

## BENEFICIAL USE OF ZINC SULFATE FOR IMPROVING SEMEN CHARACTERISTICS AND FERTILITY OF RABBIT BUCKS DURING SUMMER SEASON

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### SUMMARY

Three groups of New Zealand White rabbit bucks were fed *ad libitum* with basal diet containing 50 µg Zn/kg DM. Fresh tap water was also available at all times for three consecutive months without or with 50 or 100 mg Zn/liter in the form of zinc sulfate ( $ZnSO_4 \cdot 7H_2O$ ) to study the influence of Zn on semen quality and fertility of male rabbits during summer months. The results showed that Zn supplementation caused an increase ( $P < 0.01$ ) in semen volume, sperm cell concentration, ejaculate mass and advanced motility than control animals. Whereas decreases ( $P < 0.01$ ) in the percent of dead and abnormal spermatozoa were recorded in Zn supplemented groups. Semen pH was not significantly affected by the level of Zn supplementation.

Sperm cell concentration, sperm mass motility and advanced motility % increased ( $P < 0.01$ ) with advanced summer months, while, percentage of dead and abnormal spermatozoa declined ( $P < 0.01$ ). Ejaculate volume and semen pH were not affected by the time of sampling during summer season. At the end of the experiment (after 90 days of treatment with additional Zn), serum testosterone levels were insignificantly higher in both treated groups than in control one.

Indices of reproductive efficiency such as conception rate and the number of services per conception improved ( $P < 0.05$ ) in the rabbit bucks which received supplementary Zn in their drinking water as compared to the control. The improvement in litter size at birth was only significant ( $P < 0.05$ ) in the rabbits given 100 mg Zn/liter of drinking water.

**Keywords** : Rabbit, zinc, semen, fertility.

### INTRODUCTION

The high concentration of zinc (Zn) which is found in the male reproductive system suggests that this element has an important role in male reproduction and fertility (Stanwell-Smith *et al.*, 1983 and Madding *et al.*, 1986). There is evidence that Zn is required for normal testicular development (Prasad, 1978), spermatogenesis (Underwood and Somers, 1969 and Abbsi *et al.*, 1980) and sperm motility (Stankovic and Mikac-Deyic, 1976 and Shandhan *et al.*, 1978). Also a significant positive

correlation was found between Zn concentration in seminal plasma and in blood (Xu *et al.*, 1994). Serum Zn has been reported to be below normal in infertile oligozoospermic men (Hartoma, 1977 and Stanwell-Smith *et al.*, 1983).

Some studies indicated that supplementary zinc sulfate may be useful in the treatment of some infertile men (Marmar *et al.*, 1975 and Caldamone *et al.*, 1979). Such studies are limited in animal however.

In Egypt, many foreign breeds of rabbits were imported during the recent years to fulfill the over growing demand for animal protein. Several reports indicated that rabbits exhibited temporary marked defects in semen traits during the hot summer season (Amin *et al.*, 1987 and El-Fouly *et al.*, 1987). It has been suggested that July to September are the poorest breeding months for domesticated rabbits (Eckstein and Zuckerman, 1960). Also, serum testosterone values appeared to be lower in the summer months (Moor and Younglar, 1975).

This study was designed to show what extent that zinc sulfate supplementation can alter semen characteristics and its possible relationship to fertility of New Zealand White male rabbits during hot summer months, since, semen quality is considered a reliable guide to potential fertility.

## MATERIALS AND METHODS

All bucks used in this study were 15 mature New Zealand White rabbits of proven fertility, weighing about 3.5 kg in average. They were individually housed in wire cages in the Experimental Animal House, Faculty of Agriculture, Ain Shams University. The animals were fed *ad libitum* on balanced pelleted feed contained 50 µg Zn/kg DM, 18% crude protein, 2850 kcal/kg diet and provided with all required vitamins and minerals as recommended by NRC (1977). At the beginning of the trial, the rabbits were randomly allotted into three groups of five ones. The first group received fresh tap water without any additional zinc and served as control. The other two groups were given supplementary zinc sulfate dissolved in tap water at the rate of 50 or 100 mg Zn/liter respectively. For all groups, drinking water was available at all times during the experimental period, which extended for three consecutive months during the hot summer season (July through September). At the end of this period, blood samples were collected from each rabbit via marginal ear vein. The blood was allowed to clot, then sera were separated and kept frozen at -20°C until use. Serum testosterone (T) concentration was determined by radio-immunoassay technique using a commercial kit (COAT Count, total testosterone, Diagnostic Products Corporation, Los Angeles, U.S.A.).

All bucks were trained for artificial collection of semen. Two successive ejaculates were collected twice weekly by means of an artificial vagina. Ejaculate volume was determined after the removal of the gell mass if present. The further physical semen characters studied were sperm concentration/ml., pH, mass motility, progressive motility, dead and abnormality percentages.

After the end of Zn supplementation period, all bucks used in the study were naturally mated with 30 does to evaluate their reproductive performance. Fertility traits were recorded in terms of conception rate, number of services per conception and litter size at birth.

Data were subjected to statistical analysis of variance according to Snedecor and Cochran (1982). Differences among means were tested by multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

Zinc supplemented groups showed increase ( $P < 0.01$ ) in ejaculate volume compared with the control one, although the difference between the two levels of supplemented Zn was not significant. Also, time of sampling induced no significant effect on this character (Table, 1). Similar findings have been reported previously in goat and rabbit bucks received supplementary Zn in either drinking water or diet (Mekawey, 1988 and El-Masry *et al.*, 1994). This increase in semen volume could be attributed to the influence of abundant Zn on the activity of some accessory glands of the male reproductive system such as prostate gland and seminal vesicles (Underwood, 1977). The secretions of these glands together form about 73-93% of the semen volume and these organs are very rich in their Zn contents (Tortora and Anagnostakos, 1987).

In this study, the mean volume of ejaculates collected in summer season from Zn supplemented groups was very close or some what greater than the mean volume of ejaculates collected in winter from New Zealand White rabbit bucks in Egypt (being 0.45 ml.) as reported by Fawzy *et al.*, (1990).

Neither addition of Zn nor sampling occasion in the summer months had any significant effect on semen pH (Table 1). Amin *et al.*, (1987) found that when rabbit bucks from various breeds were exposed to high environmental temperature, the semen pH was not significantly changed during the various periods of heat exposure. In this study, the mean values of semen pH were within the normal range of semen pH in New Zealand White rabbit bucks (between 6.74 to 7.60) as reported by (More O'Ferrall and Meacham, 1968 and Macari and Machado, 1978). From Table (1), it seems that in the presence of sufficient or excessive amounts of Zn, semen reaction tends to neutrality due to its metabolism in the accessory sex glands of the male genitalia which produce the seminal plasma, particularly, the prostate gland which is known by its high affinity for Zn uptake.

Table 1 shows that sperm cell concentrations were higher ( $P < 0.01$ ) in both Zn supplemented groups as compared to control one, while no significant difference in sperm count was noted between treatment groups. Similar effects of supplementary Zn were found in men, goats and rabbits received supplementary Zn (Marmar *et al.*, 1975; Caldamone *et al.*, 1979; Mekawey, 1988; El-Masry *et al.*, 1994 and Abd El-Rahim *et al.*, 1995). Perhaps, this increase in sperm cell concentration was due to the role of Zn in the maintenance or activation of the testicular germinal epithelium in the seminiferous tubules which are responsible for the formation of spermatozoa as suggested by Underwood and Somers (1969). It seems that a certain local level of testicular Zn may be required for the development of spermatozoa, since Zn is essential for DNA synthesis and cell division (Hambidge *et al.*, 1972 and Antoniou *et al.*, 1977).

Both groups given Zn supplementation showed greater ( $P < 0.01$ ) proportion of progressively motile sperm and higher ( $P < 0.01$ ) grade of semen mass motility (wave



motion) than those of control group (Table, 2). These data agree in principle with those reported by Marmar *et al.* (1975) and Caldamone *et al.* (1979) in men and Mekaway (1988) and El-Masry *et al.* (1994) in goats and rabbits treated with supplementary zinc. In fact, the relationship between Zn and motility of sperm remains unclear. It could be suggested that Zn may play an active role in the development of the flagellar system of the sperm which is reflected on sperm motility through its passage and stay in the epididymis for complete maturation; or Zn may activate enzymes controlling flagellar system. This needs further studies. Devenson (1993) claimed that high amounts of Zn are incorporated into spermatozoa during the final phase of their maturation. Saito *et al.* (1967) found that when canine epididymal spermatozoa were bathed in  $ZnCl_2$  at 0.02 or 0.2 mM concentration, the motility of these spermatozoa was enhanced to the level of motile ejaculated spermatozoa. Furthermore, the increasing mass motility may be due to the increment in sperm cell concentration (Table, 1) as a result of Zn supplementation.

Table 1. Effect of zinc supplementation during summer on ejaculate volume, sperm cell concentration and semen pH during summer months in New Zealand White rabbits ( $X \pm SE$ ).

Semen Characters	Month	Control	Treatments		Overall $X \pm SE$
			+50 ppm Zn	+100 ppm Zn	
Ejaculate Volume (ml.)	July	0.48±0.07	0.52±0.04	0.50±0.07	0.50±0.03
	Aug.	0.40±0.04	0.50±0.04	0.56±0.02	0.49±0.03
	Sept.	0.40±0.04	0.56±0.05	0.58±0.06	0.51±0.03
Overall $X \pm SE$		0.48±0.03 <sup>A</sup>	0.53±0.02 <sup>B</sup>	0.55±0.03 <sup>B</sup>	
Sperm Cell conc. ( $\times 10^6$ /ml.)	July	154.0±12.5	180.0±6.4	173.3±10.4	169.1±6.1 <sup>A</sup>
	Aug.	157.4±9.7	198.0±7.3	181.0±5.3	178.8±6.0 <sup>A</sup>
	Sept.	170.0±11.5	228.0±9.3	236.0±3.8	211.3±9.2 <sup>B</sup>
Overall $X \pm SE$		160.4±6.3 <sup>A</sup>	202.0±6.7 <sup>B</sup>	196.8±8.4 <sup>B</sup>	
Hydrogen Ion conc. (pH)	July	7.3±0.1	7.2±0.1	7.3±0.1	7.3±0.1
	Aug.	7.2±0.2	7.1±0.1	7.1±0.2	7.1±0.1
	Sept.	7.4±0.1	7.0±0.2	7.0±0.1	7.2±0.1
Overall $X \pm SE$		7.3±0.1	7.1±0.2	7.1±0.1	

Means bearing different superscripts (A,B) within the same row or column differ significantly ( $P < 0.01$ ).

\* Averages were obtained from ninety samples in each treatment

In the present study the mean percentage of advanced motility in Zn supplemented groups were within the limits of the progressive motility of the New Zealand White rabbit semen (being 60-70%) as reported by (Blume *et al.*, 1977 and Dubiel *et al.*, 1979).

The percent means of dead spermatozoa in the New Zealand White rabbit semen in samples collected during hot summer were 19.1 (Amin *et al.*, 1987) and 18.5 (El-Masry *et al.*, 1994). Almost the same percentage was found in the control group of this study (animals without Zn supplementation) which were exposed to similar environmental conditions in Egypt (Table 2). However, dead sperm have declined

( $P < 0.01$ ) in rabbits given additional Zn (50 ppm) and also decreased ( $P < 0.05$ ) in those received 100 ppm Zn than controls.

Likewise dead spermatozoa, the percentage of abnormal sperm followed the same trend to be lower in Zn supplemented groups than in non supplemented animals, but the difference between the two treatment groups was not significant (Table 2). Similar reduction of abnormal sperm was observed in the semen of rabbits when their diet was supplemented with 35 and 170 mg Zn/kg diet on dry matter basis (El-Masry *et al.*, 1994 and Abd El-Rahim *et al.*, 1995).

Table 2. Effect of zinc supplementation on semen mass motility, percentages of advanced motility, dead and abnormal spermatozoa during summer months in New Zealand White rabbits ( $X \pm SE$ ).

Semen Characters	Month	Control	Treatments		Overall $X \pm SE$
			50 ppm Zn	100 ppm Zn	
Mass motility (Score)	July	2.26±0.25	2.48±0.23	2.26±0.22	2.33±0.13 <sup>A</sup>
	Aug.	2.40±0.10	2.90±0.19	3.10±0.19	2.80±0.11 <sup>Ba</sup>
	Sept.	2.30±0.37	3.70±0.20	3.70±0.12	3.23±0.22 <sup>Bb</sup>
Overall $X \pm SE$		2.32±0.14 <sup>A</sup>	3.03±0.17 <sup>B</sup>	3.02±0.18 <sup>B</sup>	
Advanced motility %	July	50.0±5.2	54±5.1	46±2.9	50±2.6 <sup>A</sup>
	Aug.	57.0±2.0	69±3.7	66±2.9	64±2.1 <sup>B</sup>
	Sept.	46.0±6.0	77±3.4	78±3.0	67±4.6 <sup>B</sup>
Overall $X \pm SE$		51.0±2.8 <sup>A</sup>	66.7±3.4 <sup>B</sup>	63.3±3.9 <sup>B</sup>	
Dead spermatozoa %	July	20.2±0.5	19.2±0.8	18.0±0.5	19.1±0.4 <sup>A</sup>
	Aug.	19.4±0.7	12.8±1.2	13.8±0.4	15.3±0.9 <sup>B</sup>
	Sept.	17.6±0.4	11.2±1.0	12.8±0.9	13.9±0.8 <sup>B</sup>
Overall $X \pm SE$		19.1±0.4 <sup>A</sup>	14.4±1.1 <sup>B</sup>	14.9±0.7 <sup>B</sup>	
Abnormal spermatozoa%	July	16.9±0.3	15.8±0.7	18.1±0.2	17.0±0.4 <sup>A</sup>
	Aug.	19.1±0.8	12.2±0.8	11.1±0.7	14.1±1.0 <sup>B</sup>
	Sept.	15.6±1.0	6.7±0.8	8.3±0.9	10.2±1.1 <sup>C</sup>
Overall $X \pm SE$		17.2±0.6 <sup>Aa</sup>	11.5±1.1 <sup>Bb</sup>	12.5±2.0 <sup>b</sup>	

Means bearing different superscripts (A,B,C) within the same row or column differ ( $P < 0.01$ ).

Means bearing different superscripts (a,b,c) within the same row or column differ ( $P < 0.05$ ).

\* Averages were obtained from ninety samples in each treatment

In the current study, almost all parameters of semen analysis were gradually improved as the experiment progressed throughout the summer months. These improvements were markedly obvious in the semen specimens collected in September which showed the greatest number of sperm cell concentration, the most enhanced mass or advanced motility and the lowest percentages of abnormal or dead sperm (Table 1 and 2). These effects might be attributed to changes in some environmental factors which are known to have adverse effects on semen quality such as high ambient temperature (Amin *et al.*, 1987) and long photoperiod (Soad *et*

*et al.*, 1993). While the progress of time during hot summer, the decline in ambient temperature and photoperiod duration paralleled the observed improvement in the physical characters of the semen in September. This is logical since physiological phenomena are genetically determined but modified by environment, (Kotby, 1994).

The data presented in Table 3 show that the two levels of added Zn had a significant ( $P < 0.05$ ) effect on conception rate and number of services per conception, while litter size at birth increased ( $P < 0.05$ ) only in the group received 100 mg Zn/liter of drinking water as compared with those given lower level of additional Zn (50 mg) or control group (without any supplementary Zn). Similar improvements in these traits were observed in New Zealand White rabbits when the bucks were given extra Zn in their diet (El-Masry *et al.*, 1994 and Abd El-Rahim *et al.*, 1995). However, it is possible that better fertility was obtained as a result of the improvement of semen characteristics in Zn supplemented groups, since semen quality is thought to be a reliable guide for potential fertility. This assumption has been ascertained in human beings, when eleven patients with oligospermia (sperm count less than 60 million/ml.) were given supplementary zinc sulfate for six months to one year, the mean values increased significantly for sperm count, motility index and three pregnancies were recorded during the period of treatment. (Marmar *et al.*, 1975). They speculated that the prostate gland appears to be responsive to supplementary zinc sulfate and it may hold key to some yet unknown factors necessary for successful fertilization.

Table 3. Effect of zinc supplementation for three summer months duration on some fertility traits and serum testosterone.

Traits	Treatments			Significance
	Control	50 ppm Zn	100 ppm Zn	
Number of does	10	10	10	
No. of does kidding	4 <sup>a</sup>	6 <sup>b</sup>	7 <sup>b</sup>	$P < 0.05$
% of does kidding (conception rate %)	40 <sup>a</sup>	60 <sup>b</sup>	70 <sup>c</sup>	$P < 0.05$
ToNo. of young born	25 <sup>a</sup>	39 <sup>b</sup>	59 <sup>c</sup>	$P < 0.05$
No. of service/conception	2.5 <sup>a</sup>	1.7 <sup>b</sup>	1.4 <sup>b</sup>	$P < 0.05$
Litter size at birth (kids/doe)	6.3 <sup>a</sup>	6.5 <sup>a</sup>	8.4 <sup>b</sup>	$P < 0.05$
Serum testosterone (T) ng/ml.	1.72	2.08	2.23	N.S

In the same row any two means having the same letter do not differ significantly.  
N.S. = nonsignificant.

Several reports showed that peripheral testosterone values appeared to be lower than norms in summer months (Carson and Amann, 1972; Moor and Younglai, 1975 and El-Masry *et al.*, 1994). This suggests that a concomitant impairment of testicular function may occur during the exposure to higher temperature. But, as regard to the effect of zinc on the levels of circulating testosterone, the published reports are inconsistent. In the present study, after 90 days on the drinking water supplemented with Zn, both treated groups showed insignificant higher levels of serum testosterone than control rabbits (Table 3). El-Masry *et al.* (1994) reported a significant rise in serum testosterone concentration when the diet of rabbit bucks was supplemented with 35 mg Zn/kg on the base of dry matter. These findings suggest that Zn may be implicated in testicular steroidogenesis. It is possible that Zn has important functions in Leydig cells by increasing their capacity to produce testosterone.



On the one hand, Hesketh (1982) reported that Leydig cells in the zinc-deficient boars were smaller than those from zinc-supplemented animals, also their cytoplasm stained weakly and contained many fat droplets. On the other hand, injection of neutralized zinc (zinc gluconate + arginine) intraprostatically in rats (Fahim *et al.*, 1993a) or into the cauda epididymis in dogs (Fahim *et al.*, 1993b) did not show any significant effect on serum testosterone level between controls and treated animals. So, difference in route and dose of Zn administration and species of animal may be responsible for these unexpected data. The role of Zn in male testicular physiology needs further studies.

#### CONCLUSION

These results indicate that zinc sulfate supplementation improves the quality of New Zealand White rabbit semen and hence their fertility during hot summer months. This improvement occurred in a dose-dependent fashion. The exact mechanisms of these effects still unclear, but, it is possible to explain the apparent beneficial effect of supplementary Zn on semen parameters through its active role in the metabolism of testicular tissue. While Zn has been recognized as a cofactor or constituent of certain metalloenzymes (Zn activated and Zn dependent enzymes). Zinc is also complexed with other intracellular organic compounds such as nucleic acids. So, in relation to the fact that sperm structure is very rich in proteins and enzymes, sperm therefore, require an abundance of Zn for normal development and physiological activity.

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## إستخدام نافع لكبريتات الزنك لتحسين صفات السائل المنوى وخصوبة ذكور الأرانب فى فصل الصيف

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استخدم فى هذه الدراسة ثلاث مجموعات من ذكور الأرانب النيوزيلندى الأبيض البالغه . كانت جميعها تتغذى إلى حد الشبع على عليقة واحدة تحتوى على ٥٠ميكروجرام زنك/كجم علف . وكان ماء الشرب النقى متاحا أمامهم وبحريه طوال وقت التجربة التى استمرت لمدة ثلاثة أشهر متتابعة خلال فصل الصيف الحار . أضيف إلى ماء الشرب للمجموعتين الثانية والثالثة خمسون ومائة ملليجرام من عنصر الزنك لكل لتر ماء شرب فى صورته كبريتات الزنك على التوالى . ولم يتم إضافة أى مقدار من الزنك إلى ماء شرب المجموعة الأولى التى استخدمت كمجموعة ضابطة وذلك لإختبار أثر إضافة الزنك على نوعيه السائل المنوى وخصوبه ذكور الأرانب خلال أشهر الصيف. وكانت النتائج كالتالى : أدت إضافة الزنك إلى زيادة معنوية فى حجم السائل المنوى وعدد الحيوانات المنويه والحركة الكلية لها وكذلك الحركة التقدميه بينما قلت نسب الحيوانات المنويه الميتة والشاذة معنويا فى كلتا المجموعتين المعاملتين عن مثيلاتها فى المجموعه الضابطة. ولم تتأثر قيمة الإس الهيدروجينى للسائل المنوى بإضافة الزنك إلى ماء الشرب ومع التقدم فى وقت التجربة خلال أشهر الصيف زاد تركيز الحيوانات المنويه ودرجات ونسب الحركة الكليه والتقدميه معنويا فى كل المجموعات بينما نقصت نسب الحيوانات المنويه الشاذة والميتة معنويا . ولكن لم يؤثر توقيت جمع العينات على كل من حجم القذفه وقيمة الإس الهيدروجينى للسائل المنوى معنويا فى نهاية تجربته وبعد ٩٠ يوما من المعامله أظهرت المجموعتان المعاملتان بالزنك الإضافى زياده غير معنويه فى مستوى الهرمون الذكري الرئيسى (التستوستيرون) عنه فى مجموعته المقارنه . وبقياس بعض دلائل الكفاءة التناسليه وجد أن عدد التلقيحات اللازمه للأخصاب قد انخفضت معنويا فى المجموعتين المعاملتين بالزنك فى ماء الشرب عن مجموعته المقارنه. بينما زادت نسبة الأخصاب وعدد المواليد من الأمهات التى لقحت بذكور معاملة بالزنك فى ماء الشرب .

وبقياس عدد المواليد لكل أم وجد أنها زادت معنويا فقط فى المجموعه التى تناولت ١٠٠ ملجم زنك/لتر ماء شرب.