Effect of Different Doses of Cerium Oxide Nanoparticles (CeO_2 NPs) on *In vitro* Maturation, Cleavage and Embryo Development of Buffalo Oocytes

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Abstract: The present study investigated the effects of different concentrations of cerium oxide nanoparticles (CeO_2 NPs) on viability, *in vitro* maturation (IVM), fertilization (IVF), and subsequent embryo development of buffalo oocytes. Cumulus-oocyte complexes (COCs) were collected from slaughtered female buffalo and cultured in IVM medium. COCs were incubated in an *in vitro* maturation (IVM) medium supplemented with different concentrations of cerium oxide nanoparticles (CeO_2 NPs) (10, 20, and 100µg/ml), alongside an untreated control group(0), for 22-24 hours under controlled conditions (39 °C, 5% CO_2, and 95% relative humidity). At 0, 8, and 24 hours of incubation, oocytes from all experimental groups were collected, and their viability was determined using the trypan blue exclusion method. Following maturation, oocytes from both control and CeO_2 NPs -treated groups were subsequently used for *in vitro* fertilization with frozen-thawed buffalo spermatozoa. The gametes were co-cultured for an additional 24 hours at 39°C in 5% CO_2, following the protocol. Fertilization success was initially determined by the appearance of a second polar body and further verified by cleavage to the two-cell stage. The viability of developing embryos was assessed using the trypan blue exclusion method. The results demonstrated that supplementation of the IVM medium with 20 µg/ml CeO_2 NPs significantly enhanced nuclear maturation of oocytes, yielding the highest value (95%) compared with the control and other treated groups. Moreover, the development of fertilized oocytes, as evidenced by the progression into the two-cell stage, was significantly higher at the 20 µg/ml concentration (85%) than in the other treatments. Additionally, the percentage of non-developed (degenerated) zygotes was significantly reduced at this concentration, with the lowest value recorded (15%). In conclusion, the addition of 20 µg/ml cerium oxide nanoparticles to the IVM medium appears to improve the *in vitro* developmental competence of buffalo oocytes.

Keywords: CeO_2 NPs, buffalo oocytes, IVM*,* IVF.

1. INTRODUCTION

Domestic water buffaloes (*Bubalus bubalis*) are among the most economically important livestock species worldwide. According to FAOSTAT [1], the global buffalo population exceeds 10 million, with nearly 98% in Asia, followed by 0.8% in Africa, 0.9% in South America, and 0.2% in Europe. Buffalo milk is considered nutritionally richer than cow milk, containing approximately 7.64% fat and 4.36% protein, compared to 3.9% fat and 3.47% protein in cow milk. In Egypt, the buffalo population is estimated at about 1.42 million head, contributing roughly 49.70% of total milk production and 40.95% of overall meat production [2]. Buffaloes are highly adaptable to tropical and subtropical environments, which enhances their role in global food security, particularly amid population growth and climate change. Moreover, they have a remarkable ability to convert low-quality roughages and agricultural byproducts into high-value milk and meat [3]. Despite these advantages, buffaloes generally exhibit lower reproductive efficiency than cattle. This is mainly due to weak estrus expression, a reduced ovarian follicular reserve, and a higher rate of follicular atresia [4]. Such limitations restrict the full utilization of their productive potential. *In vitro* embryo production (IVEP) has emerged as a promising assisted reproductive technology to improve genetic advancement and productivity in water buffaloes [5]. Although current IVEP systems in Buffalo have achieved *in vitro* maturation (IVM), cleavage, and blastocyst rates of 95.8%, 75.2%, and 33.4%, respectively, these outcomes are still lower than those reported in cattle [5]. This reduced efficiency is largely attributed to the lower developmental competence of oocytes matured *in vitro* compared with those matured *in vivo* [6]. Therefore, enhancing the oocyte maturation process is critical for improving oocyte quality and subsequent embryonic development. *In vitro* maturation (IVM) represents the initial and most crucial step in IVEP, forming the foundation for advanced applications such as *in vitro* fertilization (IVF), cloning, transgenesis, and stem cell research [7]. Oocyte maturation is a complex process by which oocytes acquire the ability to support embryonic development and activate embryonic DNA. It involves two phases:

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nuclear and cytoplasmic [8]. Nuclear maturation is characterized by chromosomal condensation and proper segregation, while cytoplasmic maturation involves the redistribution of organelles and the accumulation of essential biomolecules, including mRNA, proteins, and transcription factors necessary for successful fertilization and early embryonic development [9]. The composition of the maturation medium plays a critical role in regulating these processes. In particular, supplementation with proteins and key reproductive hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) has been shown to significantly enhance maturation efficiency and subsequent embryonic competence. During *in vitro* manipulation, gametes are frequently exposed to elevated levels of reactive oxygen species (ROS), exceeding their intrinsic antioxidant capacity and leading to oxidative stress [10]. Buffalo oocytes are especially susceptible to oxidative damage during *in vitro* maturation (IVM), largely due to their high lipid content [5]. Additionally, factors such as increased oxygen tension, light exposure, and environmental contaminants further exacerbate ROS generation under *in vitro* conditions [11]. Excessive ROS levels can disrupt meiotic spindle formation, cause chromosomal misalignment, and trigger apoptosis and DNA fragmentation, ultimately impairing embryo development during IVM, *in vitro* fertilization (IVF), and subsequent culture stages [12]. Enhancing the efficiency of *in vitro* embryo production (IVEP) requires optimizing IVM conditions for both oocytes and spermatozoa. Ideally, *in vitro* culture systems should replicate the physiological environment of the oviduct and uterus to better support gamete functionality and embryonic development. In this context, nanotechnology has recently emerged as a promising approach in assisted reproductive technologies (ARTs). Nanoparticles (NPs) possess unique physicochemical properties, including nanoscale dimensions, a high surface area-to-volume ratio, and increased reactivity, which make them suitable for biomedical applications [13, 14]. Recent studies have demonstrated the potential of nanoparticles to improve oocyte maturation and embryonic development in species such as buffaloes, cattle, and pigs [15-17]. Of particular interest are plant-derived or biosynthesized nanoparticles, which are considered environmentally sustainable and enriched with bioactive compounds exhibiting strong antioxidant properties. These characteristics make them especially advantageous for ART applications aimed at mitigating oxidative stress during IVM and enhancing gamete quality. Green synthesis approaches utilizing medicinal plant extracts offer a safer alternative to conventional chemical methods, minimizing toxicity and environmental impact. Plant extracts contain natural reducing and stabilizing agents, including flavonoids, phenolic acids, terpenoids, and alkaloids, which facilitate the synthesis of metal nanoparticles such as gold, silver, copper, and zinc [18]. These biosynthesized nanoparticles exhibit enhanced bioavailability and biological activity and have been increasingly explored for their potential to improve sperm quality and overall reproductive performance in livestock species. Sugarcane (*Saccharum officinarum*), a widely cultivated tropical crop, was selected as a biological source for the green synthesis of cerium oxide nanoparticles (CeO_2 NPs) owing to its rich phytochemical composition. This includes fatty acids, alcohols, phytosterols, terpenoids, flavonoids, and phenolic compounds [19]. Cerium oxide nanoparticles are well known for their potent antioxidant activity, which arises from their reversible redox cycling and efficient reactive oxygen species (ROS) scavenging capacity [20]. These properties suggest a potential role in protecting spermatozoa and enhancing capacitation during *in vitro* fertilization (IVF). Despite these promising characteristics, the effects of CeO_2 NPs on the *in vitro* fertilization of buffalo oocytes have not yet been investigated. Current knowledge is largely based on studies conducted in mouse models, with limited research on large animal models. This gap is significant, as large animals provide a closer physiological approximation to human reproductive systems, particularly in terms of follicular dynamics and oocyte maturation. Therefore, further investigation in such models is essential to assess the applicability of CeO_2 NPs in assisted reproductive technologies (ARTs), especially in addressing reduced fertility linked to compromised oocyte competence.

2. MATERIALS AND METHODS

2.1. Ethical Committee Approval

The present study was conducted at the Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Egypt. All animal-related procedures were performed in compliance with institutional ethical standards and were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Cairo University. Unless otherwise specified, all chemicals and reagents used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Synthesis of Sugarcane (CeO_2) Nanoparticles

CeO_2 nanoparticles (NPs) were synthesized utilizing *Saccharum officinarum* extract. Initially, 1g of cerium nitrate hexahydrate was dissolved in 20 ml of water. Following this, 10 ml of the plant extract was added to the solution, and the mixture was heated to 80°C for 3-4 hours until the solution reached a viscous consistency. The viscous solution was then heated in a muffle furnace at 300°C. The resultant yellowish nanoparticles were carefully collected and stored for subsequent usage and analysis. Sugarcane-derived cerium oxide nanoparticles were synthesized at the Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi, India.

2.3. Characterization and Particle Size of CeO_2

The structural analysis of synthesized CeO_2 nanoparticles was conducted using a Rigaku Ultima IV X-ray diffractometer (XRD) operating at a scan rate exceeding 8 scans per minute, with a monochromator, Cu Kα radiation at a wavelength of 1.54 angstroms, and a 2θ angle range of 20 to 85 degrees. Morphological assessment was performed using a SEM (Model No. EVO18 Zeiss) operating at an accelerating voltage of 10-12.5 kV. Fourier Transform Infrared Spectroscopy (FTIR) spectra of CeO_2 nanoparticles were acquired using a Perkin-Elmer BX FTIR spectrometer, covering the range of 400-4000 cm−1, by preparing KBr pellets from the samples. The optical properties of the synthesized nanoparticles were investigated using UV Diffuse Reflectance Spectroscopy (UV DRS).

2.4. XRD Analysis

The crystalline structure and size of the green-synthesized CeO2 NPs were analyzed by using the powder XRD technique. Figure **1** shows the XRD pattern of CeO_2 NPs in the range of 20-80°. The results demonstrate the diffraction peaks at angle 30,35,40,50,60,80,84 that corresponds to (111), (200), (220), (311), (222), (400), (311), and (400) lattice plane of F.C.C crystal structure for CeO_2 NPs [21]. The data obtained exactly matches the JCPDS file no 34-0994. This confirms the synthesis of CeO_2 NPs.

The crystalline size was calculated using the Debye-Scherrer formula (equation 1)

$$d = \frac{0.89\lambda}{\beta \cos\theta}$$

Where λ, θ and β are the wavelength of X-rays, Bragg diffraction angle, and full width half maximum, respectively. The mean crystalline size was 26.5 nm.

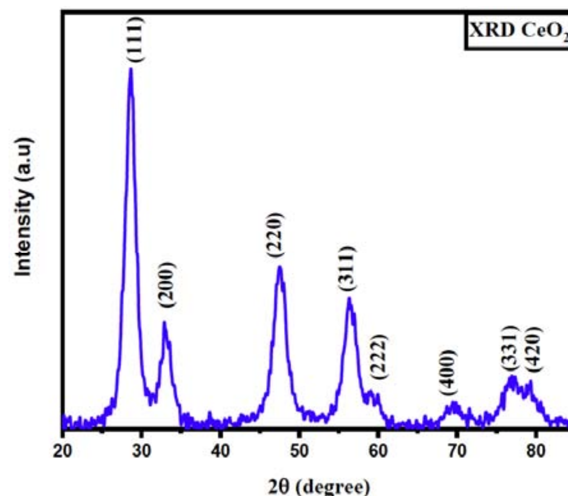

Figure 1: XRD pattern of CeO_2 NPs synthesized using Saccharum Officinalis plant extract.

2.5. Preparation of Different Concentrations of Sugarcane Nano Particles

The original suspension of chitosan nanoparticles was prepared by weighing 5 mg of CeO_2 NPs using a digital balance and dissolving them in 5 ml of Tissue culture Medium (TCM-199). This suspension was sterilized before use by passing through a 0.22 µm Millipore membrane filter fixed to a 10 ml plastic syringe to remove bacteria, and then ultrasonicated using a BRANSON Sonifier 250 device for Homogenization and dispersion. Three different concentrations of CeO_2 NPs were prepared (10, 20, and 100 µg/mL). 10 µl from the previously prepared suspension was withdrawn by an automatic pipette into a sterile Falcon tube. A sterile syringe was used to pull 1 ml of TCM-199, discarding 10 µl, and then 990 µl was added to the 10 µl of sugarcane Nano suspension to reach the final concentration (10 µg/ml). The other 2 concentrations were prepared in the same manner, but 20µl of TCM-199 was replaced by 20µl of original Nano suspension to reach the final concentration (20µg/ml), and to prepare (100µg/ml), 100µl of TCM-199 was replaced by 100µl of original Nano suspension.

2.6. Preparing Maturation Media with CeO_2 Nanoparticles

Maturation media was prepared for the culture of COCs, which was divided into four groups: control group C and treatments T1, T2, and T3, each with the addition of 0, 10, 20, and 100ul of CeO_2 NPs/1 ml of maturation media.

2.7. Collection of the Buffalo Ovaries and Recovery of Oocytes

Two hundred ovaries were collected from clinically normal buffalo females within 30 minutes post-slaughter at Moneib, Giza, Egypt, between March and October 2024. The ovaries were immediately placed in a thermos containing pre-warmed 0.9% isotonic saline maintained at 25-30 °C and transported to the laboratory within 2 hours. Upon arrival, the ovaries were rinsed several times with warm saline to eliminate residual blood and tissue contaminants, then placed in a glass beaker and kept at 37 °C until oocyte collection [22]. Cumulus-oocyte complexes (COCs) were retrieved by aspirating follicles with diameters ranging from 4 to 8 mm using an 18-gauge needle connected to a 10 ml disposable syringe. The follicular fluid containing the COCs was transferred into 15 ml conical tubes and allowed to settle at 37 °C for 15 minutes. The supernatant was then gently discarded, and approximately 5 ml of sediment containing the COCs was placed in a 90 mm Petri dish containing oocyte washing medium composed of HEPES-buffered TCM-199 (Thermo Fisher Scientific, Wilmington, DE, USA) supplemented with 10% (v/v) fetal bovine serum (FBS). The collected oocytes were inspected under a stereomicroscope (AmScope, Irvine, CA, USA) and classified into 3 grades based on morphological criteria [22].

A. Grade (I): Completely cumulus-enclosed oocytes.

B. Grade II: Partially cumulus-enclosed oocytes. C. Grade III: cumulus-Free oocytes.

2.8. Buffalo Oocyte IVM

For *in vitro* maturation (IVM), only Grade I and Grade II oocytes were selected, while those classified as Grade III were excluded. The selected oocytes were washed twice in maturation medium to remove debris and potential contaminants that could impede maturation. The maturation medium consisted of TCM-199 (9 ml) supplemented with fetal calf serum (FCS; 1 ml), follicle-stimulating hormone (FSH; 40 μl), luteinizing hormone (LH; 20 μl), gentamicin (10 μl), and antimycotic solution (100 μl). Prior to use, the medium was sterilized by filtration through a 0.22 μm Millipore membrane filter attached to a sterile 10 mL syringe to eliminate microbial contaminants. Droplets of the maturation medium (100 μl each) were prepared in sterile Petri dishes, and groups of 15-20 oocytes were placed in each droplet. To prevent evaporation and maintain sterility, the droplets were overlaid with mineral oil that had been previously sterilized using a 0.45 μm Millipore membrane filter. The oocytes were then incubated at 39 °C in a humidified atmosphere containing 5% CO_2 for 22-24 hours to complete nuclear maturation.

2.9. Evaluation of Viability of Oocytes and Cumulus Expansion

At 0, 8, and 24 hours of incubation, oocytes from all experimental groups were collected, and their viability was determined using the trypan blue exclusion method. Based on the degree of dye penetration, immature oocytes were evaluated as follows: those that did not absorb the stain were considered viable, whereas those that were completely stained were regarded as non-viable. After 22-24 hours of maturation, the extent of cumulus cell expansion was examined under a stereomicroscope, and the oocytes were categorized into three groups following the criteria of [23].

A. CE+: Full expansion of cumulus layers.

B. CE+/-: Moderate expansion of cumulus layers, where 70% of cumulus cells were homogeneously spread.

C. CE-: Slight or no expansion.

2.10. Buffalo Oocyte IVF

IVF was performed by incubating mature oocytes with sperm under controlled laboratory conditions. Cumulus-oocyte complexes (COCs) were first subjected to *in vitro* maturation for 24 hours at 39°C in an atmosphere containing 5% CO_2. Following maturation, the oocytes were exposed to frozen-thawed bull semen that had been cryopreserved in a Tris-egg yolk-glycerol extender enriched with cerium oxide nanoparticles (CeO_2 NPs) at concentrations of 0 (control), 10, 20, and 100μg/ml. The gametes were co-cultured for an additional 24 hours at 39°C in 5% CO_2, following the protocol described by [23]. Fertilization success was initially determined by the appearance of a second polar body and further verified by cleavage to the two-cell stage. The viability of developing embryos was assessed using the trypan blue exclusion method. The study was repeated 12 times, utilizing a total of 600 oocytes, with 120 oocytes assigned to each treatment group.

2.11. Statistical Analysis

Data normality was assessed using the Kolmogorov-Smirnov test. Results were expressed as

Mean ± standard error of the mean (SEM). Differences among treatments were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Recovery rates and comparisons between incubated and non-incubated cumulus-oocyte complexes (COCs) were analyzed using an independent-samples t-test. Nuclear maturation data were expressed as percentages and analyzed using the chi-square test. Statistical significance was set at $P < 0.05$ for all analyses. Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., New York, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of CeO_2 Nanoparticles on Viability and IVM of Buffalo Oocytes

The findings of the present study indicated that there were no significant differences in the proportion of viable oocytes among all experimental groups at the initial time point (0 h). However, after 8 and 24 hours of incubation, significant differences ($P < 0.05$) emerged between the control group and those supplemented with varying concentrations of cerium oxide nanoparticles (CeO_2 NPs), as shown in (Table **1**; Figure **2**). Notably, supplementation of the maturation medium with 20 µg/ml CeO_2 NPs (treatment T2) resulted in the highest maturation rate (95%), compared to 65% in the control group (Table **2**; Figure **3**). A progressive decline in oocyte viability over time was observed across all groups; however, this reduction was more pronounced in the control group than in the nanoparticle-treated groups. Importantly, CeO_2 NP supplementation did not exhibit any cytotoxic effects on buffalo oocytes, even at the highest tested concentration (100µg/ml). After 24 hours of incubation, statistically significant differences ($P < 0.05$) among treatments were observed, with T2 consistently demonstrating superior oocyte maturation. Overall, the inclusion of CeO_2 NPs in the culture medium, regardless of concentration, had a positive impact on oocyte viability and maturation and was associated with enhanced cumulus cell expansion. Furthermore, supplementation with 20 µg/ml CeO_2 NPs significantly improved nuclear maturation, fertilization rates, and subsequent embryonic development. These beneficial effects are likely attributable to the strong antioxidant properties of CeO_2 NPs. CeO_2 NPs possess strong antioxidant properties attributed to their reversible redox cycling and ability to scavenge ROS [20]. These characteristics suggest a potential role in protecting sperm cells and enhancing sperm capacitation during IVF procedures. To date, however, no studies have examined the effects of CeO_2 NPs on *in vitro* fertilization of buffalo oocytes. Supporting this interpretation, previous studies in sheep have demonstrated that low concentrations of CeO_2 NPs (44 µg/ml) during *in vitro* maturation significantly increased blastocyst yield, total cell number, inner cell mass, and trophectoderm cell populations, compared with both the control and high-dose groups. Additionally, genes associated with apoptosis and oxidative stress in cumulus cells were found to be downregulated following treatment with low concentrations of CeO_2 NPs [24]. These findings are consistent with earlier reports highlighting the redox-regulatory properties of CeO_2 NPs, which act as effective scavengers of reactive oxygen species (ROS) and provide protection against oxidative stress in both *in vivo* and *in vitro* systems [25]. Cerium oxide nanoparticles have gained considerable attention among engineered

Table 1: Effect of Different Concentrations of CeO_2NPs on Number and Percentages of Viable Buffalo Oocytes at Different Cultured Times during IVM.

Concentrations of CeO_2NPs	No. of collected oocytes	Trypan blue exclusion test		
		0 h	8 h	24 h
Control (0 µg/ml)	100	100%^{Aa} (100/100)	75%^{Bb} (75/100)	65%^{Bb} (65/100)
10 µg/ml	100	100%^{Aa} (100/100)	85%^{Aa} (85/100)	80%^{Aa} (80/100)
20 µg/ml	100	100%^{Aa} (100/100)	98%^{Aa} (98/100)	95%^{Aa} (95/100)
100 µg/ml	100	100%^{Aa} (100/100)	90%^{Aa} (90/400)	85%^{Aa} (85/100)

*Values within parenthesis indicate no. of cultured oocytes/no. of collected ones.
Percentages that carry different letters (a,b) within a column are significantly different at $P < 0.05$.
Percentages that carry different letters (A, B) within a row are significantly different at $P < 0.05$.

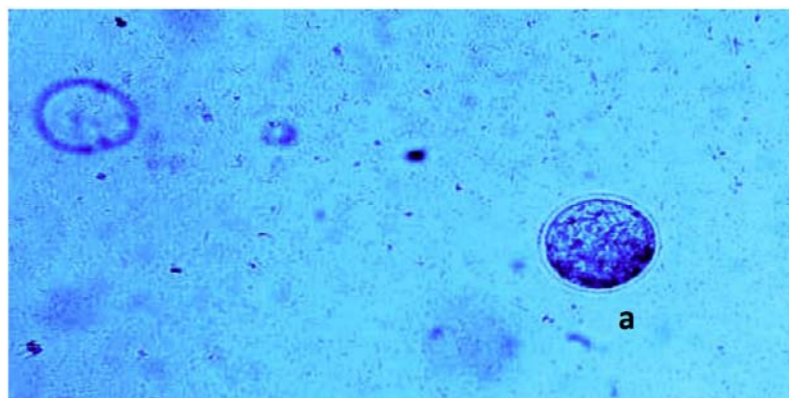

Figure 2: Viability assessment of buffalo oocytes cultured in TCM-199 with CeO_2 nanoparticles using Trypan Blue staining, showing dead oocytes in blue (a).

Table 2: Effect of Different Concentrations of CeO_2NPs Supplemention on Cumulus Expansion Percentages and Maturation Rates during IVM of Buffalo Oocytes

Concentrations of CeO_2 NPs	No. of oocytes cultured	Grades of cumulus expansion			Maturation rate (%)
		+	++	+++	
Control (0 µg/ml)	100	15%^{Bb} (15/100)	25%^{Bb} (25/100)	60%^{Bb} (60/100)	65%^{Bb} (65/100)
10 µg/ml	100	5%^{Aa} (5/100)	15%^{Aa} (15/100)	80%^{Aa} (80/100)	80%^{Aa} (80/100)
20 µg/ml	100	3%^{Aa} (3/100)	7%^{Aa} (7/100)	90%^{Aa} (90/100)	95%^{Aa} (95/100)
100 µg/ml	100	5%^{Aa} (5/100)	10%^{Aa} (10/100)	85%^{Aa} (85/100)	85%^{Aa} (85/100)

*Values within parenthesis indicate no. of cultured oocytes/no. of collected ones.
Percentages that carry different letters (a,b) within a column are significantly different at $P < 0.05$.
Percentages that carry different letters (A, B) within a row are significantly different at $P < 0.05$.

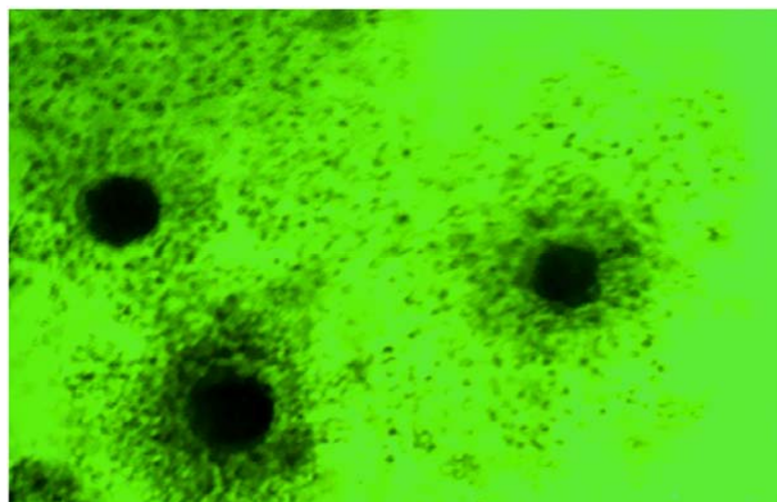

Figure 3: Cumulus expansion of buffalo oocytes following *in vitro* maturation of COCs in IVM medium supplemented with CeO_2 Nanoparticles (400x by stereo microscope).

nanomaterials due to their unique redox behavior, driven by surface crystalline defects that allow the coexistence of Ce^{3+} and Ce^{4+} oxidation states [26]. This redox flexibility underlies their potent antioxidant capacity. Their ROS-scavenging activity has been extensively documented across various biological models, where they mitigate oxidative damage and reduce apoptosis. For instance, in PC12 cells,

Table 3: Effect of Different Concentrations of CeO_2 NPs Supplemention to the Capacitation Media on IVF Parameters of Buffalo Oocytes

Concentrations of CeO_2 NPs	IVF Parameters				
	No. of inseminated Oocytes	Trypan blue exclusion test (Live zygotes)	Zygotes Developed With 2^{nd}Pb (2 cell stage)	Non-Developed zygotes	Fertilization rate
Control (0 µg/ml)	65	$60\%^{Bb}$	60%	40%	60%
10 µg/ml	80	$75\%^{Aa}$	70%	30%	70%
20 µg/ml	95	$90\%^{Aa}$	85%	15%	85%
100 µg/ml	85	$80\%^{Aa}$	75%	25%	75%

Percentages that carry different letters (a,b) within a column are significantly different at P <0.05.
Percentages that carry different letters (A, B) within a row are significantly different at P <0.05.
2nd Pb = Second Polar bodies; 2 PN = two pronuclei; 2 cell = Fertilized oocytes (2 cell stage).

exposure to CeO_2 NPs (20 and 50 µg/ml for 72 hours) resulted in a general downregulation of antioxidant-related gene expression, suggesting that these nanoparticles function as effective exogenous ROS neutralizers, thereby reducing dependence on endogenous cellular antioxidant systems [24,25].

3.2. Effect of CeO_2 Nanoparticles on IVF of Buffalo Oocytes

The present study revealed a significant impact of CeO_2 nanoparticles on IVF of buffalo oocytes. The highest number of viable zygotes (90%) was observed in the group treated with 20 µg/ml CeO_2 nanoparticles, compared with the other experimental groups (Table **3**). Likewise, key developmental parameters of cultured zygotes, at this concentration relative to the other treatments (Figures **4-6**). In contrast, the proportion of degenerated (non-developed) zygotes was significantly reduced (15%) in this group. Buffalo oocytes are especially susceptible to oxidative stress during *in vitro* maturation (IVM) due to their high lipid content. Several factors, including elevated oxygen levels, light exposure, and environmental contaminants, contribute to the generation of reactive oxygen species (ROS) during *in vitro* culture. Excessive oxidative stress can disrupt spindle formation and chromosomal alignment, trigger apoptosis, and lead to DNA fragmentation, ultimately impairing oocyte developmental competence during IVM, IVF, and subsequent culture stages [27-30]. Therefore, incorporating an effective antioxidant strategy during *in vitro* procedures is crucial for maintaining oocyte quality. In this study, we examined the role of CeO_2 nanoparticles, a well-known antioxidant, in enhancing the *in vitro* maturation and developmental potential of buffalo oocytes. Cerium oxide nanoparticles (CeO_2 NPs) are widely recognized for their ability to scavenge ROS across different biological systems. They have demonstrated protective

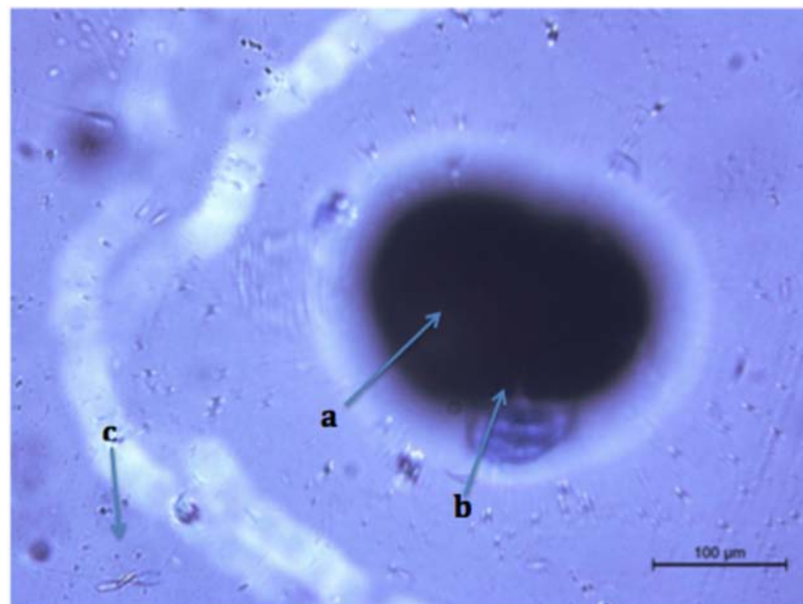

Figure 4: Two- cells embryo (a) with the 2nd polar body (b) and some of rest of sperm cells (c).

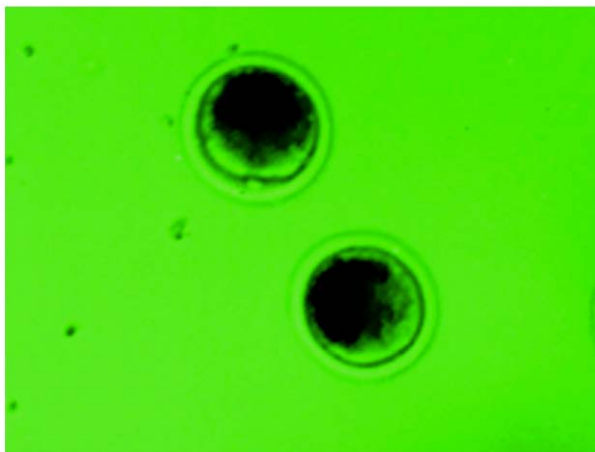

Figure 5: A fertilized oocyte exhibiting a second polar body at 22–24 hours post-fertilization.

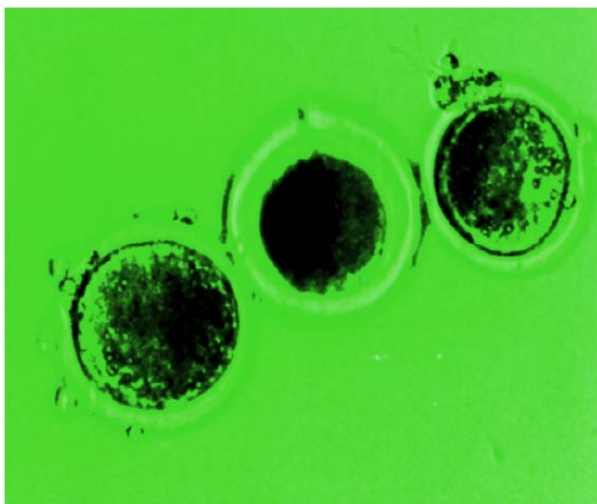

Figure 6: A fertilized oocyte reaching the two-cell stage at 36 h post-fertilization.

effects and reduced apoptosis under oxidative stress conditions, both *in vivo* and *in vitro*. For instance, in PC12 cells, treatment with CeO_2 NPs at concentrations of 20 and 50 µg ml^{-1} for 72 hours led to a general decrease in the expression of genes involved in cellular antioxidant defense [25]. This finding indicates that CeO_2 NPs act as powerful exogenous ROS scavengers, thereby reducing the need to activate endogenous antioxidant pathways.

4. CONCLUSION

Our findings indicate that supplementing the maturation medium with CeO_2 nanoparticles at a concentration of 20 µg/ml enhances both the viability and maturation rates of buffalo oocytes *in vitro*. These findings highlight the potential of CeO_2 nanoparticles as a promising antioxidant additive to culture media for improving the developmental competence of buffalo oocytes under *in vitro* conditions.

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DATA AVAILABILITY

Data sharing is not applicable.

DECLARATION OF INTEREST

All authors declare any conflict of interest.

AUTHORSHIP CONTRIBUTION STATEMENT

Saber Abd-Allah and Khaled El-Shahat: Data curation, Formal analysis, Investigation, Methodology, Writing-review & editing. Yehia R. EL-Baghdady and Shrouk H. Hamed: methodology, drafting, and supervision.

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