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IMPACT OF METAL, ORGANIC AND NANO-PARTICLES COPPER ON SEMEN QUALITY, FERTILIZING CAPACITY AND LIVER FUNCTION IN RABBIT BUCKS

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ABSTRACT: A total of 24 APRI line rabbit bucks having 2.65±0.11 kg live body weight at 20 weeks of age were used in this study. Bucks were divided into four similar groups, 6 bucks in each. Bucks in the 1st group were fed basal diet without any supplements as a control. While those in the 2nd, 3rd and 4th groups were fed the same diet supplemented with 50 mg nano-Cu (nano-Cu), 200 mg Cu sulfate (metal-Cu) or 200 mg Cu-Methionine (organic-Cu) per kg diet, respectively. Semen was collected biweekly at 28 – 36 wks of age for evaluation. APRI does were naturally mated by treatment bucks (24 does/group) at the last week of the experimental period. Results showed that bucks fed diet with nano-Cu had numerically higher ($P<0.01$) values for live body weigh and body weight gain, followed by those fed diet containing Cu as organic and metal form than a control group. The ALP activity and serum testosterone hormone concentration increased ($P<0.05$), being the highest values in the 2nd group. Supplemented rabbit bucks with nano-Cu in their diets showed the best ($P<0.05$) sperm characteristics including progressive motility, livability, and abnormality percentages of spermatozoa and sperm concentration. Sperm membrane integrity, fructose test, LDH activity and peroxidase activity in seminal plasma were better in nano-Cu group as compared to other treatment groups. The examination of buck semen by scanning electron microscope revealed lower types and proportion of sperm abnormality in the 2nd group compared to the other groups**.** The highest fertility rate (91.7%) was produced in nano-Cu dietary bucks' group, but did not differ significantly from that in metal-Cu (83.3%) and organic-Cu (87.5%) compared to 70.8% in control group. It can conclude that supplementation of the copper in Nano-particles form at a level of 50 mg/kg in the diet of rabbit bucks is recommended for improving semen quality and fertility for artificial insemination and natural breeding in rabbit production field.

Keywords: Rabbit, copper, nano- particles, semen quality, fertility, liver function.

INTRODUCTION

Rabbit are characterized by smaller body size, higher feed utilization, faster growth rate, higher quality nutritious meat, early maturity, and high genetic selection potential **i**n comparing with other livestock **(**Kumar *et al.,* 2018). APRI line rabbit is Egyptian line selected for litter weight at weaning according to Abou Khadiga *et al.* (2010).

Semen quality is the guarantee of successful insemination in breeding rabbits. Artificial insemination (AI) is widely employed and this diffusion has contributed to the increase in knowledge of sperm metabolism and management of rabbit bucks. There are many endogenous and exogenous factors affecting reproduction of rabbit bucks and thus it is crucial to define suitable additives to improve sperm characteristics (Brun *et al.,* 2002). Copper (Cu), as a trace element, plays an important role as superoxide dismutase (SOD) co-factor (Cu/Zn SOD, Tvrdá *et al.,* 2015). Live organisms regulate the cellular Cu concentration because of Cu deficiency reduces or eradicates body Cu-dependent enzymes, leading to inhibition of the cellular life processes. Also, Cu triggers large amounts of free radicals' production, which will consequently damages proteins and DNA (Ogórek *et al*., 2017).

In male fertility, Cu had antioxidative properties and improves sperm characteristics (Mirnamniha *et al*., 2019). In this respect, Cu appears to affect sperm motility and acts at the receptors of the pituitary gland such as LH (Eidi *et al.,* 2010). Spermatozoa of mammals contain high polyunsaturated fatty acids level, so they are very susceptible to oxidative stress and reactive oxygen species (ROS) production, mainly superoxide anion and hydrogen peroxide (Tvrdá *et al.,* 2015). At sites of gamete production, sperm cells are vulnerable to the harmful effects of ROS and may thus need to protection of antioxidants (Eidi *et al.,* 2010). The SOD protects sperm cells from the oxidative stress and this process is required to Cu for the live organisms to normally develop.

The form of Cu that most commonly added to animal feed is CuSO4. According to the literature, however, copper from this compound is relatively poorly absorbed by the body and substantial quantities are excreted into the environment (Karimi *et al*., 2011). The gastrointestinal absorption of Cu is influenced by a number of factors, including its chemical form: soluble Cu compounds (oxides, hydroxides, citrates and sulphate) are readily absorbed, but water‐insoluble compounds (sulphides) are poorly absorbed. Absorbed Cu binds to plasma albumin and amino acids in the portal blood and is transported to the liver, where it is incorporated into ceruloplasmin and later released into the plasma (Rosmarie, 1992). Many studies showed that Cu bioavailability was higher when it was fed in organic form, such as metal amino acid chelates and metal proteinates (Du *et al*., 1996). Minerals in organic forms are have more efficient utilization in the body for increasing their bioavailability absorption resulting in improving ejaculate volume, motility of spermatozoa and then fertility of male (Rowe *et al*., 2013). Several methods have been developed for the preparation of Cu nano-particles (Ognik *et al*., 2019) included thermal reduction, metal vapor synthesis rendition method, micro-emulsion techniques, mechanical attrition

and chemical reduction (Dorton *et al*., 2003). Therefore, the search continues for an alternative source of Cu which would increase the absorption of this element, decrease the level of supplementation, and reduce emissions of Cu to the environment (Majewski *et al*., 2017).

It was demonstrated that rabbit semen is specific for high concentration of Cu. In this respect, Lukac *et al.* (2009) found negative correlation (r= -0.75) between Cu concentration and acrosomal damage. It was found that semen quality positively correlated with Cu concentrations in blood or semen (Tvrdá et *al.,* 2015). As available in the literature, there are no information on the effect of different Cu sources (nano-Cu, Cu sulfate and organic Cu) on semen quality of rabbit bucks. Therefore, the present study was designed to investigate the efficacy of different dietary Cu sources on semen quality, ejaculated sperm characteristics, fertilizing capacity and enzyme activities of APRI rabbit bucks.

MATERIALS AND METHODS

The present study was carried out at Rabbit Farm of Sakha Station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt. The field work lasted for five consecutive months, during the period from February to June, 2018.

A total of 24 APRI line rabbit bucks having 2.65±0.11 kg live body weight (LBW) at 20 weeks of age were randomly allotted into four similar groups, 6 bucks in each. Bucks in the 1st group were fed basal diet without any supplements as control. While those in the 2nd, 3rd and 4th groups were fed the same basal diet supplemented with 50 mg nano-Cu (nano-particles Cu), 200 mg Cu sulfate (metal-Cu) or 200 mg Cu-methionine (organic-Cu) per kg diet. Levels of different Cu sources were performed according to Easa *et al.* (2018).

Bucks were individually housed in wire cages supplied with nipple drinkers and fed *ad libitum* on the experimental diets up to 36 wks of age (February-May). The ingredient basal diet contains 30.05% berseem hay, 24.6% barley, 21.5% wheat bran, 17.5% soybean meal, 3% molasses, 1.6% di-calcium phosphate, 0.95% limestone, 0.3% sodium chloride, 0.3% vitamin and mineral mixture and 0.2% DL-methionine. The calculated chemical compositions of the basal diet were 17.75% CP, 12.38% CF, 2.27% EE and 2500 Kcal/kg diet digestible energy.

Three sources of supplemental Cu were used in the current study, including copper sulphate, $CuSO_4.5H_2O$, 98% (metal-Cu; contains 25% Cu) as an inorganic form, copper-methionine 56.7% (organic-Cu; contains 10% Cu) as an organic form and nano particles of copper (nano-Cu; contains 37.38% Cu) as a nano form with a particle size of 60-70 nm. The colloid of copper nanoparticles was prepared at room temperature using $Cu(NO_3)_2.3H_2O$ (S.d. Fine-Chem. Ltd) as a source for Cu metal. Typically, 2.41 gm of Cu precursor were added to 100 solution of 75 ml deionized water and 25 ml ethylene glycol under strong magnetic stirring. The deep blue solution was subjected to additional stirring for 30 min to attain higher homogeneity. Thereafter, 300 µl of ice cold (1 M) NaBH4 (Merck) was quickly injected into the above mixture. The solution turned colorless after about 1 min, and then turned burgundy, indicating the growth of Cu nanoparticles (Zhang *et al.* (2009). Average live body weight (LBW, kg) of bucks was recorded monthly in all experimental groups, and total body weight gain (TBG) was calculated.

At 28 wks of age, semen was collected biweekly during the interval from April to May using artificial vagina from all the bucks in each treatment group up to end experimental period (36 wks of age). Semen was collected (before feeding at 8 a.m) and evaluated for volume, pH value, percentages of progressive motility, livability and abnormality of spermatozoa and sperm cell concentration. At the last semen collection week, semen samples were taken from each buck in all experimental groups into tubes and incubated for one hour in water bath at 37ºC, centrifuged at 1500 rpm for 15 minutes, and then seminal plasma was separated and frozen at -20 ºC until assaying peroxidase and fructose tests by determination of peroxidase-positive white blood cells and fructose concentration in seminal plasma according to Aitken (1989) and Foreman *et al.* (1973), respectively. LDH was determined using commercial kits (Diagnostic Products Corporation Kits). The response of spermatozoa to Hypo-Osmotic Swelling-test (HOS-t) was conducted at osmolarity level of 150 mOsm/l for 30 min, in term of determining curled sperm percentage (El-Desoky *et al*., 2017).

At the end of experimental period, blood samples were taken from the ear vein of each buck in all experimental groups into tubes. The samples were centrifuged at 3000 rpm for 15 minutes and blood serum was separated and frozen at -20 ºC until assaying the testosterone concentration using a double antibody radioimmunoassay (Diagnostic Products Corporation Kits)**.** For evaluating liver function, activity of aspartate aminotransferase (AST), and alanine aminotransferase (ALT), alkaline phosphatase (ALP), and Lactate dehydrogenase (LDH) were determined using commercial kits (Diagnostic Products Corporation Kits).

At the last week of the experimental period, 96 matured APRI female does were naturally mated by treatment bucks (24 does in each treatment group). Fertility rate and prolificacy (kits born alive/doe) were recorded in each group.

Scanning electron microscopy evaluation of semen samples:

At the last week of semen collection period, specimens (0.2 ml) from semen were taken, fixed in modified Karnovsky's fixative solution containing glutaraldehyde 7.5% and paraformaldehyde 3% according to Romeis (1989), and post-fixed with 1% osmiumtetroxide (OsO4) buffered with cacodylate (pH 7.2). Specimens were dehydrated in graded series of ethanol alcohol after washing several times in cacodylate buffer (0.1 M), and dried overnight by hexamethyldisilazane solution (HMD) (Co. Roth, Karlsruhe, Germany). The specimens were mounted with Leit-C glue (Co. Plano, Marburg, Germany) onto aluminum stubs and sputter-coated with gold (2 min, 30-40 nm). The SEM examination by a scanning electron microscope (DSM 950, Co. Zeiss, Oberkochen, Germany) at an accelerating voltage (10 kV) at magnification of 30-7500 x. Digital photos were directly taken from SEM to the personal computer.

Statistical analysis:

The obtained data were statistically analyzed by analysis of variance (ANOVA) and Pearsons correlation coefficients were determined using SAS (2004) computer program. Data were statistically analyzed using the following model: $Y_{ij} = U + A_i + e_{ij}$.

Where: Y_{ij} = observed values, U = overall mean, A_i = Cu source (1, 2, 3 and 4) and e_{ij} = random error. Data of physical semen characteristics were analyzed by two-way using the following model: $Y_{ijk} = U + A_i + B_j + AB_{ij} + e_{ijk}$.

Where: B_j = collection week (28, 30, 32, 34 and 36 wks of age), AB_{ij} = Interaction between Cu source and collection week. Significant differences among means were set at $P<0.05$ using Duncan's multiple range test **(**Duncan, 1955). The percentage values 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

Body weight:

Average final body weight (at 36 wks of age) and body weight gain (20 - 36 wks of age) were increased (P<0.05) by all Cu dietary treatments (Table 1). The impact of the Cu in improving the body weight was reported by Zhang *et al*. (2008). Also, Arangasamy *et al*. 2018) found that dietary Cu supplementation at a level of 37.5 mg/kg resulted in higher body weight of goats than that in the control. In the present study, bucks in all treatment groups showed significantly (P<0.05) higher live body weight (LBW) and total body gain than control group, being the highest in nano-Cu, followed by organic-Cu and sulfate-Cu (metal form), respectively (Table 1). These results are in agreement with Easa *et al.* (2018), who found that feeding growing rabbits a diet containing Cu-nano (50 mg Cu/kg diet) achieved the best growth performance compared to other Cu forms and the control. The effects of Cu-nano have been attributed to their small particle size and large surface to volume ratio, which allows interacting closely with microbial membranes (Ramyadevi *et al.*, 2012). The obtained results revealed insignificant improvement in LBW and body weight gain of bucks in Cu organic group as compared to Cu metal group (Table 1). Generally, the supplementation of organic forms of Cu has impact on animal LBW which may be attributed to better absorption and bioavailability of Cu in organic as compared to inorganic forms (Rowe *et al*., 2013).

Liver function indicators and testosterone profile:

Activity of AST, ALT and LDH in blood serum of bucks as hepatic indicators was not significantly affected by different Cu sources. While, activity of ALP significantly (P<0.05) increased, being the highest in bucks fed diet supplemented with Cu as a nano-particls form (Table 2). Aspartate amino transaminase (AST) is a plentifully found in heart and liver muscles and plays an essential part in amino acid metabolism and pathological changes in these organs. The AST impacts the development of glutamate and oxaloacetate attributable to the transfer of the amino group from aspartate to ketoglutaric corrosive (Abdelhamid *et al.*, 2012). It has an effect on glutamate and pyruvate due to the transfer of the amine group from alanine to ketoglutaric acid. Increased activity of the ALT leads to the occurrence of viral hepatitis or liver damage or metabolic disorders (Bagnicka *et al.*, 2014). The observed insignificant effect of Cu treatment on activity of AST and ALT indicated normal liver function of all treatment rabbit groups. Increasing serum ALP activity significantly in buck groups fed different Cu sources diets may indicate a vital role of ALP on body energy production, especially energy required for spermatogenesis. In this respect, Isitua and Ibeh (2013) showed a positive relationship between the activity of ALP in blood and health status of rabbits. LDH converts hydrogen between molecules and stimulates the last step of the glycolic path, which is the conversion from pyruvate to lactate and repetition (Bagnicka *et al.*, 2014). Level of blood LDH activity may be important in determining tissue damage and disease within the living body.

The present results (Table 2) revealed significant (P<0.05) increase in serum testosterone level in bucks of treatment groups as compared to control one, being the highest in nano-Cu group, followed by organic-Cu and metal-Cu groups, respectively (Table 2). Testosterone is the major secretary hormone of the mature testis. Both accessory sex glands function and spermatogenesis are controlled directly by testosterone concentration in males. Testosterone supplementation has previously been shown to improve sexual function and libido, increasing blood flow to the male reproductive organs, leading to stimulation of the nervous system and enhancing the sexual desire (Monera *et al.*, 2008). Increasing blood testosterone level was associated with rapid growth of the testes of animals fed diets supplemented with trace minerals (Rowe *et al*., 2013). Based on the obtained results, feed supplemented with Cu as nano particles or organic form might have improved testicular activity comparing with metal-Cu. The superiority of nano-Cu on testosterone level may be attributed to the activity of nano-particles with a large surface area, exposing their atoms to direct contact with target cells (Majewski *et al*., 2017). In accordance with the present results, Arangasamy *et al*. (2018) showed that feeding organic Cu to growing male goats improved (P<0.01) testosterone level from 1.63±0.07 to 6.17±0.06), indicating that, organic Cu in a dose dependent manner, might increase the activity of testes via increasing activity and function of the hypothalamus and the pituitary gland.

Semen quality:

Volume of ejaculate, pH value, concentration of spermatozoa, percentages of progressive motility and livability of spermatozoa were significantly (P<0.05) increased, whereas sperm abnormality decreased (P<0.05) by Cu addition with any form in buck diet compared to control one (Table 3). Bucks in group fed nano-Cu diet showed the highest semen volume and the best sperm characteristics in semen, followed by bucks fed organic Cu diet and metal Cu diet. Semen ejaculate volume, sperm concentration, sperm livability percentages were significantly (P<0.05) increased by advancing collection weeks. However, sperm abnormality percentages were decreased (P<0.05, Table 3). The effects of interaction between treatment and collection week were significant (P<0.01) only on semen volume and sperm cell concentration (Table 3 and Fig. 1), reflecting observed increase in semen volume and sperm cell concentration by advancing collection week in all groups. Except that of organic Cu group, which showed inconsistent trend of change (Fig. 1, a & c). The insignificant interaction effect on sperm motility, livability and abnormality indicated the best percentages in semen of nano-Cu group at most collection weeks (Fig. 1, d, e, f), respectively.

The present results revealed that Cu supplementation in buck diet increased semen volume and improving ejaculated sperm characteristics compared to control one. In this line, Fayed (2009) found very important role of Cu in the process of spermatogenesis of rabbit bucks. It was demonstrated that level of Cu in the seminal plasma had positive relationship with semen quality. Akinloye *et al*. (2011) also showed that Cu had significant positive effects on semen volume. In Egyptian Baladi rams, volume of ejaculate, sperm motility, and individual motility percentage were increased by dietary Cu supplementation

(Abd El-Monem *et al*., 2015). Recently, Mirnamniha *et al*. (2019) found that Cu has antioxidative properties and has a positive effect on sperm quality. Elevation of total semen volume and improving semen quality due to nano-Cu, followed by organic Cu in the present study, which may indicate pronounced effect of nano-Cu treatment and organic Cu, directly on function of accessory sex (seminal vesicles, prostate and Cowper's glands) or indirectly on testosterone secretion (see Table 2), which has vital role on accessory sex gland function in rabbit bucks**.** In this way, Walker (2009) found that testosterone was required for the maturation of male germ cells and sperm quality. Moreover, the observed findings are in corroboration with Arangasamy *et al*. (2018) who found that goat bucks fed organic Cu reached puberty 28-35 days earlier than control group with improving ($P<0.01$) semen production capacity and semen quality in comparing with the control bucks, due to improved steroidal hormone synthesis and spermatogenesis.

Sperm membrane integrity and seminal plasma parameters:

Feeding rabbit bucks on nano-Cu diet significantly ($P<0.05$) increased sperm membrane integrity in semen, amount of fructose consumed in seminal plasma (fructose test) and seminal plasma LDH activity. While, significantly ($P<0.05$) decreased peroxidase activity in seminal plasma (peroxidase test) as compared to those fed the control diet (Table 4). Both organic and metal Cu diets showed higher improvement in all previous parameters comparing with the control, but were lower than nano-Cu diet. Percentages of membrane integrity, acrosomal status and live spermatozoa were used directly to predict the fertilization rate and supplementation of trace elements is found to have vital role in the sperm structure and function (Geary *et al*., 2016). In our study, the observed elevation of integrity of plasma and acrosomal membranes of sperm cells of bucks in organic Cu group was reported in goats. In this respect, Narasimhaiah *et al*. (2018) found that organic Cu and zinc supplemented bucks produced improved plasma membrane and acrosome integrities. He added that, the increased antioxidant enzyme activities, reduced oxidative stress and lowered lipid peroxidation were positively correlated ($P<0.05$) with the sperm functional attributes. In goats, Arangasamy *et al*. (2018) also reported that supplementation of organic Cu improved the function of spermatozoa in terms of membrane and acrosomal integrities in fresh semen.

Lactate dehydrogenase (LDH) is a tetrameric enzyme, belonging to the 2-hydroxy acid oxidoreductase family, which increases the rate of the simultaneous inter-conversion of pyruvate to lactate and nicotinamide adenine dinucleotide (NAD)H to NAD+ by 14 orders of magnitude (Burgner and Ray, 1984). The reaction involves the transfer of a hydride ion from NADH to the C_2 carbon of pyruvate (Pineda *et al*., 2007) and is commonly used by cells for anaerobic respiration.

The recorded increase in fructose consumption and the reduction in peroxidase activity in seminal plasma and of nano-Cu group were in association with improving most of sperm characteristics comparing with seminal plasma of bucks in other groups. Furthermore, high quality semen should contain higher glycolytic or fructolytic rates than in semen with weak immobile sperms (Mann, 1964). In addition, Yousef

et al. (2003) assumed that the increases in sperm motility in semen could in part be attributed to the concomitant induction in semen fructose. Also, Mostakhdem *et al*. (2016) found that LDH activity had significant ($P<0.0001$) positive correlation with sperm concentration as well as between total sperm count and LDH activity.

Sperm morphology:

Examination of buck semen in different experimental groups by scanning electron microscopy (SEM) revealed that semen of all groups showed intact sperm cells with normal structure of head and tails (Plate 1 a & b), in term of normal sperm measurement of tail (Plate 1 c) and head (Plate 1 d). Also, normal sperm cells with intact acrosomal and plasma membranes and natural attached sperm neck were seen in semen of all groups (Plate 1 r, f & g). The proportion of intact spermatozoa morphologically was in a negative relationship with sperm abnormality percentage in each group (Table 3). The morphological examination by SEM for sperm abnormalities in semen of all groups showed different types of abnormalities including complete tail curling (Plate 2 A and B) for semen of all groups, being in more proportion in the control group, moderate in metal Cu and organic Cu groups, while the lowest in nano-Cu group. Also, this was marked harmful in acrosomal membrane (Plate 2 C), acrosomal top breakdown (Plate 2 D) and broken sperm neck (Plate 2 E) with the highest proportion only in the control group. Based on these observations, dietary treatment with different types of Cu supplementation showed impact on sperm morphology of rabbit bucks, being the best for nano-Cu supplementation.

These results may be attributed to a direct effect of Cu sources on spermatogenesis within the seminiferous tubules of the teses or/and maintaining the extracellular media (seminal plasma), regarding the pH value of semen and osmolarity level via an indirect effect on production of the seminal plasma from the accessory sex gland. In rams, deficiency in Cu level showed undeveloped seminiferous tubules as compared to the control. In case of Cu deficiency, Sertoli cells were seen to be with small volume of cytoplasm (Van Niekerk and Van Niekerk, 1989).

Fertilizing capacity:

Fertility rate was significantly ($P<0.05$) increased by Cu supplementation in the diet of rabbit bucks (Table 5). Does mated by bucks fed nano-Cu diets showed significantly ($P<0.05$) the highest pregnancy rate as compared to those fed control diet, but did not differ significantly from that in organic Cu and metal Cu groups. All Cu-source diets showed insignificantly higher prolificacy (kits born alive per doe) than control, being the highest in nano-Cu and organic Cu groups (Table 5). In this context, Cu as a potential element in fertility of male affects the distribution of integral androgen through the hypothalamic-pituitary-testes axis. The hypo- or hyper levels of Cu causes marked reduction in fertility of males (Ogórek *et al*., 2017)

The obtained results concerning the sperm fertility are in parallel with improving most sperm characteristics and seminal plasma parameters in semen of bucks fed nano-Cu diet. Among all characteristics of spermatozoa, motility is critical in enabling the sperm to ascend the female reproductive tract to the site of fertilization which is necessary if fertilization is to be achieved (Aitken, 1989). It is conceivable that the increase in sperm concentration might lead to

higher fertility (Oyeyemi *et al*., 2008). The present result demonstrates that addition Cu as organic form to the diet of rabbit bucks in most traits studied, has more efficiency than metal-Cu form, reflecting positive impact on fertilizing capacity. Similarly, Rowe *et al*. (2013) mentioned that sperm motility and consequently male bull fertility was improved as affected by organic minerals supplementation. Decreased level of some trace elements (copper, manganese, magnesium, zinc and selenium) can negatively affect human reproductive health, semen quality, sperm normal function and as the result, fertility potency in men (Mirnamniha *et al*., 2019).

Correlation coefficients:

Data in Table (6) revealed positive correlation ($P<0.05$) between testosterone hormone concentration and each of semen volume, sperm cell concentration, motility, livability and fertility rate. Results showed negative correlation ($P<0.05$) between testosterone level and each of mount of fructose consumed and peroxidase activity. The obtained results revealed positive correlation between fertility rate and each of sperm cell concentration, livability ($P<0.05$) and motility ($P<0.01$). However, fertility rate negatively ($P<0.05$) correlated with sperm abnormality, fructose consumed and peroxidase activity. In this way, Sun *et al.* (1990) stated that testosterone and FSH hormones are responsible for spermatogenesis and all stages of spermatogenesis are stimulated by testosterone. Boiti *et al.* (1992) found a positive correlation between testicle and seminal vesicle weight and testosterone levels in rabbits. In addition, Castro *et al.* (2002) found that plasma testosterone level was correlated significantly with any of the following parameters: seminiferous tubular diameter, number of Sertoli cells per tubular cross-section, ratios between germ cells and Sertoli cells and ratios among germ cells. Based on the obtained correlation coefficients, feeding rabbit bucks on nano-Cu diet markedly affect accessory sex glands leading to increase ejaculate volume (semen volume), and spermatogenesis yielding higher sperm cell concentration, beside the effect of nano-Cu on increasing testosterone concentration that required for accessory sex gland functions and spermatogenesis within the seminiferous tubules of the testicular mass of rabbit bucks (Awoniyi, 2010).

CONCLUSION

Based on the previous findings, the current study can conclude that supplementation of the copper in Nano-particles form at a level 50 mg/kg in diet of rabbit bucks is recommended for improving semen quality and fertility artificial insemination and natural breeding to consequently increase in the economical return in rabbit production field.

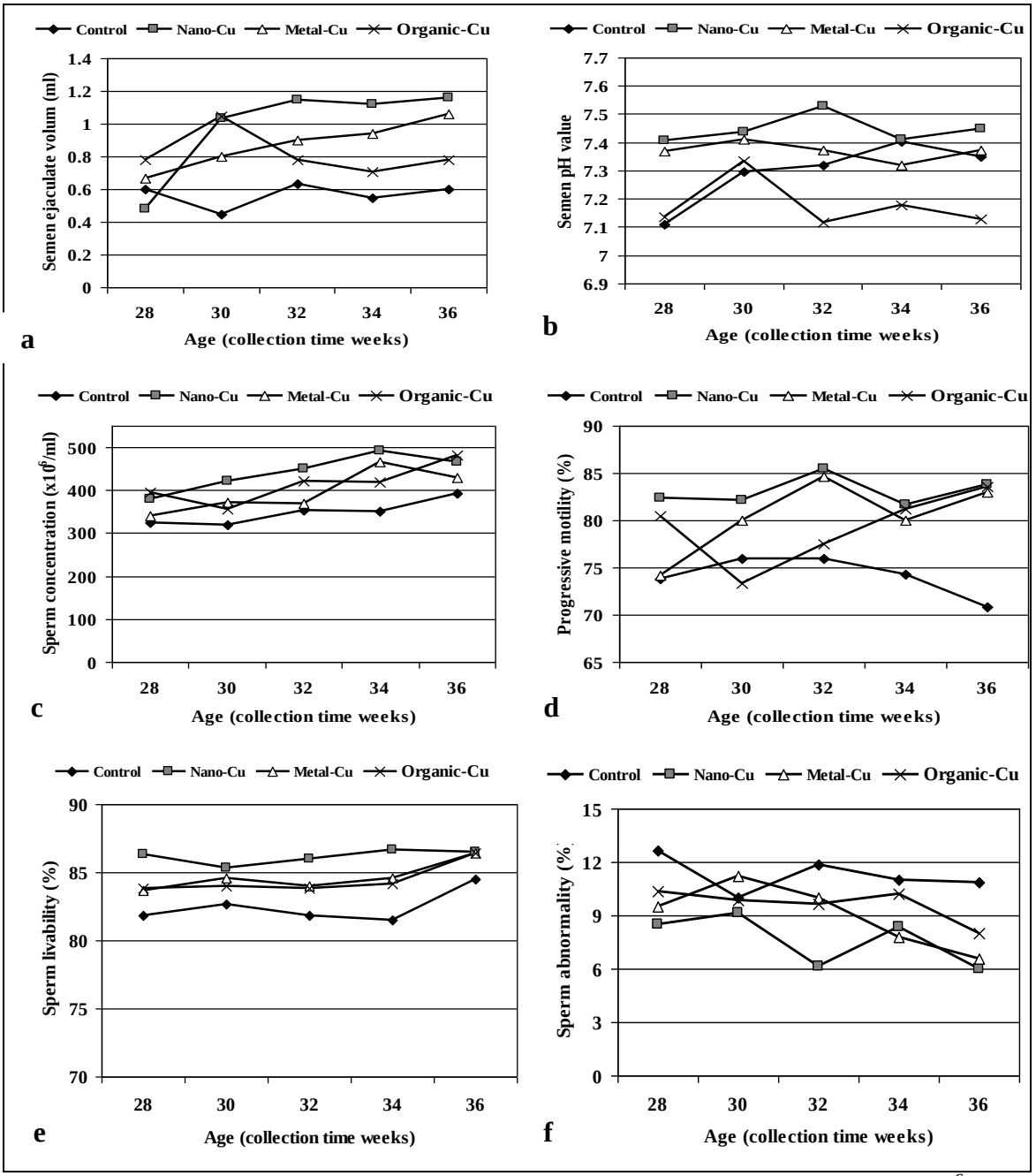

Fig. (1): Changes in semen volumes (ml), pH value, sperm cell concentration (x10^6/ml), percentages of progressive sperm motility, livability and abnormality in rabbit semen bucks of all experimental groups at different collection weeks.

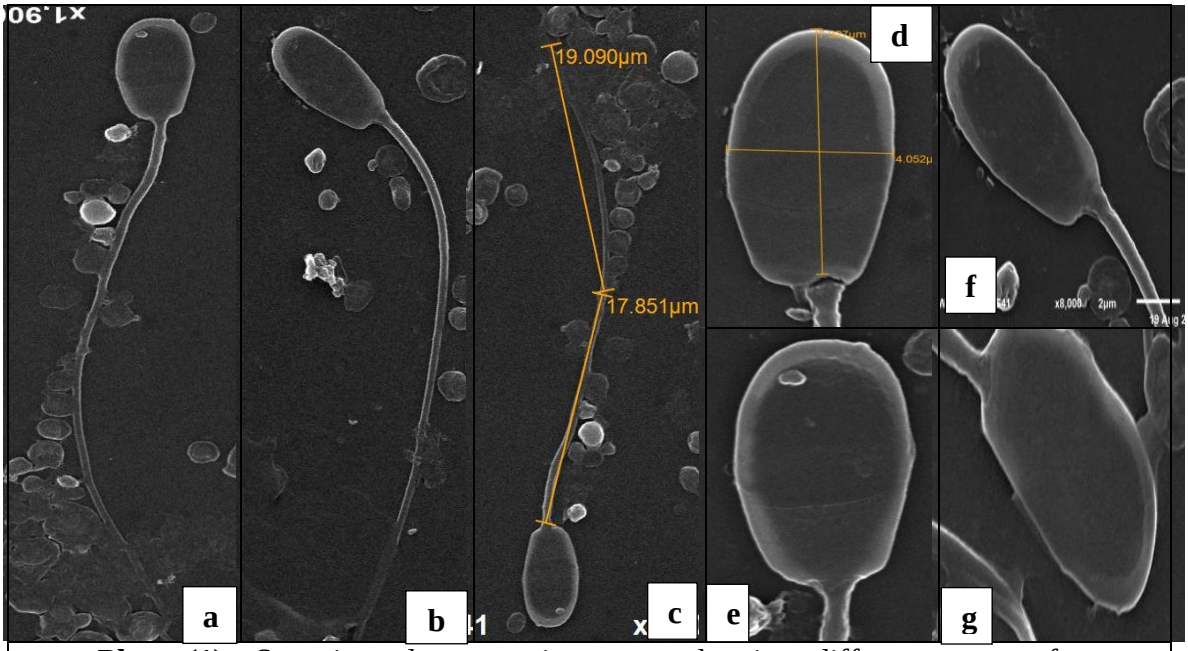

Plate (1): Scanning electron microscopy showing different types of sperm normality in semen of the experimental buck groups, including normal and intact spermatozoa in the control bucks (a) and nano-Cu group (b) with normal sperm measurements for mid- and main tail pieces (c), length and width of sperm head (d), normal acrosomal membrane and neck in the control group (e), nano-Cu group (f) and organic Cu group (g).

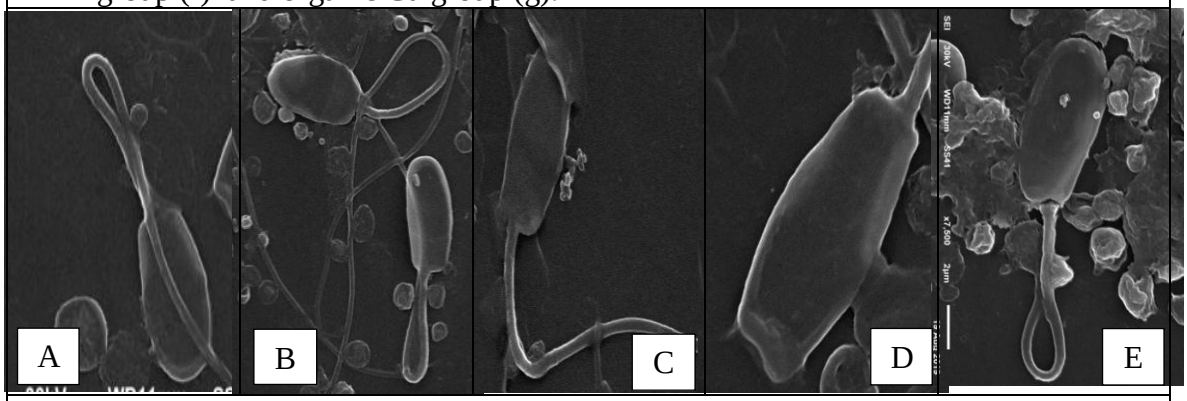

Plate (2): Scanning electron microscopy showing different types of sperm abnormality in the experimental buck groups, including abnormalities in sperm tail (complete tail curling, A and B) for semen of all groups, and in sperm head (harmful in acrosomal membrane, C; acrosomal top breakdown, D; and unattached neck, E) in control bucks group.

Table (1): Initial and final live body weight and body weight gain of rabbit bucks fed different copper (Cu) sources from 20- 36 wks of age.

Body weight (kg)	Control	Nano- Cu	Metal- Cu	Organic- Cu	±SEM
At 20 wk of age	2.64	2.66	2.63	2.64	0.031
At 36 wk of age	3.28^{c}	3.59^{a}	3.41^{b}	3.48^{ab}	0.029
Total gain	0.647^{c}	0.933^{a}	0.776^{b}	0.842^{ab}	0.042

$^{a, b}$ and ^{c}: Means in the same row with different superscripts are significantly different at P<0.05.

Table (2): Enzymes activity and testosterone concentration in blood serum of rabbit bucks fed different copper (Cu) sources at the end of collection semen period.

Copper sources	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	LDH (IU/l)	Testosterone (ng/ml)
Control	58.3	63.7	57.7^{c}	1123.3	6.14^{c}
Nano-Cu	60.7	58.0	74.7^{a}	1457.1	7.34^{a}
Metal- Cu	59.3	61.3	66.7^{b}	1325.6	6.70^{b}
Organic- Cu	59.0	63.0	69.3^{ab}	1315.6	7.17^{ab}
Stander errore	±3.4	±3.2	±1.96	±78.6	±0.29

$^{a, b \& c}$ Means in the same column with different superscripts are significantly different at P<0.05.

Table (3): Semen quality and ejaculated sperm characteristics of rabbit semen as affected by Cu sources supplementation in the diet, semen collection time and their interaction (LSM±SE).

Variable	Ejaculate volume (ml)	Semen pH value	Sperm cell concentration (×10^{6}/ml)	Sperm motility (%)	Sperm livability (%)	Sperm abnormality (%)
Effect of Cu source (S):						
Control	0.567^{c}	7.23^{b}	348.2^{c}	74.2^{c}	82.5^{c}	11.28^{a}
Nano- Cu	0.995^{a}	7.43^{a}	439.8^{a}	83.1^{a}	86.2^{a}	7.63^{c}
Metal- Cu	0.849^{b}	7.39^{a}	393.3^{b}	77.6^{bc}	84.2^{b}	9.02^{b}
Organic- Cu	0.871^{b}	7.27^{b}	416.2^{ab}	79.2^{b}	84.6^{b}	9.61^{b}
Stander error	±0.039	±0.042	±8.74	±1.09	±0.32	±0.43
Effect of semen collection time (week of age):						
28	0.633^{b}	7.25	360.6^{b}	77.7	83.9^{b}	10.25^{a}
30	0.803^{a}	7.39	363.1^{b}	77.4	84.2^{b}	10.05^{a}
32	0.866^{a}	7.31	425.7^{a}	80.9	83.9^{b}	9.41^{a}
34	0.833^{a}	7.33	416.1^{a}	78.3	83.8^{b}	9.35^{a}
36	0.906^{a}	7.26	442.8^{a}	78.6	85.9^{a}	7.86^{b}
Stander error	±0.053	±0.054	±10.2	±1.42	±0.45	±0.56
Effect of interaction (S*W)						
P-value	0.01**	0.449	0.01**	0.076	0.781	0.144

$^{a, b}$ and ^{c}: Means in the same column for each factor with different superscripts are significantly different at P<0.05. ** Significant at P<0.01.

Table (4): Sperm membrane integrity, fructose and peroxidase tests and LDH activity in seminal plasma of rabbit bucks fed different Cu sources.

Treatment (Cu source)	Sperm membrane integrity (%)	Fructose test (mg/ dl)	Peroxidase test ($U\times10^3$)	LDH activity (U/l)
Control	54.33^{b}	283.33^{a}	0.967^{a}	50.95^{c}
Nano- Cu	76.67^{a}	103.67^{b}	0.500^{b}	118.88^{a}
Metal-Cu	67.67^{ab}	121.67^{b}	0.633^{b}	86.87^{b}
Organic- Cu	74.00^{a}	110.00^{b}	0.600^{b}	106.93^{a}
Stander error	±4.89	±12.86	±0.104	±7.23

[a, b] and [c] Means in the same column with different superscripts are significantly different at P<0.05.

Table (5): Fertilizing capacity of rabbit bucks fed different Cu sources.

Items	Control	Nano- Cu	Metal- Cu	Organic- Cu
Fertility rate No. (%)	17/24 (70.8^{b})	22/24 (91.7^{a})	20/24 (83.3^{ab})	21/24 (87.5^{a})
Prolificacy (kits born alive/doe)	6.58±0.39	7.47±0.33	7.15±0.34	7.46±0.34

[a] and [b]: Means in the same row with different superscripts are significantly different at P<0.05.

Table (6): Pearsons's correlation coefficients (r) between testosterone level and semen characteristics and reproductive performance of rabbit bucks fed different Cu sources.

	Testosterone concentration (ng/ml)	Semen volume (ml)	Sperm cell concentration ($x10^6$/ml)	Sperm motility (%)	Sperm livability (%)	Sperm abnormal (%)	Sperm membrane integrity	Fructose test (mg/dl)	Peroxidase test ($Ux10^3$)	LDH Activity (U/l)	Fertility (%)
Testosterone (ng/ml)	1.000										
Semen volume (ml)	0.579*	1.000									
Sperm cell ($x10^6$/ml)	0.538*	0.445**	1.000								
Sperm motility (%)	0.524*	0.185*	0.210*	1.000							
Sperm livability (%)	0.419*	0.296**	0.371***	0.208*	1.000						
Sperm abnormal (%)	-0.518	-0.362**	-0.388***	-0.245**	-0.560**	1.000					
Sperm membrane I.	0.440	0.342	0.447	0.302	0.402	-0.651*	1.00				
Fructose test	-0.591*	-0.614*	-0.454	-0.355	-0.496	0.897**	-0.665*	1.00			
Peroxidase test	-0.605*	-0.733**	-0.361	-0.579*	0.153	0.725**	-0.473	0.698**	1.00		
LDH activity	0.325	0.253	0.542*	0.503*	0.532*	0.172	0.189	-0.488	-0.221	1.00	
Fertility (%)	0.575*	0.259	0.467*	0.559**	0.450*	-0.386*	0.238	-0.554*	-0.515*	0.426	1.00

Sperm membrane I = Sperm membrane integrity,

* Correlation is significant at the 0.05 level, ** at 0.01 level and *** at 0.001 level.

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الملخص العربى

تأثير جزيئات النحاس النانو والمعدني والعضوي علي جودة السائل المنوي والقدرة الاخصابيه ووظائف الكبد فى ذكور الارانب

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أستخدم فى هذه الدراسه عدد 24 من ذكور الأرنب الأبرى (2,65 ± 0,11 كجم وزن جسم حي وعمر 20 أسبوع)، تم تقسيمها إلى أربعة مجاميع متماثلة، 6 ذكور في كل منها. تم تغذيه المجموعة الاولي على عليقه متكاملة دون أي إضافات (الضابطه)، بينما تم تغذية الأرانب فى المجموعات الثانية والثالثة والرابعة على نفس العليقة مضافا إليها مصدر للنحاس ممثلا فى صورة 50 ملجم نانو نحاس (مجموعة النانو نحاس)، 200 ملجم كبريتات النحاس (مجموعة نحاس معدنى) وصورة عضويه 200 ملجم ميثونين النحاس (مجموعة نحاس عضوى) لكل كيلوجرام عليقه. تم بدء جمع السائل المنوى أسبوعيا عند عمر 28 – 36 اسبوع لتقييمه. تم تلقيح عدد من الاناث الابرى بالزكور المعامله (24 أنثى/مجموعه معامله) فى الاسبوع الاخير من االفتره التجريبيه. أظهرت النتائج أن ذّكور الارانب المغذاه على عليقه بها نانو نحاس تمتلك أعلى معنوبا (مستوى 1%) وزن جسم وعائد وزن جسم، تليها مجموعه النحاس العضوي والمعدني مقارنه بالمجموعه الضابطه. زاد نشاط انزيم الفوسفاتيز القاعدي وهرمون التستستيرون معنويا (مستوى 5%) في مجموعات المعامله وكان اعلاها فى مجموعة النانو نحاس. أظهرت تغذية ذكور الأرنب على عليقة نانو النحاس أفضل خصائص للحيوانات المنوية في السائل المنوي معنويا (مستوى 5%) بما في ذلك نسبه الحركة التقدمية، والحيوية، والشواذ وكذلك تركيز الحيوانات المنويه. كانت سلامة الغشاء البلازمى للحيوانات المنويه، اختبار الفركتوز، نشاط انزيم LDH وأختبار البيروكسيديز في البلازما المنوية أفضل في المجموعة النانو نحاس مقارنة بالمجموعات الأخرى. أظهر فحص السائل المنوي للذكور في المجموعات التجريبية المختلفة باستخدام المسح بواسطة الميكروسكوب الالكتروني أنواع ونسب أقل من الشواذ فى الحيوانات المنوية في مجموعة النانو نحاس مقارنة مع المجموعات الأخرى. كان أعلى معدل خصوبه (91,7٪) تم الحصول عليه فى مجموعه الذكور المغذاه بالنانو نحاس، ولم تختلف بشكل معنوي ذكور مجموعة النحاس المعدني (83,3%) ومجموعة النحاس العضوي (87,5%) مقارنه ب 70,8% للمجموعه الضابطه. وبناء على نتائج هذه الدراسه نوصى باضافة النحاس على شكل جزيئات النانو بمستوى 50 مليجرام/كجم عليقة حيث تؤدى هذه الاضافه الي تحسين جودة وخصائص السائل المنوى وزيادة خصوبة ذكورالارانب المستخدمه فى التلقيح الطبيعي أو التلقيح الاصطناعي وبالتالي زيادة العائد الاقتصادي في مجال إنتاج الأرانب.