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RESEARCH ARTICLE

Effects of Calcium Nanoparticles on Male Rat Fertility and Sperm Function

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Abstract

Calcium is an essential regulating factor in a variety of biological functions including reproduction. It is widely required for different physiological activities in spermatozoa including spermatogenesis, sperm motility, capacitation, acrosome response, and fertilization. Recent advancements in nanotechnology have broadened its potential applications in biomedicine, including improving animal reproductive aspects. Our research was planned fundamentally to examine the influence of calcium deficiency and calcium administration using Nano and commercial calcium in two doses for each type of calcium (1000 mg/Kg body weight (BW) and 500 mg/Kg BW once daily for 64 days orally) to reveal their effects on male reproductive function as well as regulatory mechanisms connected to male fertility. Semen examination, biochemical analyses, enzymatic antioxidant, lipid peroxidation, and testis histopathology were all evaluated after 64 days. Our results revealed that Serum calcium, testosterone, and ABP levels, sperm count, motility, and percentage of intact acrosomes, as well as testicular antioxidant enzymes, were all considerably lower in the calcium-free diet group, whereas sperm abnormalities and testicular Malondialdehyde were significantly higher. In calcium (Nano and commercial) administered male rats, serum calcium, testosterone, and ABP levels, sperm count, motility, percentage of intact acrosomes, and testicular antioxidant enzymes all increased significantly, whereas sperm abnormalities and testicular Malondialdehyde dropped dramatically. Overall, these results point to a strong link between Ca^{2+}, sperm function, and fertility outcomes. what's more, Ca^{2+} supplementation, particularly nanoparticles could efficiently improve male reproductive function and fertility.

Keywords: Calcium Nanoparticles, Male Rat Fertility, Semen Quality, Testosterone, Oxidative Stress.

Introduction

Calcium is the 5^{Th} of the most plentiful components in the earth and the most bountiful element in the body [1]. It is found in some foods, added to others, available as a dietary supplement, and present in some medicines [2]. Through communicating with various proteins dispersed in various cell compartments, calcium is associated with many physiological processes, for example, muscle contraction, enzyme activation, cell differentiation, immune reaction, programmed cell death and neuron activity [3-5].

Calcium homeostasis is the controlled system by which the body keeps up with satisfactory calcium levels. Disturbances of this system lead to hypercalcemia or hypocalcemia, either of them can have significant hazards for health [6]. Ca^{2+} homeostasis is fundamentally constrained by three physiological processes, including intestinal calcium absorption, renal calcium reabsorption, and bone arrangement resorption, which is principally controlled

by parathormone, calcitonin and vitamin D_3 secretion [7-10].

Many investigations have revealed a correlation among calcium and male infertility [11, 12]. Concerning semen of mammals, one of the most broadly studied elements is calcium [13]. It is pivotal for perfect motility of sperm cells, capacitation, acrosome reaction and fertilization [14].

Semen quality is affected by dietary deficiency of some trace elements which has a significant effect on the reproductive function of male. Trace elements like calcium, manganese, magnesium, copper, zinc, and selenium are constituents of semen that are essential for normal sperm function and metabolic processes [15, 16]. So, one of the most important reasons for poor quality of semen and also male fertility is the diminished level of them [15-18].

Additionally, regulation of testosterone synthesis occurs in a pulsatile way under LH. Binding of LH to its receptors on Leydig cells highly elevates cAMP and cytoplasmic Ca^{2+} levels which are both needed for steroidogenesis [19].

Recent studies have detailed that Ca^{2+} is fundamental for occurrence of steroidogenesis in Leydig cells of the testis [20]. Ca^{2+} deficiency induces male infertility through inadequate sperm motility, disturbance of chemotaxis, capacitation, acrosome reaction and steroidogenesis. Therefore, in order to fortify sperm function and all steps toward successful fertilization, adequate Ca^{2+} concentration in semen is needed [21].

Nanoparticles (NPs) are characterized by very small size, at the nanometer scale, with adaptable manufacture and high surface-area ratio. Nanoparticles can be produced from different materials including metals, polysaccharides, and proteins. Late advances in nanotechnology have widely extended its potential applications in several branches of science including medicine. This is basically credited to the designing of nanoparticles with different chemical and physical properties to be steadier, dissolvable and more biologically efficient contrasted with their relating un engineered homologues. Moreover, NPs have been progressively used for production of therapeutic formulations in the field of drug industry [22].

Drug efficiency is increased by development of nanoparticles or nanoparticle-loaded drug through a) protecting against processing and breakdown in the digestive tract and consequently boosting intestinal reabsorption and expanded oral bioavailability [23]; b) elongation of the half-life of medications available for use; c) transmitting blood-tissue barriers and conveyance to certain target tissues, or even at the level of cell; d) fast beginning and long duration of therapeutic action; and e) diminished effective dose and side effects. Several studies give far reaching outlines on the extending utilizations of nanotechnology in the field of drug production [24-26]. Late advances in technologies of nanoparticles lead to improvement of NPs characterized by anti-inflammatory, antioxidant, and antimicrobial effects [27-29]. Utilizations of NPs dependent on their antioxidative effects can be especially significant for male fertility and sperm functions [30].

Due to the great medical benefits of Nano therapy applications recent researches begin to focus its experiments on Nano therapy and physiological function especially reproduction in a hope to treat reproductive diseases and improve these important physiological functions more efficiency. Therefore, the aim of this study was to investigate the impacts of calcium and calcium nanoparticles supplementation on male reproductive function by evaluating semen picture, acrosomal membrane integrity, serum testosterone level and testicular antioxidant capacity. Also, a histopathological assessment of testicular tissue was performed.

Material and Methods

Experimental Animals

A total of 60 adult male healthy albino rats weighing from 140-170 g were obtained from laboratory animal center of military Veterinary Hospital, Nasr City, Cairo, Egypt. All animals were held in cages of stainless steel at an ambient temperature of 25±2 ˚C on a 12-hour light/ dark system with free access to food and water. Before the beginning of experiment, rats were maintained 2 weeks for acclimation to the laboratory conditions. The protocol used in this study was accepted by the institutional animal care and use committee at Zagazig University (approval No. ZU- IACUC/2/f/571 2021).

Nano calcium preparation and characterization

Nano calcium carbonate was prepared in the biochemical laboratory of military Veterinary Hospital, Nasr City, Cairo, Egypt according to Hariharan *et al.* [31] for cockle shell $CaCO_3$ Nano powder preparation. Nano calcium carbonate was characterized using transmission electron microscope in Cairo University. $CaCO_3$ Nano powder surface morphology was examined by SEM **(**JEOL-JSM 5800) scanning electron microscope operating at 25 kV accelerating voltage, Calcite is hexagonal Cube-like crystals, Figure 1.

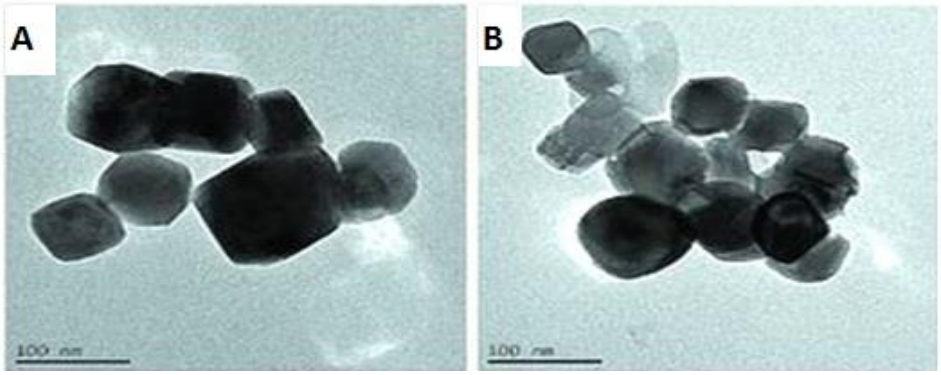

Figure 1: A) and **B)** scanning electron microscope (SEM) morphology of Nano calcium carbonates Nano powder (Calcite is hexagonal Cube-like crystals)

Commercial calcium carbonate

Calcimate 500 mg capsules purchased from El Nasr Pharmaceutical co., Cairo, Egypt.

Experimental design

The animals were separated into equal six groups, 10 animals for each as following: Group A: or control group, the animals were fed on purified rat protein diet according to Coman and Vlase [32] for 64 days (Table 1) which is prepared in the biochemical lab. of military Veterinary hospital, Nasr city, Cairo, Egypt. Blood and tissue samples were taken after 64 days. Group B: or Ca^{2+}-deficient group, the animals were fed on the same purified rat protein diet which is deficient in calcium for 64 days. Group C: Animals were fed on purified rat protein diet deficient in calcium and administered Nano calcium carbonate using stomach tube in (1000 mg/1 kg body weight (BW)) once daily for 64 days. Group D: Animals were fed on purified rat protein diet deficient in calcium and administered Nano calcium carbonate using stomach tube (500 mg/1 kg BW) once daily for 64 days. Group E: Animals were fed on purified rat protein diet deficient in calcium and administered commercial calcium carbonate, where 140 mg commercial calcium dissolved in 1cm of distilled water and administered using stomach tube (1000 mg/1kg BW), once daily for 64 days. Group F: Animals were fed on purified rat protein diet deficient in calcium and administered commercial calcium carbonate where 70 mg commercial calcium dissolved in 1cm of distilled water and administered using stomach tube (500 mg/1 kg BW) once daily for 64 days.

Table 1: Purified rat protein diet

Purified rat protein diet			
Ingredient	**g/kg diet**	**Ingredient**	**g/kg diet**
Cornstarch	465.692	**Mineral mix**	35.000
Casein (>85% protein)	140.000	**Vitamin mix**	10.000
Dextrinized cornstarch (90-94% tetrasaccharides)	155.000	**L-Cystine**	1.800
Sucrose	100	**Choline bitartrate (41.1% choline)**	2.500
Fiber	90	**Tert-butylhydroquinone**	0.008

Sampling and analysis

Blood samples were taken after 64 days after slaughtering into clean tubes, maintained for 2 hours at room temperature then centrifuged at 3000 rpm for 20 minutes, serum samples were taken into epindoorf tubes and kept at -20°C until used. Serum level of calcium was measured according to Robertson *et al.* [33] using the VITROS Calcium Slides and the VITROS 4600 chemistry systems, the VITROS 5600 integrated systems for the quantitative measurement of Calcium serum, purchased from VITROS chemistry products co**.** Bridgend, United Kingdom. Rat testosterone measurement was performed using the VITROS ECI/ECIO/ 3600 immunodiagnostic systems, the VITROS 5600 integrated systems for the quantitative measurement of testosterone hormone purchased from VITROS Immunodiagnostic Co. Bridgend, United Kingdom according to Hassan *et al.* [34]. Androgen binding protein was measured according to Rommerts *et al.* [35] using double-sandwich ELISA kit (MBS1600536) purchased from CUSABIO BIOTECH CO. Wuhan City, China. Seminal fluid were taken from tail of epididymis and assayed for semen picture according to Ikawa *et al.* [36], acrosomal membrane integrity according to Andraszek *et al.* [37]. Testicular tissue samples were taken for analysis of antioxidant activity (glutathione, catalase, superoxide dismutase and Malondialdehyde) according to Aebi [38] using double-sandwich ELISA technique. Specimens from testis were taken and fixed in 10% neutral buffered formalin for histopathological examination [39]

Statistics

One-way ANOVA using SPSS 20 was conducted on all experimental data. Post-hoc multiple comparisons were performed by Duncan's Multiple Range Test according to Kim [40].

Results

Serum calcium, Testosterone hormone and Androgen binding protein (ABP) levels

As displayed in (Table 2), calcium-deficient group exhibited a significant decrease ($p<0.05$) in serum calcium, testosterone hormone and ABP levels relative to the control group. In contrast to the calcium-deficient group, administration of Nano and commercial calcium significantly increased serum calcium, testosterone, and ABP levels (Table 2, $p< 0.05$). However, their levels were markedly increased following Nano calcium administration than in commercial calcium administration compared with control group.

Table (2): Calcium, Testosterone hormone and Androgen-binding protein levels in serum of control, Ca^{2+}-deficient, Nano and commercial calcium administered male rats.

Groups	Ca^{2+} mg / dl	Testosterone ng / ml	Androgen binding protein ng / ml
A: Control group	9.12±0.19 [b]	3.78±0.12 [c]	2.60±0.20 [e]
B: Ca^{2+}-deficient group	6.13±0.15 [a]	1.64±0.30 [d]	0.82±0.08 [f]
C: Nano Ca^{2+} (1000 mg/kg BW)	10.78[e]±0.13 [e]	4.67±0.08 [a]	18.60±1.67 [a]
D: Nano Ca^{2+} (500 mg/kg BW)	10-36±0.32 [d]	4.50±0.13 [a]	15.60±1.34 [b]
E: Commercial Ca^{2+} (1000 mg/kg BW)	9.74±0.18 [c]	4.25±0.17 [b]	11.40±0.89 [c]
F: Commercial Ca^{2+} (500 mg/kg BW)	9.26±0.24 [b]	3.99±0.12 [c]	7.56±0.40 [d]

Values (Mean ± Standard Deviation) with different small letters in the same column are significantly different at ($p<0.05$).

Table (3): Semen picture of control, Ca^{2+}-deficient, Nano and commercial calcium administered male rats.

Groups	Sperm cell Concentration x 10^6	Motility %	Abnormal sperms %
A: Control group	74.400±2.50 [d]	62.20±2.68 [e]	17.60±2.50 [b]
B: Ca^{2+}-deficient group	52.200 ±1.60 [c]	50.60±0.89 [f]	33.60±4.03 [a]
C: Nano Ca^{2+} (1000 mg/kg BW)	164.20±01.24 [e]	74.60±0.54 [a]	7.60±2.50 [d]
D: Nano Ca^{2+} (500 mg/kg BW)	138.2 ±1.54 [c]	70.40±0.54 [b]	11.40±1.14 [c]
E: Commercial Ca^{2+} (1000 mg/kg BW)	115±0.5 [bc]	68.00±2.23 [c]	15.20±1.92 [b]
F: Commercial Ca^{2+} (500 mg/kg BW)	89.20±02.24 [e]	65.00±0.70 [d]	15.40±1.14 [b]

Values (Mean ± Standard Deviation) with different small letters in the same column are significantly different at ($p<0.05$).

Sperm characteristics in rat semen

As presented in (Table 3) the calcium deficient group demonstrated a significant decrease ($p<0.05$) in sperm cell concentration and sperm motility percentage when compared to the control. However, the percentage of sperm abnormalities was considerably higher in the calcium deficient rats relative to the control group. In contrast, calcium supplementation, particularly Nanoparticles, significantly ($p<0.05$) enhanced sperm count and motility while decreasing the percentage of sperm abnormalities when compared to the calcium deficient group.

Acrosome membrane integrity

The results from this study demonstrated that calcium deficiency significantly decrease ($p<0.05$) intact acrosome % but significantly increase detached and loose acrosome % compared to control group (Table 4, Figure 2). Whereas Calcium (Nano and commercial) administration restored their concentrations to approximate the normal levels found in control group. This was better observed in Nano calcium treated rats than commercial calcium treated rats.

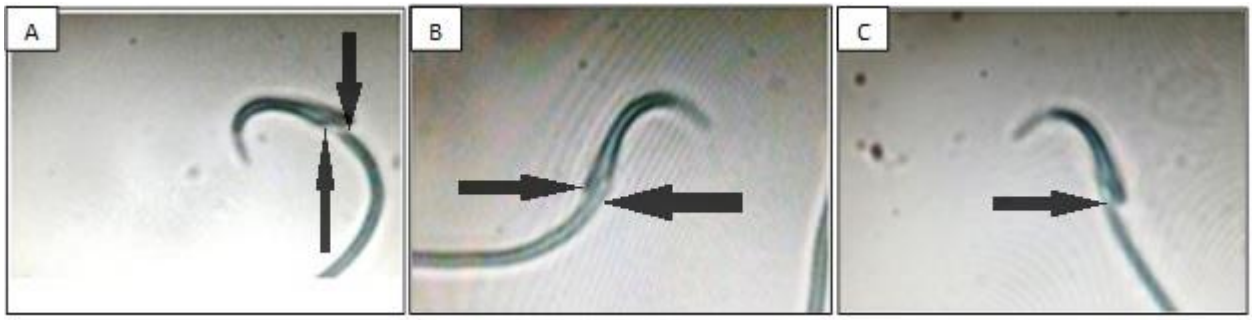

Figure (2): ***Acrosome membrane integrity***

A). Detached: sperm shows staining over the anterior portion of sperm head and unstained white band over the entire postacrosomal region. **B). Intact acrosome:** sperm has uniform staining over the sperm head**. C). Loose:** sperm shows unstained white band under the postacrosomal region.

Table (4): Acrosome membrane integrity in rat semen of control, Ca^{2+}-deficient, Nano and commercial calcium administered male rats.

Groups	Intact % of acrosome	Detached % of acrosome	Loose % of acrosome
A: Control group	88.40±1.14 ^{d}	9.20±1.09 ^{bc}	2.60±0.54 ^{b}
B: Ca^{2+}-deficient group	82.40±0.54 ^{e}	14.20±0.44 ^{a}	3.40±0.54 ^{a}
C: Nano Ca^{2+} (1000 mg/kg BW)	93.20±0.83 ^{a}	4.80±0.83 ^{e}	1.60±0.54 ^{c}
D: Nano Ca^{2+} (500 mg/kg BW)	91.20±0.44 ^{b}	6.60±0.54 ^{d}	2.20±0.44 ^{bc}
E: Commercial Ca^{2+} (1000 mg/kg BW)	89.60±0.54 ^{c}	8.40±0.54 ^{c}	2.00±0 ^{bc}
F: Commercial Ca^{2+} (500 mg/kg BW)	88.40 ±0.54 ^{d}	9.60±0.54 ^{b}	2.00c±0^{b}

Values (Mean ± Standard Deviation) with different small letters in the same column are significantly different at ($p<0.05$).

Table (5): Antioxidant capacity in testes of control, Ca^{2+}-deficient, Nano and commercial calcium administered male rats.

Groups	SOD U/mg	MDA nmol/mg	GSH ng/mg	CAT ng/mg
A: Control group	13.20±1.09 ^{e}	102.80±4.65 ^{b}	2.37±0.27 ^{e}	33.40±3.78 ^{d}
B: Ca^{2+}-deficient group	6.40±0.54 ^{f}	124.20±3.42 ^{a}	1.19±0.20 ^{f}	19.00±1.22 ^{e}
C: Nano Ca^{2+} (1000 mg/kg BW)	30.80±1.30 ^{a}	42.40±2.50 ^{f}	9.64±0.40 ^{a}	64.20±1.30 ^{a}
D: Nano Ca^{2+} (500 mg/kg BW)	27.60±1.14 ^{b}	64.00±2.34 ^{e}	8.33±0.39 ^{b}	46.80±1.30 ^{b}
E: Commercial Ca^{2+} (1000 mg/kg BW)	23.80±3.56 ^{c}	82.80±2.58 ^{d}	7.43±0.21 ^{c}	41.40±3.36 ^{c}
F: Commercial Ca^{2+} (500 mg/kg BW)	17.80±2.16 ^{d}	90.80±3.96 ^{c}	4.68±0.32 ^{d}	36.60±2.60 ^{d}

Values (Mean ± Standard Deviation) with different small letters in the same column are significantly different at ($p<0.05$).

Oxidative stress findings

In testicular tissue samples, levels of malondialdehyde (MDA, the marker of lipid peroxidation), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were evaluated (Table 5). Our findings showed that there is significant decrease of testicular tissue concentrations of SOD, CAT and GSH and increase in MDA in calcium-deficient group in comparison to the control group ($p<0.05$). Whereas supplementation of Nano and commercial calcium resulted in significant increase in SOD, CAT and GSH and decrease in MDA compared to calcium-deficient group ($p<0.05$), and the best improvement was for the group administrated Nano calcium than commercial calcium administrated group.

Histopathological findings

The control rat testes have normal histological structure of seminiferous tubules (Figure 3A) and the present study showed that the histopathological examination of rat testes of Nano and commercial calcium administered male rats were better than that of calcium-deficient group. The results represented by microscopic examination of the testis in the calcium-deficient group (Figure 3B) revealed many seminiferous tubules with degeneration and disorganized germinal epithelium. Spermatogonia were vacuolated with small dark nucleus and few spermatozoa were present in the lumen. Primary spermatocytes were reduced in number and had a small dark nucleus. Spermatogenesis was reduced with few late spermatids. Interestingly, microscopic examination of the testis in the Nano calcium groups (Figure3C) revealed high stimulatory response, normal morphology, normal histological structure of seminiferous tubules and full spermatogenesis. Many late spermatids and spermatozoa were present, complete spermatogenesis and well-organized germ cells. Microscopic examination of the testis in the groups treated with commercial calcium (Figure 3D) showing a moderate stimulatory response where many seminiferous tubules showed full spermatogenesis. Many late spermatids and spermatozoa were present. However, germ cells were slightly disorganized.

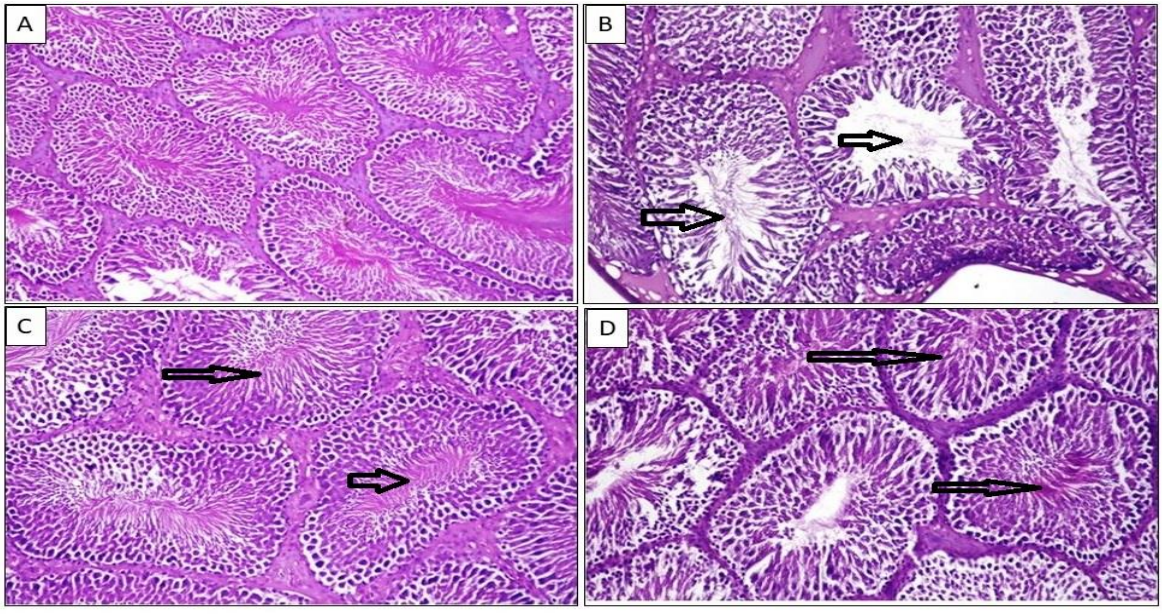

Figure 3: Testicular Histopathology: A). Testis of rat in control group, showing normal histological structure of seminiferous tubules (H&E, X200). **B).** Testis of rat in the Ca^{2+} deficient group showing many seminiferous tubules with degeneration and disorganized germinal epithelium. Spermatogonia were vacuolated with small dark nucleus and few spermatozoa present in the lumen (H&E, X200). **C).** Testis of rat in the nano calcium administered group with high stimulatory response showed almost normal histological structure of seminiferous tubules and full spermatogenesis. Many late spermatids and spermatozoa were present (H&E, X200). **D).** Testis of rat in the commercial calcium administered group showed moderate stimulatory where improved spermatogenesis in which many seminiferous tubules showed full spermatogenesis. Many late spermatids and spermatozoa were present (H&E, X200).

Discussion

The main objective of this study was to evaluate the effects of calcium deprivation and calcium (Nano particles and commercial) administration on male rat reproductive functions. Multiple investigations have shown that Ca^{2+} plays a major role in sperm control and fertilization events [41, 42]. Our results revealed a significant decrease in serum calcium, testosterone hormone and ABP levels in Ca^{2+}-deficient group as a consequence of receiving purified rat protein diet deficient in calcium for 64 days, on the other hand it revealed a significant increase in their levels in serum of Nano and commercial calcium administered male rats.

Our findings are consistent with a previous study which detailed that Ca^{2+} is required for steroidogenesis activation in testis Leydig cells [43]. Ca^{2+} chelators block steroid synthesis, whereas an expanded Ca^{2+} levels are linked to enhanced testosterone production and release in activated Leydig cells [44]. In addition, Cinar, *et al.* [45] revealed that Athletes who consume a lot of calcium and train hard have greater concentration of free and total testosterone in their blood. Also, Janszen [46] revealed that Ca^{2+} may be implicated in steroidogenesis outside the luteinizing hormone receptor-adenylate cyclase-protein kinase system,

Larriva-Sahd et al [47] was able to show that in addition to testosterone and FSH, progestins induce ABP release into the blood by kinetic examination of the disappearance of ABP from blood following testes excision and so factors affecting testosterone release and secretion reflected on ABP levels. Lee et al. [48] demonstrated that using Nano-Ca^{2+}, enhance the bio-availability or absorption of calcium.

The improvement of semen picture with administration of Nano and commercial calcium in our study is consistent with Harchegani *et al.* [21] who suggested that Calcium is a vital nutrient that functions as an intracellular second messenger. Spermatogenesis, sperm motility, capacitation, acrosome response, and fertilization are all dependent on it. Ca^{2+} shortage causes male infertility by impairing sperm motility, chemotaxis, capacitation, acrosome response, and steroidogenesis. In addition, Morton *et al.* [49] reported that lower seminal Ca^{2+} levels are linked to lower sperm motility in humans. Bassey *et al.* [50] noted that in oligospermic, azoospermic infertile men, seminal plasma Ca^{2+} was relatively smaller than in normospermic males. The much more recent report demonstrated a favorable association between the levels of seminal plasma Ca^{2+} and semen characteristics such as pH, volume and sperm numbers [51].

During sperm contact, the acrosome reaction is crucial. Spermatozoa penetrate and merge with the oocyte membrane during this phase. Many investigations have found that Ca^{2+} influx through the sperm plasma membrane's Ca^{2+} channels is required to trigger the acrosomal response and sperm fruitfulness [52]. The results from this study demonstrated that intact sperm ratio of rat semen of Nano and commercial calcium administered male rats was better than that of calcium restricted group. Furthermore, both the plasma and acrosomal membranes of mammalian spermatozoa include Ca^{2+} pumps that function as a Ca^{2+} storage system during acrosome response [53]. Moreover, Benoff *et al.* [54] revealed that Cadmium inhibits Ca^{2+} channels, which lowers sperm's capacity to undergo acrosome reaction.

The present study revealed a substantial rise in antioxidant capacity (SOD, GSH and CAT) in rat testes in Nano and commercial calcium administered male rats and significant decrease in MDA level, whereas calcium deficiency resulted in decrease antioxidant defense. This study adds to the growing body of evidence that Ca^{2+} has an antioxidant function and may protect tissues

from lipid oxidation-induced harm. Therefore, Antioxidant effects of NPs can be especially beneficial to sperm capacities and male reproductively [30]. Our findings are in line with Das *et al.* [55] who revealed that supplementing calcium and Vitamin E with fluoride resulted in a considerable improvement in testicular diseases and oxidative damage in the testis. Itoh *et al* [56] suggested that dietary calcium restriction significantly down regulated the actions of superoxide dismutase and glutathione peroxidase through decreasing Gpx mRNA expression and the expression of SOD mRNA. Also, one possible explanation is that Calcium boosts metal ion protein carriers production such as Zn and Cu lead to improvements in the cell's bioavailability. These metal ions function as co-factors necessary for many enzyme activities, especially antioxidant enzymes [57]. On the other hand, Heffner and Storey [58] concluded that calcium exhibits strong relevance to male fertility by increasing the defense of antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and by reducing oxidative stress (malondialdehyde, nitric oxide).

Furthermore Sun *et al.* [59] concluded that lower intracellular calcium levels reduced sperm count and movement, as well as the occurrence of testicular histopathological alterations. Decreasing the expression of cyclin E and CDK2, as well as up regulation of p53 and p21 expression, reduced spermatogenic cell proliferation, whereas up regulation of Bax and p-caspase 3 expressions, as well as down regulation of Bcl-xl expression, promoted spermatogenic cell apoptosis. The deficient reproductive characteristic in male mice, including hypogonadism, decreased sperm count and motility, histological defects of the testis, and dysfunctional spermatogenesis, was corrected at the point when serum calcium was standardized by the salvage diet.

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الملخص العربي

تأثير جزيئات الكالسيوم النانوية على خصوبة ذكور الجرذان ووظيفة الحيوانات المنوية

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*المعامل المركزيه للبحوث الطبيه وبنك الدم للقوات المسلحه

يعد الكالسيوم عامل تنظيمي أساسي في مختلف الوظائف البيولوجية بما في ذلك التكاثر. فالكالسيوم يستخدم على نطاق واسع في الأنشطة الفسيولوجية المختلفة في الحيوانات المنوية بما في ذلك تكوين الحيوانات المنوية ، حركة الحيوانات المنوية ، السعة ، تفاعل الأكروسوم ، والإخصاب. ولقد أدت التطورات الحديثة في تكنولوجيا النانو إلى توسيع نطاق تطبيقاتها المحتملة في مجال الطب الحيوي ، بما في ذلك تحسين الجوانب التناسلية للحيوانات.لذلك تم التخطيط لبحثنا بشكل أساسي لفحص تأثير نقص الكالسيوم وإعطاء الكالسيوم باستخدام النانو والكالسيوم التجاري في جرعتين لكل نوع من أنواع الكالسيوم (1000 مجم / كجم من الوزن الطبيعي و 500 مجم / كجم من الوزن الطبيعي مرة واحدة يوميًا لمدة 64 يومًا. عن طريق الفم) للكشف عن آثارها على الوظيفة الإنجابية للذكور فضلا عن الآليات التنظيمية المرتبطة بخصوبة الذكور. بعد 64 يومًا، تم تقييم كلا من السائل المنوي ،التحاليل الكيميائية الحيوية ،مضادات الأكسدة ، بيروكسيد الدهون والتشريح المرضي للخصية. ولقد أظهرت نتائجنا أن مستويات الكالسيوم في الدم ، والتستوستيرون ، و ABP ، وعدد الحيوانات المنوية ، والحركة ، ونسبة الأكروسومات السليمة ، وكذلك إنزيمات الخصية المضادة للأكسدة ، كانت جميعها أقل بكثير في مجموعة النظام الغذائي الخالي من الكالسيوم ، في حين كانت تشوهات الحيوانات المنوية و Malondialdehyde في الخصية أعلى بكثير. بينما في مجموعات الكالسيوم (النانو والتجاري) التي يتم تناولها في ذكور الجرذان ، زادت مستويات الكالسيوم في الدم والتستوستيرون و ABP ، وعدد الحيوانات المنوية ، والحركة ، ونسبة الأكروسومات السليمة ، وإنزيمات الخصية المضادة للأكسدة بشكل ملحوظ ، في حين انخفض تشوهات الحيوانات المنوية و Malondialdehyde في الخصية بشكل كبير. بشكل عام ، تشير هذه النتائج إلى وجود صلة قوية بين الكالسيوم ووظيفة الحيوانات المنوية ونتائج الخصوبة. علاوة على ذلك ، يمكن لمكملات الكالسيوم ، وخاصة الجسيمات النانوية ، تحسين وظيفة الإنجاب والخصوبة لدى الذكور بكفاءة..