**Table(2):** Effects of age and vitamin E and selenium supplementation on some semen characters and plasma levels of fructose and testosterone of NZW rabbit bucks **(**mean).

| **Main effects** | **SCC/ml ($x10^6$)** | **TMS/ml ($x10^6$)** | **TMS/EJ ($x10^6$)** | **SMI (%)** | **Fructose (mg/dl)** | **Testosterone (ng/dl)** |
|---|---|---|---|---|---|---|
| **Age (A)** | | | | | | |
| Young | $277.11^{b}$ | $199.05^{b}$ | $113.39^{c}$ | 89.76 | $222.98^{ab}$ | $354.51^{a}$ |
| Middle | $327.65^{a}$ | $243.73^{a}$ | $194.65^{a}$ | 90.59 | $201.32^{b}$ | $242.55^{b}$ |
| Old | $343.42^{a}$ | $239.19^{a}$ | $153.79^{b}$ | 92.01 | $245.66^{a}$ | $283.85^{ab}$ |
| MSE | 9.93 | 8.17 | 6.921 | 0.45 | 6.12 | 8.15 |
| *P.Value* | 0.017 | 0.048 | 0.001 | 0.129 | 0.011 | 0.045 |
| **Treatment (T)** | | | | | | |
| Control | $261.48^{b}$ | $175.60^{b}$ | $132.50^{b}$ | $89.87^{b}$ | 214.97 | 309.41 |
| Vit E+ Selenium | $369.71^{a}$ | $278.36^{a}$ | $172.63^{a}$ | $91.86^{a}$ | 229.95 | 281.75 |
| MSE | 9.89 | 8.13 | 6.89 | 0.45 | 6.09 | 10.26 |
| *P.Value* | 0.001 | 0.001 | 0.003 | 0.001 | 0.222 | 0.596 |
| **Interaction effect (A*T)** | | | | | | |
| Young * Control | $230.57^{c}$ | $155.45^{c}$ | $92.32^{c}$ | $88.77^{b}$ | $225.65^{a}$ | $358.05^{a}$ |
| Young *Vit E+ Selenium | $323.65^{b}$ | $242.66^{b}$ | $132.59^{bc}$ | $90.63^{ab}$ | $219.92^{ab}$ | $351.78^{a}$ |
| Middle * Control | $262.42^{bc}$ | $181.55^{c}$ | $170.37^{b}$ | $89.61^{b}$ | $178.96^{b}$ | $263.94^{bc}$ |
| Middle *Vit E+ Selenium | $392.88^{a}$ | $305.91^{a}$ | $215.47^{a}$ | $91.44^{ab}$ | $220.49^{ab}$ | $220.83^{c}$ |
| Old * Control | $291.44^{bc}$ | $189.79^{c}$ | $135.81^{bc}$ | $90.28^{ab}$ | $241.73^{a}$ | $306.94^{ab}$ |
| Old *Vit E+ Selenium | $392.61^{a}$ | $286.50^{ab}$ | $168.70^{b}$ | $93.45^{a}$ | $247.16^{a}$ | $273.02^{bc}$ |
| MSE | 9.89 | 8.13 | 6.89 | 0.45 | 6.09 | 11.36 |
| *P.Value* | 0.001 | 0.001 | 0.001 | 0.050 | 0.022 | 0.042 |

a,b,c Mean within a column and within a source not sharing a common superscript differed significantly ($p \leq 0.05$ or $p \leq 0.001$). MSE= Mean of standard error.
SCC/ml= sperm cell concentration/ml, TMS/ml= total motile sperm/ml, TMS/EJ= total motile sperm/ejaculate, SMI %= Sperm membrane integrity percent.

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## الملخص العربي

## تأثير العمر والمعاملة بفيتامين هـ والسلينيوم علي صفات السائل المنوي لذكور الأرانب النيوزيلندي الأبيض

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تهدف هذه الدراسة إلي بحث تأثير العمر والمعاملة بكلاً من فيتامين هـ والسلينيوم علي صفات السائل المنوي لذكور الأرانب النيوزيلندي الأبيض. تم استخدام ٣٦ ذكر أرانب بصحة جيدة وتم تقسيمهم إلي ثلاثة مجاميع علي حسب العمر، المجموعة الأولى ذكور صغيرة العمر (٤-٥ شهور)، والمجموعة الثانية ذكور متوسطة العمر (٩-١٠ شهور) والمجموعة الثالثة ذكور كبيرة العمر (٢٠-٢٤ شهر). تم تقسيم كل مجموعة إلي تحت مجموعتين (معاملة و كنترول). المجموعة المعاملة تم حقنها ب ٥٠ مجم فيتامين هـ و ٠,١ مجم سلينيوم لكل كجم وزن حي أما الثانية تم حقنها بمحلول ملحي كمجموعة مقارنة. تم حقن الحيوانات مرة كل أسبوع لمدة ١٢ أسبوع. بعد ستة أسابيع من المعاملة تم جمع السائل المنوي من الذكورلمدة ستة أسابيع متتالية. تم قياس الرغبة الجنسية كما تم تقدير صفات السائل المنوي ومستوي هرمون التستستيرون.

أشارت النتائج إلي أن مجموعة الحيوانات الصغيرة كانت أقل معنوياً ($p<0.05$) في تركيز الحيوانات المنوية، عدد الحيوانات المنوية الحية المتحركة أما نسبة الحركة والحيوية كانت أقل ولكن الاختلافات غير معنوية. في مجموعة العمر المتوسط كان الحجم الكلي للقذفة أعلي معنوياً ($p<0.05$) مقارنة بالمجاميع الأخري. وأيضا كانت أعلي ($p<0.05$) في نسبة الحيوانات المنوية سليمة الغشاء البلازمي بينما كان مستوي الفركتوز أقل معنويا ($p<0.05$) مقارنة بمجموعة العمر الكبير بينما لم تختلف معنويا مع مجموعة العمر الصغير. الحيوانات المعاملة تحسنت في معظم صفات جودة السائل المنوي. فكان متوسط حجم السائل المنوي ونسبة الحركة والتركيز وعدد الحيوانات المنوية المتحركة في المليليتر والرغبة الجنسية وسلامة الغشاء البلازمي أعلي معنويا ($p<0.05$) في الذكور المعاملة بفيتامين هـ والسيلينيوم عن الغير معاملة. أيضا لوحظ أن أفضل القيم كانت في المجموعة المعاملة ذات العمر المتوسط بينما كانت أقل القيم في المجموعة الغير معاملة ذات العمر الصغير.

من هذه الدراسة يمكن استخلاص أن المجموعة ذات العمر المتوسط وتليها المجموعة الكبيرة كانت أفضل معنويا في معظم خصائص جودة السائل المنوي مقارنة بالمجموعة الصغيرة. يمكن استخدام المعاملة بفيتامين هـ والسلينيوم لتحسين الأداء التناسلي لذكور الأرانب في موسم الصيف. أفضل النتائج لوحظت في الأعمار المتوسطة لكن خصائص السائل المنوي في الأعمار الصغيرة والكبيرة لم تنخفض بشكل حاد وظلت في المستوى المقبول. بمقارنة ذكور الأرانب في الأعمار المختلفة يمكن استنتاج أن الذكور الصغيرة والمتوسطة في العمر يمكن أن تستخدم في تربية الأرانب. بالاضافة إلى ذلك فإن ذكور الأرانب يمكن أن تستخدم بكفاءة في موسم الصيف بالمعاملة بفيتامين هـ والسيلينيوم.